Published in final edited form as:

Domest Anim Endocrinol. 2011 January ; 40(1): 30–39. doi:10.1016/j.domaniend.2010.08.004.

Maternal obesity in the ewe results in reduced fetal pancreatic β -cell numbers in late gestation, and decreased circulating insulin concentration at term

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Abstract

About 30% of U.S. women of reproductive age are obese, a condition linked to offspring obesity and diabetes. This study utilized an ovine model of maternal obesity, where ewes are overfed to induce obesity at conception, and throughout gestation. At mid-gestation, fetuses from these obese ewes are macrosomic, hyperglycemic and hyperinsulinemic, and exhibited markedly increased pancreatic weight, and β -cell numbers compared to fetuses of ewes fed to requirements. This study was conducted to establish fetal pancreatic phenotype and function in late gestation and at term in this ovine model. Multiparous ewes were fed a control (C, 100% NRC recommendations) or obesogenic (OB, 150% NRC) diet from 60 days before conception to necropsy at day 135 of gestation, or to lambing. No differences were observed in fetal size or weight on day 135, or in lamb birth weights between C and OB ewes. In contrast to our previously published results at mid-gestation, pancreatic weights ($P < 0.01$) and β -cell numbers ($P < 0.05$) of OB fetuses were markedly lower than those from C fetuses, whereas β -cell apoptotic rate was increased ($P < 0.05$) in day 135 OB versus C fetuses. At birth, blood insulin concentration was lower ($P < 0.05$), and glucose higher ($P < 0.05$) in new born lambs from OB versus C ewes. These data demonstrate differential impacts of maternal obesity on fetal pancreatic growth and β -cell numbers during early and late gestation. During the first half of gestation there was a marked increase in pancreatic growth, β -cell proliferation, and insulin secretion, followed by a reduction in pancreatic growth and β -cell numbers in late gestation, and resulting in reduced circulating insulin at term. It is speculated that the failure of the pancreas to return to a normal cellular composition and function postnatally could result in glucose/insulin dysregulation, leading to obesity, glucose intolerance and diabetes in postnatal life.

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Keywords

obesity; gestation; fetus; β -cell; apoptosis; sheep

1. Introduction

Obesity is rapidly increasing worldwide [1–3], with approximately 65% of the U.S. adult population classified as overweight, and one-half of those considered obese [2]. Obesity is associated with predisposition to metabolic diseases including decreased pancreatic β -cell function, glucose intolerance and type-2 diabetes [4,5]. Over 80% of people with diabetes are also obese or overweight [3], suggesting a strong association between these conditions. Significant evidence has accumulated indicating that maternal nutritional status affects the intrauterine environment during gestation, inducing abnormal fetal growth and development and resulting in offspring obesity, decreased pancreatic β -cell function, diabetes and other metabolic disease in later life [6–9]. Recent human studies report that even when birth weights were similar, newborns from obese mothers exhibited a higher body fat percentage, and were more prone to insulin resistance and diabetes than newborns from nonobese mothers [10,11].

Increased apoptosis and/or decreased proliferation of β -cells can lead to insulin insufficiency, resulting in diabetes [12,13]. Exposure to higher concentrations of glucose, free fatty acid and other metabolites have been linked to β -cell apoptosis [14,15]. Maternal obesity induced by high fat feeding in rodents resulted in offspring with glucose intolerance and significantly higher triglycerides level in postnatal life, which could lead to various metabolic syndromes such as obesity and diabetes [16,17].

To date, few studies have been conducted to investigate the impacts of maternal obesity at conception and throughout gestation on the trajectory of fetal pancreatic growth, development and function. We have established an ovine maternal obesity model [18–21] and previously reported that fetuses of obese ewes were ~30% larger at midgestation compared to those of control ewes fed to requirements using the same experimental paradigm utilized in the present study [19,20]. Both obese ewes and their enlarged fetuses at midgestation exhibited greater blood concentrations of glucose, insulin and cortisol than ewes fed to requirements [19,20]. Further, fetuses from obese ewes exhibited a markedly increased pancreatic weight and β -cell proliferation than fetuses from control fed ewes at midgestation [20]. The objective of the current research was to establish the impact of diet-induced preconceptional and gestational maternal obesity on fetal growth and pancreatic β -cell development and insulin secretion in late gestation and at term in the sheep.

