The effect of prenatal maternal infection on respiratory function in mouse offspring: evidence for enhanced chemosensitivity

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1Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton, Victoria, Australia; 2Division of Sleep Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts; 3Department of Allergy Immunology and Respiratory Medicine and Central Clinical School, The Alfred and Monash University, Melbourne, Victoria, Australia; and 4Kentucky Children’s Hospital/UK Healthcare, Department of Pediatrics, University of Kentucky College of Medicine, Lexington, Kentucky

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Samarasinghe TD, Sands SA, Skuza EM, Joshi MS, Nold-Petry CA, Berger PJ. The effect of prenatal maternal infection on respiratory function in mouse offspring: evidence for enhanced chemosensitivity. J Appl Physiol 119: 299–307, 2015. First published May 28, 2015; doi:10.1152/japphysiol.01105.2014.—Systemic maternal inflammation is implicated in preterm birth and bronchopulmonary dysplasia (BPD) and may induce morbidities including reduced pulmonary function, sleep-disordered breathing, and cardiovascular disorders. Here we test the hypothesis that antenatal maternal inflammation per se causes altered alveolar development and increased chemoreflex sensitivity that persists beyond infancy. Pregnant C57BL/6 mice were administered lipopolysaccharide (LPS) (150 μg/kg ip) to induce maternal inflammation or saline (SHAM) at embryonic day 16 (randomized). Pups were weighed daily. On days 7, 28, and 60 (D07, D28, and D60), unrestrained wholebody plethysmography quantified ventilation and chemoreflex responses to hypoxia (10%), hypercapnia (7%), and asphyxia (hypoxic hypercapnia). Lungs were harvested to quantify alveolar number, size, and septal thickness. LPS pups had reduced baseline ventilation per unit bodyweight (~40%, \( P < 0.001 \)) vs. SHAM. LPS increased ventilatory responses to hypoxia (D07: 66% vs. 28% increase in ventilation; \( P < 0.001 \)) hypercapnia (170% vs. 88%; \( P < 0.001 \)), and asphyxia (249% vs. 154%; \( P < 0.001 \)); hypersensitive hypoxic responsiveness persisted until D60 (\( P < 0.001 \)). LPS also increased apnea frequency (\( P < 0.01 \)). LPS caused thicker alveolar septae (D07, \( P < 0.001 \)), diminished alveolar number (D28, \( P < 0.001 \)) vs. SHAM, but effects were minimal by D60. Pups delivered from mothers exposed to antenatal inflammation exhibit deficits in lung structure and hypersensitive responses to respiratory stimuli that persist beyond the newborn period. Antenatal inflammation may contribute to impaired gas exchange and unstable breathing in newborn infants and adversely affect long-term health.

respiratory function; chemosensitivity; prenatal infection

**PRETERM BIRTH (PTB), defined as delivery occurring at <37 completed weeks of gestation, is a major risk factor for neonatal mortality and morbidity. Of the four million neonatal deaths worldwide each year, an estimated 27% are attributable to PTB (45). Although we have a poor understanding of the aetiology of PTB, it is strongly associated with advanced maternal age (42), poor socioeconomic status (2, 23), maternal smoking (72), a previous history of PTB, and multiple gestations (44). Animal studies have shown that infection is implicated in PTB, as blockade of maternal inflammation substantially reduces PTB in the mouse (71). Yet it remains unclear whether maternal inflammation is directly responsible for two of the most common sequelae of PTB, namely bronchopulmonary dysplasia (BPD) and unstable breathing (periodic breathing and apnea), two conditions that by promoting hypoxemia reduce survival in the newborn period (10) and increase morbidity thereafter (30, 57, 66).

