Intrafetal glucose infusion alters glucocorticoid signaling and reduces surfactant protein mRNA expression in the lung of the late-gestation sheep fetus

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1Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, Australia; and 2Molecular and Evolutionary Physiology of the Lung Laboratory, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, Australia

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McGillick EV, Morrison JL, McMillen IC, Orgeig S. Intrafetal glucose infusion alters glucocorticoid signaling and reduces surfactant protein mRNA expression in the lung of the late-gestation sheep fetus. Am J Physiol Regul Integr Comp Physiol 307: R538–R545, 2014. First published July 2, 2014; doi:10.1152/ajpregu.00053.2014.—Increased circulating fetal glucose and insulin concentrations are potential inhibitors of fetal lung maturation and may contribute to the pathogenesis of respiratory distress syndrome (RDS) in infants of diabetic mothers. In this study, we examined the effect of intrafetal glucose infusion on mRNA expression of glucose transporters, insulin-like growth factor signaling, glucocorticoid regulatory genes, and surfactant proteins in the lung of the late-gestation sheep fetus. The numerical density of the cells responsible for producing surfactant was determined using immunohistochemistry. Glucose infusion for 10 days did not affect mRNA expression of glucose transporters or IGFs but did decrease IGF-1R expression. There was reduced mRNA expression of the glucocorticoid-converting enzyme HSD11B-1 and the glucocorticoid receptor, potentially reducing glucocorticoid responsiveness in the fetal lung. Furthermore, surfactant protein (SFTP) mRNA expression was reduced in the lung following glucose infusion, while the number of SFTP-B-positive cells remained unchanged. These findings suggest the presence of a glucocorticoid-mediated mechanism regulating delayed maturation of the surfactant system in the sheep fetus following glucose infusion and provide evidence for the link between abnormal glycemic control during pregnancy and the increased risk of RDS in infants of uncontrolled diabetic mothers.

out gestation, obese women are less insulin-sensitive than lean and overweight women prior to conception and remain this way throughout pregnancy (7, 8). As a result, they are at an increased risk of developing gestational diabetes. Worldwide, 3–10% of pregnancies are complicated by abnormal glycemic control, caused mostly by gestational diabetes (39). Uncontrolled diabetes in pregnancy results in exposure of the developing fetus to increased plasma glucose concentrations as a result of increased placental transfer, as well as increased secretion of insulin through fetal pancreatic activity in response to the increased fetal plasma glucose concentrations. Furthermore, exposure to maternal diabetes can alter fetal glucose transport due to changes in the expression of the solute carrier family 2 (facilitated glucose transporter, SLC2A), which is expressed in the lung (49). In addition, insulin plays an important role in lung development (28), and its effects are mediated by IGF signaling, including IGF-1, IGF-2, and IGF-1 receptor (IGF-1R). These growth factors have been associated with altered lung development (17) and respiratory complications, such as respiratory distress syndrome (RDS) following birth in neonates (10).

Infants of diabetic mothers were at a six-fold increased risk of developing RDS compared with infants of nondiabetic mothers (47). RDS is associated with both structural and biochemical immaturity of the neonatal lung; of particular importance is the delay in the maturation of the surfactant system (2). The presence of pulmonary surfactant, a complex mixture of lipids and proteins, at the air-liquid interface of the lung is required to prevent alveolar collapse throughout the breathing cycle and aids in the transition to air breathing at birth (12). The surfactant complex is synthesized, stored, and secreted from type II alveolar epithelial cells (AECs), which line the alveoli (34). Maturation of the surfactant system occurs in late gestation, in parallel with the prepartum cortisol surge and plays a vital role in preparing the fetus for the transition to extraterine life (55). The lipid component of surfactant is primarily responsible for reducing surface tension; however, surfactant proteins (SFTP)-B and SFTP-C aid in their adsorption to the air-liquid interface and in the dynamic regulation of the functional surfactant film (43). SFTP-A and SFTP-D play important roles in innate immunity within the lung (24).

