Early View

Original research article

Hyperoxic ventilatory response in infants is related to nocturnal hypoxemia

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Title: Hyperoxic ventilatory response in infants is related to nocturnal hypoxemia

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Summary: Hyperoxic ventilatory response is abnormal in infants <2 years with nocturnal hypoxemia. Hyperoxia response time is negatively associated with time spent with oxygen haemoglobin saturation <90% during sleep suggesting hyperactivity of their carotid bodies.
Abstract

**Background:** The carotid bodies primarily serve as oxaemia sensors that affect tidal breathing. Their function has not been studied in infants with nocturnal hypoxaemia yet. This cross-sectional study aimed to characterise the hyperoxic ventilatory response (HVR) in infants and its relationship to nocturnal hypoxaemia.

**Methods:** The HVR was analysed in term infants aged <24 months with childhood interstitial lung disease (chILD), those with severe recurrent wheezing (wheeze), and non-respiratory controls. The HVR timing was characterised using hyperoxia response time (HRT1) and HVR magnitude was characterised by the relative change in minute ventilation between normoxia and 30-s hyperoxia (VE_dH30). Time spent with an arterial haemoglobin oxygen saturation (SpO₂) <90% during overnight monitoring (t90) was estimated.

**Results:** HVR data were available for 23 infants with chILD, 24 with wheezing, and 14 control infants. A significant decrease in minute ventilation under 30 s of hyperoxia was observed in all patients. HRT1 was shorter in chILD (5.6±1.2 s) and wheeze (5.9±1.5 s) groups than in the controls (12.6±5.5 s) (ANOVA p-value <0.001). VE_dH30 was increased in the chILD group (24.3±8.0%) compared with that in the controls (14.7±9.2%), p=0.003. T90 was abnormal in the wheeze (8.0±5.0%) and chILD (32.7±25.8%) groups and higher in the chILD group than in the controls (p<0.001). HRT1 negatively correlated with t90 in all groups.

**Conclusion:** Significant differences in HVR timing and magnitude were noted in the chILD, wheeze, and control groups. A relationship between nocturnal hypoxaemia and HRT1 was proposed. HVR characterisation may help identify patients with abnormal nocturnal SpO₂.

**Keywords:** carotid bodies, control of breathing, multiple breath washout test, pulmonary function in infants, hyperoxia, oxygen saturation
Introduction

Carotid bodies are peripheral chemoreceptors that play an important role in control of breathing. They predominantly serve as oxaemia sensors, hypercapnaemia has additional stimulating effect. Decreased oxygen partial pressure in arterial blood (PaO$_2$) swiftly increases carotid body afferent signalling to the breath centre in the brainstem and raises minute ventilation within seconds [1]. Increased minute ventilation leads to normalisation in blood gases and activity of carotid bodies returns to the baseline level (feedback loop). Children with chronic respiratory disease may have this homeostatic mechanism distorted. Although the clinical implications are not fully understood, impaired carotid body function may lead to higher susceptibility to respiratory insufficiency. The baseline activity of carotid bodies may be assessed using Dejours test [2] or its later modifications [3], which quantify ventilation decline under hyperoxic conditions (100% oxygen).

Peripheral chemoreceptor function has been studied mainly in newborns when carotid body function is being ‘reset’ during the first few days of life [4]. Various factors, such as prematurity, hypoxaemia, postnatal exposure to hyperoxia, bronchopulmonary dysplasia (BPD), and chronic lung disease of immaturity (CLDI), potentially alter their function or delay their natural development [4–7], with possible roles in various pathological situations, such as sudden infant death syndrome (SIDS), obstructive sleep apnoea (OSA), or apnoea of prematurity [8–11]. However, no studies have reported on peripheral chemoreceptor activity in older patients with chronic respiratory disease and impaired oxygenation.

We hypothesised that the hyperoxic ventilatory response (HVR) is exaggerated in infants with nocturnal hypoxaemia and peripheral chemoreceptor activity may thus reflect long-term oxygenation. This observational study aimed to compare peripheral chemoreceptor activity during chloral hydrate-induced sleep among infants with normal awake arterial haemoglobin oxygen saturation (SpO$_2$), but suffering from nocturnal hypoxaemia of different severities.