2. Materials and Methods

2.1 Animals

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. From 60 days before conception (first day of mating = day 0) and throughout gestation, 3–4 year old multiparous (2 to 3 prior successful pregnancies) Rambouillet/Columbia cross ewes carrying twin fetuses were fed either a highly palatable ration at 100% (control, C; n=11) of National Research Council (NRC) [22] recommendations or 150% (obesogenic, OB; n=13) of NRC recommendations as previously described [18,20,23]. All ewes were weighed weekly and diets adjusted for changes in body weight. On day 135 of gestation, 4 C and 6 OB ewes were euthanized and exsanguinated to collect tissues. The remaining ewes in each group (7 C and 7 OB) remained on their respective diets and were allowed to lamb.

2.2 Blood and tissue collection

Immediately prior to necropsy, ewes were weighed and sedated with an intravenous injection of ketamine (22 mg/kg) and maintained under inhalation anesthesia (isoflurane, 2.0 to 2.5%). Maternal blood was collected via jugular venipuncture into a chilled tube (heparin plus sodium fluoride; 2.5 mg/mL; Sigma, St. Louis, MO), centrifuged, and plasma collected and frozen at -80°C until assayed for glucose and insulin. After opening the gravid uterus, umbilical venous plasma samples were collected from each fetus and processed as described for maternal blood.

After exsanguination under isoflurane anesthesia, fetal weights, crown rump lengths, and sex were determined for all twin fetuses (8 C and 12 OB). The fetal pancreas from one twin (selected at random) of each set (4 C and 6 OB) was immediately collected, weighed and the tail portion of pancreas was placed in a tissue cassette (Tissue Tek, Miles Labs, Elkhart, IN), fixed with 4% (w/v) paraformaldehyde in a phosphate buffer (0.12 M; pH 7.4) and paraffin embedded as previously described [20]. The pancreas from the other twin fetus (4 C and 6 OB) was used for determination of islet insulin content as describe below. Weights of other selected fetal organ and tissues were also recorded.

2.3 Glucose and Hormone Analyses

Plasma glucose was analyzed via the glucose hexokinase reagent (Liquid Glucose Hexokinase Reagent Set, Pointe Scientific, Inc., Canton MI) colorimetric assay using 96-well plates as previously described [9]. Samples were run in triplicate. The intra-assay and inter-assay CVs were 3% and 5%, respectively, and the sensitivity was 25mg/dl. Serum insulin was measured by RIA (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA) as previously validated in our laboratory [9]. Intra-assay CV was < 3%, while inter-assay CV was < 5%, assay sensitivity was 0.10 ng/mL. Serum Cortisol was measured by RIA kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA) as previously validated in our laboratory [24]. Intra-assay CV for cortisol was < 3% and sensitivity was 2.5ng/mL.

2.4 Isolation of pancreatic islets and measurement of insulin content

At necropsy, the pancreas was carefully dissected from the fetuses, weighed, and islets were isolated as previously described [25]. Briefly, the pancreas was slowly infused with an islet separation solution until fully distended. The solution was made up of Krebs Ringer buffer (KRB; 118 mm NaCl, 2.5 mm CaCl_2 , 4.8 mm KCl, 25 mm NaHCO_3 , 1.2 mm MgSO_4 , KH_2PO_4 , pH 7.3) with Liberase BlendZyme III (0.175mg/mL; Roche, Indianapolis, IN) and 0.2% DNase I (Roche). The pancreas was then put into an additional islet separation solution at 37.5°C for 20–30 min. Digested tissue was then diluted with KRB (on ice) which contained 0.25% BSA by allowing the islets to settle for 10 min. Islets were purified by centrifugation at 800 g for 20 min with a gradient solution of 2:1 Histopaque (1.119 g/mL; Sigma) and KRB with BSA. After centrifugation, the cell clusters were removed from the interface and washed with KRB with BSA media followed by a 1min centrifugation. Islets were then hand picked and collected. The islets were washed in KRB with BSA media and equilibrated to 37.5°C and 5% $\text{CO}_2/95\% \text{O}_2$. Ten to 15 fetal islets from C and OB fetuses were hand picked and placed in a 1.5 mL tube ($n \geq 3$ per animal). Islet insulin content was extracted by incubating with ICB (70% ethanol and 1 mol/liter HCl) at -20°C for 12 h and debris removed by centrifugation and collected for DNA concentration test. Islet DNA concentration was determined using the PicoGreen dsDNA Quantitation Kit (Invitrogen, Carlsbad, CA). Concentration of insulin was determined by RIA as described above and normalized for both islet number and DNA concentration.

