Maternal high-fat diet is associated with impaired fetal lung development

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Submitted 10 April 2015; accepted in final form 8 June 2015

The Barker hypothesis established that in utero conditions, such as poor maternal nutrition, give rise to restricted fetal growth and multiorgan impairments (2, 5). The typical American diet is one where >38% of calories are derived from fat. This adoption of diets rich in fat has led to an obesity epidemic in women of childbearing age. Importantly, it has not been established if the maternal diet, specifically a diet high in fat (HFD), impacts fetal lung development. There are other nutritional components that have been shown to impact fetal lung development (24, 36), but the focus of this manuscript will be on the maternal HFD. The fetal lung is particularly susceptible to in utero insults (27). For example, chorioamnionitis (inflammation of the fetal placental membranes) is associated with reduced microvascular and alveolar development in the lungs of fetal sheep (22, 23). Maternal consumption of a HFD induces maternal obesity and leads to a state of maternal systemic inflammation (3, 29). However, it is unclear if maternal systemic inflammation induces an inflammatory response in the placenta, thus altering placental function, fetal growth, and lung development.

Diets rich in saturated fatty acids, such as palmitic acid, are associated with increased tissue inflammation by means of direct activation of the innate immune system via Toll-like receptors (34). Lifelong consumption of HFD leads to elevations in inflammatory markers in the maternal circulation such as serum amyloid A3 (SAA3), an acute-phase reactant protein (1, 29). Furthermore, fetal exposure to maternal HFD, independent of maternal obesity and hyperinsulinemia, induces placental inflammation and increases the frequency of stillbirths in nonhuman primates (15).

Placental function is critical for optimal fetal growth, and placental insufficiency is the most common cause of FGR (16). FGR is a pathological reduction in fetal growth potential (16). It is a major complication of pregnancy and increases the risk of fetal, perinatal, and lifelong morbidity and mortality (27). Recently, it has been demonstrated that maternal HFD leads to a reduction in utero-placental blood flow in primates (15), and FGR is associated with structural changes and cellular dysfunction in the fetal lung in sheep (33).

Because placental inflammation and FGR are independently associated with abnormalities in lung development (23, 27), we characterized the impact of maternal HFD on the placenta and fetal lung development. We hypothesized that maternal HFD induces fetal-placental inflammation, resulting in FGR and inhibition of fetal lung development. We further sought to determine the mechanism by which the maternal HFD inhibits fetal lung development. Given the critical role of steroids in fetal lung development, we evaluated the influence of maternal HFD on aspects of the glucocorticoid pathway in the placenta and fetal lungs. We show that maternal HFD is associated with delayed fetal lung development as well as postnatal alveolarization and that expression of the glucocorticoid receptor (GR) is decreased in both the placenta and fetal lungs. This is the first study to determine a potential mechanism by which the maternal HFD inhibits fetal lung development. These findings are critically important given the rising obesity epidemic.
MATERIALS AND METHODS

Animals. All animal study protocols were approved by the Institutional Animal Care and Use Committee at the University of Texas (UT) Southwestern Medical Center. C57Bl/6j mice (6–8 wk old) were purchased from The Jackson Laboratory. All mice were housed in a temperature-controlled environment in groups of two to five at 22–24°C using a 12:12-h light-dark cycle with ad libitum access to water. Male mice had ad libitum access to standard chow (no. 2916; Harlan-Teklad). Female mice (also 6–8 wk of age) were placed on either HFD (42% of the calories derived from fat, no. 8813T, n = 30; Harlan Teklad) or normal chow (21.2% of the calories derived from fat, no. 2916, n = 25; Harlan-Teklad) for 3–5 mo before mating.

Animal mating. Males and nulliparous females were paired for 36 h and monitored. Maternal weight gain was assessed weekly. Pregnant females were killed on embryonic day 18 (E18), and tissues were collected.