Methods

Patients

Patients referred for infant pulmonary function testing (iPFT) to the Department of Paediatrics, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, were recruited in this study. Recruitment period was June 2017 to June 2022. The inclusion criteria were as follows: i) age <2 years, ii) full-term birth (i.e., completion of 37 gestational weeks), iii) detailed perinatal-history data availability, and iv) fulfilment of
the diagnostic criteria for one of the following patient groups: severe recurrent wheeze (wheeze), childhood interstitial lung disease (chILD), or non-respiratory control group. Severe recurrent wheezing was defined as at least three physician-documented episodes of wheezing with dyspnoea and bronchodilator treatment within the preceding 9 months. Infants aged <9 months required two such episodes in their lives for them to participate in this study. At least one episode required treatment with supplemental low-flow oxygen, but SpO2 normalised after treating the acute phase of infection. ChILD was diagnosed according to international standards [12, 13] using high-resolution chest computed tomography (CT). Patients with post-infectious bronchiolitis obliterans (PIBO) were also included. Infants without lower-airway or cardiac pathologies who had undergone the same procedural sedation for other purposes (echocardiography, detailed abdominal ultrasonography, and CT) were recruited as controls. A major cardiac defect involving a right-left shunt was an exclusion criterion, and the foramen ovale aperture was not a significant finding. The participants’ parents provided written informed consent for all study procedures and data analyses. This study was approved by the Institutional Ethics Committee (EK-576/14).

Measurements

All recruited infants underwent continuous overnight SpO2 monitoring at room air using a PalmSAT® 2500 (Nonin Medical Inc., Minnesota, USA) with a finger probe on the upper extremity. Monitoring was performed in inpatient settings before iPFT (maximum interval: 7 days). The minimum acceptable monitoring time was 8 h, and the time spent with a saturation <90% (t90%) was the outcome parameter. T90>5% was considered abnormal, as proposed by the American Thoracic Society (ATS) guidelines [14]. No patient in the study had been on long-term home oxygen therapy prior to study.

Perinatal history with respect to Apgar score, delayed postnatal adaptation, and supplemental oxygen or ventilatory support requirement (noninvasive/invasive) during the first 28 days was recorded. An abnormality rendered the perinatal history complicated. Supplemental oxygen requirements outside the neonatal period and any diagnostic or therapeutic procedures under general anaesthesia were retrieved from medical records.

Resting awake SpO2 at room air was measured in all infants before sedation for iPFT using the same pulse oximeter (PalmSAT® 2500). The ATS-proposed normal range was used [14]. Subsequently, all infants underwent at least three nitrogen multiple breath washout (N2-MBW) trials. Testing was performed according to international recommendations [15] in the supine position during chloralhydrate-induced sleep (80–100 mg/kg). An Exhlayzer D device
(Ecomedics, Duernten, Switzerland) was used. A face mask (Render-Barker No. 1 or 2; to keep dead space under 2 mL/kg body weight) was gently placed and sealed around the nose and mouth. All measurements were initiated ≥1 min after placing the facemask when the breathing pattern stabilised [16]. The children were free of acute respiratory infection for ≥3 weeks before testing and received their regular long-term treatment. Room temperature was maintained at 21–23 °C. The end-tidal expiratory CO$_2$ levels (etCO$_2$), SpO$_2$, and pulse rate were continuously monitored during testing and for 30 min thereafter. Data quality was extensively checked, with special attention paid to regular breathing pattern with a stable end-expiratory level and no leaks. The breath-to-breath coefficient of variation for tidal breath parameters was acceptable at <15%. The pre-washout (patient breathing room air) and washout (100% oxygen) periods lasted ≥30 s. Measurements that did not fulfil the abovementioned criteria were excluded. At least two trials of sufficient quality were required to analyse the HVR.

Acceptable raw N$_2$-MBW data were processed offline using custom-made software, enabling a detailed analysis of the tidal breathing parameters under normoxia (N) and the first 30 s of hyperoxia (H30). The outcome parameters included minute ventilation (VE$_N$, VE$_{H30}$), tidal volume (Vt$_N$, Vt$_{H30}$), respiratory rate (RR$_N$, RR$_{H30}$), and mean etCO$_2$ (etCO$_2$$_N$, etCO$_2$$_{H30}$). VE and Vt were normalised to body weight (VE/kg, Vt/kg), and RR was expressed as the z-score of the available norm [17]. The relative change in VE between the N and H30 period (VE$_{dH30}$) was calculated to quantify the magnitude of HVR. The hyperoxia response time 1 (HRT1) was the time from hyperoxia onset to a significant VE decrease (i.e., under the 5$^{th}$ percentile of normoxic values). Details of the software are provided in Appendix 1.

Statistics

Data are presented as the mean±standard deviation (SD) or median [interquartile range (IQR)], as appropriate. As there were no heavy outliers among the study participants, parametric test use was justified by their robustness against the violation of data normality in such circumstances [18]. Paired t-tests were used to compare tidal breath parameters (VE, Vt, RR, and etCO$_2$) under normoxia and 30 s of hyperoxia in the respective patient groups. Comparisons among patient groups were performed using analysis of variance (assumptions for its use were confirmed using Levene’s test). The Bonferroni test was used for subsequent multiple comparisons between mutual pairs of patient groups. The t-test was used to compare the wheeze peri 0 and wheeze peri + subgroups. The Pearson correlation coefficient (r) was used to evaluate the relationship between relevant outcome parameters. Sample size (one-
correlation t-test, one-tailed) was estimated to minimum of 15 patients (r=0.65 and power =0.85). Statistical analyses were performed using Statistica 14.0.0.15 (Tibco Software, Inc.). Statistical significance was set at P<0.05.

