The Journal of Physiology

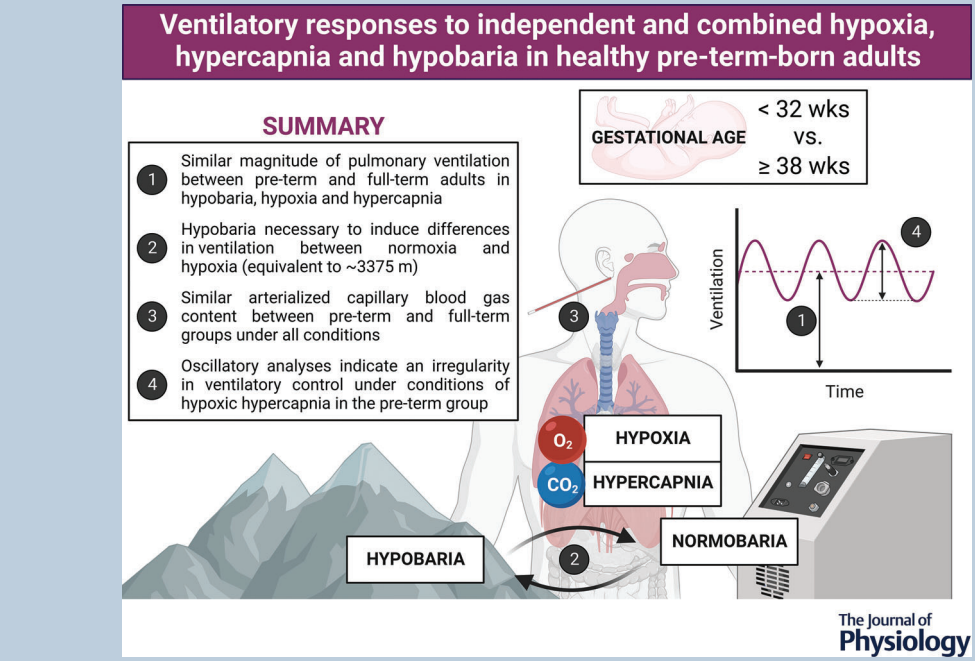
Ventilatory responses to independent and combined hypoxia, hypercapnia and hypobaria in healthy pre-term-born adults

Benjamin J. Narang[1,2], Giorgio Manferdelli[3], Nicolas Bourdillon[3], Grégoire P. Millet[3] and Tadej Debevec[1,2]

[1]*Department of Automatics, Biocybernetics and Robotics, Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*Faculty of Sport, University of Ljubljana, Ljubljana, Slovenia*
[3]*Institute of Sport Sciences, University of Lausanne, Lausanne, Switzerland*

Handling Editors: Harold Schultz & Mike Stembridge

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP285300#support-information-section).



Abstract Pre-term birth is associated with physiological sequelae that persist into adulthood. In particular, modulated ventilatory responsiveness to hypoxia and hypercapnia has been observed in this population. Whether pre-term birth *per se* causes these effects remains unclear. Therefore, we aimed to assess pulmonary ventilation and blood gases under various environmental conditions, comparing 17 healthy prematurely born individuals (mean ± SD; gestational age, 28 ± 2 weeks; age, 21 ± 4 years; peak oxygen uptake, 48.1 ± 11.2 ml kg^{-1} min^{-1}) with 16 well-matched adults born at term (gestational age, 40 ± 1 weeks; age, 22 ± 2 years; peak oxygen uptake, 51.2 ± 7.7 ml kg^{-1} min^{-1}). Participants were exposed to seven combinations of hypoxia/hypobaria (equivalent to ~3375 m) and/or hypercapnia (3% CO_2), at rest for 6 min. Pulmonary ventilation, pulse oxygen saturation

B. J. Narang and G. Manferdelli have joint first authorship.
G. P. Millet and T. Debevec have joint last authorship.

 DOI: 10.1113/JP285300

and the arterial partial pressures of O_2 and CO_2 were similar in pre-term and full-term individuals under all conditions. Higher ventilation in hypoxia compared to normoxia was only observed at terrestrial altitude, despite an equivalent (normobaric) hypoxic stimulus administered at sea level (0.138 F_{IO_2}). Assessment of oscillations in key variables revealed that combined hypoxic hypercapnia induced greater underlying fluctuations in ventilation in pre-term individuals only. In general, higher pulse oxygen saturation fluctuations were observed with hypoxia, and lower fluctuations in end-tidal CO_2 with hypercapnia, despite similar ventilatory oscillations observed between conditions. These findings suggest that healthy prematurely born adults display similar overall ventilation to their term-born counterparts under various environmental stressors, but that combined ventilatory stimuli could induce an irregular underlying ventilatory pattern. Moreover, barometric pressure may be an important factor when assessing ventilatory responsiveness to moderate hypoxic stimuli.

(Received 14 July 2023; accepted after revision 18 September 2023; first published online 6 October 2023)
Corresponding author B. J. Narang: Department of Automatics, Biocybernetics and Robotics, Jožef Stefan Institute, Jamova Cesta 39, 1000 Ljubljana, Slovenia. Email: benjamin.narang@ijs.si

Abstract figure legend The long-term physiological effect of pre-term birth is a growing area of research. In particular, existing evidence shows unique ventilatory responses to various environmental stimuli in prematurely born adults, suggesting that pre-term birth modulates chemosensitivity. In this study, a group of young, healthy and physically active prematurely born adults were recruited and intentionally well-matched to a term-born group of control participants. The two groups were exposed to seven different combinations of hypoxia, hypercapnia and hypobaria across testing visits conducted near sea level (295 m) and at altitude (3375 m). Frequency-domain analysis of relevant cardiorespiratory data suggested that combined hypoxic hypercapnia elicited a ventilatory instability in the pre-term group. However, the magnitude of the ventilatory responses to all conditions, and the corresponding arterialized capillary partial pressures of oxygen or carbon dioxide were similar between groups. This study indicates that a combination of advancements in neonatal treatment strategies and the health/fitness status of individuals within this population are critical factors underpinning the manifestation of long-term physiological sequelae. Created with BioRender.com.

Key points

- Evidence exists for unique pulmonary and respiratory function under hypoxic conditions in adult survivors of pre-term birth. Whether pre-term birth *per se* causes these differences requires a comparison of conventionally healthy prematurely born adults with an appropriately matched sample of term-born individuals.
- According to the present data, there is no difference between healthy pre-term and well-matched term-born individuals in the magnitude of pulmonary ventilation or arterial blood gases during independent and combined hypobaria, hypoxia and hypercapnia.
- Terrestrial altitude (hypobaria) was necessary to induce differences in ventilation between normoxia and a hypoxic stimulus equivalent to ∼3375 m of altitude. Furthermore, peak power in pulse oxygen saturation was similar between hypobaric normoxia and normobaric hypoxia.
- The observed similarities between groups suggest that ventilatory regulation under various environmental stimuli is not impaired by pre-term birth *per se*. Instead, an integrated combination of neonatal treatment strategies and cardiorespiratory fitness/disease status might underlie previously observed chemosensitivity impairments.

