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Ventilation-induced lung injury is not exacerbated by growth restriction in preterm lambs

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The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia; Department of Obstetrics and Gynecology, Monash University, Clayton, Victoria, Australia; The Australian and New Zealand Neonatal Network 2012. The initiation of mechanical ventilation elicits a profound inflammatory response within the immature lung (21, 27, 44) and may, in turn, induce ventilation-induced lung injury (VILI), which causes airway remodeling and potential long-term pulmonary consequences (10, 60). Increased lung inflammation and injury following mechanical ventilation has been demonstrated in preterm experimental models (3, 14, 44) and preterm infants (63). An increased requirement for ventilator support, such as higher tidal volumes (VT) and/or pressures, increases lung inflammation or injury or alter lung parenchymal and vascular structure compared with AG fetuses. The authors suggest that increased respiratory requirement in IUGR infants compared with AG infants include altered fetal lung development, fetal lung inflammation, increased respiratory requirements, and/or increased ventilation-induced lung injury. IUGR was surgically induced in preterm fetal sheep (0.7 gestation) by ligation of a single umbilical artery. Four weeks later, preterm lambs were euthanized at delivery or delivered and ventilated for 2 h before euthanasia. Ventilator requirements, lung inflammation, early markers of lung injury, and morphological changes in lung parenchymal and vascular structure and surfactant composition were analyzed. IUGR preterm lambs weighed 30% less than AG preterm lambs, with increased brain-to-body weight ratio, indicating brain sparing. IUGR did not induce lung inflammation or injury or alter lung parenchymal and vascular structure compared with AG fetuses. IUGR and AG lambs had similar oxygenation and respiratory requirements after birth and had significant, but similar, increases in proinflammatory cytokine expression, lung injury markers, gene expression, and surfactant phosphatidylcholine species compared with ventilated controls. IUGR does not induce pulmonary structural changes in our model. Furthermore, IUGR and AG preterm lambs have similar ventilator requirements in the immediate postnatal period. This study suggests that increased morbidity and mortality in IUGR infants is not due to altered lung tissue or vascular structure, or to an altered response to early ventilation.

SGA; IUGR; lung injury; lung inflammation
flapmation and injury in IUGR preterm neonates may underlie the increased risk of respiratory impairments.

In this study, we examined the origins of the increased risk of adverse respiratory outcomes in preterm IUGR neonates, by exploring the separate and combined effects of altered antenatal development in IUGR, followed by preterm delivery and ventilation. Specifically, we aimed to determine whether IUGR preterm lambs had 1) altered fetal lung development, 2) sustained fetal lung inflammation, 3) increased respiratory requirements acutely after preterm delivery, and/or 4) increased ventilation-induced lung inflammation and VILI. We hypothesized that placental insufficiency resulting in growth restriction alters fetal lung development and function, increasing the requirement for respiratory support in the immediate newborn period and causing increased VILI in IUGR preterm lambs.

METHODS

Ethics statement. The experimental protocol was performed in accordance with guidelines established by the National Health and Medical Research Council of Australia and was approved by the Monash Medical Centre animal ethics committee at Monash University.

Animals. Twin-bearing Border-Leicester pregnant ewes (n = 27) underwent surgery on days 103–105 of pregnancy (term ~148 days) for the procedure of single umbilical artery ligation (SUAL), as previously described (55). Briefly, anesthetized ewes underwent surgery to instrument each fetus with a femoral artery catheter and an amniotic catheter for determination of blood gases and to administer antibiotics, respectively. In one fetus (randomly selected), one of the two umbilical arteries was ligated (SUAL fetus); in the other fetus the umbilical cord was manipulated but not ligated (appropriately grown [AG] fetus). The fetuses were returned to the uterus, and a maternal jugular vein catheter was inserted for antibiotic administration before the ewe recovered.

Experimental procedures. For 3 days after surgery, antibiotics were administered to both the fetus (ampicillin, 1 g via the amniotic catheter) and the ewe (engemycin 5 ml iv). The maternal and fetal catheters were flushed daily with heparinized saline, and a fetal blood catheter (55). Briefly, anesthetized ewes underwent surgery to instrument each fetus with a femoral artery catheter and an amniotic catheter for determination of blood gases and to administer antibiotics, respectively. In one fetus (randomly selected), one of the two umbilical arteries was ligated (SUAL fetus); in the other fetus the umbilical cord was manipulated but not ligated (appropriately grown [AG] fetus). The fetuses were returned to the uterus, and a maternal jugular vein catheter was inserted for antibiotic administration before the ewe recovered.