Results

Between January 2018 and October 2022, 26 infants with chILD, 29 with recurrent severe wheezing, and 17 controls were recruited. Data of sufficient quality for HVR analysis were available for 23 chILD cases, 24 wheeze cases, and 14 controls (study sample further analysed). All controls were born at term with normal birth weight and uncomplicated postnatal adaptation. Outside the neonatal period, they did not require supplemental oxygen, were not hospitalised for respiratory symptoms, and did not undergo any procedure under general anaesthesia. Patients in the chILD group were born at term, had normal birth weight, and had uncomplicated postnatal adaptation. The mean±SD age at respiratory symptom onset was 6.2±2.1 months. Symptoms leading to chILD suspicion and iPFT included persistent tachypnoea (65.2%), abnormal auscultatory findings (56.5%), retraction (52.2%), cough (47.8%), increased work of breathing (39.1%), and parent-reported breath sounds (13.0%). Failure to thrive was present in 26.1% of patients. Long-term home oxygen therapy was initiated in 52.1% of chILD patients after the initial workup (including study examinations). In these cases oxygen treatment was required during sleep only. A definitive diagnosis was established in 19 patients (neuroendocrine cell hyperplasia syndrome in 6 patients, PIBO in 5, post-aspiration chILD in 4, exogenous allergic alveolitis in 3, and surfactant protein C deficiency in 1). The wheeze group included normal-birth-weight term infants; 66.7% had uncomplicated postnatal adaptation (wheeze peri 0 subgroup), and 33.3% had a complicated history (wheeze peri + subgroup). The median (IQR) number of severe wheezing episodes requiring supplemental oxygen was two (1, 2). The asthma predictive index[19] was positive in 58.3% of patients. On testing, 4 infants had no antiasthma medication, 2 were administered leukotriene receptor antagonists only, 7 were treated with inhaled corticosteroids, and 11 with inhaled corticosteroids combined with long-acting beta-agonists. Long-term home oxygen therapy was initiated in no infant in wheeze group. The patient characteristics are shown in Table 1.

In normoxia, the chILD group had greater VE/kg and Vt/kg values than did the control and wheeze groups; RR [l/min], RR [z-score], and etCO2 did not differ among the groups (Table 2). Under 30 s of hyperoxia, VE/kg and Vt/kg significantly decreased in all groups, whereas both RR [l/min] and RR [z-score] decreased exclusively in the chILD and wheeze groups. No
statistically significant changes in etCO₂ were noted in any group during the first 30 s of hyperoxia. The data are summarised in Table 3 and Diagram 1. HRT1 was significantly reduced in both the chILD and wheeze groups compared with that in the controls; HRT1 did not differ between the chILD and wheeze groups (p=0.919). VE_dH30 was higher in the chILD group than in the control group (p=0.007). VE_dH30 did not differ between controls and patients with wheezing or between patients with chILD and those with wheezing (Table 4).

Awake SpO₂ was within the ATS-proposed normal range in all groups and did not vary among them. The mean t90 was in the ATS-proposed normal range, slightly increased, and markedly increased in the control, wheeze, and chILD groups, respectively. T90 in the chILD group was significantly higher than that in the control and wheeze groups and did not differ between the control and wheeze groups. Significant correlations were found between t90 and HRT1 in all three groups (control: r=−0.590, p=0.027; chILD: r=−0.793, p<0.001; wheeze: r=−0.726, p=0.002) but not between t90 and VE_dH30 (control: r=0.140, p=0.634; chILD: r=0.227, p=0.297; wheeze: r=−0.108, p=0.701) (Diagrams 2 and 3).

Within the wheeze group, VE_dH30 was significantly higher in the wheeze peri + subgroup than in the wheeze peri 0 subgroup (intergroup difference Δ=92.8 (29.7; 155.9), p=0.006). No other parameters (HRT1, VE_N/kg, Vt_N/kg, RR_N, RR_N z-score, t90, or awake SpO2) varied between the two groups.

**Discussion**

Our study demonstrates that minute ventilation per body weight decreases significantly after 30 s of hyperoxia in all study groups, thus revealing that an HVR occurs in both diseased and control infants aged <24 months. HVR timing and magnitude differences were noted in the study groups, and a possible relationship with overnight hypoxaemia was identified. Patients with chILD who had normal awake SpO₂ but suffered from severe nocturnal hypoxaemia at testing had shorter HRT1 and greater VE_dH30 than did the controls. This potentially indicates the baseline hyperactivity of peripheral chemoreceptors in infants with overnight hypoxaemia. Patients in the wheeze group had mild nocturnal hypoxaemia at testing (mean t90 outside the norm but not significantly higher than that of the controls) with normal awake SpO₂. These infants had a significantly shorter HRT1 than did the controls but similar VE_dH30. Interestingly, HRT1, but not VE_dH30, strongly and negatively correlated with t90 in all study groups.