Maternal diabetes has been associated with a reduction in SFTP-A protein (51) and lipid profiles (35) in amniotic fluid, suggesting a delay in fetal lung maturation. In vitro and in vivo studies using rodents have shown that increased glucose and/or insulin concentrations result in a reduction of both the protein...
and lipid components of pulmonary surfactant (19, 20, 22, 23, 45). The molecular mechanism regulating this delayed maturation of the surfactant system has not been investigated. It was proposed that insulin may act indirectly by antagonizing the stimulatory effects of cortisol (14), the endogenous glucocorticoid (GC), in the lung, which normally plays a major role in stimulating lung and surfactant maturation. The bioavailability of cortisol in the lung may be affected, for example, through alterations in the regulatory enzyme isoforms, hydroxysteroid (11β) dehydrogenase (HSD11B-1), which catalyzes the conversion of inactive cortisone to cortisol, or HSD11B-2, which catalyzes the conversion of bioactive cortisol to cortisone (56). Alternatively, GC signaling may be affected by alterations in the levels of the GC receptor (nuclear receptor subfamily 3, group C, member 2; NR3C2), the intracellular mediators of GC activity (37).

With the exception of one study examining factors, including glycogen regulation, surfactant lipid content (65), β-cell receptor binding (62), and surface-active material flux in tracheal fluid (60, 63) in the lung of the fetal sheep following exposure to hyperglycemia and hyperinsulinemia (analogous to the conditions experienced by a fetus in an uncontrolled diabetic pregnancy), no studies have evaluated the effect on regulation of SFTP expression in a large animal model with a developmental lung pattern similar to that of humans. Furthermore, no molecular mechanism has been identified to explain the historical link between uncontrolled diabetes in pregnancy and RDS in infants of diabetic mothers. Here, we investigate the impact of intrafetal glucose infusion on pathways that may regulate maturation of the surfactant system in the lung of the late-gestation sheep fetus.

METHODS

All procedures were approved by the University of Adelaide Animal Ethics Committee.

Animals and Surgery

Twenty-one pregnant Merino ewes were housed in individual pens in animal holding rooms, with a 12:12-h light-dark cycle, and fed once daily with water ad libitum. At 118–120 days of gestation (term 150 ± 3 days), general anesthesia was induced in the ewe with an intravenous injection of sodium thiopentone (1.25 g; Pentothal, Rhone Merieux, Pinkenba, QLD, Australia) and maintained with 2.5–4% halothane inhalation anesthesia (Fluothane; Imperial Chemical Industries (ICI) Ltd., Melbourne, VIC, Australia) in oxygen. Vascular catheters were implanted in the ewe’s jugular vein, a carotid artery, and jugular vein of the fetus, and in the amniotic cavity, as previously described (38). Ewes received an intramuscular injection of antibiotics [3.5 ml of Norocillin (150 mg/ml procaine penicillin and 112.5 mg/ml benzathine penicillin); Norbrook Laboratories, Gisborne, Australia] and 2 ml of 125 mg/ml dihydrostreptomycin in sterile saline (Sigma, St. Louis, MO) for 3 days following surgery. Antibiotics (500 mg; sodium ampicillin; Commonwealth Serum Laboratories, Melbourne, VIC, Australia) were administered intra-amniotically to all fetal sheep daily for 4 days postoperatively. Ewes were allowed at least 4 days to recover from surgery prior to the experimental protocol.

Arterial Blood Gas Measurements

Fetal arterial blood was collected throughout the infusion period, and at each time point, whole blood arterial partial pressure of oxygen (PaO2), arterial partial pressure of carbon dioxide (PaCO2), pH, oxygen saturation (SaO2), and Hb content were measured using an ABL 520 analyzer (Radiometer, Copenhagen, Denmark) with the temperature corrected to 39°C.

Intraplatal Infusion Regime

Glucose-infused fetuses received an intravenous infusion of 50% dextrose in saline from 130–140 days of gestation (n = 9). Infusion began at an initial rate of 1.9 ml/h for 24 h and was then increased in a stepwise manner by 1.9 ml/h per day for the next 3 days. The final infusion rate of 7.5 ml/h obtained on the 4th day of the infusion was maintained until post mortem. Saline-infused fetuses received saline intravenously from 130 to 140 ± 1 day of gestation (n = 12). The timing of this infusion regime coincides with the time during which the surfactant system is undergoing maturation. The glucose infusion protocol resulted in a significant increase in both fetal plasma glucose and insulin concentrations (38), with mean values throughout the 10-day infusion period for plasma glucose (saline-infused, 1.10 ± 0.09 mmol/l; glucose infused, 2.37 ± 0.19 mmol/l; P < 0.05) and insulin (saline-infused, 4.93 ± 1.04 mmol/l; glucose infused, 10.26 ± 1.50 mmol/l; P < 0.05).

