Fetal growth restriction in mice modifies postnatal airway responsiveness in an age
and sex-dependent manner

Running title: Airway responsiveness after IUGR

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ABSTRACT

Epidemiological studies demonstrate an association between intrauterine growth restriction (IUGR) and asthma, however the underlying mechanism is unknown. We investigated the impact of maternal hypoxia-induced IUGR on airway responsiveness in male and female mice during juvenility and adulthood. Pregnant BALB/c mice were housed under hypoxic conditions for gestational days 11-17.5 and then returned to normoxic conditions for the remainder of pregnancy. A control group were housed under normoxic conditions throughout pregnancy. Offspring were studied at 2 weeks (juveniles) and 8 weeks (adults), where lung volume was assessed by plethysmography, airway responsiveness to methacholine determined by the forced oscillation technique and lungs fixed for morphometry. IUGR offspring were lighter at birth, exhibited “catch-up growth” by 2 weeks, but were again lighter in adulthood. IUGR males were “hyper-responsive” at 2 weeks and “hypo-responsive” as adults, in contrast to IUGR females who were hyper-responsive in adulthood. IUGR males had increased inner and total wall thickness at 2 weeks which resolved by adulthood, while airways in IUGR females were structurally normal throughout life. There were no differences in lung volume between Control and IUGR offspring at any age. Our data demonstrate changes in airway responsiveness as a result of IUGR that could influence susceptibility to asthma development and contribute to sexual dimorphism in asthma prevalence which switches from a male dominated disease in early life to a female dominated disease in adulthood.
ABBREVIATIONS LIST

ASM, airway smooth muscle; GD, gestational day; IUGR, intrauterine growth restriction; LFOT, low-frequency forced oscillation technique; MCh, methacholine; P_{bm}, perimeter of the basement membrane; Zrs, impedance

PERSPECTIVE

• Intrauterine growth restricted and low birth weight are associated with asthma development, however the underlying mechanism is unknown. This study used a mouse model of intrauterine growth restriction to show changes in airway responsiveness that may impact susceptibility to asthma and the prevalence of disease between males and females throughout life.

• We found that while growth restricted male mice exhibited "hyper-responsiveness" at 2 weeks, they became "hypo-responsive" at 8 weeks. In contrast, growth restricted females, while normal at 2 weeks, became “hyper-responsive” at 8 weeks.

• These findings suggest that changes in the prevalence of asthma throughout life between males and females (male dominated early in life and female dominated late in life) may be explained by temporal changes in airway responsiveness that originates from disrupted fetal growth.
INTRODUCTION

Reduced lung function in early infancy is associated with the development of asthma in childhood (1, 2) and in young adults (3). Subsequently, in postnatal life, there is little (4) to no (5) deterioration in lung function relative to non-asthmatic subjects. These observations support a mechanism relating to impaired lung growth and development beginning in prenatal life. One likely contributor to abnormal organ growth of the fetus is intrauterine growth restriction (IUGR), often attributed to fetal hypoxia (6), and a precursor to low birth weight (7). Asthma is statistically linked with both IUGR (8) and low birth weight (9) although the mechanism is unknown. We propose that IUGR gives rise to abnormalities in airway function from birth, thereby predisposing individuals to subsequent asthma development.

Although growth trajectories are to some degree established before birth, growth dynamics are subject to change and/or are potentially modifiable in the postnatal period. For instance, some IUGR affected-individuals display accelerated postnatal growth and reach normal age-matched developmental milestones, a phenomenon termed “catch-up growth” (10). It is also well established that male and females display different patterns in somatic growth (11), and if similar changes occur with respect to respiratory system development, this may impact the relative prevalence of asthma between males and females, which shifts from a male-dominated disease early in life to a female-dominated disease following puberty (12, 13). Sexual dimorphism of asthma prevalence is attributed to changes in lung volume (14), airway structure (e.g., size) (15) and airway responsiveness (16), potentially mediated through hormonal pathways (17). If the relationship between IUGR and asthma does indeed occur due to alterations in lung
function initiated by abnormal development prior to birth, it is therefore reasonable to propose this occurs in a sex-dependent manner that manifests at different stages of postnatal life.