Introduction

Pre-term birth, defined as any birth occurring prior to the 37th week of gestation, results in long-term physiological effects that persist beyond maturity (Crump, 2020). Specifically, prematurely born adults demonstrate differences in respiratory (Näsänen-Gilmore et al., 2018), cardiac (Schuermans & Lewandowski, 2022) and vascular (Barnard et al., 2020) function, which appear to manifest as exercise capacity reductions (Duke et al., 2022) and augmented chronic disease risk (Crump, 2020). Given that the human body is required to adapt to external perturbations to maintain homeostasis, it is important to identify whether pre-term birth might

influence the physiological responses to environmental stimuli.

One such stimulus is hypoxia: a decrease in the partial pressure of inspired oxygen (P_{IO_2}) typically induced by increasing altitude. An increasing number of individuals live at high altitude or travel to these regions recreationally, so a comprehensive understanding of hypoxic physiology is becoming increasingly important. Hypoxic exposure elicits several integrated effects to attenuate the reduction in the arterial partial pressure of O_2 (Bärtsch & Saltin, 2008). A transient increase in pulmonary ventilation typically occurs to mitigate reductions in alveolar O_2 pressure, and thus preserve the alveolar-to-arterial O_2 pressure gradient. Given that hypoxaemia is associated with altitude-induced pathophysiology (Hohenhaus et al., 1995; Richalet et al., 2012), an appropriate hypoxic ventilatory response is considered fundamental to healthy altitude acclimatization.

Research investigating the effect of pre-term birth on the resting hypoxic ventilatory response is equivocal (Narang et al., 2022). Blunted ventilatory responses to hypoxic stimuli have been observed in healthy prematurely born adults (Bates et al., 2014; Debevec et al., 2019), although paediatric data suggest that bronchopulmonary dysplasia is a necessary precursor of these effects (Calder et al., 1994; Katz-Salamon et al., 1995). A likely cause may be peripheral chemoreceptor dysfunction induced by neonatal hyperoxic treatment in these individuals (Bates et al., 2013). However, given the wide methodological variety in hypoxic ventilatory response investigations (Duffin, 2007), and the biphasic nature of the phenomenon (Teppema & Dahan, 2010), a more in-depth assessment in pre-term individuals seems warranted. In addition, there is growing evidence to suggest that normobaric and hypobaric hypoxia induce distinct physiological responses (Millet & Debevec, 2020). In particular, 40 min of hypobaric hypoxia appeared to stimulate a higher respiratory frequency and lower tidal volume than normobaric hypoxia when matched for P_{IO_2} (Savourey et al., 2003). These differences were accompanied by greater hypoxaemia and hypocapnia in hypobaric hypoxia. In another study, lower minute ventilation ($\dot{V}_E$) was observed in hypobaric relative to normobaric hypoxia across a more prolonged 10 h exposure (Loeppky et al., 1997). Together, these differential responses suggest that barometric pressure *per se* could mediate the physiological responses to hypoxia and, given the practicality of acclimatizing individuals to altitude using simulated (normobaric) hypoxia (Fulco et al., 2013; Millet et al., 2013), it is important to understand whether gas density modifies the observed responses in pre-term individuals.

In addition to the ventilatory response to hypoxia, hypercapnia is also known to stimulate chemoreceptors to increase ventilation. Mechanistically, the ventilatory response to hypercapnia primarily originates in central chemoreceptors, whereas the hypoxic ventilatory response is triggered by carotid body (peripheral) chemoreceptors (Duffin, 2011). An appropriate balance between these two afferents is necessary to maintain blood gas homeostasis. The only study to have assessed the hypercapnic ventilatory response in prematurely born (but healthy) adults observed similar $\dot{V}_E$ in response to 3% CO_2, but a higher $\dot{V}_E$ in response to a 6% CO_2 stimulus relative to a well-matched full-term control group (Manferdelli et al., 2021). Further research is, however, necessary to corroborate these findings, and to assess whether pre-term individuals may be more (or less) sensitive to combined hypoxia and hypercapnia.

In addition to the *magnitude* of the ventilatory response to various stimuli, ventilatory oscillations can also be quantified as an indication of the ventilatory response *pattern*. For example, periodic breathing may be identified through the conversion of ventilatory data to the frequency domain (Hermand et al., 2015). Periodic breathing is the cyclic result of a transient hypopnea/apnoea causing an increase in arterial CO_2, which is compensated for by a brief period of increased ventilation. This causes a decrease in arterial CO_2, and the cycle is then restarted with a short apnoea. Periodic fluctuations in pulmonary ventilation indicate an instability in the feedback sent to the respiratory control system, and cause arterial and alveolar partial pressures of O_2 and CO_2 to fluctuate beyond their typical steady-state levels. Given the association of periodic breathing with clinical outcomes (Mortara et al., 1997), and external stimuli

Benjamin J. Narang graduated from the University of Bath, UK, with an MSci in Sport and Exercise Science in 2020. During his undergraduate degree he completed an exercise physiology internship at the Western Australian Institute of Sport, and subsequently returned to Bath to lead research projects in exercise metabolism and data analysis methods. He is now completing his PhD at the University of Ljubljana, Slovenia, investigating the interactive effects of pre-term birth, exercise and hypoxia. **Giorgio Manferdelli** received his undergraduate degree and Master of Science from the University of Milan, and his Master of Research at the University of the West of Scotland under the supervision of Dr Simone Porcelli and Dr Chris Easton. He is currently a PhD candidate in Life Sciences at the University of Lausanne under the supervision of Professor Grégoire P. Millet. His research interests include the cardiovascular and cerebrovascular responses to exercise and environmental stress.

including exercise and hypoxia (Hermand et al., 2015), they could be pertinent to the long-term physiological sequelae associated with premature birth.

Overall, evidence assessing the ventilatory responses to variations in environmental stimuli in prematurely born individuals is necessary, and the independent and combined effects of hypoxia, hypercapnia and hypobaria are yet to be thoroughly assessed. Moreover, ventilatory oscillations have not been investigated in this population, but could reveal some mechanisms by which the respiratory system responds to these stimuli. The primary aim of this study, therefore, was to quantify the magnitude and pattern of the ventilatory responses to hypoxia, hypercapnia and hypobaria, independently and together, in pre-term adults compared to a term-born control group. Pre-term individuals were hypothesized to demonstrate blunted ventilatory responses, and a greater peak power of ventilation (indicative of greater fluctuations), under each condition. A secondary aim of this study was to explore the effects of each environmental condition *per se*, as well as their integrated effects, to better characterize the interactive ventilatory effects of hypobaric, hypoxic and hypercapnic stimuli.

Methods

Participants and ethical approval

Seventeen prematurely born and 17 term-born male adults, matched for age, anthropometric characteristics and exercise capacity, were initially recruited for this study. Participants were eligible for the pre-term group if their gestational age and mass were <32 weeks and <1500 g, respectively, and if they had received neonatal supplemental oxygen therapy. Control group participants were born after a minimum of 38 weeks of gestation and with a minimum 2500 g mass at birth. Potential pre-term candidates were identified using the Slovenian National Pre-term Register and received personal postal invitations containing information about the planned study. Participants for the control group were recruited using online advertisements. None of the participants had been a habitual smoker in the previous 5 years, and all were free from any haematological and cardiorespiratory diseases which could influence the study outcomes, including bronchopulmonary dysplasia. The study was pre-registered at ClinicalTrials.gov (NCT04739904), and ethical approval was obtained from the ethics committees at the University of Ljubljana Faculty of Sport (8/2020-316) and the Aosta Hospital (06/05/2021.0038781.I). Written informed consent was provided by all participants prior to any testing procedures. All procedures were subsequently conducted in accordance with the ethical principles outlined in the *Declaration of Helsinki*. Participant characteristics are presented in Table 1.