Post Mortem Procedures

At 140 ± 1 day gestation, ewes were humanely killed with an overdose of sodium pentobarbital administered via the jugular vein (Virbac, Peakhurst, NSW, Australia), and fetal sheep were delivered by hysterotomy. The lungs were removed, weighed, snap frozen in liquid nitrogen, and stored at −80°C for molecular analysis. A section of lung tissue was fixed in 4% paraformaldehyde for immunohistochemical analysis. Neuroendocrine function data from these fetuses have been published previously (38).

Quantification of mRNA Transcripts Within the Fetal Lung

Total RNA extraction. Total RNA was extracted from fetal lung samples (~50 mg) using Invitrogen TRIzol reagent solution and Qiagen miRNex purification columns as per the manufacturer’s guidelines (Invitrogen, Carlsbad, CA) (18, 34). Total RNA integrity from all extracted tissue samples was assessed by running samples on an agarose gel stained with ethidium bromide. Total RNA was quantified by spectrophotometric measurements at 260 and 280 nm and checked for protein and DNA contamination. cDNA was synthesized using Superscript III First Strand Synthesis System (Invitrogen) using 2 μg of total RNA in a final volume of 20 μl as per the manufacturer’s guidelines. Controls containing either no RNA transcript or no Superscript III were used to test for reagent contamination and genomic DNA contamination, respectively.

Quantitative real-time RT-PCR. Initially, the geNorm component of qbaseplus 2.0 software (Biogazelle, Zwijnaarde, Belgium) was used to determine the most stable reference genes from a panel of candidate genes (57) and the minimum number of reference genes required to calculate a stable normalization factor, as previously described (34, 52). For quantitative RT-PCR (qRT-PCR) data output normalization, three stable housekeeping genes—β-actin (ACTB; U39357), peptidylprolyl isomerase A (PPIA; AY251270) (42), and tyrosine 3-monooxygenase (TYMS; AY970970) (34)—were run in parallel with target genes, as previously described (34, 52). The gene expression of the glucose transporters [SLC2A1 (U89029.1) and SLC2A4 (AB005283), IGFB signaling [IGF1 (DQ152962), IGF2 (M98789), and IGF1R (AY162434) (18)], GC regulatory genes [HSD11B-1, NM_001009395.1; HSD11B-2, NM_001009460.1; NR3C1, NM_001141861; NR3C2, AF349768.1 (34)], surfactant proteins [SFTP-A, AF211856; SFTP-B, AF07544; SFTP-C, AF076634; and SFTP-D, AJ133002.1 (34, 41)] were measured by qRT-PCR using Fast SYBR Green master mix (Applied Biosystems, Foster City, CA) in a
Table 1. Quantitative RT-PCR primer sequences for designed target genes

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence 5' → 3'</th>
<th>Primer Concentration, µM</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC2A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>ATGGTGCCCACTTCTTGCGTGG</td>
<td>0.45</td>
<td>U89029.1</td>
</tr>
<tr>
<td>Reverse</td>
<td>TGAGAACATGGCAGCAAAAGAA</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>SLC2A4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>TGCGACTCTTGGAATTCCTCC</td>
<td>0.45</td>
<td>AB005283</td>
</tr>
<tr>
<td>Reverse</td>
<td>AGGTCTGGAATGATGAGAATTTG</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

Accession numbers refer to the published cDNA sequences from which the primer sequences were designed.

final volume of 6 µl on a Viia7 fast real-time PCR system (Applied Biosystems), as previously described (34). Each qRT-PCR well contained 3 µl of Fast SYBR Green master mix (2X), 2 µl of forward and reverse primer mixed with H2O to obtain final primer concentrations and 1 µl of diluted relevant cDNA. The abundance of each transcript relative to the abundance of stable housekeeping genes (27) was calculated using DataAssist 3.0 analysis software (Applied Biosystems) and was expressed as mRNA mean normalized expression (MNE) ± SE (34, 52).

