The airway smooth muscle layer is structurally abnormal in low birth weight infants: implications for obstructive disease

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To the Editor:

This research letter concerns known associations between intrauterine growth restriction (IUGR), low birth weight (LBW) and increased risk of obstructive disease [1, 2]. Our prior animal simulations, specifically a mid-gestation maternal hypoxia mouse model of IUGR, has forwarded the hypothesis that increased risk of obstructive disease is due to abnormalities of the airway wall. In mice, IUGR causes airway smooth muscle (ASM) thickening of the fetus [3], and in postnatal life, increases airway wall stiffness [4] and modifies bronchoconstrictor response to inhaled methacholine [5]. These forerunner findings support airway pathophysiology (*i.e.* ASM) as a possible contributor to adverse respiratory function in growth-restricted individuals born with LBW, a proposal that has not been tested in human subjects.

The purpose of the present study was to assess in a human population whether LBW at term is associated with structural abnormalities of the ASM layer. Approval for this study was obtained from the institutional ethics committees of the participating centres and from the Sir Charles Gairdner Hospital Human Research Ethics Committee (HREC 2015-53). Tissues were derived from infants who died at birth ("term" ⩾37 weeks gestational age) with normal weight (2.51–3.99 kg, control; n=7) or LBW (1.50–2.50 kg; n=6). In view of the association between high birth weight (⩾4.0 kg) and risk of asthma [6], the upper limit for weight in the control group was 3.99 kg. Reported causes of death are listed verbatim from hospital and coroner files: still birth, meconium aspiration, asphyxia, osteogenesis imperfecta and pleural effusion.

Quantitative assessment of airway dimensions using histological sections has been previously described [7]. In airway cross sections, ASM layer thickness was calculated from the square root of the ASM area divided by the perimeter of basement membrane (P_{bm}, "airway size"), determined by planimetry. The number of ASM cells per airway length (N_L) was measured using the optical dissector approach and facilitated estimation of mean ASM cell volume (V_C). Proportions of ASM (V_{VASM}), extracellular matrix (V_{VECM}) and "other" (V_{VOTHER}; spaces between cells, blood and inflammatory constituents) within the ASM layer were measured by point counting. Data are presented as mean±SEM or median (interquartile range (IQR)), and unless otherwise stated, analysed by unpaired t-test or Mann-Whitney U-test. Data were analysed using SigmaPlot (version 13, Chicago, IL, USA), Prism (version 7, San Diego, CA, USA) and R software, with statistical significance defined as $p<0.05$.

The LBW group had a lower birth weight as per classification (control, 3.14±0.16 kg; LBW, 2.21±0.10 kg; $p<0.01$) with no difference in gestational age (control, 39.7±0.5 weeks; LBW, 38.3±0.4 weeks; $p=0.07$) compared with controls. There were four males and three females in the control group, and four males and two females in the LBW group ($p=0.73$, Fisher's exact test). The median (IQR) number of transverse airways section studied per case was 9 (6–13), which were averaged before group analysis. The P_{bm} was similar between groups (control, 1.22±0.13 mm; LBW, 1.30±0.13 mm; $p=0.67$), indicating no significant difference in airway size. There was also no difference in mean (IQR) ASM layer thickness (control, 0.10 (0.03); LBW, 0.10 (0.01); $p=0.45$) between groups.

However, N_L was increased ($p=0.03$; figure 1a) in the LBW group compared with controls. In figure 1b, the output of a linear mixed effects model is shown for all airways from all cases (subject identification is included as a random variable), demonstrating that N_L increases with P_{bm} ($p<0.01$), a relationship that is upward shifted in the LBW group ($p=0.02$). These data are consistent with an effect of LBW that

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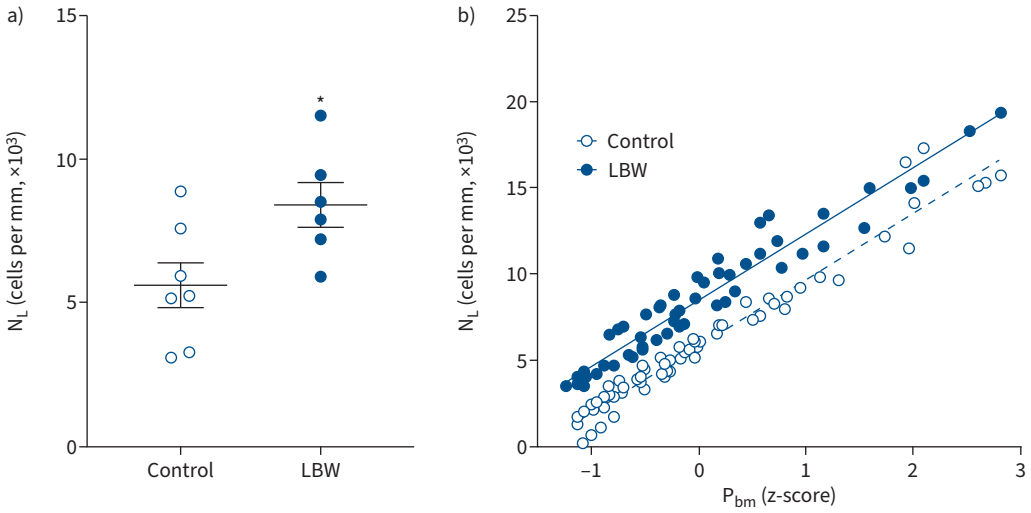