Intrauterine inflammation is present in 25% of all babies who develop severe and chronic lung disease in the form of BPD (40), a condition that causes significant morbidity and mortality in preterm babies. Acute and chronic intrauterine infection/inflammation associated with PTB can adversely influence lung development by exposing the developing fetus to cytokines, chemokines, and lipid mediators (24, 46). In a number of experimental models (29, 33, 36, 43), antenatal inflammation caused by intra-amniotic lipopolysaccharide (LPS) influences lung development and maturation via elevated expression of proinflammatory cytokines resembling what is seen in premature infants (25). An acute infection of the fetal and maternal membranes predisposes to BPD, which leads to the infant being given supplemental oxygen, a treatment that itself adversely affects lung development by creating a phenotype with fewer and larger alveoli (3, 32).

Unstable breathing in the form of apnea and periodic breathing is extremely common in preterm infants and has long been considered to be the consequence of immature ventilatory control (82, 83). Despite a high prevalence of inflammation in pregnancy, how it affects ventilatory control over extended periods of infancy is unknown. What is known is that the acute effect of intravenous infusion of LPS in anesthetized cats diminishes hypoxic ventilatory responses mediated by the carotid body (19). Likewise, in newborn mice, rats, and piglets, a proinflammatory cytokine such as IL-1β acutely depresses respiration, prolongs the duration of apnea, and modifies autotorsuscitation (21, 52, 73). In infants, acute respiratory infection is commonly associated with apnea and unstable breathing (5, 8, 49, 58). Mechanistically, unstable breathing reflects an elevated “loop gain” (sensitivity of the feedback loop controlling ventilation) due to hypersensitive ventilatory chemoreflex responses to hypoxia and hypercapnia. Indeed, increased loop gain via exaggerated chemoreflexes is implicated in periodic breathing and apnea in infants (1), newborn animals (17, 18), and adults (20, 31, 84).

We aimed to improve our understanding of the effects of prenatal maternal infection on postnatal respiratory structure

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and function in offspring. We hypothesized that newborn mice exposed to an in utero maternal inflammatory insult have enhanced chemosensitivity and impaired lung development. Accordingly, we administered pregnant mice with LPS at a dose that elevates expression of proinflammatory cytokines similar to that present in preterm human infants (51). We then compared the ventilatory responses to hypoxia, hypercapnia, and asphyxia of pups from LPS-treated and saline-injected pregnancies. Finally, we quantified the propensity of the two sets of pups for spontaneous apneas and postsigh apneas during recovery from hypoxic, hypercapnic, and asphyxic inspired gas.

MATERIALS AND METHODS

Animals

All experimental procedures conformed to the guidelines established by the National Health and Medical Research Council of Australia and had the approval of the standing committee in Ethics in Animal Experimentation of Monash University, Melbourne, Victoria, Australia. Time-mated C57BL/6 mice were used for the experiments. The morning of finding the vaginal plug was considered day 1 of pregnancy. All animals were maintained under standard environmental conditions (12:12 h light:dark cycle) with free access to food and water.

Experimental Design

At embryonic day 16, C57BL/6 pregnant mice randomly received a single intraperitoneal injection of either LPS (Escherichia coli serotype 0127:B8; Sigma-Aldrich, Castle Hill, Australia) at a dose of 150 μg/kg, or an equal volume of saline. Mice were then allowed to deliver naturally, and the pups were randomly allocated to one of three sets of pups for spontaneous apneas and postsigh apneas during recovery from hypoxic, hypercapnic, and asphyxic inspired gas.