This is one of the few studies on HVR in infants aged approximately 1 year. To date, the HVR has been studied mainly in term and preterm newborns. Hertzberg et al. [4]
demonstrated that the HVR is not present several hours postnatally but appears between the 2nd and 6th postnatal days in healthy-term newborns. The mean±SD VE decrease was 9.8±7.7% after 30 s of 100% hyperoxia, which was slightly less than that in our older control infants. HRT1 was not estimated in their study. In preterm infants, HVR emergence is slightly delayed. Katz-Salamon et al. revealed that up to 60% of very preterm infants with BPD still lacked a significant HVR by the 40th postconceptional week [7]. The magnitude of the HVR (VE_dH30) was negatively correlated with the time spent on a ventilator and closely related to BPD severity, suggesting a possible association of VE_dH30 with lung disease severity and past hypoxaemia. In another study, the same research group found that HVR developed in preterm infants who initially lacked it [5]. It appeared at a mean postnatal age of 14 weeks, and in severe CLDI cases even later. Bouferache et al. [3] studied babies born at 36.6 weeks free from neurological, cardiac, and respiratory symptoms at a postnatal age of 3 weeks. They reported an HRT1 of 13±4 s. After 30 s of 100% hyperoxia, a 15±7% VE decrease was observed. These data correspond to those of our controls.

Based on our results and those of previous studies, we speculate that once established, the HVR persists in infants throughout the first 24 months with similar characteristics (HRT1 and VE_dH30). This is consistent with recent findings by Freislich et al., who demonstrated that a HVR is present in extremely preterm infants at their postnatal age of 12–15 months [20]. However, this response may be blunted in up to 44% of cases. Singer et al. investigated the ventilatory response to N2-MBW in infants with cystic fibrosis and controls aged 3–57 weeks [21]. They reported significant Vt (–8.7%) and VE (–11.2%) changes under 100% hyperoxia. These findings are similar to ours, although the exact hyperoxia duration was not specified, rendering the comparison imprecise. Moreover, they did not yield any data on HVR timing (no HRT1 data). They also did not identify any HVR differences between controls and infants with cystic fibrosis. This contradicts our findings; however, the infants in their study might not have experienced nocturnal hypoxia. Whether HVR disappears later in life remains unclear. While Jost et al. found no systematic effect of 100% oxygen on the breathing pattern of young school-aged children during N2-MBW [22], Becker et al. reported VE increases under different hyperoxic levels [23, 24] and noted the attenuating effect of hypocapnia on VE increase. However, Becker used a longer exposure to hyperoxia than Jost.

Peripheral chemoreceptor function is involved in control of breathing and contributes to maintaining stable PaO2 under different external conditions (e.g., high altitude, exercise, and hypoxia) [1]. Abnormal peripheral chemoreceptor function has been proposed as one of the pathogenic mechanisms underlying apnoea of prematurity and SIDS[9, 11, 25, 26]. The carotid
bodies’ blurred response to hypoxaemia was speculated to leave infants unprotected from prolonged apnoea and hypoxaemia, possibly leading to death. Hyperactivity of peripheral chemoreceptor has also been detected in preterm infants with apnoea and ventilation instability. Cardot et al. found that the frequency of short apnoeic episodes in late preterm infants was related to the magnitude of the HVR (VE_dH30) [27]. Similarly, Nock et al. [28] found that preterm infants with a greater number of apnoeic episodes exhibited an increased HVR and speculated that repeated hypoxia during apnoea leads to peripheral chemoreceptor hyperactivity. With respect to these studies, our data suggest that infants with normal awake SpO2, but nocturnal hypoxaemia, may have increased peripheral chemoreceptor activity as a probable compensatory mechanism for impaired oxygenation. In the chILD group, which suffered from severe nocturnal hypoxaemia, the HVR differed from that of the controls, not only in timing (shorter HRT1) but also in magnitude (greater VE_dH30), whereas in the wheeze group with milder nocturnal hypoxaemia, only HVR timing differed from that of the controls. This suggests higher sensitivity of HRT1 to nocturnal hypoxaemia than that of VE_dH30.

Our study demonstrates that raw N2-MBW data may be used to derive HRT1, VE_dH30, and other parameters characterising the HVR using custom-made software and offline analyses. Because of the strong correlation between HRT1 and t90, such an analysis may help identify patients at risk of nocturnal hypoxaemia or latent respiratory insufficiency. This could significantly increase the clinical yield of iPFT; moreover, we propose incorporating our HVR analysis into the current N2-MBW software packages. HVR evaluation during iPFT potentially serves as a complementary examination to overnight pulse oximetry in different respiratory and non-respiratory conditions (sleep-disordered breathing, BPD, CLID, chILD, pulmonary hypertension, and cardiac defects) and does not bring any additional burden for the patient.