We hypothesised that IUGR modifies airway responsiveness and the susceptibility to subsequent disease and that this occurs in a sex-dependent manner. To address this hypothesis, we used a mouse model of maternal hypoxia to produce IUGR through fetal hypoxia. The hypoxic exposure was limited to the period of airway development (pseudoglandular – canalicular phases) and male and female offspring were reared to 2 weeks (juveniles) and 8 weeks (adults) for assessment of lung volume, airway responsiveness to methacholine (MCh) challenge and finally airway structure. Findings suggest that IUGR alters functional properties of the airway that may affect susceptibility to the development of asthma. These abnormalities are impacted by sex in a manner consistent with the change in asthma prevalence between males and females throughout life.

MATERIAL AND METHODS

Ethical Approval

Our experimental approach was to subject pregnant mice to a hypoxic or normoxic environment and, in both male and female offspring to assess the thoracic gas volume by plethysmography and airway responsiveness by the forced oscillation technique at 2 and 8 weeks of age. After euthanasia, lungs were fixed for morphometry. This study was carried out in strict accordance with the recommendations of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition). The
protocol was approved by the Telethon Kids Institute Animal Ethics Committee (Project Number 264).

Maternal hypoxia-induced IUGR mouse model

Pregnant female BALB/c mice at gestational day (GD) 7 were obtained from Animal Resources Centre (Murdoch, WA, Australia) and housed at the Telethon Kids Institute in specific pathogen-free environments. Mice were maintained on a 15:9 hour light:dark cycle and supplied with an allergen free diet (Specialty Feeds, Glen Forrest, WA, Australia) and water ad libitum. One group of pregnant mice were housed under hypoxic conditions (10.5% O₂) from GD 11 – GD 17.5 (pseudoglandular – canalicular stage in the mouse lung development) and then returned to a normoxic environment (21% O₂) until birth (GD 21; n=11). A control group of pregnant mice was housed under normoxic conditions throughout pregnancy (n=13). Offspring from dams exposed to hypoxia are referred to as IUGR offspring and offspring from dams exposed to normoxia are referred to as Control offspring. Weights of offspring were recorded at birth and prior to \textit{in vivo} assessment at 2 or 8 weeks.

Plethysmography and Forced Oscillation Technique

At 2 weeks of age and 8 weeks of age, offspring were anaesthetised by an i.p. injection of ketamine and xylazine (2 weeks: 0.2 and 0.01 mg/g body weight respectively; 8 weeks: 0.4 and 0.02 mg/g body weight respectively), tracheostomised (18) and mechanically ventilated at 400 breaths/min with a tidal volume of 10 mL/kg and 2 cmH₂O positive end-expiratory pressure in a plethysmographic chamber. During pauses in ventilation, the trachea was occluded and the intercostal muscles electrically stimulated to induce
inspiratory efforts. Thoracic gas volume was calculated by Boyle’s law using tracheal and chamber pressure as previously described (19) and presented as raw volume (mL) or normalised to body weight (mL/g).

After performing lung volume measurements, mice were transferred to a flexiVent system (FX module 1, flexiWare version 7.5, SCIREQ, Montreal, QC, Canada) to assess responsiveness to MCh (β-methacholine chloride, Sigma–Aldrich, St. Louis, MO). Mice were ventilated at 250 breaths/min with a tidal volume of 8.8 mL/kg and positive end expiratory pressure of 2 cmH\textsubscript{2}O. Lung volume history was standardised via 3 slow inflation-deflation manoeuvres up to 20 cmH\textsubscript{2}O transrespiratory pressure. Respiratory impedance (Z\textsubscript{rs}) was measured by low-frequency forced oscillation technique (LFOT) (20) and the constant phase model (21) used to partition Z\textsubscript{rs} into airway and parenchymal components; allowing calculation of Newtonian resistance, inertance, tissue damping and elastance. In mice, Newtonian resistance is equivalent to airway resistance because of the high compliance of the chest wall. The calibration procedure in the flexiVent FX software was used to correct for the resistance of the tracheal cannula. Inertance values were negligible and are not reported.