Ventilatory Measurement

The ventilatory responses to hypoxia (10% O2), hypercapnia (7% CO2), and asphyxia (10% O2 + 7% CO2) were measured by whole-body plethysmography as previously described (12). Air was drawn through the measurement chamber at 2 liters/min with a bias-flow regulator (Buxco Electronics) in a circuit connected to vacuum. The pressure difference between the chamber containing the pup and the reference chamber was measured with a differential pressure transducer (range, ±0.1 mbar; EFFA, Asnières, France). Care was taken to minimize the impact of random pressure fluctuations in the laboratory by isolating the chamber from environmental vibrations and by incorporating a capacitative filter in series within the bias-flow circuit. Gas leaving the chamber passed through an O2 analyzer (model S-3A/I, Ametek Process Instruments, Pittsburgh, PA) and a CO2 analyzer (Novametrix NICO CO2 Monitor) to determine fractional inspired concentrations of O2 (FiO2) and CO2 (FiCO2). Signals from the pressure transducer and gas analyzers were recorded at 400 Hz (LabChart 7.0, ADInstruments, Sydney, Australia). The differential pressure signal was bandpass filtered (0.4-15 Hz) and used to calculate respiratory frequency (f), tidal volume (VT), and minute ventilation (VE = VT × f) for each breath as described previously (14). The system was calibrated prior to each study by repetitive injection of 50 μl and then 100 μl of air into the chamber with a precision syringe (Hamilton Bonaduz AG, Switzerland); volume deflections made at the respiratory frequency were used to calibrate pressure measurements.

On the day of the experiment, mice were placed inside the measurement chamber. We assumed body temperature was constant at 37°C (67), and the measurement chamber was maintained within the thermoneutral range for young, adult mice as described previously (11). After 15 min of acclimatization, a 30-min period of baseline breathing was recorded, followed by exposure to hypoxia, hypercapnia, and asphyxia for 10 min each. Each challenge was presented in random order and separated by a 15-min recovery period (air).

Data Analysis

Ventilation and ventilatory responses. Analysis of ventilatory parameters was performed with MATLAB (The MathWorks, Natick, MA). Ventilatory variables VT, f, and VE were calculated by carrying out breath-by-breath analysis of traces free from movement artefact. The baseline values of ventilatory variables were obtained by averaging 10 min of stable recording in the control period at the start of the experiment. Earlier measurement established that gas within the chamber takes 2.5 min to reach a steady state once the inspired gas is changed; thus the first 2.5 min of respiratory data for each test was discarded, leaving the last 7.5 min for quantitation of responses to hypoxia, hypercapnia, and asphyxia. The ventilatory response data are presented as a percent increase from baseline since the relative changes are most important for blood gas homeostasis and also due to distinct changes in ventilatory responses across a range of ages where baseline VE changed markedly. The time taken to reach 50% of the peak ventilatory response was also calculated.

Apnea analysis. Apnea was defined as a cessation of ventilatory airflow for at least two respiratory cycles (50) and was classified as either a spontaneous (SA) or postsigh apnea (PSA). To be adjudged a PSA, an apnea had to have been preceded by a breath with double the amplitude of resting tidal volume (Fig. 1), whereas an apnea that was not preceded by a sigh was scored as SA. SA and PSA frequencies were determined from the number of apneas during the 10-min baseline period, during the last 7.5 min of the three challenge phases, and during the 15-min recovery phase between challenges. All apnea numbers were divided by the number of minutes in the sample period. Apneas were assessed during the recovery phase from hypoxic, hypercapnic, and asphyxic challenges because of their prevalence at this time (and absence at baseline and during the challenges). The recovery phase from hypoxia is an established period of instability (27, 62, 81), likely due to the presence of arterial hypoxia (which increases chemoreflex responsiveness) and an enhanced PO2 gradient for gas exchange (inspired—alveolar PO2; due to a lower alveolar PO2) (38, 69). Likewise, the recovery phase from hypercapnia predisposes...
to instability possibly via an increased PCO₂ gradient for gas exchange (while PICO₂ is zero and alveolar PCO₂ is still elevated).

**Histology.** Pups were humanely killed by an intraperitoneal injection of a mixture of a ketamine (100 mg/ml) xylazine (100 mg/ml). A catheter was then inserted into the trachea to fill the lung with 10% formalin at a constant pressure of 20 cmH₂O for 5 min before the trachea was ligated distal to the catheter tip. The lungs were excised and further fixed by immersion in 10% formalin for 48 h, followed by storage in PBS. Lungs were processed, embedded in paraffin cassettes, and sectioned at 5-µm thickness before staining with hematoxylin and eosin (HE) by standard techniques.