Study design

The present study consisted of a baseline testing phase followed by three experimental testing phases during which participants were exposed to seven environmental conditions. These conditions included various combinations of hypoxia, hypercapnia and hypobaria as described below. Participants were asked to avoid altitude exposure >2000 m for at least 2 months prior to each laboratory visit. In addition, they avoided caffeine and alcohol ingestion and intense exercise for 24 h prior to each testing session. All testing sessions were carried out at the same time of day (between 09.00 and 11.00 h)

Baseline testing

Prior to the experimental trials, all participants underwent a series of baseline tests. These included tests of lung function and diffusion capacity, a routine blood sample analysis, an acute hypoxic ventilatory response test and an incremental exercise test to exhaustion. Details of the latter test, which was used to determine maximal aerobic capacity in this cohort, are provided elsewhere (Manferdelli et al., 2023).

Baseline lung function was assessed using standard spirometry procedures (Miller et al., 2005) with a portable pneumotachograph (Cardiovit AT-2plus, Schiller, Baar, Switzerland). The forced vital capacity test was performed until three successful trials were complete, with any trials influenced by insufficient effort, insufficient exhalation duration or hesitation/coughing repeated. Forced vital capacity, forced expiratory volume in 1 s and peak expiratory flow rate were extracted from the resulting maximal flow volume loop. The variables from the highest forced vital capacity result were taken for the analysis. Predicted lung function was calculated using standardized equations (Quanjer et al., 2012). Participants also performed a maximal hyperventilation test during which they were asked to 'breath maximally by increasing both the rate and depth of breathing for 12 s'. This test was performed twice in a seated position, and the highest recorded result taken to represent maximal voluntary ventilation.

Diffusion capacity for carbon monoxide was also measured using standardized procedures (MacIntyre et al., 2005), and predicted values were calculated using the most recent Global Lung Initiative reference equations (Stanojevic et al., 2017). Participants were seated, and instructed to take a few regular breaths before exhaling maximally. They then inhaled a gas mixture containing 0.3% carbon monoxide and a trace amount of methane

Table 1. Participant characteristics

	Pre-term (n = 17)	Term born (n = 16)
Gestational age (weeks)	28 ± 2	40 ± 1*
Gestational mass (g)	1132 ± 265	3653 ± 421*
Age (years)	21 ± 4	22 ± 2
Height (cm)	178 ± 9	182 ± 6
Body mass (kg)	72.4 ± 14.4	75.4 ± 7.1
Body mass index (kg m^{-2})	22.5 ± 2.7	22.8 ± 1.9
Peak oxygen uptake (ml kg^{-1} min^{-1})	48.1 ± 11.2	51.2 ± 7.7
Maximal aerobic power (W kg^{-1})	3.6 ± 0.7	3.9 ± 0.6
Haemoglobin (g l^{-1})	154 ± 18	151 ± 6
Haematocrit (%)	45 ± 4	44 ± 2
Normobaric normoxia (295 m above sea level)		
Forced vital capacity (l)	5.4 ± 0.9 (98 ± 10)	5.6 ± 0.7 (97 ± 10)
Forced expiratory volume in 1 s (l)	4.2 ± 0.6 (91 ± 9)	4.6 ± 0.7 (94 ± 13)
Peak expiratory flow rate (l min^{-1})	8.8 ± 1.4	8.6 ± 1.5
Maximum voluntary ventilation (l min^{-1})	180 ± 28	194 ± 33
Lung CO diffusion capacity (mmol kPa^{-1} min^{-1})	11.1 ± 2.5 (103 ± 17) §	11.9 ± 1.1 (104 ± 11) §
Alveolar volume (l)	6.5 ± 1.0 (102 ± 17) §	7.0 ± 0.7 (104 ± 6) §
Hypobaric hypoxia (3375 m above sea level)		
Forced vital capacity (l)	5.4 ± 0.8	5.6 ± 0.7
Forced expiratory volume in 1 s (l)	4.3 ± 0.6	4.7 ± 0.6
Peak expiratory flow rate (l min^{-1})	10.0 ± 1.5	10.0 ± 2.0
Maximum voluntary ventilation (l min^{-1})	190 ± 38	225 ± 34§*

All values are means ± SD. Data presented in brackets are expressed as the percentage of the predicted values based on participant characteristics. Reference values for spirometry and lung diffusion capacity variables were calculated using prediction equations from Quanjer et al. (2012) and Stanojevic et al. (2017), respectively. The exercise protocol used to determine peak oxygen uptake and maximal aerobic power in this cohort has been published elsewhere (Manferdelli et al., 2023).
§n = 15. *P < 0.05 *vs.* Pre-term. CO, carbon monoxide.

as deeply as possible, held their breath for 10 s, and then again exhaled fully (for at least 4 s). Measurements were considered acceptable if the differences in inspiratory vital capacity and alveolar volume between consecutive tests were ≤150 ml and ≤300 ml, respectively, and the breath hold duration was between 9 and 11 s.

The acute hypoxic ventilatory response was quantified using a nitrogen chemosensitivity test. Participants were seated in a comfortable chair and were instructed to stay relaxed and breath normally until they were informed that the test had been completed. A pulse oximeter (Xpod 3012LP, Nonin Medical Inc., Plymouth, MN, USA) was placed on the left earlobe, and participants breathed through a two-way T-shape non-rebreathing valve (2700 series, Hans Rudolph, Kansas City, MO, USA) connected to a securely and comfortably fitted low dead-space facemask (7400 oronasal series, Hans Rudolph). A three-way T-shape manual directional control valve (2100 series, Hans Rudolph) was used to transition between ambient air and a bag containing pure nitrogen out of sight of the participants. Throughout the test, $\dot{V}_E$ and pulse oxygen saturation (S_{pO_2}) were recorded breath-by-breath using a metabolic cart (Ergocard Professional, Medisoft, Sorinnes, Belgium). After a 3-min baseline period breathing ambient air, the participants were transiently exposed to the pure nitrogen source on 10 occasions. Each exposure lasted between one and eight consecutive breaths, to induce a wide range of S_{pO_2} reductions (∼99% to 54%), in a randomized order and in a single-blinded fashion. Each transient exposure was interspersed with a minimum 2 min period of breathing ambient air to allow S_{pO_2} and $\dot{V}_E$ to return to baseline levels. The peak $\dot{V}_E$ value induced by each nitrogen exposure was plotted against the minimum S_{pO_2} value from the corresponding exposure. The gradient of the linear regression line through these points was used to quantify the acute hypoxic ventilatory response for each participant. If the values for a specific pure nitrogen exposure appeared to deviate considerably from the general trend of the other plotted data, this particular exposure (number of breaths) was repeated before the test was terminated.

Experimental testing sessions

Three experimental testing sessions were conducted to assess the participants' responses to various combinations of hypoxia, hypercapnia and hypobaria. Each session

Table 2. Summary of the seven conditions under which pulmonary ventilation, pulse oxygen saturation and arterial blood gases were assessed.