Delivery of preterm lambs. At 123–127 days gestation, when lung maturation is similar to the human very preterm infant (<32 wk) (2), each ewe was anesthetized, as above, and the fetus exposed via caesarean section. A pulse oximeter probe (Masimo, Irvine, CA) was placed on the right forearm for preductal measurement of transcutaneous oxyhemoglobin saturation levels. The fetus was placed on the right forelimb for preductal measurement of arterial oxygen saturation, pH, hematocrit, glucose, transcutaneous oxyhemoglobin saturation levels. The fetus was exposed via caesarean section. A pulse oximeter probe (Masimo, Irvine, CA) was placed on the right forearm for preductal measurement of transcutaneous oxyhemoglobin saturation levels. The fetus was placed on the right forelimb for preductal measurement of arteriolar wall thickness (light microscopy) by 10.220.33.5 on October 20, 2017 http://ajplung.physiology.org/ Downloaded from

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an airway were randomly selected for morphometric measurements, including external and internal vessel area and area of smooth muscle (vessel lumen external area — internal area).

**Biochemical analysis of bronchoalveolar lavage fluid.** Samples of bronchoalveolar lavage fluid (BALF) were snap frozen at postmortem for subsequent measurements of total protein concentration, which were determined using a protein assay, and surfactant phosphatidylcholine (PC) concentration and composition, as described below. Only one sample of BALF was taken per lamb; therefore, IUGRUV C and AGUVC samples are before ventilation, and IUGRVENT and AGVENT (ventilated IUGR and AG) are following 2-h ventilation.

**PC analysis.** For PC analysis, dried cellular extracts from BALF samples were resuspended in butanol-methanol (1:1 vol/vol) 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, Australia), and eluted at 0.2 ml/min over a 5-min gradient of water-methanol-tetrahydrofuran (50:20:30 vol/vol/vol) to water-methanol-tetrahydrofuran (5:20:75 vol/vol/vol), with the final buffer held for 3 min. Lipids were analyzed by electrospray ionization-mass spectrometry using an Agilent Triple Quad 6460 (Mulgrave, Australia). Lipid species presence in the PC lipid class was identified using precursor ion scanning from 100 to 1,000 m/z, in positive ion mode, PC, and lysophosphatidylcholines (precursors of m/z 184.1), and sphingomyelins (m/z 184.1). Identified lipid species were quantified using multiple reaction monitoring with 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 4,000 V, 140–380 V, and 15–60 V, respectively. In all cases, the collision gas was nitrogen at 7 l/min. Electrospray ionization-mass spectrometry data were processed using Agilent Mass Hunter (Mulgrave, Victoria, 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 relating 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 milligrams of protein in BALF.

**Statistical analysis.** Data are expressed as means ± SE. Two-way ANOVA (SigmaStat 3.5) was used to compare histological and morphological, molecular, and biochemical indexes, and two-way repeated-measures ANOVA (SigmaStat 3.5) with Tukey’s post hoc comparison used to compare the physiological data, including ventilator output data. *P < 0.05 was accepted as statistically significant.

**RESULTS**

**Baseline characteristics and physiological parameters.** IUGR fetuses had a significantly decreased arterial oxygen saturation following surgery for SUAL; however, 12 days postsurgery, this was not significantly different from AG fetuses (Fig. 1). Glucose was significantly decreased in IUGR compared with AG fetuses (Fig. 1). Fetal hematocrit was significantly increased at 20 days after surgery in IUGR compared with AG fetuses (Fig. 1). Fetal pH, PaO2, and lactate were significantly increased at 20 days after surgery in IUGR compared with AG fetuses (Fig. 1). Fetal pH, PaO2, and lactate were not different between groups or throughout the experimental period. PaCO2 increased significantly in both groups over time (Fig. 1).

Circulating cortisol levels in IUGRUV C were significantly higher than those in AGUVC (*P = 0.015). Cortisol levels in
IUGRVENT and AGVENT lambs were significantly higher than those in AGUVC (P = 0.007); ventilation significantly increased cortisol levels in AGVENT lambs compared with AGUVC, but ventilation of IUGR lambs did not result in a further increase in cortisol levels (Fig. 2).

Body weight was significantly reduced and brain-to-body weight ratio was increased in IUGR lambs compared with AG groups (Fig. 4). Relative lung, kidney, heart, liver, and splenic weights were not different between groups. The ratio of males to females was not different between groups (Table 1).