**Digital imaging.** HE-stained slides were digitally scanned at ×400 magnification by Aperio ScanScope XT (Aperio Technologies) to produce high-resolution whole-slide images. The images were analyzed with Aperio ScanScope software (Aperio Technologies). In each lung, five nonoverlapping fields of view in different sections, representing an area of 2,000 × 2,000 µm², were used for counting alveolar number and estimating alveolar area. Septal thickness was assessed by linear measurements of the septum, using fifteen measurements per field, and five fields per lung. Total alveolar number, alveolar area, and septal thickness were measured using ImageJ software (1.47v, National Institutes of Health). ImageJ measurements such as perimeter and surface area were used to calculate a surrogate value representing an area of 2,000 µm².

**Statistical Analysis**

All results are presented as means ± SE. Statistical analyses were performed on raw data for all variables by SigmaPlot 12 (SyStat, San Jose, CA). The impact of LPS on body weight, growth rate, baseline physiological values, chemoreflex responses, apnea frequency, apnea duration, and lung histology parameters were assessed by a two-way ANOVA with Student-Newman-Keuls post hoc test. *P < 0.05 was considered significant.

**RESULTS**

**Body Weight**

Pups from 3–5 separate litters in LPS and saline (SHAM) groups were used at each time point for birth weight analysis. Despite the trend toward reduced body weight in LPS pups at D07, there were no significant differences in body weight at any age. However, LPS-exposed pups had a significantly greater growth rate than SHAM pups at D28 (ANOVA main effect; Table 1).

**Baseline Ventilation**

Baseline V̇e was lower in LPS pups vs. SHAM pups across all three age groups (ANOVA main effect *P < 0.001; Table 1). Table 1. *Baseline respiratory parameters in SHAM and LPS mice from D07 to D60*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 07</th>
<th>Day 28</th>
<th>Day 60</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM (19)</td>
<td>LPS (22)</td>
<td>SHAM (16)</td>
<td>LPS (12)</td>
</tr>
<tr>
<td>Body weight, g*</td>
<td>3.7 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>11.6 ± 0.5</td>
<td>12.6 ± 0.3</td>
</tr>
<tr>
<td>Growth rate, g/day</td>
<td>0.34 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.48 ± 0.06</td>
<td>0.73 ± 0.05†</td>
</tr>
<tr>
<td>Ti, s</td>
<td>0.10 ± 0.09</td>
<td>0.11 ± 0.09†</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Te, s</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.09 ± 0.05</td>
<td>0.12 ± 0.01†</td>
</tr>
<tr>
<td>Tm, s</td>
<td>0.23 ± 0.007</td>
<td>0.22 ± 0.004</td>
<td>0.16 ± 0.007</td>
<td>0.19 ± 0.014†</td>
</tr>
<tr>
<td>V̇e, µl/g</td>
<td>7.9 ± 0.7</td>
<td>5.5 ± 0.8†</td>
<td>8.3 ± 0.5</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>V̇e, µl·s⁻¹·g⁻¹</td>
<td>28.6 ± 2.1</td>
<td>17.9 ± 1.2</td>
<td>49.6 ± 2.6</td>
<td>37.3 ± 2.6†</td>
</tr>
</tbody>
</table>

*The data are presented as means ± SE. Numbers are calculated over a 10-min control period from >2000 breaths for each variable. Growth rate was calculated using body weight only before the study time-point calculated as the difference/day. Age had a significant ANOVA main effect *P < 0.001 on all variables. SHAM, saline; LPS, lipopolysaccharide; Ti, inspiratory time; Te, expiratory time; V̇e, tidal volume; V̇e, minute ventilation; Tm, breath duration. *Log-transformed to provide equal variance before statistical comparisons. †P < 0.001 LPS vs. SHAM, post hoc comparisons.*