Laboratory visit	Location (Altitude)	Barometric pressure (mmHg)	Condition	Composition of inspired gas (%)		Measured partial pressure (mmHg)	
				O_2	CO_2	O_2	CO_2
Visit 1	Ljubljana (295 m)	738 ± 2	Nb_Nx	Ambient air		152.7 ± 0.6	0.9 ± 0.2
			Nb_Nx+CO_2	20.94	3.00	156.8 ± 0.9	17.9 ± 1.9
Visit 2	Rifugio Torino (3375 m)	503 ± 3	Hb_Hx	Ambient air		103.5 ± 1.0	0.6 ± 0.3
			Hb_Nx	32.00	0.03	149.7 ± 5.2	0.4 ± 0.4
			Hb_Nx+CO_2	32.00	3.00	151.9 ± 3.7	13.7 ± 0.9
Visit 3[§]	Ljubljana (295 m)	742 ± 3	Nb_Hx	13.80	0.03	109.2 ± 11.3	0.5 ± 0.4
			Nb_Hx+CO_2	13.80	3.00	106.9 ± 9.7	19.8 ± 5.5

Gas compositions were calculated *a priori* using the expected difference in atmospheric pressure between the two locations based on their altitude. Composition of inspired gas (%) presents the value reported on the gas bottles used during testing. Measured partial pressure values were calculated using the fraction of inspired gases measured by the metabolic cart and the barometric pressure recorded during each pre-test calibration. Values are means ± SD ($n = 33$).
[§] Reduced sample size ($n = 28$) due to participant availability for Visit 3. Hb, hypobaria; Hx, hypoxia; Nb, normobaria; Nx, normoxia.

was preceded by a minimum 2-month washout period. Visit 1 and Visit 3 were both performed in Ljubljana, Slovenia (295 m above sea level), and therefore included the normobaric conditions. Visit 2 was conducted at the Rifugio Torino mountain hut (3375 m above sea level) on the Mont Blanc massif, and included all hypobaric conditions. For Visit 2, participants were driven for ∼7 h from Ljubljana, Slovenia (295 m above sea level) to Courmayeur, Italy (1300 m above sea level), before ascending to Rifugio Torino via a 20-min cable car journey. Participants subsequently spent the night living at altitude before completing the hypobaric conditions listed below the following morning. The amount of time spent at Rifugio Torino prior to the commencement of the experimental protocol in Visit 2 was 21.3 ± 0.2 h (range 20.9–21.8 h). Additionally, 60 to 90 min prior to this experimental protocol, participants repeated the spirometry testing protocol described above.

Experimental protocol

Participant preparation for these tests was similar to that described above for the nitrogen chemosensitivity test. Briefly, participants were seated in a comfortable chair and instructed to remain relaxed and breath normally. A three-way valve was used to transition participants between the conditions described below in a single-blinded manner. Each exposure lasted 6 min. Participants were again fitted with the low dead-space facemask and had a pulse oximeter attached to their earlobe. An arterialized capillary blood sample was collected from the other earlobe in the final 30 s of each exposure, and analysed using an arterial blood gas analyser (ABL-90 FLEX, Radiometer, Copenhagen, Denmark) for determination of arterialized capillary O_2 (P_{aO_2}) and CO_2 (P_{aCO_2}). Ventilatory variables and S_{pO_2} were recorded breath-by-breath using a metabolic cart.

On Visit 1, participants completed the normobaric normoxic normocapnic (ambient air; Nb_Nx) and normobaric normoxic hypercapnic (20.94% O_2, 3.00% CO_2; Nb_Nx+CO_2) conditions. On Visit 2, the hypobaric hypoxic normocapnic (ambient air; Hb_Hx), hypobaric normoxic normocapnic (32.00% O_2, 0.03% CO_2; Hb_Nx) and hypobaric normoxic hypercapnic (32.00% O_2, 3.00% CO_2; Hb_Nx+CO_2) conditions were completed. Finally, Visit 3 included the normobaric hypoxic normocapnic (13.80% O_2, 0.03% CO_2; Nb_Hx) and normobaric hypoxic hypercapnic (13.80% O_2, 3.00% CO_2; Nb_Hx+CO_2) conditions. The O_2 concentrations were determined *a priori* by calculating the F_{iO_2} required to induce an equivalent P_{iO_2} at 3375 m above sea level (Visit 2) and at 295 m above sea level (Visit 1 and Visit 3). A direct quantification of all experimental conditions is presented in Table 2.

Data processing

Data exported from the metabolic cart were initially processed in Microsoft Excel. An arithmetic mean from the final 60 s of each exposure was calculated from the breath-by-breath data to quantify response magnitude. Variables included in this analysis were $\dot{V}_E$, tidal volume, respiratory frequency and S_{pO_2}. Ventilatory oscillations were subsequently identified independently for each participant and condition using established methods (Hermand et al., 2019). The following steps were applied to $\dot{V}_E$, S_{pO_2} and P_{ETCO_2} data. First, the raw breath-by-breath data from each 6 min data trace were spline-interpolated

using cubic polynomial functions, and resampled to a 1 Hz sampling frequency, to ensure a consistent sampling rate for further analysis. The data corresponding to the final 3 min of each exposure were extracted, and a third order high-pass Butterworth filter, with a cut-off frequency of 0.02 Hz, was then applied to remove low-frequency artefacts from the signal. The resulting data were subsequently divided into three 90-s 'windows', each corresponding to half of the extracted signal with a 50% overlap. Welch's power spectral density estimate was then calculated for each window. The power spectral density estimates from the three windows were then averaged to provide a smoother and more reliable representation across the frequency range. The frequency at which the largest peak occurred was considered to represent the oscillatory 'period', when converted back to the time domain and expressed in seconds. The area under the curve at this peak, ±0.02 Hz on the frequency axis (Hermand et al., 2019), was considered to represent the power of this peak and is expressed in l^2 min^{-2}, $\%^2$ and $mmHg^2$ for $\dot{V}_E$, S_{pO_2} and P_{ETCO_2}, respectively. Figure 1 shows some representative examples of these data processing steps. All data processing for oscillatory outcomes was performed using MatLab (R2019b, The MathWorks, Natick, MA, USA).

Statistical analysis

The primary outcome in the present study was defined as the resting hypoxic ventilatory response, and an appropriate sample size was therefore determined *a priori* using this outcome and the G*Power statistical power analysis tool (version 3.1.9.4, Düsseldorf, Germany). As per this analysis, 13 participants were required per group to detect a previously observed effect (Cohen's *d*) of 1.18 (Debevec et al., 2019), with independent groups, a two-tailed α-value of 0.05 and a minimum statistical power of 0.80.