2.5 Immunohistochemistry

2.5.1 Determination of insulin positive cells—As previously described [20], six 5 μ m sections were obtained from paraffin-embedded blocks of C and OB fetal pancreatic tissue, maintaining a 50 μ m distance between the sections. Sections were then deparaffinized and rehydrated by routine methods before heating in 95° C water bath for antigen retrieval [20]. Briefly, nonspecific antigenic sites were blocked by a 60 min incubation in 1.5% normal goat serum (Vector Laboratories, Burlingame, CA) in PBS with 0.05% Tween 20 (Bio-Rad Laboratories Inc., Hercules, CA) before the sections were incubated with guinea pig anti-porcine insulin (Dako, Carpinteria CA, 1:500) antibodies at 4° C overnight, followed by fluorescent labeled secondary antibodies: Rhodamine labeled goat anti-guinea pig (Millipore, Billerica, MA 1:500) for 60 min at 22° C. Images were visualized using an Olympus BX50 microscope and captured digitally using a Retiga EXiFast camera. The areas of pancreatic islets were measured in a 400 $\times$ magnification were taken using QED Imaging software (Media Cybernetics, Silver Spring, MD) for 20 fields of view from 6 sections per fetus. Numbers of insulin positive cells were counted per field of view by two experienced individuals blinded to treatment and the counts averaged as previously described [20].

2.5.2 Measurement of β -cell apoptosis—Following dewaxing, rehydrating, and antigen retrieval as described above, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick translation end labeling (TUNEL) were performed on three pancreatic sections per fetus with the In Situ Cell Death Detection POD Kit (Roche Molecular Biochemicals, Mannheim, Germany) per manufacturer's instructions [20]. The sections were incubated with 50 μ l of TUNEL reaction mixture or 50 μ l of label solution alone (negative control) for 60 min at 37 °C. Sections were incubated with guinea pig anti-porcine insulin (Dako, Carpinteria, CA; 1:500), at 4 °C overnight, and Rhodamine labeled goat anti-guinea pig (Millipore, Billerica, MA, 1:500) at 22°C for 60 min. Slides were mounted in VECTASHIELD mounting medium with DAPI as previously described [20].

2.5.3 Measurement of β -cell proliferation and mitosis—Three sections were incubated with guinea pig anti-porcine insulin (1:500 dilution; Dako, Carpinteria CA), and rabbit anti-proliferating cell nuclear antigen (PCNA) (1:250 dilution; Santa Cruz Biotechnology (Santa Cruz, CA) for the DNA replication assay, or incubated with guinea pig anti-porcine insulin (1:500; Dako, Carpinteria, CA) and rabbit anti-phospho-histone H3 (pHH3) (1:400 dilution; Upstate, Lake Placid, NY) for the mitosis assay at 4 °C overnight [20]. Sections were incubated with fluorescent labeled secondary antibodies: Rhodamine labeled goat anti-guinea pig for insulin (Millipore, Billerica, MA, 1:500) and AlexaFluor 488 labeled goat anti-rabbit IgG (1:500) for PCNA and pHH3 by incubating for 60 min at 22°C. Finally, sections were mounted in VECTASHIELD mounting medium with DAPI (4',6 diamidino-2-phenylindole; Vector Laboratories, Burlington, CA) to identify fluorescing cell nuclei. Insulin and PCNA positive cells (for DNA replication assay) or insulin and pHH3 positive cells (for mitosis assay) were counted as previously described [20].

2.5.4 Morphometric analysis—Ten to 20 fields of view were photographed at 400 $\times$ magnification using QED Imaging software (Media Cybernetics, Silver Spring, MD) for each section as previously described [20]. The percentage of PCNA stained insulin positive cells were determined by evaluating >2,000 cells within 3 pancreatic sections per fetal pancreas, with each section separated from the next by 100 μ m. The percentage of pHH3/insulin positive cells was determined by evaluating >3,000 cells within 3 pancreatic sections per fetal pancreas and TUNEL positive cells determined by evaluating >2,000 cells within 3 pancreatic sections per fetal pancreas separated from each other at least by 50 μ m as previously described [20].

