Pediatric RESEARCH

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BASIC SCIENCE ARTICLE

Fetal growth restriction is associated with an altered cardiopulmonary and cerebral hemodynamic response to surfactant therapy in preterm lambs

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BACKGROUND: Efficacy of surfactant therapy in fetal growth restricted (FGR) preterm neonates is unknown.
METHODS: Twin-bearing ewes underwent surgery at 105 days gestation to induce FGR in one twin by single umbilical artery ligation. At 123–127 days, catheters and flow probes were implanted in pulmonary and carotid arteries to measure flow and pressure. Lambs were delivered, intubated and mechanically ventilated. At 10 min, surfactant (100 mg kg^{-1}) was administered. Ventilation, oxygenation, and hemodynamic responses were recorded for 1 h before euthanasia at 120 min. Lung tissue and bronchoalveolar lavage fluid was collected for analysis of surfactant protein mRNA and phosphatidylcholines (PCs).
RESULTS: FGR preterm lambs were 26% lighter than appropriate for gestational age (AGA) lambs and had baseline differences in lung mechanics and pulmonary blood flows. Surfactant therapy reduced ventilator and oxygen requirements and improved lung mechanics in both groups, although a more rapid improvement in compliance and tidal volume was observed in AGA lambs. Surfactant administration was associated with decreased mean pulmonary and carotid blood flow in FGR but not AGA lambs. No major differences in surfactant protein mRNA or PC levels were noted.
CONCLUSIONS: Surfactant therapy was associated with an altered pulmonary and cerebral hemodynamic response in preterm FGR lambs.

Pediatric Research (2019) 86:47–54; https://doi.org/10.1038/s41390-019-0398-4

INTRODUCTION

Introduction of surfactant replacement therapy in the 1990s marked the beginning of a new era in the management of neonatal surfactant deficiency and its clinical sequelae, respiratory distress syndrome (RDS).[1] Today, surfactant therapy along with antenatal glucocorticoid use is recognized as one of the principal interventions that decrease overall mortality in preterm infants. The rationale for surfactant therapy is supported by robust scientific evidence. Surfactant administration reduces inspired oxygen and ventilation requirements of the neonate, as well as the incidence of severe respiratory distress, pneumothorax, pulmonary interstitial emphysema, and death.[2–4]

Fetal growth restriction (FGR) and prematurity are two important pregnancy complications that lead to high rates of mortality and morbidity in neonates. Unfortunately, they also commonly co-exist to increase the risk of adverse outcomes.[5,6] FGR complicates 10–25% of preterm births and its incidence increases with decreasing gestational age at delivery.[7,8] Abnormal intrauterine cardiovascular, neuroendocrine, metabolic, and oxidative stress states in FGR infants make them susceptible to both short- and long-term complications. Compared to appropriately grown age-matched counterparts, FGR babies have higher perinatal mortality rates, are at significant risk for reduced postnatal growth and impaired development, and have increased incidence of morbidities, such as RDS, bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis.[9–11] Despite clear differences in postnatal sequelae for FGR, current respiratory management of lung disease in preterm FGR infants is not different to that for preterm appropriate for gestational age (AGA) infants that includes supportive respiratory care, early initiation of continuous positive airway pressure, and early surfactant replacement therapy. In fact, there is paucity of data on surfactant therapy and its effects in the preterm FGR neonate.[12] Most studies related to surfactant use in preterm infants have not reported a sub-group analysis on the effectiveness of surfactant therapy in FGR infants.[13]

In this study, we aimed to compare the ventilator, respiratory, and hemodynamic response to early surfactant administration between growth-restricted and appropriately grown preterm lambs. Surfactant phosphatidylcholine (PC) and protein mRNA levels between groups were also assessed. We hypothesized that FGR would invoke different respiratory and hemodynamic responses to surfactant therapy in preterm lambs.

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Received: 19 February 2019 Revised: 3 April 2019 Accepted: 7 April 2019
Published online: 14 April 2019

METHODS

Ethical approval

Experiments complied with the National Health and Medical Research Council of Australia guidelines for the care and use of animals for scientific purposes and were approved by Monash Medical Centre Animal Ethics Committee (MMCA/2010/23).

Experimental design

Animals. Time-mated ewes were obtained from Monash Animal Research Platform (Monash University). Twin bearing Border–Leicester pregnant ewes underwent surgery on days 103–105 of pregnancy (term approximately 148 days) for the procedure of single umbilical artery ligation (SUAL) to induce FGR as previously described.[14] Briefly, ewes were initially anesthetized with sodium thiopentane (20 mL; Pentothal; Boehringer Ingelheim, Australia) prior to intubation and maintenance of anesthesia with inhaled isoflurane (2.0–5.0%) in titrated oxygen. Under sterile operative conditions, the uterus and each fetus were sequentially exposed, and each fetus instrumented with a femoral artery catheter and an amniotic catheter placement for determination of blood gases and to administer antibiotics, respectively. In one of the fetuses (randomly selected), one umbilical artery was ligated using two silk ties (FGR fetus); in the other fetus the umbilical cord was manipulated but not ligated (appropriately grown (AGA) fetus). The fetuses were returned to the uterus, the catheters were exteriorized through the right flank of the ewe, and the wound was secured. A catheter was inserted in a maternal jugular vein for maternal antibiotic administration.

Experimental procedures

For 3 consecutive days after surgery, antibiotics were administered to both the fetus (Ampicillin, 1 g via the amniotic catheter) and the ewe (Engemycin 5 mL intravenous (i.v.)). The maternal and fetal catheters were flushed daily with heparinized saline and a fetal blood sample was taken every second day for assessment of fetal wellbeing. The partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$), oxygen saturation (SaO_2), pH, hematocrit, glucose, and lactate were measured (ABL 700 blood gas analyzer; Radiometer, Copenhagen, Denmark). A separate cohort of animals was euthanized at birth without ventilation (unventilated AGA and FGR).