Quantification of SFTP-B-Positive Cells in the Fetal Lung

Immunohistochemistry for identification of SFTP-B-positive cells. In a subset of animals (saline-infused, n = 5; glucose-infused, n = 6), immunohistochemistry was performed (34) using a monoclonal antibody to SFTP-B (produced by Dr. Y. Suzuki, Kyoto University, Japan and kindly donated by F. Possmayer, University of Western Ontario, Canada), staining of which is restricted to type II AECs in the alveolar epithelium and Clara cells in the bronchiolar epithelium (32). Paraformaldehyde-fixed, paraffin-processed lung tissue sections of 7-µm thickness were deparaffinized and rehydrated before endogenous peroxide solution activity was blocked, and followed with antigen retrieval. Slides were incubated overnight with the aforementioned SFTP-B antibody (1:10,000) at 4°C. Negative control slides were performed in parallel with test slides. A Histostain-Plus broad-spectrum kit (Zymed Laboratories, San Francisco, CA) was utilized with horseradish peroxidase and 3,3-diaminobenzidine chromagen (metal-enhanced diaminobenzidine substrate kit; Pierce Biotechnology, Rockford, IL) for visualization of SFTP-B-positive cells. All sections were counterstained with Mayer’s hematoxylin (Sigma-Aldrich, St. Louis, MO).

Quantitative assessment of SFTP-B-positive cells in fetal lung tissue. Sections were examined using Visiopharm NewCAST software (Visiopharm, Hoersholm, Denmark), as previously described (34). Analysis was carried out by a trained individual, who was blinded to treatment groups. Sixty counting frames (x600 magnification) of the alveolar epithelium were randomly selected per section. Point-counting using an unbiased counting frame with an area of 20,000 µm² was used to determine the numerical density of SFTP-B-positive cells within the fetal lung. Using the four corners of the test frame, the reference space was estimated from the points falling on lung tissue. The numerical density of SFTP-B-positive cells expressed as SFTP-B-positive cells per mm² of lung tissue was obtained using the following equation (5, 34);

\[
\text{Density of SFTP-B-positive cells} = \frac{N}{P} = \frac{\sum Q}{\sum P}\text{ (fetal lung tissue)} \times \text{area of frame}\]

where \(\sum Q\) (SFTP-B-positive) represents the total number of SFTP-B-positive cells counted in all counting frames of one fetal lung tissue section; \(\sum P\) (lung tissue) represents the total number of points falling on lung tissue (i.e., the reference space); \(P\) is the number of points that were used to count the points hitting the reference space (i.e., four corners per counting frame); and \(a\) was the area of the counting frame. Tissue sections were photographed using a digital camera DP72 (Olympus Australia), which was connected to a BX53 Research Microscope (Olympus Australia).

Table 2. Mean arterial blood gas and pH values on the day before post mortem in saline- and glucose-infused fetuses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline-Infused (n = 12)</th>
<th>Glucose-Infused (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, mmHg</td>
<td>21.6 ± 0.5</td>
<td>21.4 ± 1.0</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>51.1 ± 1.2</td>
<td>55.3 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.383 ± 0.008</td>
<td>7.393 ± 0.007</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>64.9 ± 1.7</td>
<td>62.0 ± 2.6</td>
</tr>
<tr>
<td>Hb, ml/dl</td>
<td>10.4 ± 0.3</td>
<td>11.2 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Data were analyzed by a Student’s unpaired t-test. *P < 0.05 was considered statistically significant. PaO2, arterial partial pressure of oxygen; PaCO2, arterial partial pressure of carbon dioxide; SaO2, oxygen saturation; Hb, hemoglobin.