Tissue collection. Before death, pregnant females were fasted for 3 h. At death, maternal body weight and blood samples were collected to assess fasting glucose and insulin concentrations. Mice were killed, and the placentas were collected and weighed. Maternal and fetal portions of the placenta were separated and saved in RNAlater (Life Technologies) for RNA analysis, snap-frozen in liquid nitrogen for protein analysis, or fixed with 4% paraformaldehyde (PFA) for histological analysis. Fetal number was documented, and weights were obtained. The fetal-to-placental ratio, which is a measure of placental sufficiency, was calculated by dividing the fetal weight by the placental weight. The right and left lungs were dissected using a surgical dissecting microscope and weighed. The right fetal lung was fixed in 4% PFA for histology or frozen in liquid nitrogen for protein analysis. The left fetal lung was saved in RNAlater for RNA analysis.

Maternal fasting glucose. Serum was separated from whole blood samples, and fasting glucose was determined using the Fasting Blood Glucose kit (Millipore) according to the manufacturer’s instructions.

Maternal fasting insulin. Serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA) kits to assess insulin concentrations (Millipore) according to the manufacturer’s instructions.

Real-time quantitative RT-PCR. Placental (fetal side) and fetal lung tissues were excised and quickly placed in RNAlater (Life Technologies). Total RNA was extracted following tissue homogenization in Trizol (Invitrogen) using a TissueLyser (Qiagen) and then isolated using the RNeasy RNA extraction kit (Qiagen) according to the manufacturer’s instructions. The quantity and quality of the RNA were determined by absorbance at 260/280 nm. cDNAs were prepared by reverse transcribing 1.5 μg RNA using SuperScript III reverse transcriptase (Invitrogen) and oligo(dT) (Invitrogen). Quantitative real-time PCRs were performed with TaqMan primers for Nlrp3 (Mm00840904_m1), IL-1β (Mm01336189_m1), GR (Mm00433832_m1), 11β-hydroxysteroid dehydrogenase (HSD) 1 (Mm0047182_m1), 11β-HSD2 (Mm01215041_m1), surfactant protein A (SpA) (Mm0106170_m1), SpB (Mm0455678_m1), SpC (Mm0488414_m1), SpD (Mm00486060_m1), and β-actin (Mm00607939_s1) on an ABI Prism 7900 HT sequence detection system (Applied Biosystems) using technical duplicates. The relative amounts of all mRNAs were calculated by using the comparative threshold cycle method. β-Actin mRNA was used as the reference gene.

Protein analysis. Placental (fetal side) and fetal lung tissues were dissected and immediately frozen in liquid nitrogen. Tissues were homogenized in a 20 mM Tris buffer (pH 7.4) containing 150 mM NaCl, 1% Triton X-100, 10 mM EDTA, Complete protease inhibitor cocktail, and PhosSTOP phosphatase inhibitor cocktail (Roche Applied Science). Tissue extracts were centrifuged at 12,000 g at 4°C, and supernatants were collected. Protein concentration was measured with a bicinchoninic acid assay kit (Pierce). Proteins were then separated on 10, 4–15, or 4–20% Criterion TGX precast gels (Bio-Rad) and electrotransferred to nitrocellulose or PVDF membranes using the Trans-Blot Turbo transfer system (Bio-Rad). Transfer efficiency was determined with Ponceau red stain. Nonspecific binding sites were blocked by incubating membranes for 1–2 h in TBS supplemented with 5% nonfat powdered milk (wt/vol in 20 mM TBS), followed by overnight incubation with primary antibodies for SpB (NBPI-57977; Novus), SpD (NBPI-41050; Novus), and β-actin (sc-47778; Santa Cruz Santa Cruz). Labeling was revealed with appropriate IRDye secondary antibodies (LI-COR) using the ODYSSEY Quantitative Fluorescence Imaging Systems (LI-COR) or with the corresponding secondary antibody conjugated with peroxidase (Pierce Biotechnology) visualized by chemiluminescence (PerkinElmer Life Science). Bands were quantified using a LI-COR Odyssey scanner. Boxes were manually placed around each band of interest, which returned near-infrared fluorescent values of raw intensity with intraline background subtracted using Odyssey 3.0 analytical software (LI-COR). Data were normalized to control values. The concentration of IL-1β was determined in serum samples by ELISA (Thermo Scientific) according to the manufacturer’s instructions.

