Hyperoxic ventilatory response in infants is related to nocturnal hypoxaemia

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Shareable abstract (@ERSpublications)
Hyperoxic ventilatory response is abnormal in infants <2 years with nocturnal hypoxaemia; hyperoxia response time is negatively associated with time spent with oxygen haemoglobin saturation <90% during sleep, suggesting hyperactivity of their carotid bodies https://bit.ly/3Qk3L2G

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Abstract

Background The carotid bodies primarily serve as oxaemia sensors that affect tidal breathing. Their function has not yet been studied in infants with nocturnal hypoxaemia. 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 nonrespiratory controls. The HVR timing was characterised using hyperoxia response time (HRT1), and HVR magnitude was characterised by the relative change in minute volume between normoxia and 30-s hyperoxia (V_E_dH30). Time spent with an arterial haemoglobin oxygen saturation (S_{pO_2}) <90% during overnight monitoring (t_{90}) was estimated.

Results HVR data were available for 23 infants with chILD, 24 wheeze and 14 control infants. A significant decrease in minute volume 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<0.001). V_E_dH30 was increased in the chILD group (24.3±8.0%) compared with that in the controls (14.7±9.2%) (p=0.003). t_{90} 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 t_{90} 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 S_{pO_2}.

Introduction

Carotid bodies are peripheral chemoreceptors that play an important role in control of breathing. They predominantly serve as oxaemia sensors; hypercapnia has an additional stimulating effect. Decreased oxygen partial pressure in arterial blood (P_{aO_2}) swiftly increases carotid body afferent signalling to the breath centre in the brainstem and raises minute volume (V_E) within seconds [1]. Increased V_E leads to normalisation in blood gases and activity of carotid bodies returns to the baseline level (feedback loop). This homeostatic mechanism may be distorted in children with chronic respiratory disease. 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 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 (S_{pO_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. The recruitment period was June 2017 to June 2022. The inclusion criteria were 1) age <2 years, 2) full-term birth (*i.e.* completion of 37 gestational weeks), 3) detailed perinatal-history data availability and 4) fulfilment of the diagnostic criteria for one of the following patient groups: severe recurrent wheezing (wheeze), childhood interstitial lung disease (chILD) or nonrespiratory 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 S_{pO_2} normalised after treating the acute phase of infection. chILD was diagnosed according to international standards [12, 13] using high-resolution chest computed tomography. 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 computed tomography) were recruited as controls. A major cardiac defect involving a right–left shunt was an exclusion criterion, and 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 S_{pO_2} monitoring at room air using a PalmSAT 2500 (Nonin Medical, MN, 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% (t_{90}) was the outcome parameter. t_{90} >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 S_{pO_2} 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 (N_2-MBW) trials. Testing was performed according to international recommendations [15] in the supine position during chloral hydrate-induced sleep (80–100 $mg \cdot kg^{-1}$). An Exhlayzer D device (Ecomedics, Duernten, Switzerland) was used. A face mask (Render-Barker No. 1 or 2; to keep dead space <2 $mL \cdot kg^{-1}$ bodyweight) 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 carbon dioxide concentration ($ETCO_2$), S_{pO_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 volume (V_E_N, V_E_H30), tidal volume (V_T_N, V_T_H30), respiratory rate (RR_N, RR_H30), and mean $ETCO_2$ ($ETCO_2$_N, $ETCO_2$_H30). V_E and V_T were normalised to body weight ($V_E \cdot kg^{-1}$, $V_T \cdot kg^{-1}$) and RR was expressed as the z-score of the available norm [17]. The relative change in V_E between the N and H30 period (V_E_dH30) was calculated to quantify the magnitude of HVR. The hyperoxia response time 1 (HRT1) was the time from hyperoxia onset to a significant V_E decrease (*i.e.* <5th percentile of normoxic values). Details of the software are provided in the supplementary material.