**Response to Hypoxia**

Pups exposed to antenatal LPS exhibited a larger V̇e increase than SHAMs in response to hypoxia (10% O₂) across all three age groups (ANOVA main effect *P < 0.001; Fig. 2A). At D07, SHAM pups (n = 19) had a peak V̇e increase of 28 ± 6% above baseline, whereas in pups exposed to LPS (n = 22) the increase was 65 ± 6% (Figs. 2A and 3A). At D07, the speed of hypoxic response, as measured by time to reach 50% of the peak V̇e, was not significantly different between groups (LPS: 115.8 ± 16.3 s; SHAM: 183.2 ± 35.4 s; *P = 0.074). At D28, SHAM pups (n = 16) lowered ventilation in hypoxia (−18.4 ± 6.6%), whereas LPS pups (n = 12) had an increase in V̇e (16.2 ± 7.7%; Figs. 2A and 3A). A hypoxia-mediated increase in V̇e was present in both groups at D60, being 35.9 ± 9.4% in LPS pups (n = 8) and 7.5 ± 7.7% in SHAM pups (n = 12) (Figs. 2A and 3A). The time to 50% peak response did not differ between LPS and SHAM pups at D28 and D60.

**Response to Hypercapnia**

LPS significantly increased the ventilatory response to hypercapnia (ANOVA main effect *P = 0.006), which was most evident at D07 (Figs. 2B and 3B). While the speed of the hypercapnic response was similar in SHAM and LPS groups at each age, there was a significant difference in the size of V̇e responses of the LPS group (169 ± 15%) compared with the SHAM at D07 (87 ± 16%) (Figs. 2B and 3B). Responses to the hypercapnic challenge were similar in LPS and SHAM pups at D28 and D60.

**Response to Asphyxia**

Asphyxia caused an increase in V̇e at all three ages (Fig. 2C). LPS increased the magnitude of V̇e response (ANOVA main effect *P = 0.002), which was significantly greater at D07 and D28 (Figs. 2C and 3C). LPS increased the speed of response evident exclusively at D07 (LPS: 41.1 ± 4.9 s; SHAM: 63.5 ± 10.0 s; *P < 0.05).

**Spontaneous and Postsigh Apnea**

During baseline breathing, and during exposure to hypoxia, hypercapnia, and asphyxia, SA and PSA were infrequent and similar between groups at the three ages studied. Following return to room air after hypoxia, LPS pups exhibited a greater frequency of SAs (ANOVA group × age *P = 0.002; Fig. 4) and PSAs (ANOVA main effect *P = 0.047; Fig. 4) at D07.
compared with SHAM pups. Following hypercapnia, LPS increased the frequency of PSA (ANOVA main effect $P < 0.001$) but not SA, an effect that again was most evident at D07 (Fig. 4). Following asphyxia, LPS increased the frequency of SA (ANOVA group × age; $P = 0.03$), most clearly at D28 (Fig. 4) and increased PSA frequency (ANOVA main effect $P < 0.001$), which was most evident at D07 and D28 (Fig. 4).

At D07, LPS pups exhibited longer SA duration vs. SHAM during hypoxic (ANOVA main effect $P = 0.01$) and hypercapnic recovery (ANOVA main effect $P = 0.02$). At D28, SA durations were significantly longer in LPS than SHAM pups in the postasphyxia period ($P < 0.05$). At D60, PSA duration was significantly longer in LPS pups than SHAMs during postasphyxia (ANOVA main effect $P = 0.045$; Fig. 5).

**Lung Histology and Morphometric Analysis**

Compared with SHAM, LPS pups had a reduced alveolar number (ANOVA main effect $P = 0.02$; Fig. 6A). Alveolar size was similar between groups with a trend toward larger

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**Fig. 2.** Changes in ventilatory parameters during hypoxic (10% O$_2$) (A), hypercapnic (7% CO$_2$) (B), and asphyxic challenge (10% O$_2$ + 7% CO$_2$) (C) for 15 min. **Left:** responses at D07. **Middle:** responses at D28. **Right:** responses at D60. All values are calculated as percentage change from baseline and expressed as means ± SE. The serrated box (A, left) indicates the minute ventilation over 7.5 min of the challenge that has been used for analysis in conditions of hypoxia, hypercapnia, and asphyxia. The graph represents saline (SHAM) pups (●) and lipopolysaccharide (LPS) pups (○).