Histology. Fetal lung tissues for histology and immunohistochemistry were excised, weighed, and fixed in 4% PFA for 48 h and then rinsed and stored in PBS. Subsequent paraffin processing, embedding, sectioning, and hematoxylin and eosin (H&E) staining, as well as Periodic acid-Schiff (PAS) staining for glycogen were performed by The Molecular Pathology Core at UT Southwestern Medical Center at Dallas as described previously (23, 39).

Fetal lung cell proliferation by Ki-67 immunohistochemistry. Ki-67 is a marker of cellular proliferation. Mouse antisera used for Ki-67 immunohistolabeling of paraffin sections was obtained from Vector Laboratories (catalog no. VP-K452; Burlingame, CA). Following pH 6.0 citrate-based, heat antigen retrieval, serial sections were quenched of autofluorescence with 100 mM glycine and blocked against endogenous mouse IgG and secondary antibody host-serum affinity utilizing commercially available blocking reagents (Vector Mouse on Mouse Kit, catalog no. BMK-2202). Sections were subjected to either primary antibody (1:300 dilution of commercially supplied stock) or normal mouse serum and incubated overnight at 4°C. Subsequent biotin/streptavidin fluorescein detection of bound primary was conducted the following day according to Mouse on Mouse kit instructions. To quantify the extent of cellular proliferation, lung tissue samples were selected from four different fetuses from different mothers fed either chow or HFD. For each sample, four random, noncontiguous fields were photographed at ×40 using an Olympus FSX100 microscope. Proliferating cells (Ki-67 positive) and nuclei were manually counted in each field using ImageJ software. For each sample, the counts from the four fields were summed, and a proliferation index (proliferating cells-to-total cell nuclear ratio) was obtained. The averages of the proliferation index per diet exposure were then calculated.

Fetal lung maturation. To evaluate fetal lung maturation, H&E fetal lung slides were scored by a pathologist blinded to the study design and dietary conditions using methods described previously (8). Briefly, maturation was scored using the following scale: 0 = pseudoglandular stage of lung development (E9.5–16.0); 1 = canalicular (E16.6–17.4); 2 = canalicular/terminal saccular (E17.5–postnatal day 5); 3 = terminal saccular (17.4–18.2); and 4 = terminal saccular/alveolar (18.2 until after birth). The pseudoglandular stage of lung development was characterized by round tubules lined by a single layer of simple cuboidal epithelium with either absent or small lumens. The canalicular stage of lung development was characterized by dilated tubules, irregularly shaped and lined with cuboidal epithelium, and capillaries close to the tubules. The terminal saccular stage of lung development was classified as having more complex tubules with budding with no discernible epithelial lining. The sacs are separated from each other by smooth-walled, thickened septae. In the alveolar stage of lung development, the lung is mature, with fully developed alveoli and a thinned endothelium.
Blinded glycogen gradation. Lung maturation in the fetal lungs at E18 was also assessed using semiquantitative PAS staining for glycogen as described previously (7). Fetal lungs were rapidly excised and snap-frozen to enhance tissue quality. Quantification of cytoplasmic glycogen content in the fetal lungs was done by a pathologist blinded to the study design. The quantification protocol for glycogen was conducted as previously described (10). Briefly, the glycogen staining intensity was scored using the following scale: 0 = no detectable staining; 1 = first detectable staining; 2 = cytoplasmic glycogen granules in <30% of cells; 3 = cytoplasmic staining in 30–60% of cells; and 4 = cytoplasmic granules in >60% of cells. The average score per maternal diet was then calculated.