Delivery of preterm lambs. At 123–127 days gestation, ewes were again anesthetized as above and each fetal head and chest was sequentially exposed via cesarean section. An additional catheter was placed in the brachial artery, and ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the left main pulmonary artery (size: 4 mm) and left carotid artery (size: 3 mm) for the measurement of pulmonary and cerebral arterial blood flows (PBF and CBF, respectively), respectively. A pulse oximeter probe (Masimo, Irvine, CA) was placed on the left forelimb for measurement of transcutaneous oxyhemoglobin saturation levels (SpO_2). After closure of incisions, each fetus was intubated (4.0 mm cuffed endotracheal tube) and lung liquid was passively drained. The umbilical cord was then clamped and cut; the lambs were dried and weighed and transferred to an infant warmer (Fisher and Paykel, Auckland, NZ) where ventilation was initiated. Directly following delivery of the lambs, ewes were euthanized with 20 mL intravenous sodium pentobarbitone (325 mg mL^{-1}, Lethobarb, Virbac, Australia Pty Ltd).

Ventilation. Ventilation of the preterm FGR and AGA lambs was initiated using pressure support ventilation (Babylog 8000+, Dräger, Lüberk, Germany) with an initial peak inspiratory pressure (PIP) of 40 cm H_2O and positive end-expiratory pressure (PEEP) of 5 cm H_2O for the first 20 min. The inspired oxygen fraction (FiO_2) began at 0.21 but was adjusted to maintain SpO_2 between 90 and 95%. Lambs were subsequently ventilated for 100 min (total 120 min) with volume guarantee mode with a target tidal volume (V_T) of 7 mL kg^{-1}. All lambs received prophylactic surfactant (100 mg kg^{-1}, Curosurf; Chiesi Pharma, Italy) at 10 min. Throughout ventilation, lambs were kept sedated by continuous infusion of Alfaxane (3 mg kg^{-1} min^{-1}) through an umbilical vein catheter implanted immediately after delivery. Lamb wellbeing was assessed by regular arterial blood gas measurements (ABL30; Radiometer, Copenhagen, Denmark) via samples collected from the femoral arterial catheter. At the end of the experiment, lambs were euthanized by pentobarbital sodium overdose (100 mg kg^{-1} i.v.; Valabarb, Jurox, Rutherford, Australia). Data from the first 60 min of the experiment were used in this study.

Physiological analysis. All physiological data, including PBF and carotid blood flow and pressures, SpO_2, heart rate, and ventilator parameters (delivered airway pressure, flow, tidal volume, respiratory system resistance), were recorded continuously using LabChart (ADInstruments, NSW, Australia) and analyzed offline. Ten seconds of clean recording was captured immediately prior to surfactant administration and then every 30 s for the first 10 min after surfactant administration.

Calculations. Dynamic lung compliance was calculated as V_T (mL kg^{-1} birth weight)/ΔP (cm H_2O), where $\Delta P = (PIP - PEEP)$. Ventilation efficiency index (VEI)[15] was calculated as $3800/\Delta P \times f \times PaCO_2$, where 3800 mL kg^{-1} min^{-1} mm Hg = CO_2 production constant, ΔP (in mm Hg), and f = breathing frequency. The alveolar arterial difference in oxygen ($AaDO_2$) was calculated as $[P(\text{barometric}) - P(H_2O)] \times FiO_2 - (PaCO_2/0.85) - PaO_2$, where P(barometric) is barometric pressure and $P(H_2O)$ is water vapor pressure at 39 °C. 0.85 is the optimal respiratory quotient in newborn lambs.[16] Pulsatility index (PI) in the pulmonary and carotid artery was calculated from the blood flow waveform using the formula: (peak − systolic blood flow − end-diastolic blood flow)/mean blood flow.

Molecular analysis of lung tissue. Lungs were removed and weighed and tissue samples from the right lower lobe were frozen in liquid nitrogen for subsequent measurement of relative surfactant protein mRNA levels using quantitative reverse transcription PCR (qRT-PCR). Fetal sheep lung tissue was homogenized and total mRNA was isolated (RNeasy Midi Kit, Qiagen) and reverse-transcribed into cDNA (SuperScript III reverse transcriptase, Invitrogen). Genes of interest were measured by qRT-PCR using Applied Biosystems 7900HT Fast Real-Time PCR system. Relative mRNA expression of the surfactant proteins (*SP-A, -B, -C* and *-D*) (See Table 1 for primer details.) were measured. The expression of all genes were normalized to the *18S* rRNA for each sample using the cycle threshold (ΔC_T) method of analysis and was expressed relative to the AGA_{UVC} group.

Biochemical analysis of bronchoalveolar lavage fluid (BALF). The left lung was lavaged three times by infusion and withdrawal of a sufficient volume of 4 °C physiologic saline solution to fully fill the lung.[17] The recovered BALF was frozen in liquid nitrogen for subsequent measurements of total protein concentration and surfactant PC concentration and composition. BALF samples were centrifuged at 9280 × *g* at 4 °C (model 5415 R, Eppendorf) before a protein assay was performed (Bio-Rad). The supernatant was diluted with 0.9% saline (1:4), and 10 µL of the solution were pipetted into a 96-well plate. Dye reagent (200 µL, 1:5 dilution in distilled deionized water; Bio-Rad cat. no. 500-0006) was added to the samples in the wells. Absorbance of the samples and bovine serum albumin standards was measured at 595 nm using a plate reader (SpectraMax i3, Molecular Devices). Soluble protein concentration was calculated from the standard curve.