**Fig. 3.** Average minute ventilation ($V_E$) through D07–D60. The bar graph represent the changes in $V_E$ from baseline to the average of the last 7.5 min of 10-min exposures to hypoxia (10% O$_2$) (A), hypercapnia (7% CO$_2$) (B), and asphyxia (10% O$_2$ + 7% CO$_2$) (C) in SHAM and LPS pups through D07–D60. SHAM pups are denoted by solid bars and LPS pups by open bars. All data represented as means ± SE; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; LPS vs. SHAM, post hoc comparisons.
alveoli with LPS \((P = 0.08\) at D28; Fig. 6B). LPS pups had thicker septae (ANOVA main effect \(P < 0.001\)), which was most evident at D07 (Fig. 6C). Example lung sections are shown in Fig. 7.

Alternative Analysis

The effect of LPS on the ventilatory responses to hypoxia \((P < 0.001\) ANOVA main effect), hypercapnia \((P < 0.001)\), and asphyxia \((P = 0.004)\) maintained significance when responses were reported in absolute change from baseline (units of \(\mu\text{L} \cdot \text{s}^{-1} \cdot \text{g}^{-1}\)) rather than as a percentage of baseline.

DISCUSSION

For the first time, we demonstrate that experimentally induced prenatal inflammation in the last days of gestation in the mouse markedly increases ventilatory responses to hypoxia, hypercapnia, and asphyxia in the offspring, particularly at 7 days postnatal age. Our finding of enhanced chemical controller sensitivity is expected to increase the propensity for unstable ventilatory patterns once air breathing begins after delivery. Our finding of greater apnea frequency in LPS mice and duration at D07 and D28 confirms our proposal that the LPS-exposed ventilatory control system is indeed less stable. We also show that offspring affected by LPS exhibit evidence of disrupted lung development, with substantially increased septal thickness and alveolar size and reduced alveolar number. These anatomical features promote reduced gas transfer across the lung and may limit oxygenation of arterial blood. Together, our data demonstrate that prenatal inflammation causes ventilatory instability and structural lung impairment that could lead

Fig. 4. The effects of inspired gas on SA and PSA frequency. SA and PSA events expressed per minute. In SHAM pups SA are expressed in solid bars, PSA in grey bars. In pups exposed to LPS, SA is indicated by open bars and PSA are indicated by serrated open bars. With the abrupt return to room air following gas challenges, the pups exposed to LPS had higher incidence of SA and PSA at D07. Modest effects were observed in the LPS group at D28 in the incidence of SA and PSA upon the transition from asphyxia. Values are means ± SE. *\(P < 0.05\), **\(P < 0.01\); LPS vs. SHAM, post hoc comparisons.

Fig. 5. The effects of inspired gas on SA and PSA duration. SA and PSA events expressed per minute. In SHAM pups SA are expressed in closed bars, PSA in grey bars. In pups exposed to LPS, SA is indicated by open bars and PSA are indicated by serrated open bars. Pups exposed to prenatal LPS exhibited longer SA at D07 during hypoxic and hypercapnic recovery phases. At D28, with the abrupt return to room air following asphyxic challenge, LPS pups demonstrated longer SA duration. Values are means ± SE. *\(P < 0.05\), **\(P < 0.01\), LPS vs. sham, post hoc comparisons. N, apneas not detected.
to intermittent and chronic hypoxia, which is common in neonates and can have major adverse effects on long-term outcome (26, 30, 57, 66).