All statistical analyses were conducted using R Studio (R Version 4.2.1, posit, Boston, MA, USA). Values are presented as means ± SD in all cases. Univariate outliers, defined as values three interquartile ranges lower or higher than the first and third quartiles, were identified using boxplot methods and removed from their corresponding comparisons. As a result of this approach, the data for one full-term participant were removed entirely, resulting in $n = 17$ and $n = 16$ for pre-term and control, respectively. Data resulting from the oscillatory analyses (period and peak power for $\dot{V}_E$, S_{pO_2} and P_{ETCO_2}) were highly positively skewed and were thus (natural) log transformed prior to inferential analyses. Differences between groups and conditions, and potential interaction effects, were assessed using two-way mixed-effects ANOVA (group [preterm, term born] × condition [Nb_Nx, Nb_Hx, Nb_Nx+CO_2, Nb_Hx+CO_2, Hb_Nx, Hb_Hx, Hb_Hx+CO_2]), irrespective of minor deviations from normality in the underlying data (Maxwell & Delaney, 1990). Significant effects were subsequently investigated using the Bonferroni *P*-value adjustment. In all cases, differences were considered statistically significant if $P < 0.05$.

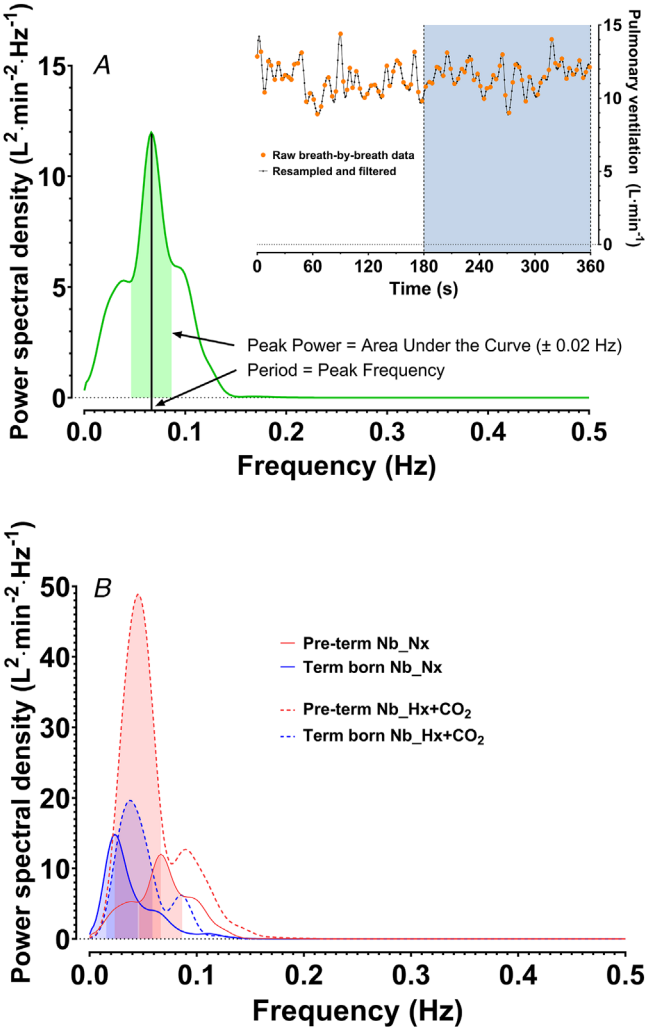


Figure 1. Example of the spectral analysis conducted for detection of oscillations in the breath-by-breath signals
A, the power spectral density curve for a representative trial, with the solid black line indicating the location of the peak, and the shaded pale green area representing the peak power (area under the curve ± 0.02 Hz). Inset shows the raw breath-by-breath data trace (orange circles) for the given participant in normobaric normoxic normocapnia. The black line is the processed data trace, after resampling (1 Hz spline interpolation) and filtering (3rd order high-pass Butterworth filter; cut-off frequency 0.02 Hz). The shaded blue area represents the 3 min window extracted for subsequent analyses. *B*, four representative power spectral density curves, representing a pre-term (red) and a term born (blue) participant, each in normobaric normoxic normocapnia (Nb_Nx; continuous line) and normobaric hypoxic hypercapnia (Nb_Hx+CO_2; dashed line).

Results

Baseline testing

The results of all pulmonary function tests are presented in Table 1. No differences were observed between groups in any spirometry variables at sea level, whether expressed in absolute or relative terms. Peak expiratory flow rate ($P < 0.001$) and maximum voluntary ventilation ($P < 0.001$) were both higher at altitude. A significant interaction effect was also observed ($P = 0.021$), whereby maximum voluntary ventilation was significantly lower in pre-term than full-term participants at altitude ($P = 0.036$), with no difference having been observed at sea level ($P = 0.888$). Absolute and relative diffusion capacity measurements did not differ between groups. No significant difference ($P = 0.083$) was observed between pre-term (0.383 ± 0.250 l min^{-1} %$^{-1}$) and term-born (0.260 ± 0.117 l min^{-1} %$^{-1}$) participants in the acute hypoxic ventilatory response. The fit quality (R^2) of the linear regression between $\Delta\dot{V}_E$ and ΔS_{pO_2} was 0.77 ± 0.19.

Pulmonary ventilation

The effects of condition and group on pulmonary ventilation are displayed in Fig. 2. There was neither a statistically significant group × condition interaction effect ($F(3.14, 78.62) = 0.558$; $P = 0.652$), nor a significant main effect of group ($P = 0.904$). A significant main effect of condition was, however, apparent ($P < 0.001$). *Post hoc* analyses revealed no difference in ventilation across the three hypercapnic conditions (all $P > 0.999$), although all hypercapnic conditions induced a higher ventilation than all normocapnic conditions (all $P < 0.001$). Ventilation in Nb_Nx (11.5 ± 2.0 l min^{-1}) was similar to both Hb_Nx (12.2 ± 1.6 l min^{-1}; $P > 0.999$) and Nb_Hx (11.0 ± 1.4 l min^{-1}; $P > 0.999$), whereas ventilation in Hb_Hx (13.2 ± 1.5 l min^{-1}) *was* significantly greater than Hb_Nx ($P < 0.001$).

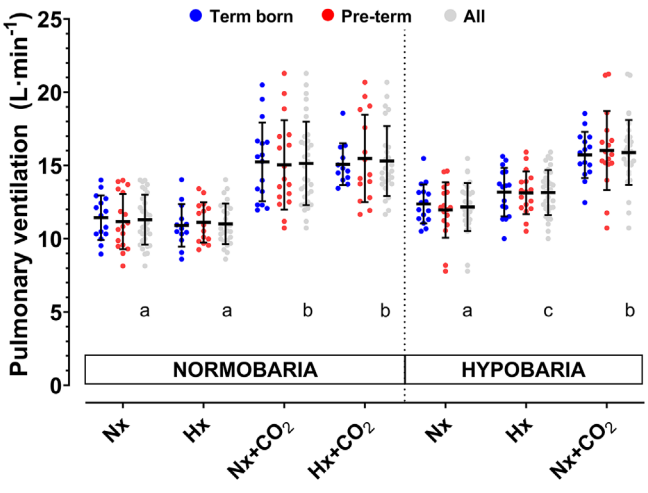


Figure 2. Pulmonary ventilation under each of the seven environmental conditions, comprising normobaria, hypobaria, normoxia (Nx), hypoxia (Hx), normocapnia and hypercapnia (+CO_2)
Values represent individual data points, superimposed by group means with error bars indicating standard deviation. Data displayed for term-born participants (blue), pre-term participants (red) and both groups combined (ALL; grey). Data were analysed using two-way ANOVA, with Bonferroni *post hoc P*-value adjustments applied to investigate significant main or interaction effects. Pairwise comparisons which did *not* differ significantly ($P \geq 0.05$) are indicated by matching letters.