Table 1. Primer sequences for quantitative real-time PCR

Gene	Species	Accession no.	Primer sequence	Amplicon length, nt
18S	Rat	X01117	5′-GTAACCCGTTGAACCCCATT-3′ 5′-CCATCCAATCGGTAGTAGCG-3′	105
SP-A	*Ovine*	AF211856	*5′* -CATCAAGTCCTGCAGTCACA-3′ 5′-GCCCATTGGTAGAGAAGACC-3′	85
SP-B	Ovine	AF211857	*5′*- GTCCTCTGCTGGACAAGATG-3′ 5′-GGAGAGGTCCTGTGTCTGAG-3′	83
SP-C	Ovine	AF076634	5′-GTGAACATCAAACGCCTTC-3′ 5′-TGTGAAGACCCATGAGCA-3′	88
SP-D	Ovine	AJ133002	5′-ATGACCGATACCAGGAAGGA-3′ 5′-GCCCAGTTGGAATAGACCAG-3′	71

PC analysis using liquid chromatography mass spectrometry (LC-MS). For PC analysis, dried cellular extracts from BALF samples were re-suspended in butanol/methanol (1:1, v/v) containing 5 µM ammonium formate. Lipids were separated by injecting 5 µL aliquots onto a 50 mm × 2.1 mm × 2.7 µm Ascentis Express RP Amide column (Supelco, Sigma, St Louis, MO) at 35 °C using an Agilent LC 1200 (Mulgrave, VIC, Australia) and eluted at 0.2 mL min^{-1} over a 5-min gradient of water/methanol/tetrahydrofuran (50:20:30, v/v/v) to water/methanol/tetrahydrofuran (5:20:75, v/v/v), with the final buffer held for 3 min. Lipids were analyzed by electrospray ionization–mass spectrometry using an Agilent Triple Quad 6460 (Mulgrave, VIC, Australia). Lipid species' presence in the PC lipid class were identified using precursor ion scanning from 100 to 1000 *m/z*, in positive ion mode, PCs and lysophosphatidylcholines (precursors of *m/z* 184.1) and sphingomyelins (*m/z* 184.1). Identified lipid species were quantified using multiple reaction monitoring with a 20-ms dwell time for the simultaneous measurements of ~20–50 compounds and a chromatographic peak width of 30–45 s; minimum data points collected across the peak were 12–16. Optimized parameters for capillary, fragmentor, and collision voltages were 4000, 140–380, and 15–60 V, respectively. In all cases, the collision gas was nitrogen at 7 L min^{-1}. MS data were processed using Agilent Mass Hunter (Mulgrave, VIC, Australia).

Internal lipid standards (Avanti Polar Lipids, Alabaster, AL) were prepared by adding 0.25 µM of dansyl-phosphatidylethanolamine to each sample. Lipid concentrations were calculated by relation of the peak area of each species to the peak area of the corresponding internal standard. Detected PC lipid species were annotated as follows: PC (sum of carbon atoms in the two fatty acid chains esterified at the sn-1 and sn-2 positions: sum of double bonds in the fatty acid chains). Total PC concentration was calculated by summation of the individual lipid species concentrations. The concentrations of total PC and individual PC species are expressed per mg of protein in BALF.

Statistical analysis. Statistical analysis was conducted using a commercial software (SigmaStat, v3.5, Systat, San Jose, CA). Physiological measures and ventilation data were analyzed with two-way repeated-measures analysis of variance (ANOVA) and Tukey's post hoc comparison. Biochemical indices were compared with two-way ANOVA using group (FGR vs. AGA) and treatment (unventilated vs. ventilated) as the factors. Where data were not normally distributed, data were normalized using a log transformation. Regression analysis was used to determine the correlation between PBF and factors (FiO_2 and PBF PI) that are most likely driving the changes observed with surfactant administration. "*n*" represents the number of animals used and $p < 0.05$ was accepted as statistically significant. Data are expressed as mean ± standard error of mean.

Table 2. Lamb baseline characteristics

	AGA_{UVC} *n* = 5	FGR_{UVC} *n* = 4	AGA_{Vent} *n* = 9	FGR_{Vent} *n* = 9
Male, *n* (%)	2 (40)	2 (50)	4 (44)	4 (44)
GA, days	125.0 ± 0.6	125.0 ± 0.6	125.6 ± 0.4	125.6 ± 0.4
Body weight, kg	3.5 ± 0.1	2.5 ± 0.2[a]	3.3 ± 0.2	2.3 ± 0.1[a]
Brain, g/kg	13.8 ± 0.5	17.7 ± 1.1[a]	14.0 ± 0.7	20.5 ± 1.3[a]
Lungs, g/kg	37.3 ± 5.1	34.3 ± 0.7	35.2 ± 1.2	36.5 ± 3.2
Heart, g/kg	7.6 ± 0.4	7.2 ± 0.2	7.1 ± 0.3	7.4 ± 0.3

Data are expressed as mean + SEM unless specified
AGA_{UVC} appropriate for gestational age unventilated controls, FGR_{Vent} fetal growth restricted ventilated animals, *GA* gestational age
[a]Indicates significant difference from the corresponding AGA group

RESULTS

Baseline characteristics

Fourteen ewes were operated on to induce SUAL. There was one fetal death in the FGR group. Baseline characteristics of included lambs are shown in Table 2. FGR lambs were 23% lighter and had increased brain:body weight ratio compared to AGA lambs. Lung and heart weights corrected for body weight were not different between groups.