Postnatal pup studies. A subset of neonatal pups remained with their mothers on their respective diets until postnatal day 15. The pups were anesthetized, the abdomen was opened, and the animal was exsanguinated by transection of abdominal vessels. A thoracotomy was performed, and the trachea was exposed. A blunt cannula was inserted and tied to the trachea. The lungs were inflation fixed by instilling PBS-buffered 4% PFA at a pressure of 25 cm water. Histological lung sections were processed for H&E stain as described above.

Statistical analyses. The distribution of data was tested for normality with the Shapiro-Wilk normality test. Means from normally distributed data were compared using an unpaired, two-tailed t-test, whereas means from nonnormally distributed data were compared using the Mann-Whitney U-test. P values <0.05 were considered statistically significant. Data are presented as means ± SE.

RESULTS

Maternal weight gain during pregnancy. Before being randomized to either HFD or chow, females did not differ in body weight (data not shown). However, 3–5 mo of HFD resulted in 23% greater weight in the HFD-exposed mice [42.8 ± 0.46 g vs. 34.8 ± 0.02 g, respectively; P ≤ 0.001 (HFD n = 30, chow n = 25)] (Fig. 1A). Although HFD-exposed mice were heavier at the time of mating, their weight gain during pregnancy was significantly less than chow-exposed mice (10.0 ± 0.20 g vs. 13.3 ± 0.2 g, respectively; P ≤ 0.001) (Fig. 1B). Maternal fasting blood glucose was elevated in the HFD-exposed mice relative to chow-fed mice (243 ± 15 vs. 178 ± 5.9 mmol/l, respectively; P = 0.01) (Fig. 1C). In addition, maternal fasting serum insulin levels were greater in the HFD-exposed mice compared with the chow-fed mice (1.40 ± 0.15 vs. 0.94 ± 0.15 ng/ml; P ≤ 0.05) (Fig. 1D).

Maternal HFD and placental inflammation. Following HFD exposure, the fetal side of the placenta demonstrated a significant increase in Nlrp3 and IL1β mRNA compared with chow diet exposure (Fig. 2, A and B). This was associated with an increase in placental IL-1β protein determined by immunoassay following in utero HFD exposure (P ≤ 0.05) (Fig. 2C).

Fetal viability after HFD exposure. Fetal death was determined by counting the number of resorbed fetal sacs observed at termination of each experiment. HFD-exposed mothers had a twofold increase in the number of resorbed sacs vs. the chow-exposed mice (HFD: 1.34 ± 0.10, chow: 0.60 ± 0.07, P ≤ 0.001) (Fig. 3A).

Maternal HFD and FGR. Fetal weights at E18 in HFD-exposed dams were significantly lower compared with fetuses from chow-fed mothers (0.64 ± 0.01 vs. 0.78 ± 0.02 g, respectively; P ≤ 0.001) (Fig. 3B). The fetal-to-placental ratio was calculated as a measure of placental sufficiency. Exposure to HFD was associated with a significantly lower fetal-to-placental weight ratio compared with mothers fed a chow diet (5.50 ± 0.17 vs. 6.76 ± 0.23, P ≤ 0.001) (Fig. 3C). Fetal lung weight was also determined, and, despite reductions in fetal weight, there were no significant differences in fetal lung weights between the diets (data not shown).

Maternal HFD and fetal lung development. To evaluate structural fetal lung maturation, a pathologist blinded to the dietary intervention examined the histology of fetal lungs obtained at E18 (Fig. 4, A and B). The most advanced phase of structural lung development reached for each fetus was determined as described earlier (5). HFD-exposed fetuses had reduced fetal lung maturation compared with chow-exposed.
fetuses (1.63 ± 0.27 vs. 2.1 ± 0.27, respectively; \( P = 0.04, n = 36 \)) (Fig. 4C).