Lung parenchymal and vascular morphology. Relative lung weights did not differ between groups (Table 1). Representative lung pictographs are shown in Fig. 3. There was no difference in tissue-to-air space ratio or septal crest density between groups (Fig. 4). There was no difference in elastin density (data not shown). Assessment of the muscle component of the pulmonary vasculature demonstrated that there was no difference in wall thickness, external wall area, or the area of smooth muscle (when corrected for vessel area) between IUGR and AG lambs in any surfactant lipid variable measured (data not shown).

Lung inflammation and injury. mRNA levels of IL-1ß, IL-6, IL-8, and the early markers of lung injury, EGR-1, CTGF, and CYR61, were assessed. IUGRVENT and AGVENT lambs had significantly increased IL-1ß (P = 0.03), IL-6 (P = 0.016), EGR-1 (P = 0.014), and CYR61 (P = 0.02) mRNA level expression compared with IUGRUVC and AGUVC groups (Fig. 5). IUGRVENT lambs had higher CYR61 mRNA level expression compared with IUGRUVC lambs (P = 0.002, Fig. 5). There was no difference in the expression of IL-8 and CTGF between any groups.

Ventilation and oxygenation parameters. PaCO₂ significantly decreased over the period of ventilation (Fig. 6) but was not different between groups. Ventilation requirements, including VT and PIP, were not different between IUGRVENT and AGVENT lambs. However, in both groups, PIP significantly decreased once the lambs were placed on volume guarantee (20 min postbirth, Fig. 6). Alveolar-arterial difference in oxygen decreased, while ventilator efficiency index increased (improved) in IUGRVENT and AGVENT groups at 30 min, relative to the initial 20 min (Fig. 6).

Surfactant composition. Lung relative SP-A, SP-B, SP-C, and SP-D mRNA levels were not different between IUGR and AG groups and were also not different following ventilation (Fig. 7). The concentrations of lipid species in PC were not different between IUGRUVC and AGUVC lambs. Ventilation increased the concentration of the PC lipid species 36:2, 34:0, 38:1, 38:2, and 38:4 similarly in IUGRVENT and AGVENT groups compared with their respective IUGRUVC and AGUVC groups (data not shown). No differences were observed between IUGR and AG lambs in any surfactant lipid variable measured (data not shown).

**DISCUSSION**

In this study, we aimed to characterize the cause(s) of poor respiratory outcome in preterm infants that were growth restricted in utero. We used a well-characterized ovine model of placental insufficiency and asymmetric growth restriction that mimics the known pathophysiology observed in human IUGR infants (37, 54, 55). Specifically, we investigated whether IUGR alters lung structure and function in preterm lambs, induces fetal lung inflammation, and whether IUGR increased the risk and severity of VILI following the initiation of ventilation in preterm IUGR lambs. We show that IUGR per se did not alter relative lung weight, lung parenchymal or vascular structure, or surfactant composition, nor was lung inflammation and injury evident. Following preterm delivery and ventilation, IUGR preterm lambs had similar respiratory requirements in the first hours of life compared with AG preterm lambs, and ventilation-induced lung inflammation and injury was not different between groups. These findings refute our hypothesis of altered pulmonary physiology in response to fetal
growth restriction and suggest that the origins of increased respiratory complications observed in IUGR preterm infants may not be due to fundamental pulmonary structural differences.

We used SUAL to cause IUGR in fetal sheep, as it results in chronic hypoxia and produces reliable asymmetric fetal growth restriction (37, 54, 55). Consistent with previous findings (37, 54, 55), the IUGR fetuses in this study were hypoxic, hypoglycemic, and had elevated cortisol concentrations (>3-fold) compared with AG fetuses, and, at preterm birth, IUGR lambs were ~30% smaller than AG offspring (corresponding to a moderate to severe IUGR in the humans) and had asymmetric growth with increased brain-to-body weight ratio, indicative of brain sparing. Several other models of IUGR are currently used, spanning many species, including chick embryos, rodents, and guinea pigs, as well as other methods in sheep. The advantages, disadvantages, and varying levels of severity of IUGR animal models have been well reviewed recently (8, 56).