Ventilation and oxygenation parameters

pH, PaO_2, $PaCO_2$, VEI,[18] and alveolar–arterial difference in oxygen ($AaDO_2$) was not different between groups throughout the study (Fig. 1a–e). Respiratory system resistance was not different between AGA and FGR lambs before (Mean (SD) AGA: 80.7 ± 2.21 vs. FGR: 110.0 ± 18.0 (cm H_2O) ml^{-1} min^{-1}) or after (AGA: 82.3 ± 22.5 vs. FGR: 88.8 ± 12.4 (cm H_2O) ml^{-1} min^{-1}) surfactant administration. Surfactant administration was associated with significantly increased pH and VEI and decreased $PaCO_2$ and $AaDO_2$ by 10 min in both groups (Fig. 1a–e).

Dynamic lung compliance was significantly higher in AGA lambs compared to FGR lambs throughout the study (Fig. 1f); surfactant administration was associated with a significant increase in lung compliance in both groups, but the improvement was more rapid in AGA lambs (by 2.5 min) than in FGR lambs (by 7.5 min). PIPs were not different between groups throughout the study; however, PIPs did decrease over the experimental time period. PIP was 40.2 ± 0.3 cm H_2O prior to surfactant administration, which decreased to 36.0 ± 2.9 cm H_2O by 10 min after surfactant ($p < 0.001$ for both groups). Despite similar settings, tidal volumes achieved were significantly higher in AGA lambs compared to FGR lambs (Fig. 1g); surfactant administration was significantly associated with an increase in

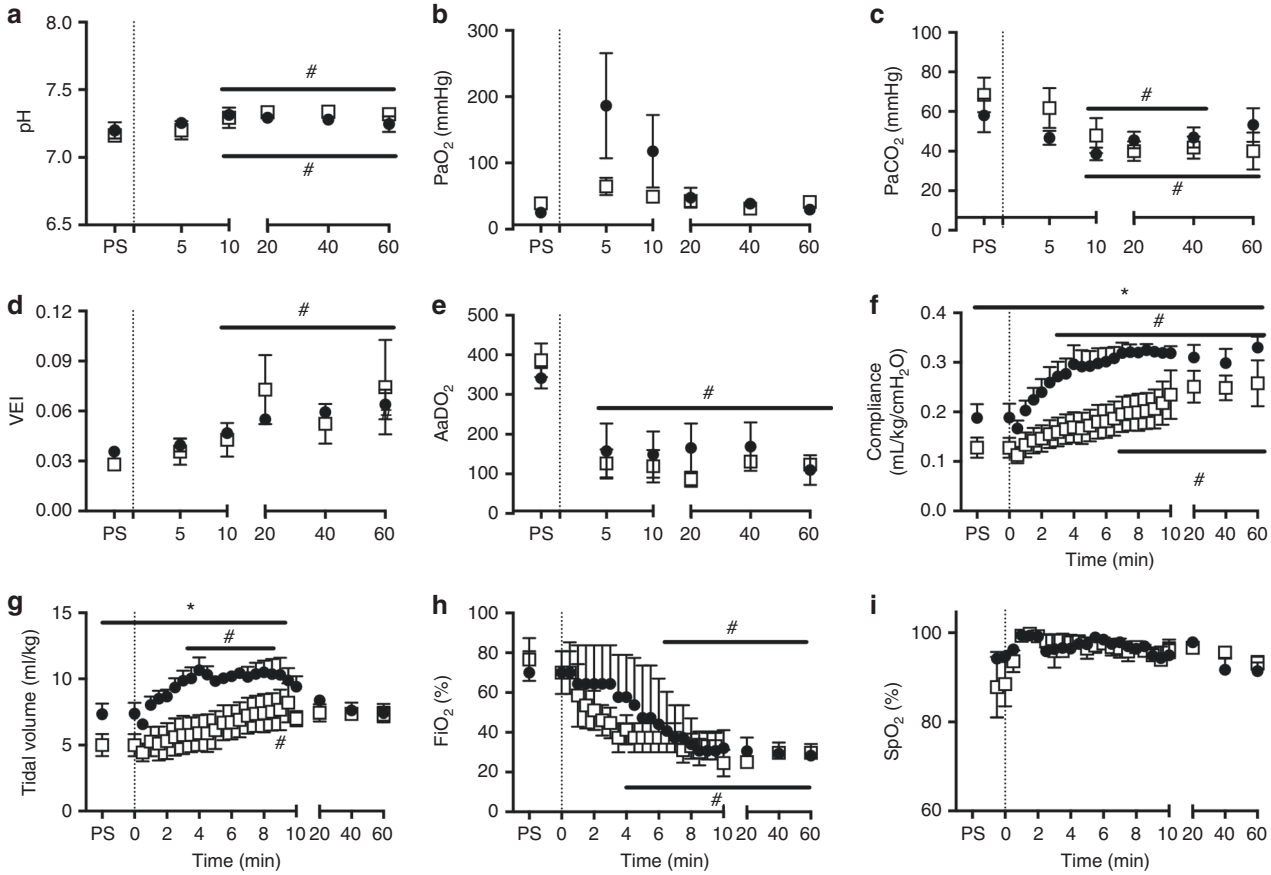

Fig. 1 Graphs showing the mean ± SEM **a** pH, **b** PaO_2 (partial pressure of oxygen in arterial blood), **c** $PaCO_2$ (partial pressure of carbon dioxide in arterial blood), **d** VEI (ventilator efficiency index), **e** $AaDO_2$ (alveolar arterial difference in oxygen concentration), **f** dynamic lung compliance, **g** tidal volume, **h** FiO_2 (fractional concentration of inspired oxygen) and **i** peripheral oxygen content as measured by pulse oximetry prior to (PS) and after surfactant administration (dashed line) in fetal growth restricted (white boxes) and appropriate for gestational age preterm lambs (black circles). Asterisk denotes significant difference between groups, hash denotes time effect from the PS value

tidal volume by 5 min in AGA lambs and by 9.5 min in FGR lambs. Tidal volumes were not different after 10 min as lambs were all in volume guarantee mode. FiO_2 requirements were not different between groups prior to surfactant administration (Fig. 1h); FiO_2 decreased 8 min after surfactant in AGA lambs and 5 min after surfactant in FGR lambs and was not different thereafter.