Because lung maturation is also associated with increased cellular proliferation (13), we evaluated fetal lung proliferation using Ki-67 immunostaining, a marker for cellular proliferation that is expressed in all stages of the cell cycle (13) (Fig. 5A). The proliferation index was calculated as the number of actively dividing cells divided by the total number of nuclei per \( \times 40 \) field. The proliferation index at E18 in the fetal lungs exposed to maternal HFD was reduced by 35\% (Fig. 5C).

Glycogen is a necessary substrate for surfactant production; thus, lung glycogen content decreases with increasing gestational age (9). Therefore, we assessed fetal lung glycogen content and surfactant apoprotein gene expression as markers for alveolar type II cell maturation (21). Fetuses exposed to maternal HFD contained 30 – 60\% cytoplasmic staining for immature SpB protein (Fig. 3). Notably, maternal HFD also resulted in a reduction in 18-kDa glycogen in developing columnar epithelial cells (Fig. 5, C and D). In contrast, there was minimal staining for glycogen in the lungs of fetuses from mothers exposed to the chow diet (Fig. 5, C and D). Notably, maternal HFD also resulted in a reduction in \( \text{SpB}, \text{SpC}, \) and \( \text{SpD} \) mRNA expression with no change in \( \text{SpA} \) (\( P \leq 0.03 \)) (Fig. 6A). We also examined expression of \( \text{SpB} \) and \( \text{SpD} \) protein by immunoblot because these are the surfactant proteins critical for surfactant function and respiratory distress. There was a significant reduction in 18-kDa mature \( \text{SpB} \) protein (\( P \leq 0.05 \)) (Fig. 6B). There was a reduction in \( \text{SpD} \) protein; however, it did not reach statistical significance.

**DISCUSSION**

In the present report, we provide evidence for the first time that fetal exposure to maternal HFD is associated with a reduction of maternal weight gain during pregnancy, elevations in maternal glucose and insulin levels, induction of a placental inflammatory response, placental insufficiency, FGR, and inhibition of normal fetal lung development. Maternal HFD exposure and postnatal pup lung development. To determine if the effect of maternal HFD on fetal lung development persisted after birth, we examined lung structure at postnatal day 15 (Fig. 8A). Structural development in the HFD-exposed animals determined by measuring the mean linear intercept was less in the HFD animals (74.9 ± 5.8 vs. 49.9 ± 1.8, respectively; \( P \leq 0.005 \)) (Fig. 8B).

**Influence of maternal HFD and GR expression in the placenta and fetal lungs.** Mice with conditional deletion of GR in alveolar type II pneumocytes have reduced fetal viability and delayed structural and biochemical lung maturation (25). Both placental and lung GR gene expression was reduced by 50\% in HFD-exposed fetuses (Fig. 7, A and B).

Maternal HFD and GR expression in the placenta and fetal lungs. Mice with conditional deletion of GR in alveolar type II pneumocytes have reduced fetal viability and delayed structural and biochemical lung maturation (25). Both placental and lung GR gene expression was reduced by 50\% in HFD-exposed fetuses (Fig. 7, A and B).

MATERNAL HIGH-FAT DIET IMPAIRS FETAL LUNG DEVELOPMENT

**A**

**Placenta: Nlrp3 mRNA Expression**

**B**

**Placenta: IL1β mRNA Expression**

**C**

**Placenta: IL1β Protein Concentration**

Fig. 2. The effect of HFD on placental inflammation. A: Nlrp3 gene expression was elevated in placentas exposed to maternal HFD. B: IL-1β mRNA gene expression was elevated in placentas exposed to maternal HFD. C: IL-1β protein concentration was elevated in HFD placentas, suggesting placental inflammation. *\( P < 0.05 \).