2.6 Statistical analysis

Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System). Means separation was performed using LSMEANS. Data are presented as least square means $\pm$ SEM, and differences considered significant at $P \leq 0.05$, with a tendency at $P \leq 0.10$.

3. Results

No differences were observed in ewe body weights prior to diet initiation for ewes assigned to C and OB groups which averaged 74.3 ± 2.6 and 73.2 ± 3.5 kg, respectively. Body weight of ewes fed the OB diet increased linearly from diet initiation to conception ($P < 0.01$), mid-gestation ($P < 0.01$), and late-gestation (Figure 1; $P < 0.01$). No difference in body weight was observed in C ewes from diet initiation to conception, a modest increase from conception to mid-gestation ($P < 0.05$) and a more marked increase from mid-gestation to late-gestation (Figure 1; $P < 0.01$). Body weights of ewes on the OB diet remained markedly greater than that of C ewes from conception through late-gestation ($P < 0.01$).

On day 135, no differences were observed in fetal weight (5.0 ± 0.2 vs. 4.7 ± 0.2 kg), empty body weight (4.0 ± 0.1 vs. 3.6 ± 0.2 kg), crown rump length (58.1 ± 0.7 vs. 56.5 ± 1.0 cm), abdominal circumference (35.4 ± 0.6 vs. 34.3 ± 0.8 cm) and thoracic circumference (36.5 ± 0.4 vs. 36.7 ± 0.6 cm) between C and OB fetuses. No differences were observed for fetal organ weights of C and OB ewes with the exception of the kidney and pancreatic weights, which were decreased both in absolute terms, and as a percent of fetal body weight in OB versus C fetuses (Table 1).

No differences were observed in insulin or cortisol concentrations in maternal blood of C or OB ewes on day 135 of gestation, whereas glucose concentrations tended to be greater ($P = 0.07$) in OB ewes compared with C ewes (Table 2). No significant differences were observed in glucose, insulin and cortisol concentration in fetal blood in C and OB groups (Table 2).

Pancreatic β -cell numbers per unit area (mm^2) of islet tissue were reduced ($P < 0.05$) in the pancreas of d 135 fetuses from C versus OB ewes (185.2 ± 37.3 vs. 101.0 ± 6.5 ; Figure 2, panel A). Fetal pancreatic β -cell apoptotic rate was markedly increased ($P < 0.01$) in OB compared with C ewes (3.6 ± 0.1 vs. 2.1 ± 0.3 %; Figure 2, panel B). In contrast, no significant differences were found in the markers for cellular DNA replication (PCNA) and mitosis (pHH3) in the pancreas of fetuses from C and OB ewes (pHH3, 1.0 ± 0.1 vs. 1.2 ± 0.3 % and PCNA, 7.3 ± 0.4 vs. 6.4 ± 0.2 % respectively; Figure 2, panel C and D). No differences were observed in numbers of pancreatic islets per unit area of pancreas.

In contrast to β -cell number, insulin concentrations of pancreatic islets were increased ($P < 0.05$) in fetuses from OB versus C ewes. When the insulin concentrations were normalized to both islet number (C vs. OB; 0.5 ± 0.1 vs. 2.1 ± 0.4 ng/mL; $P < 0.05$; Figure 3, panel A) and DNA concentration (C vs. OB; 1.6 ± 0.7 vs. 8.5 ± 2.0 ng/mL; $P < 0.05$; Figure 3, panel B) the results remained the same.

Glucose concentrations in the blood of lambs collected immediately after birth and prior to suckling were higher (105.7 ± 14.0 vs. 36.4 ± 6.3 mg/dL; $P < 0.01$; Figure 4, panel A), and insulin concentrations were lower (0.4 ± 0.1 vs. 1.8 ± 0.7 ng/mL; $P < 0.05$; Figure 4, panel B) in OB than C dietary groups. Survival rates of lambs was unaffected by maternal dietary group (C or OB), averaging 100% in both groups.