Once stabilised on the ventilator, LFOT measurements were acquired every minute for 5 min to establish baseline respiratory mechanics, followed by a 10 s saline aerosol and increasing doses of MCh (maximum dose 30 mg/mL) delivered by the Aeroneb ultrasonic nebulizer (SCIREQ). After each challenge, LFOT measurements were again acquired every min for 5 min with the peak response used for analysis.
Lung morphometry
Approximately 5 min after bronchial challenge and euthanasia, lungs were fixed by intra-tracheal instillation of 4% formaldehyde at a transrespiratory pressure of 10 cmH$_2$O (22). To obtain transverse sections of lung tissue (and airways therein), the left lobe was embedded in paraffin with the proximal-end facing down. The length of the left lung was measured and used to calculate 6 equally spaced sampling zones from which 5 micron-thick sections were obtained. This sectioning approach allowed the main bronchus to be viewed in cross-section, and since the airway extends to the base of the left lung, proximal to distal regions of the bronchial tree were measured and subsequently averaged. Sections were stained using the Masson’s Trichrome technique. The cross-sectional area of airway smooth muscle (ASM) layer, epithelium, inner, outer and total wall, and internal perimeter of the basement membrane ($P_{bm}$) (23) were measured using the newCast stereology software (Visiopharm, Hoersholm, Denmark). Since airways were fixed after MCh challenge, lumen dimensions are not reported. The square roots of areas were normalised to $P_{bm}$, the standard index of airway size (24).

Statistical analysis
Data were transformed where necessary to ensure the assumptions of normality and homoscedasticity of variances for the parametric tests were satisfied. Normally distributed data were analysed using t-tests to determine the effect of treatment (Control vs IUGR). Non-normally distributed data were analysed using equivalent non-parametric statistical analyses or transformed when necessary. Two-way ANOVA was used to assess the maturation effect on $P_{bm}$ with age (i.e., treatment and age effect). For the MCh challenges, analyses were conducted using two-way repeated measures ANOVA with
MCh concentration (saline or maximal dose of MCh i.e., 30 mg/mL) as a matched variable and treatment (Control or IUGR) as an unmatched variable. Pairwise multiple comparison procedures were used when an interaction was observed between dose and treatment. All data are presented as mean ± SEM with *$P<0.05$ considered significant. Graphical and statistical analyses were performed by SigmaPlot version 13.0 or Prism version 7.02. n refers to the number of offspring.

**RESULTS**

**Growth outcomes**

The IUGR offspring (n=48) were lighter at birth compared with Control (n=48) offspring ($P<0.001$, unsexed; Figure 1A). There was no difference in body weight between the groups at 2 weeks of age (male, $P=0.688$; female, $P=0.160$; Figure 1B) indicating that catch-up growth had occurred. However, despite early catch-up growth, the weight of mice in the IUGR group was less than mice in the Control group at 8 weeks of age (male, $P<0.01$; female, $P=0.004$; Figure 1B). Maternal hypoxia had no effect on litter size (Control, 4.00 ± 0.39 pups; IUGR, 4.45 ± 0.37 pups; $P=0.412$) or gestational period (Control, GD 19.84 ± 0.15 days; IUGR, GD 19.00 ± 1.00 days; $P=0.806$). We did not observe any incidence of stillborn pregnancy in this cohort, however, there is a possibility that some pups were not born alive and were subsequently consumed by the dam before inspection.