**A**

**Number of Miscarriages**

**B**

**Fetal Weight**

**C**

**Fetal:Placental Ratio**

Fig. 3. The effect of maternal HFD on fetal development. A: increased no. of miscarriages in HFD pregnancies. B: decreased fetal weight after exposure to in utero HFD. C: reduced fetal (F)-to-placental (P) ratio in the HFD cohort suggesting placental insufficiency. *\( P < 0.05 \).
may contribute to the observed inhibition of fetal lung development.

In our study, consumption of maternal HFD results in maternal obesity, associated with elevations in maternal glucose and insulin levels, and an induction of placental inflammation. There are many different mechanisms by which the maternal HFD model may induce placental inflammation, and two are highlighted here. First, maternal HFD induces systemic inflammation as evidenced by increased maternal SAA3 levels (data not shown), an acute-phase reactant protein and a biomarker of inflammation. Inflammatory cytokines from the maternal circulation may transmit to the placenta. Second, it is known that SAA3 and palmitate, a saturated fatty acid rich in the maternal HFD, directly activate the Nlrp3 inflammasome, inducing production of IL-1β, a master regulator of the inflammatory response (1, 21, 27, 29). Our data provide evidence that consumption of maternal HFD increases placental expression of components of the inflammasome, Nlrp3 and IL-1β, indicating increased placental inflammation.

Consumption of HFD was associated with an induction of placental inflammation, potentially resulting in reduced placental function and/or blood flow, as evidenced by the presence of FGR and decreased fetal-to-placental weight ratio. Placental function may have been impaired by placental inflammation. Impairments in uteroplacental blood flow result in reduced nutrient supply to the fetus, thus inhibiting optimal growth. In addition to impaired uteroplacental blood flow, the HFD-exposed dams in our study gained less weight over the course of pregnancy, which could have further impaired fetal growth.

Our data suggest that maternal HFD induces a placental inflammatory response and reduces fetal growth. Given the known association of placental inflammation and FGR with decreased fetal lung development, we evaluated the influence of maternal HFD on fetal maturation (13, 17). FGR is an independent risk factor for the development of chronic lung disease in the postnatal period (4, 26, 27). However, the effects of FGR on fetal lung development have not been clearly established. Although it has been suggested that intrauterine stress secondary to FGR advanced maturation of the lung (11), Tyson et al. (35) reported that neonates with FGR did not have a decreased risk for respiratory distress syndrome (RDS) when compared with normally grown neonates of the same gestational age. Rather, they had an increased risk of RDS and the occurrence of respiratory failure or death (35). Their results are consistent with our findings of reductions in functional and structural lung maturation in the fetuses exposed to maternal HFD, as well as the persistence of poor lung development, which would predispose them not only to a higher risk of RDS but also an increase in postnatal lung disease.

To evaluate fetal lung development, we compared the fetal lungs exposed to in utero maternal HFD vs. chow in terms of characteristics of fetal lung development, such as structural maturation, glycogen content, cellular proliferation, and surfactant production. We chose the E18 time point because this corresponds to the canalicular stage of lung development, which is equivalent to 26 wk gestation in a premature infant (18). Histologically, at E18, fetal lungs from chow-fed mothers had normal saccular lung development, whereas fetal lungs

![Figure 4. The effect of maternal HFD on the structural development of fetal lungs. A and B: lung sections from HFD and chow at embryonic day (E) 18 were stained with hematoxylin and eosin. Magnification, ×10 and ×40. C: structural lung maturation was graded by a pathologist blinded to the diets according to 5 distinct phases of normal mouse lung development as described in MATERIALS AND METHODS. Fetal lungs exposed to maternal HFD were significantly less mature at E18 compared with chow lungs. *P < 0.05.](http://ajplung.physiology.org/)
from mothers exposed to HFD showed lung morphology more comparable to E15-16, suggesting that maternal HFD exposure results in delayed structural lung development. At the cellular level, we found that the maternal HFD reduces fetal lung cellular proliferation compared with chow fetal lungs.