Statistics

Data are presented as the mean±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 (V_E, V_T, RR and $ETCO_2$) under normoxia and 30 s of hyperoxia in the respective patient groups. Comparisons among patient groups were performed using ANOVA (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 peri0 (*i.e.* perinatal history without complications) and wheeze peri+ (*i.e.* perinatal history with any complication) 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 be a minimum of 15 patients (r=0.65 and power=0.85). Statistical analyses were performed using Statistica 14.0.0.15 (Tibco Software). 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 birthweight 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 birthweight, 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 six patients, PIBO in five, post-aspiration chILD in four, exogenous allergic alveolitis in three and surfactant protein C deficiency in one). The wheeze group included normal-birthweight term infants; 66.7% had uncomplicated postnatal adaptation (wheeze peri0 subgroup) and 33.3% had a complicated history (wheeze peri+ subgroup). The median number of severe wheezing episodes requiring supplemental oxygen was two (IQR 1–2). The Asthma Predictive Index [19] was positive in 58.3% of patients. On testing, four infants had no antiasthma medication, two were administered leukotriene receptor antagonists only, seven were treated with inhaled corticosteroids and 11 with inhaled corticosteroids combined with long-acting β-agonists. Long-term home oxygen therapy was initiated in no infants in the wheeze group. The patient characteristics are shown in table 1.

In normoxia, the chILD group had greater $V_E \cdot kg^{-1}$ and $V_T \cdot kg^{-1}$ values than did the control and wheeze groups; RR (breaths·min^{-1}), RR (z-score) and $ETCO_2$ did not differ between the groups (table 2). Under 30 s of hyperoxia, $V_E \cdot kg^{-1}$ and $V_T \cdot kg^{-1}$ significantly decreased in all groups, whereas both RR (breaths·min^{-1}) and RR (z-score) decreased exclusively in the chILD and wheeze groups. No statistically significant changes in $ETCO_2$ were noted in any group during the first 30 s of hyperoxia. The data are summarised in table 3 and figure 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 ($p=0.919$). V_E_dH30 was higher in the chILD group than in the control group ($p=0.007$). V_E_dH30 did not differ between controls and patients with wheeze or between patients with chILD and those with wheeze (table 4).

TABLE 1 Patient characteristics

	Controls	chILD	Wheeze	ANOVA p-value
Gestational weeks	39.5±1.0	38.6±0.9	38.9±2.3	0.341
Birthweight g	3161.1±463.6	3641.5±403.4[#]	3153.8±526.8	0.001
Birthweight z-score	−0.54±1.00	0.56±1.14[¶]	−0.17±0.82	0.004
Age at testing weeks	62.7±32.3	48.8±15.6	62.2±28.3	0.282
Weight kg	9.7±4.3	8.7±1.4	10.3±2.2	0.113
Weight z-score	−0.28±1.39	−1.13±0.82[+]	−0.23±1.24	0.018
Length cm	74.9±14.8	74.6±4.6	76.8±7.4	0.665
Length z-score	−0.60±1.51	−0.24±1.01	−0.38±1.51	0.729
BMI kg·m^{-2}	16.6±2.8	15.5±1.6[§]	17.3±2.4	0.032
BMI z-score	0.15±1.50	−1.05±0.91[f]	0.16±1.41	0.003

Data are presented as mean±SD, unless otherwise stated. chILD: childhood interstitial lung disease; BMI: body mass index. [#]: 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; [f]: different from the control (p=0.039) and wheeze (p=0.005) groups.

Awake S_{pO_2} was within the ATS-proposed normal range in all groups and did not vary among them. The mean t_{90} was in the ATS-proposed normal range, slightly increased and markedly increased in the control, wheeze and chILD groups, respectively. t_{90} 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 t_{90} 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 t_{90} and V_E_dH30 (control: r=0.140, p=0.634; chILD: r=0.227, p=0.297; wheeze: r= –0.108, p=0.701) (figures 2 and 3).

Within the wheeze group, V_E_dH30 was significantly higher in the wheeze peri+ subgroup than in the wheeze peri0 subgroup (intergroup difference Δ=92.8 (29.7–155.9), p=0.006). No other parameters (HRT1, V_E_N·kg^{-1}, V_T_N·kg^{-1}, RR_N, RR_N z-score, t_{90} or awake S_{pO_2}) varied between the two groups.