**Thoracic gas volume**

Thoracic gas volume at 2 weeks (male, $P=0.379$; female, $P=0.466$) and 8 weeks (male, $P=0.195$; female, $P=0.414$) of age was not different between IUGR and Control groups.
There were also no differences between the groups at 2 weeks (male, $P=0.292$; female, $P=0.181$) and 8 weeks (male, $P=0.919$; female, $P=0.787$) when the thoracic gas volume was normalised to body weight.

Methacholine responsiveness

Sex and age of assessment (2 or 8 weeks) affected the differences observed between Control and IUGR offspring. In male offspring at 2 weeks of age, airway resistance was not different between groups prior to MCh administration ($P=0.363$; Figure 2A), but was greater in IUGR offspring after MCh ($P=0.007$; Figure 2A). There was no statistical difference in tissue damping ($P=0.930$; Figure 3A) or elastance ($P=0.558$; Figure 4A) between male groups at 2 weeks of age, either before or after MCh. The effect of IUGR in male mice was reversed at 8 weeks of age with a decrease in both airway resistance ($P=0.041$; Figure 2B) and tissue damping ($P=0.003$; Figure 3B) in IUGR offspring compared with Control after MCh. There was no difference between the male groups in airway resistance ($P=0.476$; Figure 2B) or tissue damping ($P=0.782$; Figure 3B) prior to MCh, or in elastance before or after MCh ($P=0.563$; Figure 4B) at 8 weeks of age.

The effects of IUGR in female mice and the associated changes with age were the opposite of those observed in male mice. In female mice at 2 weeks of age, there was no difference in airway resistance between IUGR and Control offspring before or after MCh ($P=0.984$; Figure 2C). However, in female mice at 8 weeks of age, while airway resistance was not different between groups prior to MCh administration ($P=0.339$; Figure 2D), resistance was increased in IUGR offspring after MCh compared with Control ($P=0.006$; Figure 2D). The effect of IUGR on tissue damping and elastance in
female mice displayed a different pattern to airway resistance. In female mice at 2 weeks of age, tissue damping was increased in the IUGR group compared with the Control group both before and after MCh ($P=0.001$; Figure 3C). The elastance at 2 weeks was greater in the IUGR group after MCh administration ($P=0.013$; Figure 4C), but not before MCh ($P=0.121$; Figure 4C). No differences were observed in tissue damping ($P=0.071$; Figure 3D) or elastance ($P=0.314$; Figure 4D) between female groups at 8 weeks, either before or after MCh.

**Morphometry**

Airway morphological measurements at 2 weeks and 8 weeks of age are provided in Table 2 and Table 3 respectively. In male offspring at 2 weeks of age, there was an increase in inner ($P=0.01$) and total ($P=0.03$) airway wall thickness in the IUGR group (Figure 5B) compared with Control (Figure 5A). There were no other differences in airway wall parameters between groups at 2 weeks of age, and any differences between male groups were no longer apparent at 8 weeks of age (Control, Figure 5C; IUGR, Figure 5D). In female mice, airway wall dimensions were comparable between groups at both 2 and 8 weeks of age.

With respect to the effect of maturation on airway size ($P_{bm}$), there was an increase in airway size in male IUGR offspring from 2 to 8 weeks of age ($P=0.03$). There was also a similar trend for an increase in airway size in female Control offspring from 2 to 8 weeks ($P=0.06$). There was no significant increase in airway size in male Control and female IUGR offspring.
DISCUSSION

Reduced expiratory flow in infancy (1, 3) is associated with subsequent asthma development, implicating an *in utero* developmental disorder as a mechanism driving abnormal postnatal lung function. Population studies have reported that IUGR and low birth weight are each associated with the development of asthma (8, 25). In a Swedish population, IUGR-affected children had an odds ratio of 1.24-1.87 for asthma (8), and in a Finnish population, the odds ratio for asthma was 2.0 in individuals that were born with low birth weight (25). We propose that the association between IUGR and subsequent low birth weight with increased risk of asthma development is due to concomitant abnormalities in postnatal airway function. Our findings show that airway responsiveness in IUGR offspring is altered compared with normal development and that this varies with the age of assessment (juvenile or adult periods) in a sex-dependent manner.