Our study has several technical limitations. First, controlling for all the factors that could affect the HVR was not possible. We did not monitor the sleep state, which affects breathing pattern and its reaction to external stimuli [29]. Instead, all infants were examined during chloralhydrate-induced sleep, which conforms to current iPFT practice. Chloralhydrate has been shown to be safe, with minimal impact on breathing pattern [30, 31] and sleep. Second, carbon dioxide levels were not kept constant during testing (poikilocapnic hyperoxia), thus potentially underestimating the HVR [23]. As etCO2 during normoxia neither varied across the groups nor significantly increased in any group under hyperoxia, it tended not to have a significant and differentiating impact on the observed HVR in the respective study groups. Third, the central chemoreceptors’ modifying effect on the HVR also could not be assessed in the present study. Notwithstanding, this study was not intended to investigate all HVR-related
physiological aspects but aimed to evaluate the HVR in clinically relevant iPFT settings and examine its relationship with overnight hypoxaemia. Perinatal history, such as prematurity, oxygen treatment during the first hours of life (‘resetting’ carotid bodies for higher oxiaemia), and various forms of ventilatory support, also affect peripheral chemoreceptor function. In the chILD and control groups, only infants with inapparent perinatal history were included; however, 33.3% of infants in the wheeze group received oxygen treatment or ventilatory support as neonates. Indeed, the wheeze peri + subgroup had significantly higher VE_dH30 values than did the wheeze peri 0 subgroup, suggesting that perinatal insults may affect the HVR even in older infants. Further research is required to address the effect of perinatal insults on later peripheral chemoreceptor function in detail.

In conclusion, an HVR was present in infants aged <24 months. Significant differences in HVR timing and magnitude were noted among the chILD, wheeze, and control groups. Furthermore, a possible association with nocturnal hypoxaemia was identified. We also demonstrated that VE_dH30 and HRT1 could be calculated using raw N2-MBW data. Characterising the HVR may potentially extend the clinical yield of iPFT, as HRT1 is strongly correlated with t90 and may help identify patients with overnight hypoxaemia.
Acknowledgements
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Conflict of interest declaration
Václav Koucký reports internal grant from the University Hospital Motol and grants from Ministry of Health, Czech Republic. Pavlína Koucká and Miroslav Koucký report no conflict of interest.

Data are available on request from the authors.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>chILD</th>
<th>Wheeze</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational week</td>
<td>39.5 ± 1.0</td>
<td>38.6 ± 0.9</td>
<td>38.9 ± 2.3</td>
<td>0.341</td>
</tr>
<tr>
<td>Birth weight [g]</td>
<td>3161.1 ± 463.6</td>
<td>3641.5 ± 403.4†</td>
<td>3153.8 ± 526.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight [z-score]</td>
<td>-0.54 ± 1.00</td>
<td>0.56 ± 1.14‡</td>
<td>-0.17 ± 0.82</td>
<td>0.004</td>
</tr>
<tr>
<td>Age at testing [weeks]</td>
<td>62.7 ± 32.3</td>
<td>48.8 ± 15.6</td>
<td>62.2 ± 28.3</td>
<td>0.282</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>9.7 ± 4.3</td>
<td>8.7 ± 1.4</td>
<td>10.3 ± 2.2</td>
<td>0.113</td>
</tr>
<tr>
<td>Weight [z-score]</td>
<td>-0.28 ± 1.39</td>
<td>-1.13 ± 0.82§</td>
<td>-0.23 ± 1.24</td>
<td>0.018</td>
</tr>
<tr>
<td>Length [cm]</td>
<td>74.9 ± 14.8</td>
<td>74.6 ± 4.6</td>
<td>76.8 ± 7.4</td>
<td>0.665</td>
</tr>
<tr>
<td>Length [z-score]</td>
<td>-0.60 ± 1.51</td>
<td>-0.24 ± 1.01</td>
<td>-0.38 ± 1.51</td>
<td>0.729</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>16.6 ± 2.8</td>
<td>15.5 ± 1.6§</td>
<td>17.3 ± 2.4</td>
<td>0.032</td>
</tr>
<tr>
<td>BMI [z-score]</td>
<td>0.15 ± 1.50</td>
<td>-1.05 ± 0.91§</td>
<td>0.16 ± 1.41</td>
<td>0.003</td>
</tr>
</tbody>
</table>

BMI, body mass index; ANOVA, analysis of variance

† Different from the control (p = 0.024) and wheeze (p = 0.002) groups
‡ Different from the control (p = 0.014) and wheeze (p = 0.040) groups
§ Different from the wheeze (p = 0.027) group
& Different from the wheeze (p = 0.027) group
$ Different from the control (p = 0.039) and wheeze (p = 0.005) groups
Table 2. Differences in normoxic ventilatory parameters among study groups