Pulmonary and cerebral hemodynamics

Prior to surfactant administration, mean and peak systolic PBF and mean and peak CBF were higher in FGR lambs compared to AGA lambs, while PBF PI was not different between groups (Fig. 2). End diastolic PBF was higher in FGR lambs compared to AGA lambs throughout the study but failed to reach significance. Surfactant administration to FGR preterm lambs was associated with a reduction in mean and peak systolic PBF ($p < 0.001$). PBF PI increased in AGA lambs after surfactant administration, resulting in higher PBF PI between 6.5 and 8.5 min compared to FGR lambs (Fig. 2d). Mean and peak CBF decreased in FGR lambs after surfactant administration (Fig. 2e, f). End-diastolic CBF was not different between groups (Fig. 2g), although ductal steal (reversed end-diastolic CBF) was present in all lambs. PI in the carotid artery was higher in FGR lambs compared to AGA for the first 20 min (Fig. 2h) indicative of a higher systemic vascular resistance. PBF and CBF in AGA lambs were not altered by surfactant administration.

To understand why surfactant administration resulted in a fall in PBF in FGR lambs, we ran regression analyses on factors that can alter PBF. We observed that FiO_2 directly correlated with PBF in FGR indicating that the decrease in PBF was most likely due to decreasing FiO_2 with improving oxygenation (Fig. 3). There was no interaction in FGR lambs between PBF PI (an indicator of pulmonary vascular resistance (PVR)) and PBF during the study, indicating that PVR did not decrease after surfactant administration. Interestingly, AGA preterm lambs had the reverse relationship between mean PBF and FiO_2 and a strong correlation was present between PBF PI and PBF.

Surfactant composition

Surfactant protein mRNA studies were done in 5 AGA_{UVC}, 4 $FGR_{UVC,}$ 6 AGA_{UVC}, and 6 FGR_{UVC} lamb lung samples. Lung relative *SP-A*, *SP-B*, *SP-C*, and *SP-D* mRNA levels were not different between the FGR and AGA groups and ventilation did not alter surfactant protein mRNA levels (Fig. 4a–d). BALF studies were available in 5 AGA_{UVC}, 3 $FGR_{UVC,}$ 5 AGA_{UVC}, and 5 FGR_{UVC} samples. Total protein content in the BALF was not different between FGR and AGA unventilated controls; ventilation and surfactant administration significantly increased total protein content in the BALF similarly in AGA and FGR preterm lambs ($p = 0.017$; Fig. 5a). The concentrations of lipid species in PC were not different between FGR and AGA UVC lambs. Ventilation and surfactant administration increased the concentration of the PC lipid species 34:0, 34:2, and 38:4 similarly in the FGR and AGA ventilated groups compared to their respective unventilated controls. No differences were observed between FGR and AGA lambs in any surfactant lipid variable (Fig. 5b).

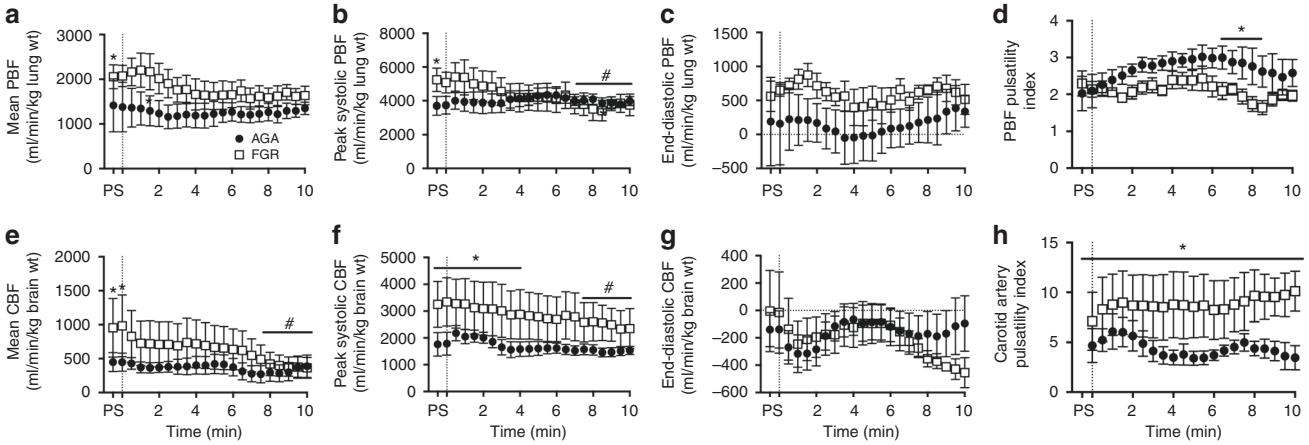

Fig. 2 Graphs showing the initial hemodynamic response to surfactant (first 10 min) of **a** pulmonary blood flow (PBF)/lung weight, **b** peak systolic PBF/lung weight, **c** end-diastolic PBF/lung weight, **d** PBF pulsatility index, **e** cerebral blood flow (CBF)/brain weight, **f** peak systolic CBF/brain weight, **g** end-diastolic CBF/brain weight, and **h** CBF pulsatility index prior to (PS) and after surfactant administration (dashed line) in fetal growth restricted (white boxes) and appropriate for gestational age preterm lambs (black circles). Data are mean ± SEM. Asterisk denotes significant difference between groups, hash denotes time effect from the PS value