Statistical Analyses

PaO2, PaCO2, pH, SaO2, and Hb were calculated as the mean of the values collected on the day before post mortem. Fetal weight, crown-rump length, lung weight, and relative lung weight were recorded at post mortem. All statistical analyses were carried out using Statistical Package for Social Sciences v20.0 (Chicago, IL). A Student’s unpaired t-test was used to compare all data between the saline- and glucose-infused fetuses. All data are presented as means ± SE. A probability level of 5% (P < 0.05) was considered significant.

RESULTS

Impact of Intrafetal Glucose Infusion on Fetal Blood Gases and Body and Organ Weight

Mean PaO2, PaCO2, pH, SaO2, and Hb were not different between saline- and glucose-infused fetuses on the day before post mortem (Table 2). Glucose infusion did not affect fetal weight, crown-rump length, lung weight, or relative lung weight-to-body weight ratio compared with saline-infused controls (Table 3).

Effect of Intrafetal Glucose Infusion on Expression of Genes Regulating Glucose Transport and Insulin Signaling in the Fetal Lung

Lung mRNA expression of glucose transporters (SLC2A1 and SLC2A4), IGF1, and IGF2 were not different between the saline- and glucose-infused fetuses. However, IGF1R mRNA expression was reduced in the lung of the glucose-infused fetus (Table 4).

Table 3. Effect of intravenous saline and glucose infusion on fetal and lung growth

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline-Infused (n = 12)</th>
<th>Glucose-Infused (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at post mortem, days</td>
<td>140 ± 1</td>
<td>140 ± 0*</td>
</tr>
<tr>
<td>Fetal weight, kg</td>
<td>4.81 ± 0.16</td>
<td>5.12 ± 0.13</td>
</tr>
<tr>
<td>Crown-rump length, cm</td>
<td>58.3 ± 1.4</td>
<td>56.9 ± 1.3</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>157.97 ± 6.7</td>
<td>158.04 ± 8.9</td>
</tr>
<tr>
<td>Relative lung weight, g/kg</td>
<td>33.1 ± 1.4</td>
<td>30.8 ± 1.3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Data were analyzed by a Student’s unpaired t-test. *P < 0.05 was considered statistically significant.
Effect of Intrafetal Glucose Infusion on Expression of Genes Regulating GC Availability and Signaling in the Fetal Lung

Lung mRNA expression of HSD11B-1 and NR3C1 was reduced in glucose-infused fetuses (Fig. 1, A and C). There was no difference in mRNA expression of HSD11B-2 or NR3C2 (Fig. 1, B and D) in the fetal lung.

Effect of Glucose Infusion on Surfactant Protein mRNA Expression and the Numerical Density of SFTP-B Positive Cells in the Fetal Lung

Glucose infusion resulted in a reduction of SFTP-A, SFTP-B, SFTP-C, and SFTP-D mRNA expression in the fetal lung (Fig. 2). The numerical density of SFTP-B-positive cells in the alveolar epithelium of the fetal lung was not different between saline- and glucose-infused fetuses (Fig. 3).

DISCUSSION

Intrafetal glucose infusion increased plasma glucose and insulin concentrations (38), resulting in a reduction in the expression of genes regulating GC availability and signaling in the lung. These findings represent a potential mechanism regulating the observed reduction of SFTP mRNA expression in the lung of the hyperglycemic and hyperinsulinemic late-gestation sheep fetus. These findings provide evidence for a molecular mechanism regulating the delay in surfactant system maturation in infants of uncontrolled diabetic mothers and may explain the increased incidence of RDS (47, 59).

While increased plasma glucose and insulin concentrations are experienced by the fetus in a diabetic pregnancy, the specific effects at the cellular level can be determined by the extent of glucose uptake due to modulation of alterations in glucose signaling or indirect action on other pathways normally regulating fetal lung development. In fetal life, SLC2A1 is the primary regulator of cellular glucose uptake across the plasma membrane by carrier-mediated facilitated diffusion in a wide variety of tissues, including the lung (49). Furthermore, in response to the secondary hyperinsulinemia induced by fetal glucose infusion, it is necessary to consider the role of the insulin-dependent glucose transporter, SLC2A4, whose action on glucose uptake has been widely characterized in insulin-responsive tissues. These factors are important as they have been implicated in the regulation of glucose signaling in the fetal lung by exposure to high or low concentrations of glucose in vitro (49). It has been suggested that a limitation in utilization of the glucose as a substrate for surfactant synthesis or