In mathematical models, unstable breathing is expected when the overall sensitivity, or loop gain, of the feedback loop governing ventilation exceeds a critical value of 1.0. Any random disturbance to resting ventilation, such as a sigh, causes a disturbance to arterial O2 and CO2, which is sensed by peripheral and central chemoreceptors whose altered discharge then elicits a compensatory change in ventilation (e.g., reduction in ventilation to apnea); ultimately the ventilatory response to the initial ventilatory disturbance depends on loop gain (controller gain × plant gain). The current study clearly demonstrates that maternal inflammation enhances the chemosensitivity to hypoxia/hypercapnia (increased “controller gain”) and thereby favors instability (particularly at D07), a prediction borne out by the increased incidence of SA and PSA in pups exposed to prenatal LPS (see Fig. 4). We also reason that the reduction in resting ventilation with LPS, in the presumed absence of a decline in metabolism, must lead to an increase in resting arterial Pco2; of note, greater Pco2 renders ventilatory disturbances more effective at changing Pco2 (raising “plant gain”), further acting to destabilize breathing (16, 68).

There are several possibilities for the reduction in ventilation observed with LPS. First, we consider the possibility that ventilation was lowered because of changes in metabolic rate. The allometric relationship between metabolic rate and body mass (y = aM^{0.7}) suggests that a smaller pup should have a greater metabolic rate (and thus greater ventilation) per unit mass than a larger control (80); however, there was only a ~10% (nonsignificant) reduction in body weight in LPS pups at 7 days, which would not account for the 37% reduction in ventilation (per unit body weight) at this time. Growth rates were also not reduced by LPS (Table 1), suggesting that a substantial reduction in metabolic rate is unlikely. Second, it is possible that the impaired or delayed lung function observed with LPS was accompanied by increased lung elastance; thus ventilation may have been reduced because of greater mechanical impedance to respiration. However, the effect of LPS on ventilation persisted after day 7 when lung function largely normalized. Third, ventilation may have been suppressed by a reduction in ventilatory drive (lowered brainstem output). A combination of reduced ventilatory drive and increased chemosensitivity is theoretically destabilizing and has been observed in infants exhibiting periodic breathing (61).

Our study shows that prenatal LPS exposure results in respiratory instability. Specifically, we observed that 1) the incidence of apneas, both SA and PSA, was greater in LPS vs. SHAM pups at D07 during hypoxic, hypercapnic, and asphyxic recovery phases. This increased frequency remained evident at D28 post asphyxia. 2) Apneas were longer in duration in LPS (D07 and D28). These findings of increased incidence and duration of apneas with LPS, combined with increased chemosensitivity and depressed mean ventilation, clearly demonstrate that perinatal inflammation destabilizes ventilatory control. That poststimulus apneas diminished in LPS by D60 is consistent with the reduced frequency of periodic breathing with age seen in infants (4, 83). However, an enhanced response to hypoxia persisted at D60; while not sufficient to cause overt

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**Fig. 6.** Effects of prenatal LPS on postnatal lung structure at D07–D60. Morphometric analyses of lung tissue samples obtained from SHAM pups (closed bars) and LPS pups (open bars). A: alveolar number. B: alveolar size (μm²). C: septal thickness (μm); n = 5–10 per group. At D28, LPS pups had a decreased alveolar number, particularly at D28. LPS pups also had thicker septae at D07. Values are means ± SE. *P < 0.05, **P < 0.01; LPS vs. SHAM, post hoc comparisons.

**Fig. 7.** Histological lung sections of pups in SHAM and LPS groups. Fixed lung tissues were stained with hematoxylin and eosin; n = 5–10 per group. Stained slides scanned at a magnification ×400. Internal scale bar 20 μm. Top: histological sections from SHAM and LPS pups at D07. Middle: sections from pups at D28. Bottom: sections from the pups at D60.
central apneas in pups, increased chemosensitivity has been implicated in the later development of hypertension (56) and obstructive sleep apnea (15, 60, 65, 79). Our results suggest that measures to avert maternal inflammation may mitigate these lasting adverse effects.