Discussion

Our study demonstrates that minute volume 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 S_{pO_2}, but suffered from severe nocturnal hypoxaemia at testing had shorter HRT1 and greater V_E_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 t_{90} outside the norm, but not significantly higher than that of the controls) with normal awake S_{pO_2}. These infants had a significantly shorter HRT1 than did the controls but similar V_E_dH30. Interestingly, HRT1, but not V_E_dH30, strongly and negatively correlated with t_{90} in all study groups.

This is one of the few studies on HVR in infants aged ∼1 year. To date, the HVR has been studied mainly in term and preterm newborns. HERTZBERG and LAGERCRANTZ [4] demonstrated that the HVR is not present

TABLE 2 Differences in normoxic ventilatory parameters among study groups

	ANOVA	Controls *versus* chILD		Controls *versus* wheeze		Wheeze *versus* chILD	
		Difference	p-value[#]	Difference	p-value[#]	Difference	p-value[#]
V_E·kg^{-1} mL·min^{-1}·kg^{-1}	<0.001	−87.3 (−131.8– −42.8)	<0.001	0.9 (−45.0–43.2)	1.000	−86.4 (−124.7– −48.1)	<0.001
V_T·kg^{-1} mL·kg^{-1}	0.010	−1.4 (−2.7– −0.2)	0.021	−0.3 (−1.6–1.0)	1.000	−1.1 (−2.2– −0.1)	0.039
RR breaths·min^{-1}	0.087	−4.4 (−12.2–3.4)	0.514	1.6 (−6.1–9.4)	1.000	−6.0 (−12.7–0.7)	0.094
RR z-score	0.080	−1.2 (−2.8–0.5)	0.285	0.1 (−1.6–1.8)	1.000	−1.3 (−2.7–0.2)	0.109
$ETCO_2$ %	0.431	0.2 (−0.2–0.6)	0.681	0.1 (−0.3–0.4)	1.000	0.1 (−0.4–0.3)	1.000

Differences between groups are presented as mean (95% CI for the difference). chILD: childhood interstitial lung disease; V_E·kg^{-1}: minute volume related to body weight; V_T·kg^{-1}: tidal volume related to body weight; RR: respiratory rate; $ETCO_2$: end-tidal expiratory carbon dioxide concentration. [#]: subsequent mutual comparisons between groups were performed using the Bonferroni method.

TABLE 3 Ventilatory parameters under normoxia and 30 s of hyperoxia

	Controls	chILD	Wheeze
V_E·kg^{-1} mL·min^{-1} ·kg^{-1}			
N	256.8±39.6	344.1±56.8	257.7±56.3
H30	226.6±50.3	259.1±47.7	209.0±52.5
Δ	−30.2 (−48.8– −11.6)	−85.0 (−98.6– −71.4)	−47.0 (−54.9– −39.0)
p-value#	0.004	<0.001	<0.001
V_T·kg^{-1} mL·kg^{-1}			
N	8.2±1.6	9.6±1.8	8.5±1.1
H30	7.4±1.8	7.6±1.6	7.2±1.0
Δ	−0.7 (−1.3– −0.2)	−2.0 (−2.4– −1.6)	−1.3 (−1.6– −0.9)
p-value#	0.011	<0.001	<0.001
RR breaths·min^{-1}			
N	33.0±7.6	37.4±9.6	31.2±9.9
H30	32.4±8.2	35.8±9.7	30.1±9.5
Δ	−0.6 (−2.9–1.7)	−1.6 (−2.9– −0.3)	−1.2 (−2.1– −0.3)
p-value#	0.597	0.022	0.014
RR z-score			
N	0.82±2.02	1.97±2.13	0.73±1.87
H30	0.68±2.14	1.62±2.22	0.46±1.82
Δ	−0.14 (−0.38–0.67)	−0.35 (−0.65– −0.05)	−0.27 (−0.46– −0.07)
p-value#	0.563	0.025	0.009
$ETCO_2$ %			
N	5.6±0.5	5.4±0.4	5.5±0.5
H30	5.8±0.7	5.4±0.6	5.6±0.5
Δ	0.2 (0.0–0.6)	0.0 (−0.2–0.2)	0.1 (−0.2–0.1)
p-value#	0.063	0.866	0.245