The site of fetoplacental unit insult resulting in IUGR varies between models, as it does in human IUGR. For example, carunclectomy directly impacts on the placenta, while chronic hyperthermia is a maternal insult. In contrast, the SUAL and chick embryo models may be considered postplacental in insult. Growth restriction modeling in fetal sheep remains advantageous because of the large fetal size and the ability to instrument and monitor fetal and maternal physiology over an extended period, and importantly, organ development in the lung, heart, and brain is well described and takes place over late gestation as per humans. SUAL, in particular, causes a reduction in fetal substrate delivery, resulting in a growth-restricted and nutrient-deprived developing fetus and subsequent growth restriction that is comparable in severity to human IUGR. Accordingly, we chose to study SUAL-induced IUGR at 105 days gestation to determine the pulmonary consequences of moderate growth restriction and the onset of neonatal ventilation.

One week of placental insufficiency and IUGR induced by SUAL in preterm fetal sheep does not alter lung development, despite a significant increase in cortisol (55), a powerful regulator of lung maturation (6). Although the exposure to SUAL was increased in the present study compared with our laboratory’s previous studies, we still did not observe gross structural changes in lung architecture. We did not measure in vivo pulmonary hemodynamics or physiology; however, it is likely that pulmonary blood flow was not altered in our IUGR model based on our laboratory’s previous work (38). This is not surprising, given that the lungs are already vasoconstricted in AG fetuses during pregnancy, and fetal hypoxia increases vasoconstrictive factors (endothelin-1 and VEGF) and decreases vasodilatory factors (endothelial nitric oxide synthase), leading to pulmonary hypertension (16). Fetal breathing movements (FBMs) are essential for normal lung development (24), and absence of FBMs leads to lung hypoplasia (32). While few studies have investigated FBMs in IUGR offspring, growth status of the fetus was found to not alter FBMs, unless the fetus was complicated by oligohydramnios, in which case FBMs were reduced (51). In the present study, oligohydramnios was not present; we can speculate, therefore, that FBMs were not dramatically altered.

Effect of IUGR on fetal lung development. Our first aim was to determine whether there were underlying structural changes to the preterm IUGR lung that may underpin worse respiratory outcomes compared with AG preterm lungs. Interestingly, IUGR per se did not alter pulmonary morphology, with no difference in the ratio of lung tissue to air space or septal crest density. We undertook this study in fetal and newborn sheep throughout the late canalicular and early alveolar stage of lung development, with delivery occurring when lung function approximates a 26- to 28-wk human infant (2). At this time, the fetal lung consists of primitive alveoli, and interalveolar wall tissue is thinning to increase the surface area for gas exchange. Our findings extend previous findings in IUGR models, from
both our group (55) and others (22, 34). However, hyperthermia-induced (50) or undernutrition-induced (31), growth-restricted offspring have demonstrated diminished vascular function and decreased pulmonary alveolarization (31, 50) and vascular density (50). Differences in the effects on pulmonary development between IUGR models appear to be heavily influenced by the methodology and timing of the insult, with earlier insult onset having a greater influence on delayed alveolarization. Our model is, however, clinically relevant, with antenatal similarities such as hypoxia and hypoglycemia, as well as similar postnatal presentations, making the changes observed in this study highly translatable. The threefold increase in cortisol observed in IUGR lambs in the present study may have been expected to induce lung maturation, as observed previously (11). However, Jobe and colleagues (28) found that much higher concentrations of cortisol were required to induce lung maturation when given as a bolus injection. Furthermore, when cortisol is infused into fetal sheep during the canalicular stage of lung development, lung maturation is not affected (35), suggesting the fetal lung at this stage of development is relatively unresponsive to cortisol. Thus it is unlikely that higher cortisol levels may have masked the effects of IUGR on alveolarization.

We did not observe a difference in pulmonary vascular size, density, or proportion of vascular smooth muscle abundance between IUGR and AG offspring. There is a clear link between poor vascular development and poor alveolar development (18, 19, 58). Given this relationship, in the present study the lack of difference in lung architecture and the vascular network in IUGR lambs is not unexpected. Cardiac output to the lungs is low (~6%) in the mid- to late-gestation fetus and is not further reduced in response to IUGR (37). Our findings suggest, therefore, that the lung vasculature is protected against known IUGR-induced cardiovascular redistribution, which is apparent in chronic hypoxia (37). However, we and others have previously shown that growth restriction causes endothelial dysfunction in other vascular beds (12, 57), and we speculate that endothelial dysfunctions likely exist in pulmonary vessels, even in the absence of gross changes in vasculature. We did not examine pulmonary vascular function in these lambs, so we cannot rule out that poor vascular function, such as reduced vasodilator response, may be an underlying mechanism for the decline in respiratory function observed in preterm IUGR infants.