Tidal volume and respiratory frequency data are displayed in Fig. 3*A* and *B*, respectively. In line with the overall ventilation data, there was neither a group × condition interaction effect in tidal volume ($F(3.54, 77.96) = 0.453$; $P = 0.747$) nor in respiratory frequency ($F(2.93, 73.16) = 0.693$; $P = 0.556$), and again no main effect of group ($P = 0.624$ and $P = 0.686$, respectively). Main effects of condition in both tidal volume ($P < 0.001$) and respiratory frequency ($P = 0.002$) were, however, observed. The significant differences observed in ventilation could solely be attributed to changes in respiratory frequency in only two comparisons: Nb_Hx *vs.* Hb_Nx ($P = 0.034$) and Nb_Hx *vs.* Hb_Hx ($P = 0.030$). The differences in ventilation observed between Nb_Hx and Hb_Nx+CO_2 were attributable to both higher respiratory frequency ($P = 0.009$) and higher tidal volume ($P < 0.001$). All other significant differences observed in ventilation were solely attributable to differences in tidal volume ($P < 0.001$ for all remaining comparisons).

Pulse oxygen saturation

There was neither a group × condition interaction effect ($F(2.69, 59.1) = 1.202$; $P = 0.315$), nor a main effect of group ($P = 0.127$), in S_{pO_2}. A main effect of condition was, however, observed ($P < 0.001$). Regarding key comparisons, S_{pO_2} values in Hb_Hx ($92.0 \pm 2.1\%$) and Nb_Hx ($92.3 \pm 2.4\%$) were statistically similar ($P > 0.999$), as were those in Nb_Nx ($99.2 \pm 0.5\%$) and Hb_Nx ($99.0 \pm 0.6\%$) ($P > 0.999$), and Nb_Nx+CO_2 ($99.7 \pm 0.4\%$) and Hb_Nx+CO_2 ($99.5 \pm 0.5\%$) ($P > 0.999$). Specific descriptive and inferential statistical comparisons for S_{pO_2} are presented in Table 3.

Arterialized blood gases

The effects of condition and group on arterialized blood gases are displayed in Fig. 4. There was neither a statistically significant interaction between the effects of group and condition on P_{aO_2} ($F(3.81, 61.03) = 1.628$;

$P = 0.181$), nor a significant main effect of group ($P = 0.289$). A significant main effect of condition was, however, apparent ($P < 0.001$). Similarly, neither an interaction effect ($F(3.19, 50.98) = 1.474$; $P = 0.231$) nor a main effect of group ($P = 0.130$) was observed in P_{aCO_2}, but there was a main effect of condition ($P < 0.001$).

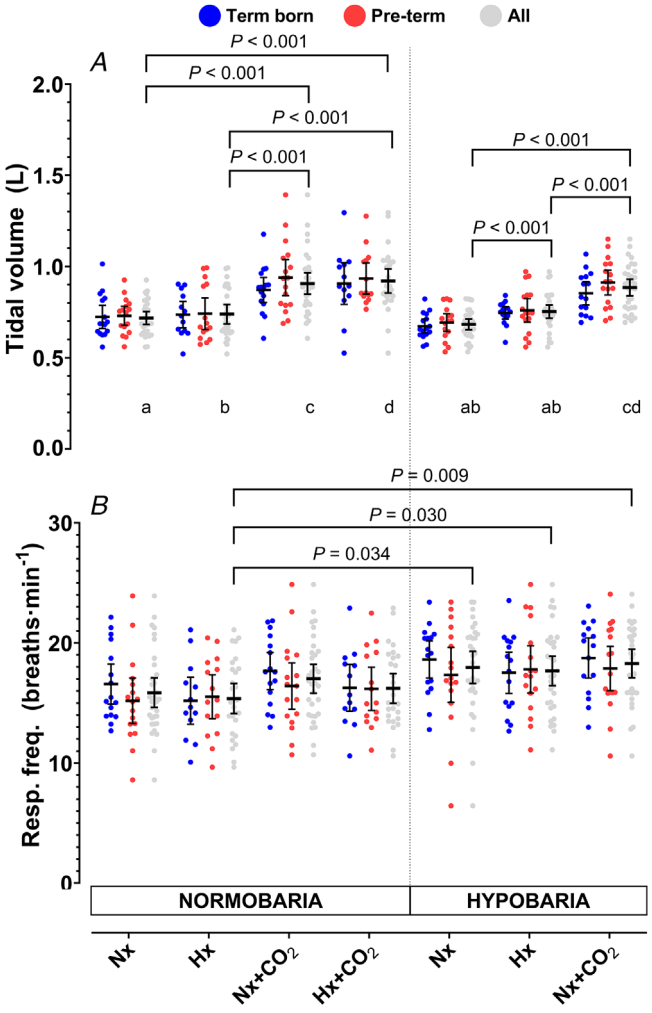


Figure 3. Tidal volume (*A*) and respiratory frequency (resp. freq.; *B*) under each of the seven environmental conditions, comprising normobaria, hypobaria, normoxia (Nx), hypoxia (Hx), normocapnia and hypercapnia (+CO_2)
Values represent individual data points, superimposed by group means with error bars indicating standard deviation. Data displayed for term-born participants (blue), pre-term participants (red) and both groups combined (ALL; grey). Data were analysed using two-way ANOVA, with Bonferroni *post hoc* *P*-value adjustments applied to investigate significant main or interaction effects. Significant pairwise comparisons indicated using their respective *P*-values. For clarity, given the number of significant differences present in panel *A*, comparisons between normobaric and hypobaric conditions are indicated using lowercase letters. Pairwise comparisons which did not differ significantly are indicated by matching letters.

Table 3. Summary of descriptive and inferential statistics for pulse oxygen saturation (%)

Condition	Group		
	Pre-term	Control	Combined
Nb_Nx	99.2 ± 0.5	99.1 ± 0.6	99.2 ± 0.5[a]
Nb_Hx	92.6 ± 2.8	92.0 ± 2.0	92.3 ± 2.4[b]
Nb_Nx+CO_2	99.8 ± 0.4	99.6 ± 0.5	99.7 ± 0.4[c]
Nb_Hx+CO_2	97.1 ± 0.9	96.7 ± 1.2	96.8 ± 1.0
Hb_Nx	99.1 ± 0.6	98.9 ± 0.6	99.0 ± 0.6[a]
Hb_Hx	92.7 ± 2.3	91.2 ± 1.6	92.0 ± 2.1[b]
Hb_Nx+CO_2	99.6 ± 0.5	99.3 ± 0.5	99.5 ± 0.5[ac]
Combined	97.2 ± 3.3	96.8 ± 3.5	97.0 ± 3.4

All values are means ± SD. Inferential statistics for the main effect of condition ($P < 0.001$) are presented in the Combined column. Pairwise comparisons which did *not* differ significantly are indicated by matching superscript letters. Neither a main effect of group ($P = 0.127$) nor an interaction effect between group and condition ($P = 0.315$) was observed. Hb, hypobaria; Hx, hypoxia; Nb, normobaria; Nx, normoxia.