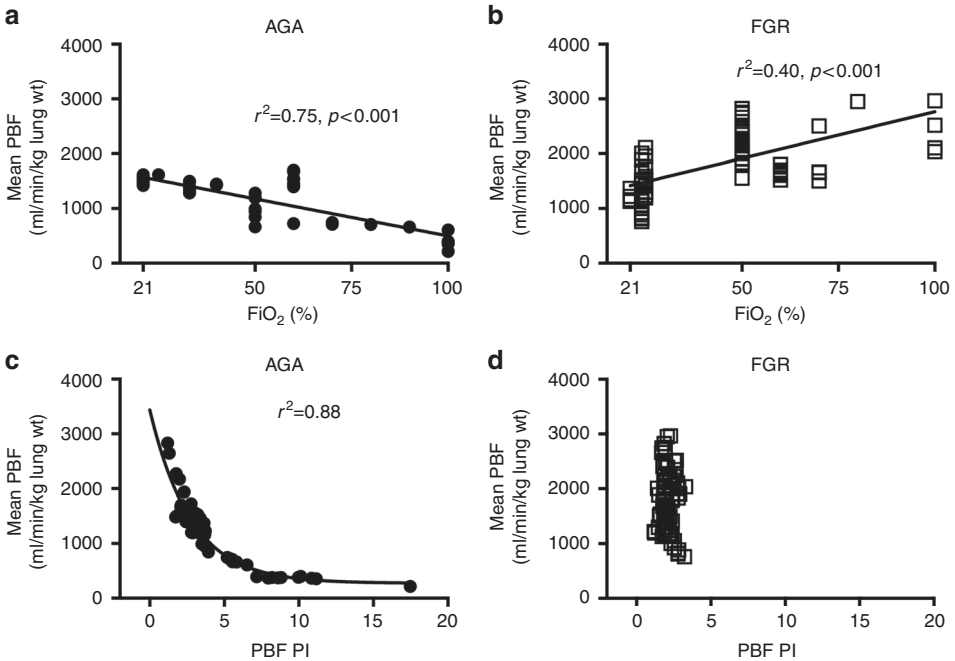

Fig. 3 Regression analysis demonstrating associations between mean pulmonary blood flow (PBF)/lung weight and FiO_2 in appropriate for gestational age (AGA) (**a**) and fetal growth restricted (FGR) (**b**) animals, and mean PBF/lung weight to PBF pulsatility index (PI) in AGA (**c**) and FGR (**d**) animals

DISCUSSION

The efficacy of exogenous surfactant administration in preterm FGR infants currently represents a significant knowledge gap in neonatal research and clinical practice.[12] Our findings in growth restricted preterm lambs demonstrated that, despite similar levels of tissue and airway surfactant mRNA and lipids, FGR preterm lambs had a delayed functional response and an altered hemodynamic response to surfactant therapy compared to appropriately grown preterm lambs in the newborn transition period in the context of an initial pressure limited ventilation strategy. Demonstration of a correlation between inspired oxygen concentration and PBF in FGR preterm lambs illustrate the pathological differences between FGR and AGA preterm neonates and how this can alter the expected response to clinical intervention.

The model

We induced placental insufficiency and growth restriction in fetal sheep using the well-established method of single umbilical artery ligation.[19] This results in placental insufficiency with the resulting growth restriction closely resembling many of the known human pathology associated with FGR, including a similar degree of chronic fetal hypoxemia, cardiac output redistribution, and asymmetric growth restriction characterized by brain sparing.[20] Importantly, lung weight relative to body weight was not different between groups indicating that gross structure was not altered by FGR, consistent with the human model and previous data from our group in preterm lambs.[14] In addition, we have also previously reported the lack of deleterious effects of the brief period of ventilation and FGR on lung histology in this model.[21]

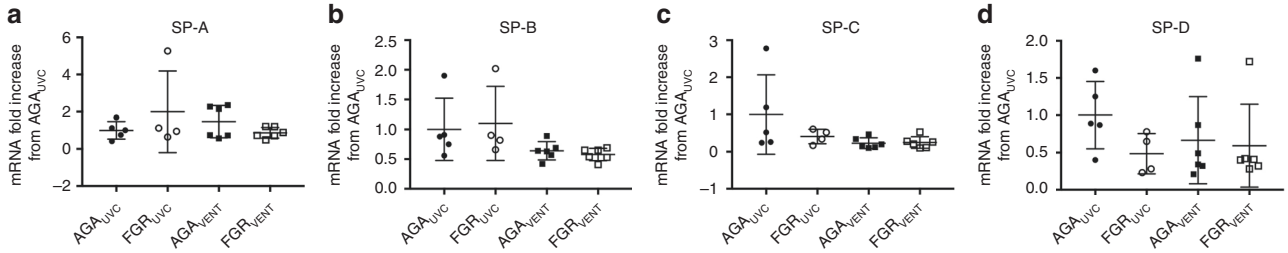

Fig. 4 mRNA levels of surfactant proteins A–D is measured in the lung tissue of appropriate for gestational age (AGA) and fetal growth restricted animals expressed as fold change from AGA$_{UVC}$. Data are expressed as mean ± SD with individual values shown. UVC unventilated controls, VENT ventilated animals