Data are presented as mean±SD or mean (95% CI for the difference), unless otherwise stated. chILD: childhood interstitial lung disease; V_E·kg^{-1}: minute volume related to bodyweight; N: normoxia; H30: 30 s of hyperoxia; Δ: difference between N and H30; V_T·kg^{-1}: tidal volume related to bodyweight; RR: respiratory rate; $ETCO_2$: end-tidal expiratory carbon dioxide concentration. #: for paired t-test comparing N and H30.

several hours postnatally, but appears between the second and sixth postnatal day in healthy term newborns. The mean±SD V_E 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*. [7] revealed that up to 60% of very preterm infants with BPD still lacked a significant HVR by the 40th post-conceptional week. The magnitude of the HVR (V_E_dH30) was negatively correlated with the time spent on a ventilator and closely related to BPD severity, suggesting a possible association of V_E_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. Bouferrache *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% V_E 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 V_E_dH30). This is consistent with recent findings by Freislich *et al*. [20], who demonstrated that a HVR is present in extremely preterm infants at their postnatal age of 12–15 months. However, this response may be blunted in up to 44% of cases. Singer *et al*. [21] investigated the ventilatory response to N_2-MBW in infants with cystic fibrosis and controls aged 3–57 weeks. They reported significant V_T (–8.7%) and V_E (–11.2%) changes under 100% hyperoxia. These findings are similar to ours, although the exact duration of hyperoxia 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*. [22] found no systematic effect of 100% oxygen on the breathing pattern of young school-aged children during N_2-MBW, Becker *et al*. [23, 24] reported V_E increases under different hyperoxic levels and noted the attenuating effect of hypocapnia on V_E increase. However, Becker *et al*. [23, 24] used a longer exposure to hyperoxia than Jost *et al*. [22].