In addition to structural maturation, we sought to determine if maternal HFD influences the maturation of the fetal lungs. Glycogen is a precursor to surfactant production and is an important marker for fetal lung maturation (12). Glycogen utilization increases with advancing gestation as surfactant production is optimized in preparation for extrauterine life. Our findings in the fetal lungs exposed to chow were consistent with previous findings indicating a reduction in cytoplasmic glycogen in fetal lungs later in gestation (9). However, fetal lungs exposed to maternal HFD had increased cytoplasmic glycogen content indicating less fetal lung maturation. These findings were quantified and supported by a pathologist blinded to the dietary intervention as described in MATERIALS 

and METHODS. HFD-exposed fetal lungs had less glycogen utilization compared with chow fetal lungs. *P < 0.05.

**Fig. 5.** The effect of maternal HFD on fetal lung cellular proliferation and glycogen utilization. A: reduced cellular proliferation in fetal lung sections immunostained for nuclear protein Ki-67 in the HFD cohort diet compared with chow. B: quantification of cell proliferation in lung sections immunostained with Ki-67 confirmed reduced cellular proliferation in the HFD cohort. C: increased histochemical staining for Periodic acid-Schiff (glycogen magenta purple). D: quantification of cytoplasmic glycogen stores in developing alveoli were scored by a pathologist blinded to the dietary intervention as described in MATERIALS AND METHODS. HFD-exposed fetal lungs had less glycogen utilization compared with chow fetal lungs. *P < 0.05.

**Fig. 6.** The effect of maternal HFD on surfactant production in fetal lungs. A: surfactant protein mRNA levels of B, C, and D were reduced in the HFD-exposed fetal lungs compared with chow controls. B: reduced surfactant protein B quantified by Western blot analysis in the HFD fetal lungs. *P < 0.05.
compared with chow fetal lungs. Clinically, this could predispose the neonatal mice to respiratory distress in the postnatal period.

Because there was an increase in glycogen, we evaluated surfactant expression in the fetal lungs. We found a significant reduction in $SpB$, $SpC$, and $SpD$ gene expression in the HFD-exposed fetal lungs compared with chow, whereas we found no difference in $SpA$ expression. At the protein level, we quantified protein concentrations of $SpB$ and $SpD$. These proteins were selected because of their critical role in surfactant production, innate immune function, and respiratory distress (38). There was a reduction in $SpB$ and $SpD$ protein in the fetal lungs following HFD exposure; however, the reduction in $SpD$ was not statistically significant. Overall, our findings suggest there may be an arrest of normal structural and functional fetal lung maturation following maternal HFD exposure. To date, this is the first study to evaluate the influence of maternal HFD on fetal lung maturation.

Glucocorticoids play a major role in the maturation of lung structure and function (31). The physiological action of glucocorticoids in the developing lungs includes stimulation of surfactant synthesis, cytodifferentiation, branching morphogenesis, vasculogenesis, and alveolarization, all of which are mediated by activation of the GR (20, 25, 28, 30, 37). Upon activation, GR translocates to the nucleus where it binds to hormone response elements and alters expression of target genes such as surfactant proteins (3, 9, 40). In the prenatal period, glucocorticoids promote surfactant synthesis in alveolar epithelial cells of the developing lung and thus are an effective antenatal treatment to accelerate lung maturation (28a). Mice with lung epithelial-specific knockout of the GR have reduced viability, decreased structural maturation, increased cytoplasmic lung glycogen content, and decreased surfactant (25). Given the similarities in our HFD phenotype, we evaluated GR expression in the fetal lung. Fetuses with exposure to maternal HFD not only had a reduction in lung GR

![Fig. 7. Effect of maternal HFD on the glucocorticoid receptor (GR) in the placenta and fetal lung. A: 2-fold reduction in GR in the HFD-exposed placenta. B: 2-fold reduction in GR in the HFD-exposed fetal lungs. *P < 0.05.](image)