4. Discussion

In contrast to our previous observations at mid-gestation (Day 75) in the same obese sheep model, where maternal obesity markedly increased fetal pancreatic weight and β -cell numbers [20], we observed a marked reduction in fetal pancreatic weight and an accelerated β -cell apoptosis at late gestation (Day 135) when maternal obesity was continued throughout pregnancy. Interestingly, even though β -cell numbers were markedly reduced in OB fetuses, higher insulin concentration were observed in pancreatic islets of OB than C fetuses on day 135, suggesting increased synthesis/storage of insulin in the remaining β -cells of OB fetuses, which is a common compensatory mechanism observed in mammals [12,26]. At term, insulin concentrations in pre-suckled newborn lambs from OB ewes were lower and glucose concentrations were higher than fetuses of C ewes, suggesting a further deterioration in pancreatic β -cell numbers and/or function in OB fetuses between days 135 of gestation and delivery. Maternal obesity in our model is consistent with an elevation in the wave of apoptosis seen in other mammalian species near term [27], which may lead to the reduced levels of circulating insulin and hyperglycemia at birth. In another study where we used the same model of maternal obesity as reported here, adult offspring were subjected to a 12-wk *ad libitum* feeding trial [28]. In response to the bout of *ad libitum* feeding, offspring from OB ewes became fatter, and exhibited a reduced acute insulin response to glucose and reduced insulin sensitivity in response to an i.v. glucose tolerance test compared with the offspring from C ewes. These data suggest that offspring subjected in utero to maternal obesity, and postnatally to food excess exhibit increased adiposity, reduced pancreatic β -cell response to increased circulating glucose, as well as insulin resistance, all of which are related to the development of diabetes [28].

Temporal patterns of prenatal pancreatic development in mammals ranging from rodents to humans are very similar [5,29,30]. In the rodent, β -cell apoptosis increases near term, and peaks at 13 days postnatal age [31]. In contrast, β -cell proliferation in the rat pup decreases from a peak of 4.7% at 2 days postnatal age to a nadir of 1.8% at 31 days of age [32]. These patterns are similar to those found in humans, where a wave of increased β -cell apoptosis occurs during the prenatal period, peaking at birth, and approaching 0 by 6 months of age, with β -cell proliferation progressively declining from early gestation to extremely low levels by 6 months postnatal age [27]. These changes in β -cell proliferation/apoptosis may be responsible for the postnatal islet remodeling seen in these species. In the human fetal and neonatal pancreas, islets are small and poorly formed, but after 6 months, the β -cell distribution approaches adult-type architecture [27,30,32]. Although the apoptotic wave of β -cell is a normal phenomenon, an increased rate of apoptosis may augment the chance of diabetes in offspring [13,33]. In 1995, Finegood et al. [13] constructed a well accepted mathematical model of dynamics of the β -cell mass in the rodent. These researchers evaluated existing studies describing a variety of factors including β -cell proliferation and apoptosis thought to mediate pancreatic growth in the rat and concluded that diabetes-prone strains exhibit an exaggerated β -cell apoptotic wave. In the present study, we report that the rate of apoptosis was increased over 70% in fetuses of OB vs. C ewes in late-gestation, and may thus predispose to diabetes postnatally.

In order to maintain the glucose levels in a normal physiological range, insulin secretion from β -cells must be tightly controlled by either altering β -cell number or insulin secretion. In non-diabetic obese humans, β -cells numbers increase by only 50%, but levels of insulin secretion can increase four to five fold in response to insulin resistance and elevated glucose concentrations in blood [12,26], indicating significant plasticity in the insulin secreting capacity of their β -cells. To maintain normal insulin secretion in late gestation with the decreased β -cells numbers observed in OB fetuses in this study, the remaining β -cells must secrete more insulin to compensate. This is consistent with the increased insulin

concentrations in pancreatic islets of fetuses from OB versus C ewes reported here, and would explain the maintenance of normal blood insulin concentration in this group into late gestation.