Lung tissue surfactant protein mRNA expression was not different between IUGR and AG preterm lambs, nor were any differences observed in the total PC or PC lipid species concentrations. This is an interesting finding in light of the threefold increased circulating levels of cortisol, but confirms earlier work that shows no change in surfactant protein mRNA expression of SP-A, SP-B, or SP-C (15, 55). Additionally, we demonstrated that fetal growth restriction did not affect the major lipid surfactant components in our model. This is in contrast to previous studies showing significantly reduced PC content and lower lung volume in growth-restricted rats (13). In sheep, growth restriction resulted in decreased surfactant protein (41), which was speculated to result from glucocorticoid and hypoxic signaling pathways (42). Importantly, none

Fig. 4. Lung parenchymal and vascular structure. Values are means ± SE of percentage of lung parenchyma composed of air space (open bars) and tissue (solid bars) (top left), secondary crest density (middle left), arteriolar vessel wall thickness (top right), external area (middle right), tissue injury (bottom left), and smooth muscle area (corrected for total tissue area; bottom right) in AGUVC (open bars) and IUGRUVVC (solid bars) and AGVENT (light shaded bars) and IUGRVENT (dark shaded bars). Values are expressed as % total tissue. Data were compared using one-way ANOVA.
of these studies have looked at levels of PC in the airway lumen (e.g., in BALF). It certainly would be of interest to investigate the composition of the surfactant produced, the number of lamellar bodies within the type II alveolar epithelial cells, or the ability of surfactant to be released from the lamellar bodies (surfactant availability) in future studies. We did not observe any effect of SUAL-induced IUGR on fetal lung airway or vascular development, or in surfactant content and composition, which would lead to worse immediate- and long-term pulmonary outcomes.

**Effect of IUGR on fetal lung inflammation.** We also investigated whether IUGR induced a persistent inflammatory response within the fetal lung. We found that markers for lung inflammation and injury were not different between IUGRUVC and AGUVC fetuses at the time of preterm delivery, indicating an absence of increased lung inflammation. We and others have previously shown an increase in inflammatory markers in the placenta and amniotic fluid, but not in maternal or fetal plasma, acutely (49), and sustained elevations in the amniotic fluid and fetus after induction of IUGR (9). Discrepancies in inflammatory markers between our present study and previous studies are likely due to differences in the time of onset of IUGR and the fetal tissue of interest studied (i.e., plasma, amniotic fluid vs. lung tissue). It is also likely that fetal lung inflammation had resolved by the time of sampling, suggesting that this model does not induce a persistent inflammatory response within the developing lungs. Similarly, the absence of any lung remodeling resultant from fetal inflammation is likely due to the time between the insult and subsequent delivery.

Studies in preterm lambs showed that the fetal lung remodels in response to intrauterine inflammation (29), but the lung recovers and develops relatively normally after a period of time (40), even if the inflammatory response persists (30).

**Effects of IUGR on postnatal respiratory requirements.** We interrogated whether preterm birth and the onset of mechanical ventilation led to lung injury, and if this was different in IUGR compared with AG lambs. We show that the ventilatory requirements, lung compliance, and oxygenation in IUGR newborn preterm lambs were similar to that in AG preterm lambs in the first hours after birth. These observations add to clinical studies showing that IUGR infants have similar, or even reduced, incidence of RDS compared with AG infants (17, 20, 23, 48). Previous studies in spontaneously breathing growth-restricted rats show significantly impaired respiratory compliance and resistance 70 days after birth, but, earlier, at 28 days after birth, respiratory function was only mildly impaired (1). Furthermore, extremely preterm IUGR infants had reduced incidence of RDS, but required more days of mechanical ventilation (17). Similar physiological outcomes between groups are perhaps not surprising in the present study, given our finding of similar lung structure and surfactant composition demonstrated between the two groups. It appears, therefore, that the progression of lung disease occurs over time and may be induced by other pathways, such as chronic inflammation or cardiovascular sequela.