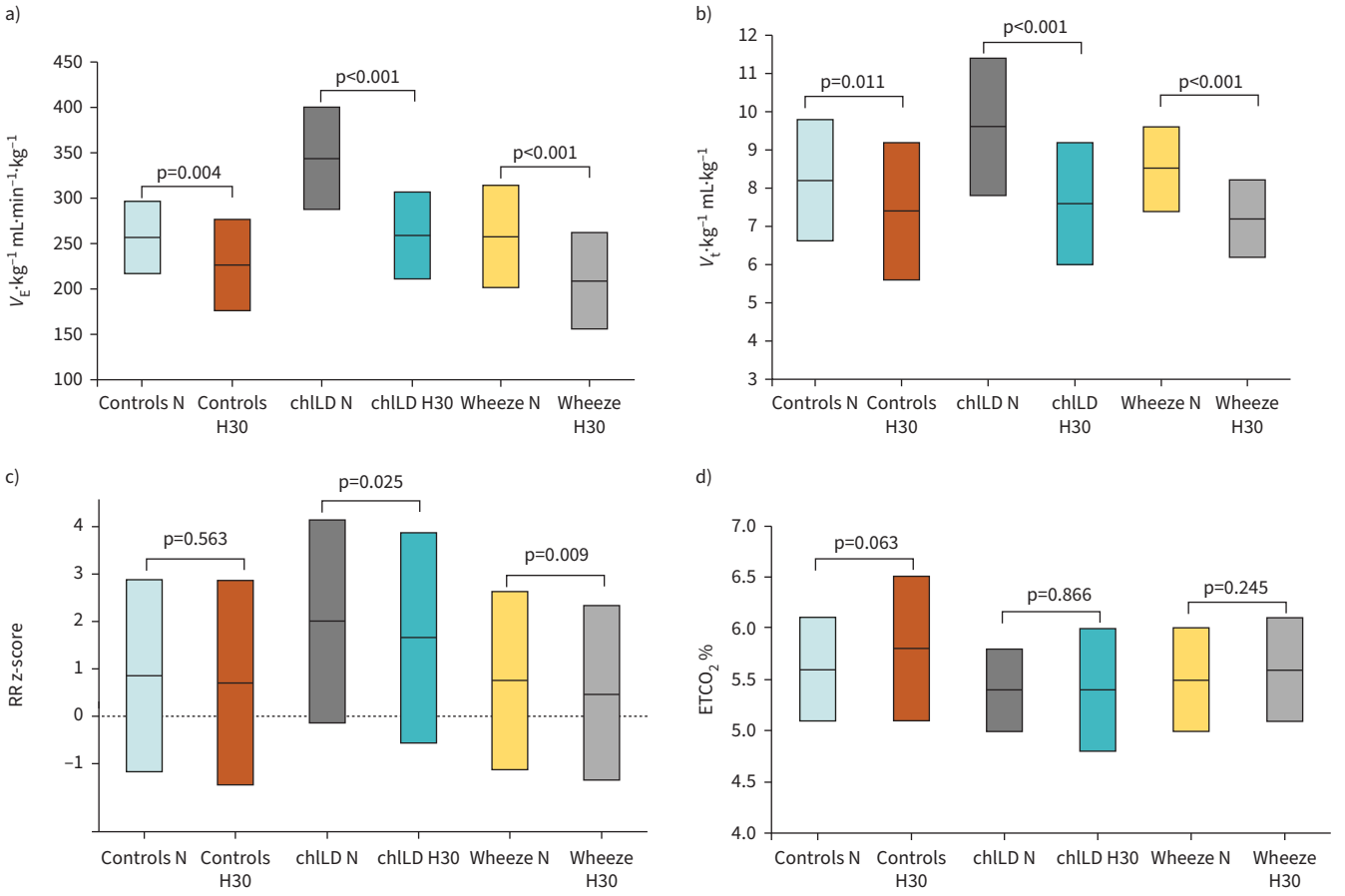

FIGURE 1 Tidal breath parameters under normoxia (N) and 30 s hyperoxia (H30). Boxes represent mean±1×SD. V_E·kg^{-1}: minute volume related to bodyweight; V_T·kg^{-1}: tidal volume related to bodyweight; RR: respiratory rate; $ETCO_2$: end-tidal expiratory carbon dioxide concentration; chILD: childhood interstitial lung disease. p-values are shown for paired t-tests comparing N and H30.

Peripheral chemoreceptor function is involved in control of breathing and contributes to maintaining stable P_{aO_2} 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.* [27] found that the frequency of short apnoeic episodes in late preterm

TABLE 4 Hyperoxic ventilatory response and haemoglobin oxygen saturation

	Controls	chILD	Wheeze	ANOVA p-value
HRT1 s	12.6±5.5	5.6±1.2	5.9±1.5	<0.001[#]
V_E_dH30 %	14.7±9.2	24.3±8.0	18.6±7.3	0.003[¶]
Awake S_{pO_2} %	97.2±1.6	96.4±1.6	98.0±1.5	0.077
t_{90} %	2.5±1.4	32.7±25.8	8.0±5.0	<0.001[+]

Data are presented as mean±SD, unless otherwise stated. chILD: childhood interstitial lung disease; HRT1: hyperoxia response time; V_E_dH30: relative change in minute volume between normoxia and 30 s of hyperoxia; S_{pO_2}: arterial haemoglobin oxygen saturation; t_{90}: time spent with S_{pO_2} <90% during sleep; Δ: difference between respective groups, stated as the mean (95% CI). [#]: 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; [+]: 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.

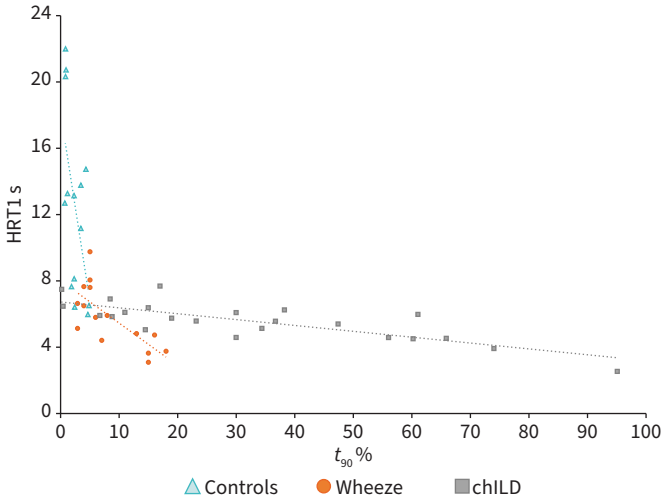

FIGURE 2 Relationship between the time spent with arterial haemoglobin oxygen saturation <90% (t_{90}) and the hyperoxia response time 1 (HRT1). chILD: childhood interstitial lung disease.

infants was related to the magnitude of the HVR (V_E_dH30). 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 S_{pO_2}, 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 V_E_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 V_E_dH30.