Table 4. Effect of intravenous infusion on the mRNA expression of genes regulating glucose uptake and insulin signaling in the fetal lung

<table>
<thead>
<tr>
<th>Gene</th>
<th>Saline-Infused (n = 12)</th>
<th>Glucose-Infused (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC2A1</td>
<td>0.0155 ± 0.0012</td>
<td>0.0138 ± 0.0008</td>
</tr>
<tr>
<td>SLC2A4</td>
<td>0.0015 ± 0.0003</td>
<td>0.0008 ± 0.0001</td>
</tr>
<tr>
<td>IGF1</td>
<td>0.0347 ± 0.0041</td>
<td>0.0393 ± 0.0042</td>
</tr>
<tr>
<td>IGF2</td>
<td>14.10 ± 0.61</td>
<td>11.91 ± 0.97</td>
</tr>
<tr>
<td>IGF1R</td>
<td>0.0837 ± 0.0029</td>
<td>0.0689 ± 0.0037*</td>
</tr>
</tbody>
</table>

Data are expressed as mean normalized expression (MNE) ± SE. Data were analyzed by a Student’s unpaired t-test between saline- and glucose-infused fetuses. *P < 0.05 was considered statistically significant.
lung liquid clearance may correlate with the historical incidence of RDS in infants of poorly controlled diabetic mothers (49). Despite these findings on SLC2A expression following exposure to increased glucose and/or insulin concentrations, we observed no change in the mRNA expression of either SLC2A1 or SLC2A4 in the lung of the sheep fetus following glucose infusion in this study. These results suggest that it is unlikely that the observed changes in the fetal lung following glucose infusion are regulated directly by glucose availability; rather, changes occur by indirect regulation by the action of these factors at the tissue level.

In addition to regulation of fetal development by glucose, insulin and IGF-1 are key mediators of normal lung development (46, 53). Within the fetal lung, IGF1 and IGF2 both bind to the IGF-1 receptor (IGF1R) to regulate normal lung development and cellular proliferation during both fetal and postnatal life (17, 28). It has previously been demonstrated that changes in expression of these growth factors between lung

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**Fig. 2.** Intrafetal glucose infusion decreased SFTP mRNA expression in the fetal lung. mRNA mean-normalized expression (MNE) of surfactant protein (SFTP)-A (A), SFTP-B (B), SFTP-C (C), and SFTP-D (D) decreased in the lung of glucose-infused fetuses. Data are expressed as means ± SE. *P < 0.05 was considered significant. Saline-infused fetuses are represented by open bars (n = 12), while glucose-infused fetuses are represented by closed bars (n = 9).

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**Fig. 3.** Evaluation of SFTP-B-positive cells in the alveolar epithelium. Micrographs demonstrating SFTP-B immunoreactivity in the alveolar epithelium in the saline-infused (A) and glucose-infused (B) fetal lung (×200 magnification; scale bar = 50 μm). Glucose infusion did not change the numerical density of SFTP-B-positive cells per mm² of lung tissue (C). Data are expressed as means ± SE. *P < 0.05 was considered significant. Saline-infused fetuses are represented by open bars (n = 5), while glucose-infused fetuses are represented by solid bars (n = 6).
increases (15), while increased insulin in combination with cortisol 

Type 2 diabetes, in which treatment with GCs decreased both 

and hyperinsulinemia involves 

changes in the fetal lung following exposure to hyperglycemia 

production, which impairs surfactant phospholipid synthesis in 

the pancreatic 

insulin signaling (16), and inhibition of insulin secretion from 

increased lipolysis and enhanced gluconeogenesis (26), altered 

expression in the lung of the glucose-infused fetuses, suggesting that there may be alterations 

to cellular proliferation and airway development in the 

lung, which may be associated with increased risk of compli- 
cations at birth in infants of poorly controlled diabetic mothers 

(17, 33).