In rodents, both maternal undernutrition and overnutrition are known to impact pancreatic β -cell numbers and function in offspring. A 50% maternal nutrient restriction from day 15 of pregnancy until weaning in rats results in reduced β -cells numbers in offspring on postnatal day 21 [34]. Due to elevated β -cell apoptosis, β -cell numbers in these offspring did not recover with increasing age, with the resultant development of glucose intolerance [34]. In mice, Gniuli et al. [35] reported that a maternal high-fat diet during gestation and lactation, produced offspring showing an initial increase in β -cell mass, resulting from a markedly elevated β -cell mitotic rate. After weaning, however, β -cell mass exhibited a marked decrease, resulting in type-2 diabetes in these offspring. These data are consistent with the data reported here suggesting that maternal obesity initially accelerates fetal β -cell mitosis, and later reduces pancreatic growth and insulin secretion during later gestation in the sheep, resulting in a markedly decreased insulin/glucose ratio at term.

We previously reported markedly higher blood glucose, free fatty acids and cortisol concentration in OB fetuses compared with C fetuses in mid-gestation [20,36], whereas these group differences disappear in late gestation in the same paradigm as reported here. To help explain these gestational age differences, Ma et al. [37] have recently reported that maternal over-nutrition and obesity from early to mid-gestation in this model results in accelerated placental vascular development, associated with increased maternal to fetal nutrient delivery and macrosomia. In contrast, these researchers [37] observed that continued overnutrition and obesity through late gestation resulted in markedly decreased placental angiogenic factor production and reduced vascular development, potentially to protect the fetus from excessive nutrient delivery and excessive weight gain.

Exposure to elevated concentrations of glucose and free fatty acid have been linked to β -cells apoptosis [14,15]. In both *in vivo* rodent studies and *in vitro* studies using β -cell lines, elevated glucose levels can cause increased β -cell apoptosis, resulting in insulin deficiency [38]. Further, the negative effect of elevated glucose exposure on β -cell function is dependent on glucose concentration and duration of exposure [38,39]. In rats, β -cells chronically exposed to elevated glucose concentrations initially show “glucose hypersensitization” associated with an enhanced expression of glycolytic enzymes leading to increased β -cell growth and survival [38,40]. However, with prolonged exposure to elevated glucose concentrations, β -cell mass decreases as a result of increased β -cell apoptosis [38]. Jansson et al. [41] reported that 4 weeks after the transplantation of human pancreatic islets to diabetic mice, the islets exhibited impaired glucose stimulated insulin secretion and increased β -cell apoptosis. In our model, the markedly increased glucose concentration observed at mid-gestation can be seen to induce an initial cell proliferation, but prolonged exposure may induce an elevated β -cell apoptosis by late-gestation.

Fatty acids are another potential stimulator of β -cell apoptosis, potentially contributing to the observed decreased β -cells mass and type-2 diabetes [14]. Obesity is associated with the loss of β -cell function [42], which suggests a potential role of increased circulating fatty acids in accelerating β -cell apoptosis, independent of peripheral insulin sensitivity [43]. Fatty acids have been shown to induce lipotoxicity in β -cells through endoplasmic reticulum (ER) stress, independent of cytokine pathways involving interleukin (IL)-1 β and NF-kappa B, and lead to apoptosis [38,44,45]. The mechanism of fatty acid induced ER stress is still not clear, but elevated levels of fatty acids in the cytoplasm of the β -cell may result in delayed processing and export of new proteins such as insulin, by impairing ER Ca²⁺ handling [38,46]. During gestation, free fatty acids are taken up from the maternal system and re-

esterified to triglycerides in the placenta and fetal liver, before being released into the fetal circulation [47–49]. In our previous studies utilizing identical dietary treatments as those reported here, we have demonstrated that OB ewes and their fetuses exhibited increased free fatty acids, cholesterol and triglyceride levels [36] by mid-gestation, which may lead to impaired β -cell development and result in increased β -cell apoptosis in late-gestation. Further, we have previously reported that fetuses from OB ewes exhibited greater adiposity at mid- and late gestation than fetuses from C ewes [50,51].

Glucocorticoids also appear to play a key role in decreasing fetal pancreatic β -cells mass in the maternal undernourished rat model by acting on specific receptors located within β -cells, and resulting in a reduced β -cell mass in early fetal development [52,53]. Ranta et al. [54] reported that exogenous administration of dexamethasone results in accelerated apoptosis of insulin-secreting pancreatic cells, through down-regulation of Bcl-2, as well as activation of calcineurin and dephosphorylation of BAD. In this model of maternal obesity, increased blood concentrations of cortisol were observed by mid-gestation in OB fetuses [20]; and while they had returned to levels of C fetuses by day 135 in the present study, when they declined from mid- to late gestation was not determined. Thus, they may have played a role in impairing β -cell development and initiating the accelerated β -cell apoptosis seen in late-gestation in this model.