Our study demonstrates that raw N_2-MBW data may be used to derive HRT1, V_E_dH30 and other parameters characterising the HVR using custom-made software and offline analyses. Because of the strong correlation between HRT1 and t_{90}, 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 N_2-MBW software packages. HVR

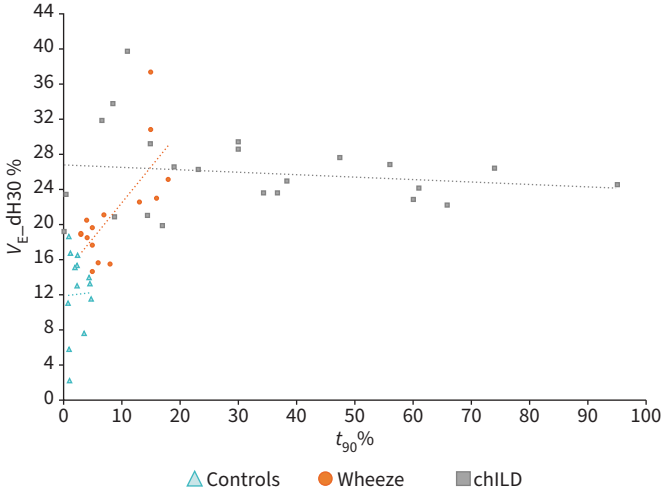

FIGURE 3 Relationship between time spent with arterial haemoglobin oxygen saturation <90% (t_{90}) and the relative change in minute volume between normoxia and the 30 s hyperoxia period (V_E_dH30). chILD: childhood interstitial lung disease.

evaluation during iPFT potentially serves as a complementary examination to overnight pulse oximetry in different respiratory and nonrespiratory conditions (sleep disordered breathing, BPD, CLDI, 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 chloral hydrate-induced sleep, which conforms to current iPFT practice. Chloral hydrate 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 $ETCO_2$ 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 oxaemia) 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 V_E_dH30 values than did the wheeze peri0 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 V_E_dH30 and HRT1 could be calculated using raw N_2-MBW data. Characterising the HVR may potentially extend the clinical yield of iPFT, as HRT1 is strongly correlated with t_{90} and may help identify patients with overnight hypoxaemia.

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References

1 Prabhakar NR, Peng Y-J. Peripheral chemoreceptors in health and disease. *J Appl Physiol* 2004; 96: 359–366.
2 Dejours P. Chemoreflexes in breathing. *Physiol Rev* 1962; 42: 335–358.
3 Bouferrache B, Filtchev S, Leke A, *et al.* The hyperoxic test in infants reinvestigated. *Am J Respir Crit Care Med* 2000; 161: 160–165.
4 Hertzberg T, Lagercrantz H. Postnatal sensitivity of the peripheral chemoreceptors in newborn infants. *Arch Dis Child* 1987; 62: 1238–1241.
5 Katz-Salamon M, Eriksson M, Jónsson B. Development of peripheral chemoreceptor function in infants with chronic lung disease and initially lacking hyperoxic response. *Arch Dis Child Fetal Neonatal Ed* 1996; 75: F4–F9.
6 Katz-Salamon M, Lagercrantz H. Hypoxic ventilatory defence in very preterm infants: attenuation after long term oxygen treatment. *Arch Dis Child Fetal Neonatal Ed* 1994; 70: F90–F95.