FIGURE 1 a) Number of airway smooth muscle (ASM) cells. Values are mean±SEM where each datapoint is a case mean of all airways examined for that subject. b) A linear mixed effects model (R software) examined the association between the number of ASM cells per airway length (N_L) and the fixed effects of airway size (perimeter of basement membrane (P_{bm})) and group (controls and low birth weight (LBW) group). Subject identification was also included as a random effect. Model output is shown demonstrating an increase in N_L with P_{bm} ($p<0.01$), a relationship that is upward shifted in the LBW group ($p=0.02$). Line of best-fit is included for visual acuity. *: $p<0.05$, significantly different between groups.

manifests globally rather than locally, and when comparing to previous data suggesting ASM growth during the perinatal period is due to cell hypertrophy [8], in the LBW infant there appears to be accelerated hyperplasia. There was no statistical effect of LBW on V_C (control, 2.38±0.13×10^5 µm^3; LBW, 1.83±0.32×10^5 µm^3; p=0.12). The V_{VECM} was increased (control, 11.8±0.9%; LBW, 16.1±1.6%; p=0.03) in the LBW group compared with controls, but there was no significant difference in V_{VASM} (control, 82.7±1.3%; LBW, 77.6±2.1%; p=0.06) and V_{VOTHER} between groups (control, 5.5±1.1%; LBW, 6.2±1.1%; p=0.68).

There is a need to consider the life-long impact of suboptimal fetal growth and lung development in the management of obstructive disease. Fetal size is inversely related to current wheeze and asthma at 5 years of age [9], while reduced lung function in infancy is associated with both asthma and COPD in adulthood [10, 11]. However, while LBW can be considered an additional characteristic of asthma, it is not amenable to the "treatable traits" approach but should not be overlooked when devising strategies to mitigate disease burden [12]. Notably, the 2023 Global Initiative for Chronic Obstructive Lung Disease guidelines now also acknowledge the importance of expanding COPD classification to non-smoking disorders, which includes abnormal lung development [13].

The present findings broaden our understanding of the relationship between IUGR and obstructive disease. Although gross changes in ASM thickness were not detected in the LBW group (present data) compared with controls, the composition of the ASM layer was found to be abnormal. In particular, there was a greater number of smooth muscle cells within the ASM layer (N_L), which is predicted to increase contractile capacity, bronchoconstriction and, in turn, the likelihood of an asthma diagnosis later in life. An increase in the number of smooth muscle cells within the ASM layer also distinguishes fatal cases of asthma from non-fatal cases [7]. Moreover, increased reactivity in infancy is associated with childhood asthma [14]. Our findings therefore provide a structural basis for previously observed relationships between LBW and asthma, and we postulate this manifests as a change in airway reactivity.

The disproportionate expansion of ECM in the LBW group compared with the control group is also worthy of discussion. Expansion of ECM proportion despite an increase in number of ASM cells can be explained by associated non-significant trends for a reduction in ASM cell size and reduced V_{VASM}. Changes in ECM protein composition within the ASM layer is reported in asthma and COPD [15, 16]. Airflow impairment following a change in ECM is potentially mediated by increased airway stiffness which would reduce airway lumen calibre for a given distending pressure. In our mouse model of IUGR,

ex vivo tracheal segments from offspring were stiffer in adulthood [4]. Alternatively, contractile capacity of ASM cells is dependent upon connections with surrounding ECM and may impact bronchoconstriction. Human ASM cells in culture are more contractile on stiff substrates and dependent on matrix protein composition, with greatest contraction when adhered to fibronectin [17]. In subjects with fixed airflow limitation (COPD), post-surgical bronchial segments exhibit greater airway narrowing to acetylcholine and a greater V_{VECM} within the ASM layer [18]. Changes to V_{VECM} in the LBW group are therefore again supportive of an increase in airway reactivity that drives airflow impairment.

The phenomena identified in this short communication should be interpreted with knowledge of the study limitations. Access to human airway tissue is difficult and there are potential variables that may affect outputs, including cause of death and maternal health [8]. Statistical power of this convenience sample is also low and restricts examination between sexes, a factor that impacts bronchoconstriction in IUGR-affected mice [5]. Regardless, these findings support the possibility that the airway wall is abnormal from the earliest stages of life and may contribute to a susceptibility for obstructive disease that presently escapes the attention of the treating physician.

In closing, we have shown that the ASM layer is vulnerable to disruption when it is still developing *in utero* and suggest that changes to ASM layer composition after LBW is evidence for a predisposition to future development of obstructive disease. Research towards elucidating the signalling pathways modifying ASM layer composition is recommended in order to develop treatments preventing abnormal ASM growth.

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