We developed a mouse model of maternal hypoxia induced-IUGR which as expected lead to low birth weight. Hypoxia is a key determinant of fetal growth and there is a positive relationship between partial pressure of oxygen in the fetal carotid artery and birth weight (26). It is noteworthy that one cause of IUGR is maternal smoking (27), which is frequently identified as a risk factor the development of asthma. In a recent sheep model of maternal asthma, fetal size (relative to maternal weight) was reduced compared with naïve Controls (28, 29) consistent with growth restriction observed in human maternal asthma (30). Other IUGR models include maternal undernutrition and placental restriction (31), each of which may have differing effects on respiratory function. However, while the upstream causes of IUGR are varied, the downstream effect is
ultimately fetal hypoxia (27, 32). A maternal model of hypoxia therefore provides a direct method of studying the functional consequences of IUGR.

Our results showed an important effect of IUGR on airway responsiveness, determined from the change in Newtonian resistance produced by MCh, which were sex and age-dependent (discussed below). Some peripheral changes were also observed, where tissue damping was decreased in male IUGR offspring at 8 weeks of age after MCh, and in female IUGR offspring at 2 weeks of age, tissue damping was increased before and after MCh, and elastance increased after MCh. Tissue damping is an index of peripheral airway function and/or tissue resistance, while elastance (that appears more variable) is a function of tissue stiffness, but is also affected by airway closure and lung volume. There were no changes in lung volume determined by plethysmography or when lung volume was normalised to body weight suggesting that lung size is not a critical factor. In rat IUGR offspring, maternal hypoxia-induced IUGR during the same gestational period did not alter alveolar volume, surface area or number of alveoli (33). That airway physiology is altered in preference to alveoli structure-function is intuitive since hypoxia was limited to the mid-gestation pseudoglandular – canalicular period when airway development is occurring, compared with alveolar development which occurs from late gestation, extending into the postnatal period. Early rather than late gestation therefore represents a window of susceptibility to IUGR and the subsequent development of airway disease. Reduced fetal size in the pseudoglandular phase is associated with asthma development in later life (34). The Dutch Winter Hunger Famine study also demonstrated that exposure to famine during early and mid-gestation resulted in an increased prevalence of obstructive airway disease in adult life (35),
whereas exposure to famine during late gestation did not show the same association with obstructive disease.

The most interesting finding of the present study was that changes in airway responsiveness with IUGR were sex- and age-dependent. Juvenile male IUGR offspring (2 weeks) had increased airway responsiveness compared with Control, consistent with an asthmatic phenotype wherein airway “hyper-responsiveness” is a primary functional abnormality (36). In contrast, juvenile female IUGR offspring had no change in airway responsiveness. The scenario in adult (8 weeks) mice was entirely inverted. While male IUGR offspring at 2 weeks of age were “hyper-responsive” compared with Control, in adulthood they became “hypo-responsive”. Instead, adult female IUGR offspring were “hyper-responsive” compared with their age-matched Control group, and are therefore more consistent with an asthmatic phenotype. Our findings on changes in airway function in IUGR mice align well those of human population studies where the prevalence of asthma is greater in males than females in early childhood (12), but subsequently greater in females than males in adulthood (13).

It is of course an oversimplification to extend findings derived from a single intervention in an animal study to human disease, particularly asthma which is attributed to a multitude of factors such as genetic predisposition and environmental exposure (37). The IUGR mouse was not exposed to any environmental stimulus (e.g., allergen) and should not be considered an animal model of asthma (if one actually exists). The purpose of this study was to instead examine the mechanism behind the increased risk of asthma development after IUGR and/or low birth weight previously reported in epidemiological studies (8,
Since airway “hyper-responsiveness” is a primary feature of asthma (36) we make the assertion that changes in airway responsiveness following IUGR alters the susceptibility of asthma development. The magnitude of the changes in airway responsiveness reported in the present study (e.g., ~35% increase in maximum resistance in IUGR males at 2 weeks and IUGR females at 8 weeks) may therefore seem small in comparison to the pronounced change in the dose-response curve of asthmatic subjects (36). However, whether an IUGR-affected individual goes on to develop asthma will ultimately be determined by other susceptibilities such as allergy that independently affects airway responsiveness (38).