![Fig. 8. The effect of maternal HFD persists into adolescence. A: inflation-fixed pup lungs were stained with hematoxylin and eosin. Magnification, ×10. Pups exposed to in utero and postnatal maternal HFD exhibited alveolar simplification. B: alveolar development as quantified by mean linear intercept (MLI) demonstrates significantly less saccular air space development at postnatal day 15 in HFD pup lungs. *P < 0.05.](image)
expression, but also the phenotype seen in the GR knockout. Our data suggest that reductions in GR gene expression in the fetal lungs may be one mechanism by which HFD inhibits fetal lung development. Decreased GR gene expression may limit the actions of glucocorticoids in maturing the fetal lungs. Importantly, there are likely several other aspects of fetal exposure to the maternal HFD that may influence fetal lung maturation such as fetal hyperglycemia and/or fetal hyperinsulinemia (factors not specifically assessed here). Maternal obesity secondary to HFD consumption appears to be deleterious to fetal development in many ways, including by constraint of fetal growth and expansion of fetal liver hematopoietic stem and progenitor cells as discussed in a recent study (19).

Collectively, the present study suggests that maternal HFD before and during pregnancy induces elevations in maternal glucose and insulin, placental inflammation resulting in placental insufficiency, FGR, and attenuation of normal fetal lung development and maturation. The reduction in structural and biochemical fetal lung development could predispose offspring to an increased risk of RDS at birth if delivered prematurely, and the development of chronic lung disease in the neonatal period, which is supported by the persistence of alveolar simplification in the HFD cohort at 2 wk of age. The airspace of HFD-exposed pup lungs morphologically were "simplified," meaning they were enlarged and elongated secondary to a reduction in alveolar septation (14). Importantly, it has been demonstrated that supplementing the maternal diet with docosahexaenoic acid lessened inflammation and improved lung growth in the offspring (32, 36). This is but one example that suggests optimizing maternal nutrition benefits both fetal and postnatal lung development. In conclusion, our data indicate that components of the HFD and/or the metabolic response to maternal obesity secondary to HFD consumption appears to be deleterious to fetal development in many ways, including by constraint of fetal growth and expansion of fetal liver hematopoietic stem and progenitor cells as discussed in a recent study (19).

Collectively, the present study suggests that maternal HFD before and during pregnancy induces elevations in maternal glucose and insulin, placental inflammation resulting in placental insufficiency, FGR, and attenuation of normal fetal lung development and maturation. The reduction in structural and biochemical fetal lung development could predispose offspring to an increased risk of RDS at birth if delivered prematurely, and the development of chronic lung disease in the neonatal period, which is supported by the persistence of alveolar simplification in the HFD cohort at 2 wk of age. The airspace of HFD-exposed pup lungs morphologically were "simplified," meaning they were enlarged and elongated secondary to a reduction in alveolar septation (14). Importantly, it has been demonstrated that supplementing the maternal diet with docosahexaenoic acid lessened inflammation and improved lung growth in the offspring (32, 36). This is but one example that suggests optimizing maternal nutrition benefits both fetal and postnatal lung development. In conclusion, our data indicate that components of the HFD and/or the metabolic response to exposure to the HFD contribute to poor fetal lung maturation and development.

GRANTS
This work was supported by a Marshall Klaus Award to R. S. Mayor and National Heart, Lung, and Blood Institute Grant HL-093535 to R. C. Savani.

DISCLOSURES
No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: R.S.M., K.E.F., B.F.P., C.R.R., R.C.S., and D.J.C. conceived and designed the research; R.S.M., C.R.R., and D.J.C. approved final version of manuscript; R.S.M., prepared figures; R.S.M., C.R.R., and D.J.C. drafted manuscript; R.S.M., A.P.F., and D.J.C. interpreted results of experiments; R.S.M. and L23–L34, 2014.

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