<table>
<thead>
<tr>
<th></th>
<th>Controls vs. chILD</th>
<th>Controls vs. Wheeze</th>
<th>Wheeze vs. chILD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANOVA difference</td>
<td>p-value‡</td>
<td>ANOVA difference</td>
</tr>
<tr>
<td>VE/kg [(mL/min)/kg]</td>
<td>&lt;0.001 -87.3 (-131.8; -42.8)</td>
<td>&lt;0.001 0.9 (-45.0; 43.2)</td>
<td>1.000 -86.4 (-124.7; -48.1)</td>
</tr>
<tr>
<td>Vt/kg [mL/kg]</td>
<td>0.010 -1.4 (-2.7; -0.2)</td>
<td>0.021 -0.3 (-1.6; 1.0)</td>
<td>1.000 -1.1 (-2.2; -0.1)</td>
</tr>
<tr>
<td>RR [/min]</td>
<td>0.087 -4.4 (-12.2; 3.4)</td>
<td>0.514 1.6 (-6.1; 9.4)</td>
<td>1.000 -6.0 (-12.7; 0.7)</td>
</tr>
<tr>
<td>RR z-score</td>
<td>0.080 -1.2 (-2.8; 0.5)</td>
<td>0.285 0.1 (-1.6; 1.8)</td>
<td>1.000 -1.3 (-2.7; 0.2)</td>
</tr>
<tr>
<td>etCO₂ [%]</td>
<td>0.431 0.2 (-0.2; 0.6)</td>
<td>0.681 0.1 (-0.3; 0.4)</td>
<td>1.000 0.1 (-0.4; 0.3)</td>
</tr>
</tbody>
</table>

Differences among groups are stated as the mean (95% confidence interval for the difference).

VE/kg, minute ventilation related to body weight; Vt/kg, tidal volume related to body weight; RR, respiratory rate; etCO₂, end-tidal expiratory carbon dioxide levels; ANOVA, analysis of variance.

‡ Subsequent mutual comparisons between groups were performed using the Bonferroni method.
Table 3: Ventilatory parameters under normoxia and 30 s of hyperoxia

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>chILD</th>
<th>Wheeze</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VE/kg [(mL/min)/kg]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>256.8 ± 39.6</td>
<td>344.1 ± 56.8</td>
<td>257.7 ± 56.3</td>
</tr>
<tr>
<td>H30</td>
<td>226.6 ± 50.3</td>
<td>259.1 ± 47.7</td>
<td>209.0 ± 52.5</td>
</tr>
<tr>
<td>Δ</td>
<td>-30.2 (-48.8; -11.6)</td>
<td>-85.0 (-98.6; -71.4)</td>
<td>-47.0 (-54.9; -39.0)</td>
</tr>
<tr>
<td>p</td>
<td>0.004</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Vt/kg [mL/kg]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8.2 ± 1.6</td>
<td>9.6 ± 1.8</td>
<td>8.5 ± 1.1</td>
</tr>
<tr>
<td>H30</td>
<td>7.4 ± 1.8</td>
<td>7.6 ± 1.6</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.7 (-1.3; -0.2)</td>
<td>-2.0 (-2.4; -1.6)</td>
<td>-1.3 (-1.6; -0.9)</td>
</tr>
<tr>
<td>p</td>
<td>0.011</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>RR [/min]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>33.0 ± 7.6</td>
<td>37.4 ± 9.6</td>
<td>31.2 ± 9.9</td>
</tr>
<tr>
<td>H30</td>
<td>32.4 ± 8.2</td>
<td>35.8 ± 9.7</td>
<td>30.1 ± 9.5</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.6 (-2.9; 1.7)</td>
<td>-1.6 (-2.9; -0.3)</td>
<td>-1.2 (-2.1; -0.3)</td>
</tr>
<tr>
<td>p</td>
<td>0.597</td>
<td>0.022</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>RR [z-score]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.82 ± 2.02</td>
<td>1.97 ± 2.13</td>
<td>0.73 ± 1.87</td>
</tr>
<tr>
<td>H30</td>
<td>0.68 ± 2.14</td>
<td>1.62 ± 2.22</td>
<td>0.46 ± 1.82</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.14 (-0.38; 0.67)</td>
<td>-0.35 (-0.65; -0.05)</td>
<td>-0.27 (-0.46; -0.07)</td>
</tr>
<tr>
<td>p</td>
<td>0.563</td>
<td>0.025</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>etCO₂ [%]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5.6 ± 0.5</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>H30</td>
<td>5.8 ± 0.7</td>
<td>5.4 ± 0.6</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>Δ</td>
<td>0.2 (0.0; 0.6)</td>
<td>0.0 (-0.2; 0.2)</td>
<td>0.1 (-0.2; 0.1)</td>
</tr>
<tr>
<td>p</td>
<td>0.063</td>
<td>0.866</td>
<td>0.245</td>
</tr>
</tbody>
</table>

N and H30 data are stated as the mean ± standard deviation; Δ is stated as the mean (95% confidence interval for the difference).