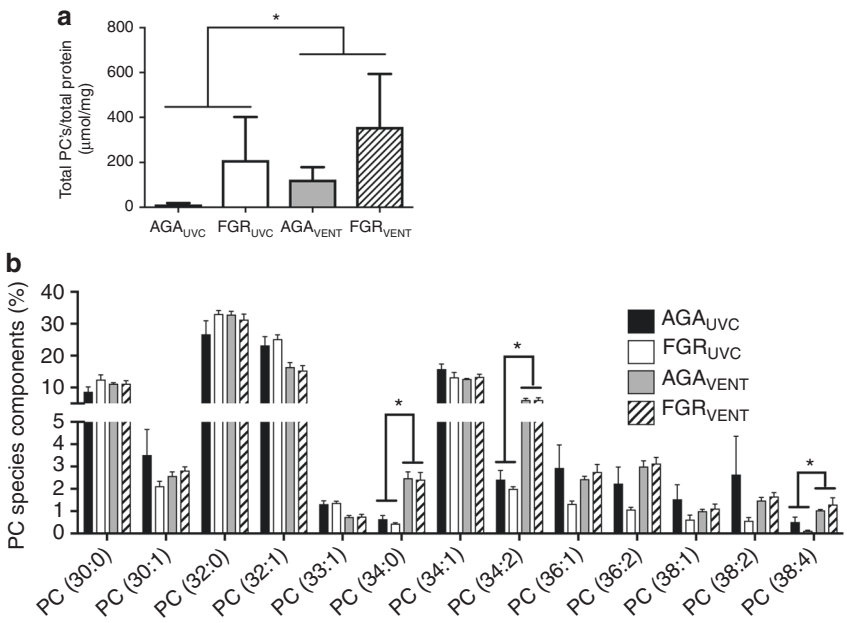

Fig. 5 Graphs showing **a** total phosphatidylcholine (PC) concentration and **b** the percentage composition of individual PC species components in the bronchoalveolar fluid of appropriate for gestational age and fetal growth restricted animals. UVC unventilated controls, VENT ventilated animals. Asterisk denotes significant difference: UVC vs. VENT

Ventilator response

We initiated ventilation in this study using positive pressure ventilation set at 40 cm H_2O for the first 10 min of ventilation. The lambs had not spontaneous breathing efforts as they were continuously sedated, and these pressures did not achieve target tidal volumes in the lamb groups, especially FGR lambs, indicative of a lower lung compliance (Fig. 1f), which is likely to be the reason for the lower achieved tidal volumes during the first 20 min. We did not observe any differences in oxygenation or VEI between the groups prior to or after surfactant administration. This result is not unexpected given our previous studies showing that lung airway development is not altered by SUAL[14,21] despite chronic exposure to higher endogenous cortisol levels.[22] Surfactant administration was significantly associated with improved $PaCO_2$, oxygenation, and ventilation efficiency in FGR and AGA preterm lambs. However, a more rapid improvement in lung compliance and an increase in tidal volumes were observed in AGA preterm lambs. These findings may suggest a better or at least more rapid recruitment of the lung post surfactant administration in the AGA compared to the FGR preterm lamb.

Hemodynamic responses

Prior to surfactant administration, PBF and CBF were higher in FGR compared to AGA preterm lambs. The increased PBF is likely a result of higher systemic vascular resistance (resistivity index), which would favor a greater volume of blood flowing across the lungs and into the pulmonary circulation (left-to-right shunt across the ductus arteriosus). This contention is further supported by the higher end-diastolic blood flow in FGR lambs—while not significantly higher, it is pertinent that 75% of AGA preterm lambs had right-to-left shunt across the ductus arteriosus prior to surfactant administration, as indicated by a negative value of end-diastolic PBF, while 100% of FGR preterm lambs had purely left-to-right shunts (positive end-diastolic PBF). The left-to-right flow across the DA means that the systemic circulation is also contributing to PBF during diastole, resulting in a greater mean PBF and consequently higher ventricular output and carotid blood flow. Higher systemic vascular resistance has been demonstrated in term and preterm FGR infants within the first days of life[23,24]; however, it is not been previously reported to manifest so early after delivery in preterm FGR neonates. Cortisol levels at birth may have been useful to delineate a cause for this early increased systemic vascular resistance.

We observed that PBF was not altered after surfactant administration in AGA preterm lambs as demonstrated previously.[25] The small changes in PBF that did occur in AGA preterm lambs throughout the study were strongly correlated with changes in PVR as expected. The negative correlation between

PBF and FiO_2 in AGA preterm lambs shown in Fig. 3a merely reflects that PBF was low prior to surfactant administration and improved with increasing compliance and oxygenation. However, PBF significantly decreased after surfactant administration in FGR lambs. This fall in PBF would result in reduced left ventricular output and subsequently the coincident fall in CBF. Interestingly, there was a strong correlation between PBF and inspired oxygen in FGR lambs, in that high PBF was associated with high FiO_2 and vice versa. This likely reflects altered inherent pulmonary vascular function in the FGR lamb making it more sensitive to changes in inspired oxygen rather than the normal relationship with PVR. Indeed, there was no evidence that PVR changed throughout the study in FGR lambs. The role of surfactant administration in influencing this difference in FGR vs. AGA lambs is debatable but it will influence the delivered FiO_2, which may have consequences for the circulation in FGR preterm neonates. We have previously demonstrated that the pulmonary vascular response to inhaled nitric oxide was enhanced in FGR preterm lambs compared to AGA lambs, indicative of altered pulmonary vascular reactivity.[26] Further, altered pulmonary vascular mechanics and cardiac performance in response to utero-placental insufficiency has been demonstrated in FGR preterm infants that was theorized as being a major contributor to the increased risk of bronchopulmonary dysplasia in FGR infants.[27] Further, systemic vascular maladaptive responses have also been identified in FGR preterm lambs[26] and infants[28] further indicating the adverse cardiovascular consequence of prolonged exposure to hypoxia.