While the maturational effects of endogenous/exogenous 

GCs on the fetal lung are widely understood, the impact of 

glucose and insulin either individually or synergistically, and 

their interaction with GC on SFTP expression, is less well 

characterized. Insulin has been shown to inhibit the GC-

stimulated incorporation of choline into the major surfactant 

phospholipid, phosphatidylcholine, by fetal rat type II AECs in 

culture (50). Furthermore, the presence of hyperglycemia and 

secondary hyperinsulinemia was found to inhibit the stimula- 
tory effects of cortisol on surface-active material flux into 

tracheal fluid (61). An antagonistic relationship between GCs 

and insulin is well established in insulin-sensitive tissues and 

includes impairment of insulin-dependent glucose uptake, 

increased lipolysis and enhanced gluconeogenesis (26), altered 

insulin signaling (16), and inhibition of insulin secretion from 

the pancreatic β-cells (4, 30). Furthermore, the opposing ef- 

fects have been characterized in clinical situations such as 

Type 2 diabetes, in which treatment with GCs decreased both 

insulin sensitivity (40) and NR3C1 expression in skeletal mus- 
cle (58). Interestingly, high plasma concentrations of insulin 

alone decrease both SFTP-A and SFTP-B mRNA expression 

(15), while increased insulin in combination with cortisol 

increases SFTP-A expression in a dose-dependent manner in 

human fetal lung explants (14). It has been suggested that a 

link between hyperinsulinemia and RDS may involve a reducti- 

on in glycerol-3-phosphate and dihydroxyacetone phosphate 

production, which impairs surfactant phospholipid synthesis in 

the lung (54). A further potential mechanism regulating 

changes in the fetal lung following exposure to hyperglycemia 

and hyperinsulinemia involves β-receptor binding, which plays 

a vital role in surfactant release and reabsorption of fetal lung 

liquid (62, 64). Interestingly, although the latter study demon- 

strated a negative impact of hyperglycemia and hyperinsulin- 

emia, similar to that experienced in this study, regarding 

β-receptor binding in the lung, there was a greater effect in 

males than females (62). This represents a potential mechanism 

for male infants to suffer disadvantage in respiratory morbidity 

following exposure to uncontrolled diabetes during pregnancy. 

Although this presents an interesting factor, a limitation of the 
current study is that there was not sufficient statistical power to 
determine differences due to fetal sex.

Despite the above-mentioned observations from previous 

studies, a molecular mechanism regulating the maturation of 

the protein component of the surfactant system under these 

conditions during fetal life has not been established. Here, we 
have demonstrated that following exposure to increased glu- 
cose and insulin concentrations in utero, there is a reduction in 

HSD11B–1 mRNA expression in the fetal lung. This result 
suggests a reduction in the rate of cortisol activation, and thus, 
glucose infusion may result in prereceptor regulation of GC 
action in the fetal lung in late gestation. In addition to regula-
tion of GC availability, there is a reduction in the mRNA 
expression of NR3C1 in the lung of the glucose-infused fetus. 
The NR3C1 is the intracellular mediator of GC function, which 
has been widely characterized by both direct action on genes 

with a GC response element and indirect mechanisms via the 

action of transcription factors and cofactors to promote both 

normal lung and surfactant system maturation (37). These 
changes in GC availability and signaling provide evidence for 
a molecular mechanism linking increased glucose and insulin 
concentrations with delayed lung maturation in utero.

Moreover, we have demonstrated that following exposure to 

increased glucose and insulin concentrations, there is a con- 
comitant reduction of SFTP mRNA in the fetal sheep lung in 

late gestation. Although a rat model has previously been 

utilized to examine the effects of induced diabetes on Stpp 

mRNA expression in the fetal lung (22, 23), we have utilized 

a more clinically relevant model to investigate the effect of 

increased glucose and insulin concentrations in utero on lung 

development and pulmonary surfactant maturation. The fetal 

sheep is an ideal model of human lung development because of 

its similar phasic pattern of development and relative propor-
tions of gestation compared with humans (34). While increased 

glucose concentrations, similar to the ones achieved in this 

study, have been associated with abnormalities in surfactant 

phospholipid content, lung stability, and glycogen regulation in 

the fetal sheep lung (65), here, we provide evidence for 

changes in the protein component of the surfactant system. A 

reduction in all four SFTPs is likely to impair both the surface 

tension regulating and the innate immune functions (24, 31) of 

the surfactant system, potentially impairing the smooth transi-
tion to postnatal life and increasing the risk of RDS following 

birth in infants of diabetic mothers.