**Effect of IUGR on postnatal inflammation and injury.** The initiation of mechanical ventilation of the preterm lung, irrespective of how it is conducted, produces lung inflammation
and injury (10, 44, 60). The key proinflammatory cytokines initiated by ventilation are IL-1β, IL-6, and IL-8. Ventilation also increases early response markers of lung injury (EGR-1, CTGF, and CYR61), which are rapidly (within minutes to hours) increased in response to ventilation (26, 61). Placental insufficiency and chronic growth restriction have been previously shown to increase inflammatory markers in the maternal (7) and fetal (9, 33) circulations, and previous studies have linked fetal inflammation to worse respiratory outcomes (25, 53, 64). However, very few studies have investigated the inflammatory cascade in IUGR offspring following ventilation. Given that we did not see an enhanced inflammatory response in the lung before ventilation, it is not surprising perhaps that ventilation resulted in a significant but similar increase in markers of lung inflammation and injury in lung tissue in both IUGRVENT and AGVENT lambs compared with IUGRUVC and

Fig. 6. Ventilation and oxygenation parameters. Values are means ± SE of PaCO2, peak inspiratory pressure (PIP), alveolar arterial difference in oxygen (AaDO2), tidal volume (VT), specific dynamic compliance, ventilator efficiency index (VEI), and minute ventilation (MV) in AGVENT and IUGRVENT newborn lambs during ventilation. #P < 0.05, differences across time (minutes) compared using two-way repeated-measures ANOVA.

Fig. 7. Surfactant protein (SP) mRNA levels. SP-A, SP-B, SP-C, and SP-D mRNA levels are shown in scatter plot showing mean (line) in AGUVC and IUGRUVC and AGVENT and IUGRVENT. Data were compared using one-way ANOVA.
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AGUVC. These findings do not preclude the possibility that serum inflammatory markers may have been upregulated. Furthermore, ventilation caused an increase in a number of surfactant PC species that was consistent between groups. Our findings suggest that the IUGR and AG preterm lung responds similarly to mechanical ventilation.

Limitations. In this study, we undertook surgery to induce placental insufficiency at 0.7 gestation. As with all experimental animal models, it is vitally important to consider the method used when interpreting results. A limitation of our study may be the timing of induction of IUGR. It is well documented that growth restriction induced in early gestation affects IUGR presentation, where offspring predominantly are symmetrically growth restricted. Initiation of placental insufficiency during the earlier stages of lung development may have resulted in simplified lung structure, indicative of delayed lung development, as demonstrated previously using the chronic hyperthermia model of IUGR and in humans (31, 50). It is, therefore, possible that the length of placental insufficiency in the present study was either insufficient, or too late in gestation to induce structural changes in the lung. Counter to this argument, human IUGR etiology is not homogenous, and, therefore, the different presentations within and between models are strengths rather than weaknesses when studying IUGR. In particular, little is know about the timing of human IUGR onset, and, therefore, as our model displays many of the common characteristics of the IUGR infant, we believe the findings in the present study are highly translatable. In this and other studies, we have shown that SUAL-induced placental insufficiency causes chronic fetal hypoxia to a similar degree to that described in human IUGR, along with fetal hypoglycemia, upregulation of inflammatory mediators, cortisol and oxidative stress, and cardiovascular adaptations that produce asymmetric growth restriction (as reviewed in Ref. 39). The clinical etiology of placental dysfunction and IUGR are not well characterized, with the exception that most IUGR infants have placental pathology, such as reduced implantation of spiral arteries (36). Whatever the mechanisms, the result is reduced substrate delivery to the human fetus, and altered physiology, as shown using this ovine model. In the present study, we only investigated the response of the lung to an initial 2-h period of ventilation. The long-term respiratory effects of the initial, or even of continued, ventilation is yet to be determined and may offer valuable insights into the progression of lung disease in IUGR preterm infants. Clinically, IUGR infants do not show profound differences to AG infants early after birth, but show later pulmonary demise. Our results support that this demise is not structural, but functional.

Conclusion. In conclusion, we have demonstrated that underlying inflammations, as well as lung parenchymal, vascular structure, and surfactant composition, are not altered following experimentally induced placental insufficiency and IUGR. Furthermore, lung inflammation and injury in response to the initiation of ventilation (i.e., VILI) was not different between AG and IUGR preterm lambs. We speculate that the long term morbidity and mortality in the IUGR population does not stem from deficiencies in the respiratory system. IUGR infants born preterm remain a vulnerable cohort, and attention should be further directed toward understanding how other physiological systems are affected by growth restriction.

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Author Contributions

References
32. McGillic EV, Orgeig S, McMullen IC, Morrison JL. The fetal sheep lung does not respond to cortisol infusion during the late canalicular phase of development. Physiol Rep 1: e00130, 2013.