Changes in airway responsiveness in male IUGR offspring can be explained by corresponding changes in airway wall structure. Inner (which included ASM thickness) and total wall thickness were increased in IUGR males compared with Controls at 2 weeks of age. Mathematical models clearly demonstrate that increased wall thickness produce geometric changes that favour lumen encroachment and reduced parenchymal afterload (39). Importantly, changes in airway resistance as a result of increased wall thickness are more pronounced after MCh with little to no change in baseline resistance (39), in agreement with our data where changes in resistance were only significant after MCh. By 8 weeks of age, airway thickness in males was no longer different between IUGR and Control groups which may be explained by airway growth. Based on the $P_{bm}$, which is widely used as a measure of airway size, there was an increase in airway size in the IUGR males from 2 to 8 weeks but not in Control males. Increased airway size with maturation in IUGR males relative to Control males favours reduced airway resistance in
adult IUGR males and is consistent with the phenotypic shift from “hyper-” to “hypo-
responsiveness”.

In comparison with males, changes in airway responsiveness in IUGR females seems to
occur through a mechanism that is independent of airway structure. Increased airway
responsiveness in IUGR females at 8 weeks was not associated with any change in airway
wall thickness. There are numerous mechanisms that could alter airway responsiveness
without changing the gross morphology of the airway wall. For instance, an increase in
ASM cell contractility would favour an increase in airway responsiveness without
necessarily altering ASM layer thickness. In culture, a gel contraction assay was used to
demonstrate increased shortening capacity of ASM cells from subjects with asthma,
which was not attributed to differences in cell size or number (40). Increased baseline
ASM tone (prior to MCh exposure) could exacerbate the response to inhaled agonist, as
was demonstrated in adult mice exposed to 3 weeks of hypoxia (41). However, postnatal
hypoxia exposure in mature animals is likely to be quite different to developmental
changes initiated by in utero exposure and the lack of difference in baseline resistance
argues against changes in neurally driven ASM tone (41). Finally, an alternative
explanation for an increase in airway responsiveness in adult IUGR females is that
changes were hormonally mediated. Relaxation of the ASM is produced by oestrogen
(42) and progesterone (43) and if down-regulated in IUGR females could account for
“hyper-responsiveness” in adult females. In other IUGR rodent models, no change in
oestrogen (44) or progesterone levels (45) of the IUGR offspring have been reported.

Whether hormone release or production is affected when IUGR is specifically limited to
the mid-gestation period of fetal development is not known.
The precise mechanisms for the sex- and time-dependent effects on airway responsiveness cannot be explicitly identified in the present study, but may be influenced by complex postnatal growth kinetics. Following birth, during the pre-weaning period of growth, catch-up growth occurred where differences in body weight were no longer observed at 2 weeks of age. At 8 weeks of age, IUGR offspring were again smaller (body mass) compared with Control offspring, suggesting that growth during the post-weaning phase (> 3 weeks) was restricted in IUGR offspring. There are conflicting data on the consequence of catch-up growth in IUGR affected individuals, with some studies reporting improved lung function (46, 47), but not others (48, 49). It is possible that the above non-linear growth kinetics interact with prepubescent maturation in males and females to exert effects on airway responsiveness. Figure 6 summarises the findings from the present study and shows that juvenile male mice (2 weeks) were “hyper-responsive” after IUGR and that was immediately preceded by a period of catch-up growth. In later life, IUGR mice became growth restricted again and females were “hyper-responsive” and males “hypo-responsive”.