To determine whether the changes in SFTP mRNA expres-
sion observed at the molecular level in this study were due to 

changes at the cellular level within the lung, we evaluated the 

number of SFTP-B-positive cells in the alveolar epithelium, 

which are able to produce the components of pulmonary 

surfactant. While it has previously been demonstrated that 

insulin inhibits type II AEC differentiation in fetal rat lung 

explants (21), the impact of high glucose and insulin at the 

cellular level has not been evaluated in vivo. In this study, we 

found no difference in the number of SFTP-B-positive cells in 

the alveolar epithelium between the saline- and glucose-in-

fused explants (21), the impact of high glucose and insulin in 

the lung is believed to impair both the surface tension 

regulating and the innate immune functions (24, 31) of the 
surfactant system, potentially impairing the smooth transition 
to postnatal life and increasing the risk of RDS following 

birth in infants of diabetic mothers. 

To determine whether the changes in SFTP mRNA expres-
sion observed at the molecular level in this study were due to 

changes at the cellular level within the lung, we evaluated the 

number of SFTP-B-positive cells in the alveolar epithelium, 

which are able to produce the components of pulmonary 
surfactant. While it has previously been demonstrated that 

insulin inhibits type II AEC differentiation in fetal rat lung 

explants (21), the impact of high glucose and insulin at the 
cellular level has not been evaluated in vivo. In this study, we 

found no difference in the number of SFTP-B-positive cells in 
the alveolar epithelium between the saline- and glucose-in-
fused explants. These results suggest that there were no struc-
tural changes in cell density within the fetal lung; however, it is 
likely that the overall reduction in SFTP mRNA expression 
observed in the glucose-infused fetuses in this study was due to 
a reduced functional capacity of the surfactant-producing cells
that are present in the lung of the glucose-infused fetus during late gestation.

Following glucose infusion in the late-gestation sheep fetus, we have identified changes in GC signaling as a mechanism regulating the observed delay in maturation of the surfactant system following exposure to increased glucose and insulin concentrations. It is likely that this effect on SFTP mRNA expression is mediated at the molecular level through an alteration to the functional output of cells able to produce surfactant, as there was no change to the number of SFTP-B-positive cells present in the alveolar epithelium.

The mRNA changes observed in this study are seen in whole lung tissue; thus, it is important to consider that changes in expression of some genes cannot be compared as simply as the expression of surfactant protein markers that are primarily restricted to the respiratory epithelium and alveolar type II cells in vivo. Despite this, the changes observed in this study highlight the multifactorial impact that exposure to increased plasma glucose and insulin concentrations has to modulate lung development on a whole, which ultimately leads to changes at the surfactant protein mRNA expression level and correlates with the historically observed increase in RDS in infants of poorly controlled diabetic mothers.

**Perspectives and Significance**

It is undisputed that there are more women of reproductive age who are classified as overweight or obese (11, 29) and that factors associated with obese obstetric populations and diabetic pregnancies contribute to an increased risk of maternal and fetal complications, preterm birth, and an increased risk of RDS following birth (6, 9). By investigating the effects in vivo of increased glucose and insulin concentrations in the lung of the late-gestation sheep fetus, we have demonstrated that conditions analogous to those experienced by a fetus in an uncontrolled diabetic pregnancy result in changes to GC signaling and are associated with downregulation of SFTP mRNA expression, which may complicate the fetus’s transition to air breathing at birth. These findings provide evidence for a GC-mediated mechanism to support the link between abnormal glycemic control in utero and RDS observed in infants of mothers with uncontrolled diabetes (47). What is clear is that glucose regulation and homeostatic feedback mechanisms throughout pregnancy are important and that early intervention to maintain tight maternal glycemic control throughout gestation is a key factor that will minimize the detrimental effects of excessive glucose and insulin concentrations on the molecular regulation of lung development and the risk of RDS in pregnancies complicated by diabetes.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

GLUCOSE INFUSION DELAYS SURFACTANT MATURATION