VE/kg, minute ventilation related to bodyweight; Vt/kg, tidal volume related to bodyweight; RR, respiratory rate; etCO₂, end-tidal expiratory carbon dioxide level; N, normoxia; H30, 30 s of hyperoxia; Δ, difference between N and H30; p, p-value for pair t-test comparing N and H30.
Table 4: Hyperoxic ventilatory response and haemoglobin oxygen saturation

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>chILD</th>
<th>Wheeze</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT1 [s]</td>
<td>12.6 ± 5.5</td>
<td>5.6 ± 1.2</td>
<td>5.9 ± 1.5</td>
<td>&lt; 0.001$^\dagger$</td>
</tr>
<tr>
<td>VE_dH30 [%]</td>
<td>14.7 ± 9.2</td>
<td>24.3 ± 8.0</td>
<td>18.6 ± 7.3</td>
<td>0.003$^&amp;$</td>
</tr>
<tr>
<td>Awake SpO2 [%]</td>
<td>97.2 ± 1.6</td>
<td>96.4 ± 1.6</td>
<td>98.0 ± 1.5</td>
<td>0.077</td>
</tr>
<tr>
<td>t90 [%]</td>
<td>2.5 ± 1.4</td>
<td>32.7 ± 25.8</td>
<td>8.0 ± 5.0</td>
<td>&lt; 0.001$^\dagger$</td>
</tr>
</tbody>
</table>

Data are stated as the mean ± standard deviation.

HRT1, hyperoxia response time; VE_dH30, relative change in VE between normoxia and 30 s of hyperoxia; SpO2, arterial haemoglobin oxygen saturation; t90, time spent with SpO2 < 90% during sleep; ANOVA, analysis of variance

$^\dagger$ Controls different from chILD: Δ = 7.0 (4.7; 9.4), p < 0.001, and from wheeze: Δ = 6.7 (4.5; 9.2), p < 0.001

$^\&$ Controls different only from chILD: Δ = 9.6 (2.8; 16.4), p = 0.003

$^\dagger$ chILD different from controls: Δ = 30.2 (15.6; 45.0), p < 0.001, and from wheeze: Δ = 24.7 (10.3; 39.1), p < 0.001

Δ, difference between respective groups; stated as the mean (95% confidence interval)
References:


Figure legend

Diagram 1: Tidal breath parameters under normoxia and 30 s hyperoxia
Caption: Boxes represent mean ± 1 standard deviation. VE/kg, minute ventilation related to bodyweight; Vt/kg, tidal volume related to bodyweight; RR z-score, z-score of respiratory rate; etCO₂, end-tidal expiratory carbon dioxide level; N, normoxia; H30, 30 s of hyperoxia; p-value for pair t-test comparing N and H30.

Diagram 2: Relationship between t₉₀ and HRT₁
Caption: t₉₀, the time spent with arterial haemoglobin oxygen saturation <90% in %; HRT₁, the hyperoxia response time 1 in seconds.

Diagram 3: Relationship between t₉₀ and VE_dH30
Caption: t₉₀, time spent with arterial haemoglobin oxygen saturation <90% in %; VE_dH30, the relative change in VE between the normoxia and 30 s hyperoxia period in %.
Diagram 1
Hyperoxic ventilatory response (HVR) analysis was performed using custom-made software that enabled a detailed offline analysis of tidal breath parameters under normoxia (20.91% oxygen) and hyperoxia (100% oxygen) and the calculation of parameters characterising the magnitude and timing of HVR.

The software was run in Microsoft Access 2016. It enables loading of nitrogen multiple breath washout (N2-MBW) raw data from the Exhalyzer D device (Ecomedics, Duernten, Switzerland) in the form of B-files. The B-files contain the following data: i) flow under BTPS conditions (body temperature, pressure, and water vapour saturation), ii) synchronised concentrations of O2 and carbon dioxide (CO2) as percentages, and iii) the calculated concentration of N2 (N2 = 100 – O2 – CO2), each sampled every 5 ms. The loaded data were stored in a database and processed as follows:

1. Individual breaths were identified in the data using a combination of threshold and smoothing algorithms for breath detection [1, 2]. Breaths could also be inspected by the operator, and those incorrectly identified were excluded from the analysis.
2. For each breath, the tidal volume (Vt), respiratory rate (RR = 60/breath duration), and minute ventilation (VE = Vt × RR) were calculated. The end-tidal expiratory CO2 concentration (etCO2) and end-tidal inspiratory O2 concentration (etiO2) were measured.
3. Normoxic (N) and hyperoxic (H) phases were identified. Hyperoxia onset was considered the first breath with an etiO2 > 95 %. This is because the residual air in the device’s dead space ‘diluted’ the 100% oxygen that had been inspired. Manual corrections were possible. As the washout phase (hyperoxia) might have had varying durations in different N2-MBW trials, a 30-s hyperoxic interval was introduced to standardise exposure to 100% oxygen.
This is consistent with the methods of most previous studies. Alternative approaches for defining breath parameters under hyperoxic conditions are available (see below).