One limitation of the present study is that experiments were not designed to identify biological pathways governing the observed physiological changes. Genes that are associated with hypoxia signalling and which are altered in the lungs of a hypoxic IUGR sheep model include the hypoxia-inducible factors complex, prolyl hydroxylase domain-containing proteins, KDM3A and SLC2A1 (50). Inflammatory signalling may also be altered in IUGR offspring, and we have previously shown that the total cell counts (predominantly macrophages) were higher in the bronchoalveolar lavage fluid of the
IUGR offspring compared with Control (33). It is also unclear whether temporal changes in airway responsiveness would continue in the ageing mouse i.e., would female IUGR mice also eventually become “hypo-responsive” in a manner similar to males? Chronic cardiopulmonary dysfunction develops in IUGR offspring at advanced age (12 - 14 months of age) (51, 52), demonstrating that the impact of IUGR is still apparent at the end of life. Now that we have established the respiratory consequences of IUGR in juvenility and adulthood, future studies are required to examine airway responsiveness in later life which is potentially relevant to asthma in the elderly (53), and to identify the underlying biological pathways.

In summation, postnatal airway responsiveness in mice following maternal hypoxia-induced IUGR is altered from early life and is subsequently modified by adulthood in a sex-dependent manner. Previously demonstrated associations between asthma and IUGR can therefore be explained by functional changes to the airways that impact the risk of asthma. Sexual dimorphism in the response to IUGR may also contribute to differences in the prevalence of asthma between males and females in early childhood and adulthood.

DECLARATION OF INTEREST

None.

FUNDING

This project was funded by the Asthma Foundation of Western Australia New Investigator Grant (K.C.W.W. and P.B.N.), National Health and Medical Research Council (NHMRC) of Australia Project Grant (1120128 (K.C.W.W.) and 1027218
AUTHORS CONTRIBUTION

K.C.W.W., A.N.L., J.S.M., S.T.D., P.B.N. designed the study, K.C.W.W. conducted the study, K.C.W.W., L.J.B. were involved in data collection, K.C.W.W., A.N.L., P.B.N. were involved in data analysis, all authors were involved in data interpretation, K.C.W.W., P.B.N. drafted the manuscript and all authors contributed and approved the final manuscript writing.
REFERENCES


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FIGURE LEGENDS

**Figure 1.** Birth weight (A) of Control (n=48) and IUGR (n=48) offspring. Body weight (B) of Control and IUGR offspring at 2 weeks (Control male, n=12; IUGR male, n=11; Control female, n=16; IUGR female, n=8) and 8 weeks of age (Control male, n=8; IUGR male, n=8; Control female, n=9; IUGR female, n=12). Sample size of birth weight is larger than experimental sample size as the birth weight of all pups in each litter was recorded. Values are mean ± SEM. *Significantly different from Control (P<0.01). Control (open circles); IUGR, intrauterine growth restriction (filled circles); W, week.

**Figure 2.** Airway resistance in males at 2 weeks (A), 8 weeks (B), and females at 2 weeks (C) and 8 weeks (D) for Control and IUGR offspring. Values are mean ± SEM. *Significantly different from Control (P<0.05). Control (open circles); IUGR, intrauterine growth restriction (filled circles); MCh, methacholine.

**Figure 3.** Tissue damping in males at 2 weeks (A), 8 weeks (B), and females at 2 weeks (C) and 8 weeks (D) for Control and IUGR offspring. Values are mean ± SEM. *Significantly different from Control (P<0.05). Control (open circles); IUGR, intrauterine growth restriction (filled circles); MCh, methacholine.

**Figure 4.** Tissue elastance in males at 2 weeks (A) and 8 weeks (B), and females at 2 weeks (C) and 8 weeks (D) for Control and IUGR offspring. Values are mean ± SEM. *Significantly different from Control (P<0.05). Control (open circles); IUGR, intrauterine growth restriction (filled circles); MCh, methacholine.
Figure 5. Representative images of airways from Control male at 2 weeks (A), IUGR male at 2 weeks (B), Control male at 8 weeks (C) and IUGR male at 8 weeks (D) groups.