Surfactant pool size and composition

No differences were observed in lung tissue surfactant protein mRNA levels between the preterm FGR and AGA animals. The scientific data for the effect of FGR on endogenous surfactant production and function are sparse and conflicting. Whereas some experimental studies in animal models report a significant alteration in maturation of type II pneumocytes,[29] resulting in reduced surfactant content and activity, others have reported a significant increase in surfactant protein synthesis.[30] Furthermore, chronic hypoxia and acidosis associated with FGR may attenuate surfactant synthesis.[31] In a sheep model of FGR induced by placental embolization,[32] no alteration in surfactant protein mRNA expression of *SP-A*, *-B*, or *-C* in lung tissue was present. In contrast, Gagnon and colleagues in a similar model reported increased lung tissue *SP-A* and *SP-B* mRNA expression in growth-restricted sheep and that the increase was closely associated with plasma cortisol levels.[30] Chronic fetal growth restriction from conception in carunclectomized sheep also showed decreased lung tissue mRNA expression of *SP-A*, *-B*, and *-C* at 133 and 141 days' gestation, with an inverse relationship between plasma cortisol concentration and *SP-A* and *SP-B* expression.[33] In contrast, previous studies utilizing the SUAL model in sheep fetuses from 108 days' gestation showed no effect on lung tissue surfactant protein mRNA levels by 115 days' gestation.[14] In addition to potential alterations in surfactant protein synthesis, fetal growth restriction could also affect the lipid components of surfactant. For example, growth-restricted rats are shown to have significantly reduced PC content and lower lung volume.[34] Importantly, none of these studies have looked at levels of PC in the airway lumen (BALF), the composition of the surfactant produced, the number of lamellar bodies within the type II alveolar epithelial cells, or the ability of surfactant release from the lamellar bodies (surfactant availability). This study is the first to report on the expression of surfactant protein mRNA levels in lung tissue as well as PC composition in airway spaces (BALF) in FGR lambs. We found that the relative composition of each of the PCs were not different between FGR and AGA preterm lambs. Further, while surfactant administration/ventilation increased total PCs compared to unventilated controls, only three surfactant PC species were increased with no differences between FGR and AGA animals were noted. The overall increase in total PCs is likely due to the administration of surfactant per se but may also likely result from the release of preformed lipids with improved and more uniform recruitment of the lung.[35] The dose of surfactant administration in this study was 100 mg kg^{-1}. This is within the recommendation of 100–200 mg kg^{-1} of surfactant for RDS in the preterm neonate.[36] It is not known whether the dose of surfactant would alter the response in FGR lambs. Overall, the lack of differences in surfactant protein mRNA coupled with some differences in PC concentrations along with the lack of more data on the surfactant protein estimation and function does not preclude that animals may have had more differences in surfactant activity.

Limitations

The study demonstrates the response to surfactant therapy in the immediate period post administration in premature lambs. Ideally, a postnatal ventilated group who was not administered surfactant therapy would be ideal to dissect specific associations of FGR with pulmonary and cerebral hemodynamics, although maintaining these lambs with serious RDS is problematic but not unsurmountable. Also, a volume guarantee mode of ventilation and surfactant administration immediately at the start of the experiment (at birth) may have prevented variation in alveolar recruitment due to differences in mechanical properties of FGR and AGA lambs; however this is not indicative of current clinical practice where volume guarantee is usually delivered once infants are in the neonatal intensive care unit. The longer-term effects of surfactant and effects of second or repeated surfactant doses have also not been investigated in this study. Notwithstanding these limitations, the study directly compares and presents data on the physiological changes following surfactant administration in the preterm FGR animal with those in the AGA animal. Further understanding of the mechanism requires a repeat of this study with volume guarantee from birth, such that the response to surfactant is a consequence of AGA/FGR rather than a consequence of differential alveolar recruitment at the start. Lastly, direct measurements of surfactant protein levels by western blot technique might help in understanding the mechanistic differences between the groups. Whether the short duration of the experiment and ventilation exposure is likely to impact of surfactant protein gene expression is also debatable.

CONCLUSIONS

Oxygenation and ventilation requirements of FGR and AGA preterm newborn lambs improved similarly after surfactant administration during initial pressure limited ventilation. However, the subsequent reduction in FiO_2 was associated with reduced PBF and CBF in growth-restricted lambs, possibly indicating a different vascular responsiveness to oxygen between FGR and AGA preterm lambs. Further preclinical studies using volume-targeted ventilation throughout and with non-surfactant ventilated comparator groups will be helpful. These results provide new insights into possible reasons for the differential transitional changes and responses in the early neonatal period seen in human FGR infants.

ACKNOWLEDGEMENTS

We thank Ilias Nitsos, Kelly Crossley, and Dalibor Stanojkovic for their help with animal experiments. This research was supported by National Health and Medical Research Council (NH&MRC) Project Grant (APP1083520), the Arthur & Mary Osborn Charitable Trust, and the Hugh D T Williamson Foundation, which are managed by ANZ Trustees; a Royal Australasian College of Physicians Foundation Fellowship (to A. M.); NH&MRC Research Fellowships (to G.R.P.: APP1105526; to S.L.M.: APP1136216; and to S.B.H.: APP545921); an Australian Research Council Future Fellowship (to S.L. M.: FT1301006); a Rebecca L. Cooper Medical Research Foundation Fellowship (to G.R. P.); and the Victorian Government's Operational Infrastructure Support Program.

AUTHOR CONTRIBUTIONS

All authors were involved in the conduct of the experiments, acquisition of data, analysis, and preparation of the manuscript. A.M. designed the experimental plan and wrote the first draft of the manuscript. G.R.P. is the senior author, critically analyzed the data, and provided statistical input to the manuscript. F.S. and V.Z. conducted the surfactant analysis.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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