Prenatal infections adversely affect lung development, and evidence to date suggests that intra-amniotic injections of endotoxin in sheep cause fetal lung inflammation, thereby reducing alveolar septation and vascularization (35); in rodents it has been shown to induce pulmonary inflammation and delayed alveolar maturation (9). In the mouse, lung development at birth is at the saccular phase, and alveolarization is initiated around postnatal day 5 and is completed during the first 2–3 wk of life (78). Because much of the lung development occurs postnatally in mice, we focused on the morphological differences in our two treatment groups across three time points. Increases in septal wall thickness have been reported previously in response to hyperoxia, with the phenomenon attributed to inhibition of pathways that lead to septal thinning (54, 63). Additionally, Velten and colleagues (77) examined the ratio of total perimeter per high-power field to septal wall thickness as a measure of surface area available for gas exchange in rat pups exposed to a combination of hyperoxia and prenatal LPS. Both these insults exhibited a remarkable increase in septal wall thickness. While confirming an effect of LPS, our study indicated that exposure to prenatal LPS had modest effects on postnatal respiratory structure. Differences in alveolar number were observed in LPS pups, primarily at D28. Thicker septae were observed in LPS pups at D07 (28% increase in septal thickness compared with SHAM pups). According to Fick’s law of diffusion, thicker septae will reduce lung diffusing capacity and potentially impede gas exchange unless a compensating rise in ventilation increases alveolar O₂; our observation that ventilation is less in LPS-treated pups shows that no such compensation occurred. Accordingly, the alterations in lung structure we induced via maternal inflammation may translate into reduced gas exchange and may contribute to the requirement for supplemental oxygen in many preterm infants to achieve adequate oxygenation (10).

The impairments we observed in lung structure and ventilatory stability due to maternal inflammation/infection may contribute to the increased risk of sudden infant death syndrome (SIDS) in preterm infants. There is a wealth of supportive evidence linking SIDS to inflammation/infection, which includes autopsy findings of infection and inflammatory markers in SIDS victims (6, 28, 47, 55) and an increased risk of SIDS in winter months (13). Unrecognized hypoxemia has also been firmly implicated in SIDS, with evidence including brain stem gliosis (39, 48, 74, 76), elevated hypoxanthine in vitreous humor (53, 64), and increased VEGF in cerebral spinal fluid (34) of SIDS victims. Dominant causes of hypoxemia in infancy are, in general, abnormalities of ventilatory control and of respiratory function. In particular, ventilatory control instability has long been suspected to play an important role in SIDS (70, 75); near-miss SIDS infants have been found to have a greater predisposition to apnea and periodic breathing (7, 37), SIDS risk is elevated with residence at higher altitude where respiratory disturbances are common (22, 41), and a high proportion of SIDS victims exhibited abnormal carotid body glomus cell mass (59). Importantly, infection and ventilatory instability are also associated and may combine to yield profound hypoxemia (5, 58). A link between SIDS and maternal inflammation/infection, while speculative, is highly feasible now that we have shown that delayed/impaired ventilatory control and lung structure are consequences of maternal inflammation/infection.

This study had limitations due to the absence of metabolic rate and arterial PCO₂ measurements. We chose to employ unrestrained wholebody plethysmography to assess the primary respiratory control effects of LPS longitudinally under maximally natural conditions; the most accurate metabolic rate measurement requires a sealed flow-through system and animal constraint, which is a considerably less natural setting (and may alter metabolic rate due to stress responses to the experimental conditions).

In summary, our study shows that prenatal fetal inflammation has a significant impact on the developing lung and on the sensitivity of respiratory chemoreflexes. LPS leads to changes in lung structure in the first month of postnatal life in the mouse that is likely to reduce gas exchange efficiency, as reported for human premature infants. The reduced resting ventilation and enhanced chemoreflexes could explain the high prevalence of periodic breathing and apnea in many preterm human infants. Since apnea and periodic breathing promote hypoxemia, prenatal maternal inflammation may provide a clue to the higher incidence of SIDS in preterm infants.

**GRANTS**

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**


**REFERENCES**