Figure 6. Changes in airway responsiveness and therefore the onset of hyper- and/or hypo-responsiveness was age- and sex-dependent, and preceded by complex postnatal growth kinetics.
## Table 1. Thoracic gas volume in the Control and IUGR offspring.

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<th>Males</th>
<th>Females</th>
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<tr>
<td></td>
<td>Control</td>
<td>IUGR</td>
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<tr>
<td>Thoracic gas volume (mL)</td>
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<tr>
<td>2 weeks</td>
<td>0.141 ± 0.011</td>
<td>0.177 ± 0.025</td>
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<td>(10)</td>
<td>(11)</td>
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<tr>
<td>8 weeks</td>
<td>0.252 ± 0.025</td>
<td>0.222 ± 0.053</td>
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<td>(8)</td>
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Values are mean ± SEM (n). IUGR, intrauterine growth restriction.

## Table 2. Airway dimensions in the Control and IUGR offspring at 2 weeks of age.

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<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>Control (n=9)</td>
<td>IUGR (n=8)</td>
</tr>
<tr>
<td>Structure</td>
<td></td>
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<tr>
<td>P_{bm} (µm)</td>
<td>1384.93 ± 101.34</td>
<td>1121.45 ± 92.40</td>
</tr>
<tr>
<td>Total airway wall</td>
<td>0.130 ± 0.004</td>
<td>0.146 ± 0.006*</td>
</tr>
<tr>
<td>(√area/P_{bm})</td>
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<tr>
<td>Outer airway wall</td>
<td>0.093 ± 0.004</td>
<td>0.099 ± 0.003</td>
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<tr>
<td>(√area/P_{bm})</td>
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<tr>
<td>Inner airway wall</td>
<td>0.089 ± 0.003</td>
<td>0.107 ± 0.005*</td>
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<td>(√area/P_{bm})</td>
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Values are mean ± SEM. *Significantly different from Control \((P<0.05)\). IUGR, intrauterine growth restriction; \(P_{\text{bm}}\), perimeter of the basement membrane; ASM, airway smooth muscle.

**Table 3.** Airway dimensions in the Control and IUGR offspring at 8 weeks of age.

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<thead>
<tr>
<th>Structure</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_{\text{bm}}) (µm)</td>
<td>Control (n=6)</td>
<td>IUGR (n=8)</td>
</tr>
<tr>
<td></td>
<td>1253.30 ± 81.00</td>
<td>1513.07 ± 134.05</td>
</tr>
<tr>
<td></td>
<td>1674.46 ± 217.12</td>
<td>1398.82 ± 99.20</td>
</tr>
<tr>
<td>Total airway wall ((\sqrt{\text{area}/P_{\text{bm}}}))</td>
<td>0.163 ± 0.008</td>
<td>0.147 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>0.154 ± 0.010</td>
<td>0.149 ± 0.004</td>
</tr>
<tr>
<td>Outer airway wall ((\sqrt{\text{area}/P_{\text{bm}}}))</td>
<td>0.114 ± 0.006</td>
<td>0.010 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>0.108 ± 0.007</td>
<td>0.104 ± 0.013</td>
</tr>
<tr>
<td>Inner airway wall ((\sqrt{\text{area}/P_{\text{bm}}}))</td>
<td>0.116 ± 0.006</td>
<td>0.107 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>0.109 ± 0.007</td>
<td>0.104 ± 0.003</td>
</tr>
<tr>
<td>Epithelium ((\sqrt{\text{area}/P_{\text{bm}}}))</td>
<td>0.088 ± 0.005</td>
<td>0.0784 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>0.079 ± 0.007</td>
<td>0.077 ± 0.004</td>
</tr>
<tr>
<td>ASM ((\sqrt{\text{area}/P_{\text{bm}}}))</td>
<td>0.053 ± 0.004</td>
<td>0.047 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>0.051 ± 0.005</td>
<td>0.045 ± 0.002</td>
</tr>
</tbody>
</table>