In summary, these findings demonstrate for the first time a biphasic impact of maternal obesity on fetal growth, as well as pancreatic development and function in an important large animal species used extensively in biomedical research to model human pregnancy. During the first half of gestation in the obese ewe we have previously reported an acceleration of fetal growth as well as increased pancreatic β -cell mitosis, with a resultant increase in β -cell numbers and hyperinsulinemia. In the second half of gestation, however, fetal growth slows, and pancreatic β -cell apoptotic rate accelerates with a resultant decrease in β -cell numbers, resulting in hypoinsulinemia at birth. If the pancreas fails to return to a normal cellular composition and function postnatally, this could lead to glucose/insulin dysregulation, glucose intolerance and diabetes in later postnatal life.

Acknowledgments

This work was supported by National Institutes of Health (NIH) INBRE P20 RR16474-04. The authors would like to thank Adam Uthlaut for animal care and management.

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Figure 1.

Ewe body weight of 11 Control (C) and 13 Obese (OB) twin bearing ewes from 60 days before conception to day 135 of gestation. The x axis depicts the interval from 60 days before conception to day 135 of gestation. ^{abcd} Means $\pm$ SEM with different superscripts differ ($P < 0.05$).

Figure 2. Insulin positive cells per unit area (mm²) (panel A), percentage of fetal pancreatic β-cells proliferating (panels B and C) and undergoing apoptosis (panel D) in fetal pancreatic islet tissue from Control (C) and Obese (OB) ewes on day 135 of gestation. *Means ± SEM differ between treatment groups (P<0.05).

Figure 3. Concentration of insulin per pancreatic islet (panel A) and per ng DNA (panel B) from 4 Control (C) and 6 Obese (OB) fetuses on day 135 of gestation. *Means $\pm$ SEM differ between treatment groups ($P < 0.05$).

Figure 4. Concentrations of blood glucose (Panel A) and insulin (Panel B) from Control (C) and Obese (OB) lambs at birth. *Means $\pm$ SEM differ between treatment groups ($P < 0.05$).

Table 1

Absolute fetal organ weight and the organ weight per unit body weight on day 135 of gestation

Organ weight (g)	Absolute value		Organ wt/fetal weight (%)	
	C (n=8)	OB (n=12)	C (n=8)	OB (n=12)
Heart	38.4 ± 1.3	35.0 ± 1.5	0.8 ± 0.02	0.7 ± 0.01
Kidney	29.5 ± 1.8	23.1 ± 1.2**	0.6 ± 0.03	0.5 ± 0.02*
Adrenal	0.5 ± 0.02	0.5 ± 0.03	0.01±0.001	0.01±0.001
Pancreas	4.5 ± 0.3	3.2 ± 0.2**	0.09 ± 0.01	0.07 ± 0.01*
Liver	123.4 ± 8.3	113.2 ± 9.1	2.4 ± 0.1	2.4 ± 0.1
Spleen	7.9 ± 0.5	7.2 ± 0.6	0.2 ± 0.01	0.2 ± 0.01
Thymus	21.9 ± 2.4	18.4 ± 1.3	0.4 ± 0.04	0.4 ± 0.01

Data are means ± SEM.

* depicts significant differences (P<0.05) and

** depicts significant differences (P<0.01) between groups.

Table 2

Maternal and fetal blood glucose, insulin and cortisol concentration on day 135 of gestation

	D135	
	C	OB
<i>Maternal</i>		
Glucose(mg/dl)	56.3±4.9	69.3±4.4 §
Insulin(ng/ml)	0.7±0.5	1.4±0.5
Cortisol(ng/ml)	94.0±10.7	106.6±6.8
<i>Fetal</i>		
Glucose(mg/dl)	27.8±2.1	29.9±1.8
Insulin(ng/ml)	0.4±0.2	0.7±0.2
Cortisol(ng/ml)	34.0±4.0	27.9±3.5

Data are means ± SEM.

* depicts significant differences (P<0.05) and

§ depicts a trend (P=0.07) between groups within a day.