With regards to specific comparisons, barometric pressure *per se* did not influence P_{aO_2}. More specifically, there were no differences in the individual comparisons between Nb_Nx and Hb_Nx ($P = 0.055$), between Nb_Hx and Hb_Hx ($P > 0.999$), and between Nb_Nx+CO_2 and Hb_Nx+CO_2 ($P > 0.999$). However, as expected, the two Hx conditions elicited a significantly lower P_{aO_2} than the two Nx conditions (and all others), and the two +CO_2 conditions elicited significantly higher P_{aO_2} than the two Nx conditions (and all others). Lastly, Nb_Hx+CO_2 induced a significantly higher P_{aO_2} than Nb_Hx ($P < 0.001$), but a significantly lower P_{aO_2} than Nb_Nx ($P < 0.001$).

Overall, the four Nb conditions resulted in higher P_{aCO_2} values than the three Hb conditions (all pairwise comparisons $P < 0.001$). The +CO_2 conditions induced a consistently higher P_{aCO_2} in relation to each of their respective Nb and Hb conditions (both pairwise comparisons $P < 0.001$). Under Nb conditions, similar P_{aCO_2} values were observed between Nx and Hx ($P > 0.999$), and also between Nx+CO_2 and Hx+CO_2 ($P > 0.999$). Conversely, under Hb conditions, P_{aCO_2} was reduced in Hx relative to Nx ($P < 0.001$).

Oscillatory parameters

There were no significant main or interaction effects in $\dot{V}_E$ period (Fig. 5*A*). A significant interaction effect between group and condition was, however, observed for $\dot{V}_E$ peak power ($F(6, 138) = 2.278$; $P = 0.040$), which could be attributed to a significantly higher $\dot{V}_E$ peak power in

Nb_Hx+CO_2 than in Nb_Nx for pre-term ($P = 0.011$) but not for control ($P > 0.999$) participants (Fig. 5*B*).

No significant interaction effects were observed in S_{pO_2} period ($F(3.97, 75.51) = 2.057$; $P = 0.095$) or peak power ($F(3.66, 69.63) = 1.393$; $P = 0.248$). However, main effects of condition were observed in both variables ($P = 0.031$ and $P < 0.001$, respectively). When adjusted for multiplicity, no pairwise comparisons for S_{pO_2} period were considered to differ significantly (all $P \geq 0.808$) (Fig. 5*C*). Several significant differences between conditions were, however, noted for S_{pO_2} peak power (Fig. 5*D*). In particular, within the Nb conditions, S_{pO_2} peak power was higher in the two Hx conditions relative to the two Nx conditions ($P < 0.001$ for both pairwise comparisons). Moreover, S_{pO_2} peak power was greater in Nb_Hx+CO_2 relative to Nb_Hx ($P = 0.046$), whereas no difference was noted between Nb_Nx and Nb_Nx+CO_2 ($P > 0.999$). Within the Hb comparisons, the Hb_Hx condition elicited the highest S_{pO_2} peak power followed by Hb_Nx and then Hb_Nx+CO_2 ($P < 0.001$ for all pairwise comparisons). Regarding comparisons across Nb and Hb conditions, Hb_Hx was similar to both Nb_Hx ($P = 0.956$) and Nb_Hx+CO_2 ($P > 0.999$). Notably, Hb_Nx and Nb_Hx were similar ($P = 0.930$). Lastly, Hb_Nx+CO_2 did not differ significantly from Nb_Nx ($P > 0.999$) and NbNx+CO_2 ($P = 0.110$).

No significant main or interaction effects were observed in P_{ETCO_2} period (Fig. 5*E*). However, a significant main effect of condition was found in P_{ETCO_2} peak power ($P = 0.005$) (Fig. 5*F*). Regarding specific comparisons, P_{ETCO_2} peak power was significantly lower in Hb_Nx+CO_2 than Hb_Hx ($P = 0.039$), Hb_Nx ($P = 0.008$), Nb_Hx ($P = 0.008$) and Nb_Nx ($P = 0.002$). In addition, P_{ETCO_2} peak power was also lower in Nb_Nx+CO_2 than Nb_Nx ($P = 0.019$).

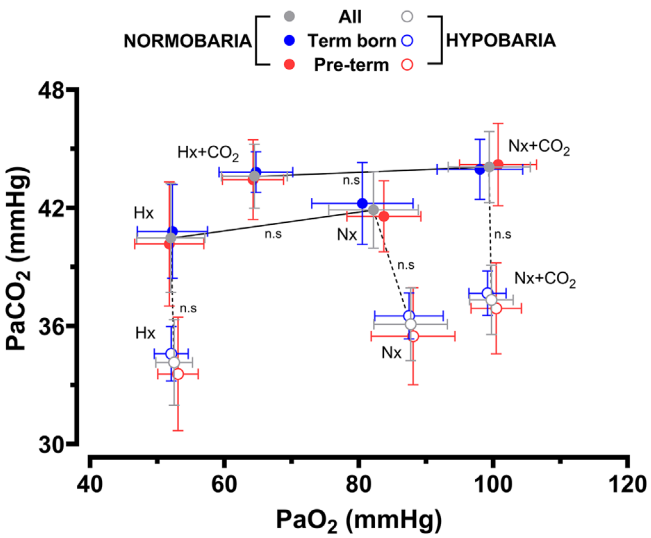


Figure 4. Arterial partial pressures of oxygen (P_{aO_2}) and carbon dioxide (P_{aCO_2}) under each of the seven environmental conditions, comprising normobaria (filled circles), hypobaria (open circles), normoxia (Nx), hypoxia (Hx), normocapnia and hypercapnia (+CO_2)
Values represent group means with error bars in both variables indicating standard deviation. Data displayed for term-born participants (blue), pre-term participants (red) and both groups combined (ALL; grey). Data were analysed using two-way ANOVA, with Bonferroni *post hoc P*-value adjustments applied to investigate significant main or interaction effects. Conditions which did not differ significantly in P_{aO_2} and P_{aCO_2} are connected by dashed and solid black lines, respectively, and labelled as such (n.s).

Discussion

The aim of this study was to compare $\dot{V}_E$, S_{pO_2} and blood gases under varying environmental conditions between healthy adults born pre-term and a group of well-matched term-born control participants. No differences were observed between the two groups in $\dot{V}_E$ magnitude under each stimulus, and this was reflected in similar P_{aO_2} and P_{aCO_2} between pre-term and full-term individuals. Independent from group, it seemed that $\dot{V}_E$ was only higher in hypoxia relative to normoxia at terrestrial altitude, with no difference at sea level. Consistently higher $\dot{V}_E$ was observed with hypercapnia, with no impact of barometric pressure, and no additional effect of hypoxia. With regard to the oscillatory analyses, the $\dot{V}_E$, S_{pO_2} and P_{ETCO_2} periods were not influenced by group or condition. However, the addition of both hypoxia and CO_2 induced a greater $\dot{V}_E$ peak power relative to Nb_Nx in prematurely born individuals only. Additionally, S_{pO_2} peak power was generally greater in the hypoxic conditions relative to their normoxic comparators, although not different between Hb_Nx and Nb_Hx. Lastly, regular fluctuations in P_{ETCO_2} appeared to stabilize when hypercapnic stimuli were applied.