7 Katz-Salamon M, Jonsson B, Lagercrantz H. Blunted peripheral chemoreceptor response to hyperoxia in a group of infants with bronchopulmonary dysplasia. *Pediatr Pulmonol* 1995; 20: 101–106.
8 Garvey JF, Taylor CT, McNicholas WT. Cardiovascular disease in obstructive sleep apnoea syndrome: the role of intermittent hypoxia and inflammation. *Eur Respir J* 2009; 33: 1195–1205.
9 MacFarlane PM, Ribeiro AP, Martin RJ. Carotid chemoreceptor development and neonatal apnea. *Respir Physiol Neurobiol* 2013; 185: 170–176.
10 Mateika JH, Ellythy M. Chemoreflex control of ventilation is altered during wakefulness in humans with OSA. *Respir Physiol Neurobiol* 2003; 138: 45–57.
11 Porzionato A, Macchi V, De Caro R. Central and peripheral chemoreceptors in sudden infant death syndrome. *J Physiol* 2018; 596: 3007–3019.
12 Kurland G, Deterding RR, Hagood JS, *et al.* An official American Thoracic Society clinical practice guideline: classification, evaluation, and management of childhood interstitial lung disease in infancy. *Am J Respir Crit Care Med* 2013; 188: 376–394.
13 Bush A, Cunningham S, de Blic J, *et al.* European protocols for the diagnosis and initial treatment of interstitial lung disease in children. *Thorax* 2015; 70: 1078–1084.
15 Robinson PD, Latzin P, Verbanck S, *et al.* Consensus statement for inert gas washout measurement using multiple- and single-breath tests. *Eur Respir J* 2013; 41: 507–522.
16 Fleming PJ, Levine MR, Goncalves A. Changes in respiratory pattern resulting from the use of a facemask to record respiration in newborn infants. *Pediatr Res* 1982; 16: 1031–1034.
17 Nguyen TTD, Hoo AF, Lum S, *et al.* New reference equations to improve interpretation of infant lung function. *Pediatr Pulmonol* 2013; 48: 370–380.
18 Rasch D, Teuscher F, Guiard V. How robust are tests for two independent samples? *J Stat Plan Infer* 2007; 137: 2706–2720.
19 Castro-Rodríguez JA, Holberg CJ, Wright AL, *et al.* A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000; 162: 1403–1406.
20 Freislich Z, Stoecklin B, Hemy N, *et al.* The ventilatory response to hypoxia is blunted in some preterm infants during the second year of life. *Front Pediatr* 2022; 10: 974643.
21 Singer F, Yammine S, Schmidt A, *et al.* Ventilatory response to nitrogen multiple-breath washout in infants. *Pediatr Pulmonol* 2014; 49: 342–347.
22 Jost K, Lenherr N, Singer F, *et al.* Changes in breathing pattern upon 100% oxygen in children at early school age. *Respir Physiol Neurobiol* 2016; 228: 9–15.
23 Becker H, Polo O, McNamara SG, *et al.* Ventilatory response to isocapnic hyperoxia. *J Appl Physiol* 1995; 78: 696–701.
24 Becker HF, Polo O, McNamara SG, *et al.* Effect of different levels of hyperoxia on breathing in healthy subjects. *J Appl Physiol* 1996; 81: 1683–1690.
25 Porzionato A, Macchi V, Stecco C, *et al.* The carotid body in sudden infant death syndrome. *Respir Physiol Neurobiol* 2013; 185: 194–201.
26 Gauda EB, McLemore GL, Tolosa J, *et al.* Maturation of peripheral arterial chemoreceptors in relation to neonatal apnoea. *Semin Neonatol* 2004; 9: 181–194.
27 Cardot V, Chardon K, Tourneux P, *et al.* Ventilatory response to a hyperoxic test is related to the frequency of short apneic episodes in late preterm neonates. *Pediatr Res* 2007; 62: 591–596.
28 Nock ML, DiFiore JM, Arko MK, *et al.* Relationship of the ventilatory response to hypoxia with neonatal apnea in preterm infants. *J Pediatr* 2004; 144: 291–295.
29 Gaultier C. Cardiorespiratory adaptation during sleep in infants and children. *Pediatr Pulmonol* 1995; 19: 105–117.
30 Lees MH, Olsen GD, McGilliard KL, *et al.* Chloral hydrate and the carbon dioxide chemoreceptor response: a study of puppies and infants. *Pediatrics* 1982; 70: 447–450.
31 Ren CL, Robinson P, Ranganathan S. Chloral hydrate sedation for infant pulmonary function testing. *Pediatr Pulmonol* 2014; 49: 1251–1252.