4. The mean ± standard deviation (SD) of VT, RR, VE, and etCO₂ under normoxia and 30 s of hyperoxia were calculated. To allow comparisons among patients of different ages, VE and VT were related to body weight (VE/kg, VT/kg); RR was expressed as the z-score of the available norm [3] – RR z-s.

5. The hyperoxia response time 1 (HRT1) was calculated. It was defined as the time from HVR onset to the first breath with VE below the 5th percentile of normoxic VE values. The moving average method was used to account for possible breath-by-breath VE fluctuations. In cases where no decrease in VE occurred under the 5th percentile of normoxic values, no HRT1 was identified.

6. The decrease in minute ventilation under 30 s of hyperoxia (VE_dH30) was calculated as the ratio between VE_N and VE_H30 and expressed as a percentage. Alternatively, as proposed by Bouferrache et al. [4], minute ventilation at HRT1 (VE_HRT1_value) and its relative change compared with that under normoxia (VE_dHRT1) were also calculated.

7. The results of the individual trials were stored in a database and availed for export to Statistica 14.0.0.15 (Tibco Software Inc.) for further statistical analysis.

In the main text, all outcome parameters are reported as the mean of all trials from a single measurement session. Inter-individual variability, that is, variability within patient groups, was characterised using SD. To evaluate intra-individual variability (variability in the respective patient), we used the mean absolute standard deviation (AbsD) as it is more robust when a low number of repeated measurements is available (two or three measurements per patient). AbsD was calculated as follows:

\[
AbsD = \frac{1}{n} \sum_{i=1}^{n} |X_i - \overline{X}|,
\]

where n is the number of repeated measurements, \(X_i\) is the i\(^{th}\) measurement, \(\overline{X}\) is the mean of all the measurements, and \(|X|\) is the absolute value.

The intra- and inter-individual variabilities of HRT1, VE_dH30, and VE_dHRT1 are presented in eTable 1. Unlike Bouferache et al. [4], we did not find any differences in the intra-individual variability (AbsD) of VE_dH30 and VE_dHRT1 (p = 0.232 from a paired t-test for the entire patient sample).
### eTable 1 Intra- and inter-individual variability of HRT1, VE_dH30, and VE_dHRT1

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>chILD</th>
<th>Wheeze</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT1 [s]</td>
<td>12.6 ± 5.5</td>
<td>5.6 ± 1.2</td>
<td>5.9 ± 1.5</td>
</tr>
<tr>
<td>HRT1 Abs D (%)</td>
<td>1.58 (12.5)</td>
<td>0.86 (15.3)</td>
<td>0.84 (14.2)</td>
</tr>
<tr>
<td>VE_dH30</td>
<td>14.7 ± 9.2</td>
<td>24.3 ± 8.0</td>
<td>18.6 ± 7.3</td>
</tr>
<tr>
<td>VE_dH30 AbsD (%)</td>
<td>3.7 (25.1)</td>
<td>3.3 (13.5)</td>
<td>3.2 (17.2)</td>
</tr>
<tr>
<td>VE_dHRT1</td>
<td>15.9 ± 10.2</td>
<td>10.1 ± 3.9</td>
<td>13.2 ± 7.8</td>
</tr>
<tr>
<td>VE_dHRT1 AbsD v (%)</td>
<td>5.5 (34.5)</td>
<td>3.8 (37.6)</td>
<td>3.7 (28.0)</td>
</tr>
</tbody>
</table>

HRT1, hyperoxia response time in seconds (expressed as the mean ± standard deviation); HRT1 AbsD (%), mean absolute deviation of HRT1 (expressed as the group mean; related to the HRT1 value as a %, i.e., \( \frac{AbsD_{HRT1}}{HRT1} \times 100\% \)); VE_dH30, relative change in minute ventilation under 30 s of hyperoxia (expressed as the mean ± standard deviation); VE_dH30 AbsD (%), mean absolute deviation of VE_dH30 (expressed as the group mean; related to the VE_dH30 value as a %, i.e., \( \frac{AbsD_{VE_{dH30}}}{VE_{dH30}} \times 100\% \)); VE_dHRT1, relative change in minute ventilation at HRT1 (expressed as the mean ± standard deviation); VE_dHRT1 AbsD (%) , mean absolute deviation of VE_dHRT1 (expressed as the group mean; related to the VE_dHRT1 value in %, i.e., \( \frac{AbsD_{VE_{dHRT1}}}{VE_{dHRT1}} \times 100\% \)).