Effect of pre-term birth

Whether pre-term birth *per se* modulates the ventilatory responses to environmental stimuli remains unclear (Narang et al., 2022). Inconsistencies within the literature could be attributed to differences in prematurity-related recruitment criteria, the nature and severity of the environmental stimulus, and data processing/analysis methods. Moreover, several studies elucidating relevant mechanisms have done so using animal models (Bisgard et al., 2003; Davey et al., 1996; Erickson et al., 1998; Lofaso et al., 2007). While many provide valuable insight into mammalian respiratory physiology, the translation of their findings to humans is unclear given interspecies differences in prenatal development (Malassiné et al., 2003). In the present study, adult pre-term participants were intentionally recruited to closely reflect a healthy

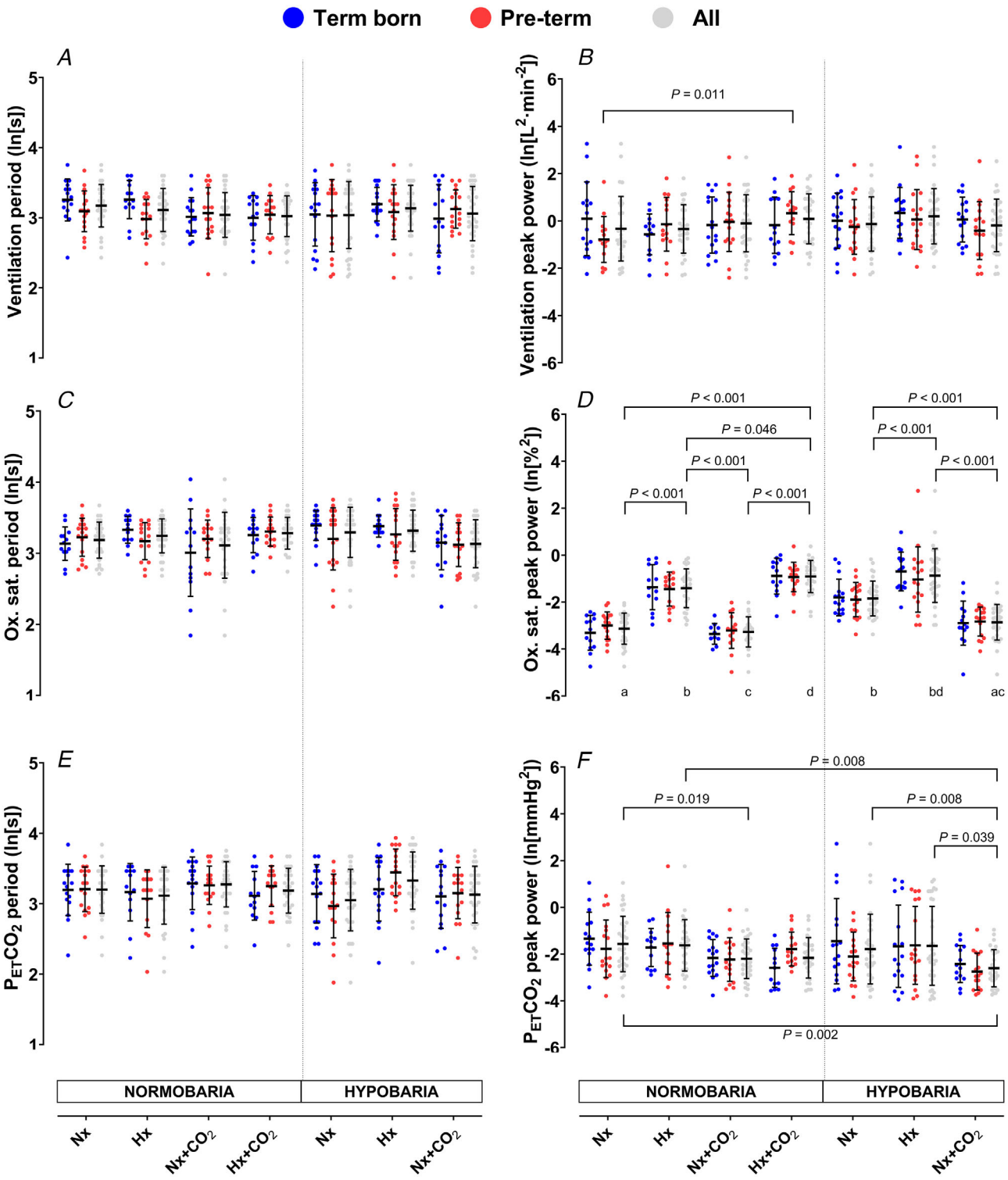


Figure 5. The period and corresponding peak power, respectively, for ventilation (AB), oxygen saturation (ox. sat.; CD), and end-tidal partial pressure of carbon dioxide (P_{ETCO_2}; EF)
Values represent individual data points, superimposed by group means with error bars indicating standard deviation. Data displayed for term-born participants (blue), pre-term participants (red) and both groups combined (ALL; grey). All values were log-transformed to stabilize variance, and are presented as such. Data were analysed using two-way ANOVA, with Bonferroni *post hoc* *P*-value adjustments applied to investigate significant main or interaction effects. Significant pairwise comparisons indicated using their respective *P*-values. Note the significant

difference in panel *B* is specific to the pre-term group after the detection of a significant interaction effect (P = 0.040). All other significant contrasts are secondary to the detection of a main effect of condition. For clarity, given the number of significant differences present in panel *D*, comparisons between normobaric and hypobaric conditions are indicated using lowercase letters. Pairwise comparisons which did not differ significantly are indicated by matching letters.

term-born control group with regards to lifestyle factors, exercise capacity, anthropometrics and (lack of) disease status. In turn, pre-term birth *per se* appeared not to influence the primary outcomes measured in this study.

In a seminal study by Bates & colleagues (2014), healthy adults born pre-term demonstrated blunted ventilatory responses across 5-min exposures to both hypoxia ($F_{iO_2} = 0.12$) and hyperoxia ($F_{iO_2} = 1.0$). Similarly, a lower resting hypoxic ventilatory response was observed in pre-term compared to term-born individuals exposed to normobaric hypoxia (F_{iO_2} = 0.13; P_{iO_2} = 91 mmHg) for 5 min (Debevec et al., 2019). Interestingly, the experimental and control groups recruited in these studies were also matched for pulmonary function and exercise capacity, so clinical differences could not explain the inconsistencies with the present results. It may be that the hypoxic dose applied in this study ($F_{iO_2} = 0.138$) did not stimulate a sufficient response to elicit between-group differences. Specifically, S_{pO_2} decreased by 7 ± 2% between the normoxic and hypoxic conditions (across both normobaria and hypobaria), whereas Debevec et al. (2019) reported decreases of 15 ± 4% and 12 ± 2% in their pre-term and full-term groups, respectively. While absolute S_{pO_2} changes were not reported by Bates et al. (2014), the more severe hypoxic stimulus applied in comparison to the Debevec et al. (2019) study suggests that hypoxaemia would have been more pronounced within their experimental protocol. In addition, the participants recruited by Bates et al. (2014) had lower cardiorespiratory fitness (∼26%) than those tested in this study, so differences between groups might more readily emerge in individuals of relatively lower training status. That being said, an investigation of prematurely born adults with diffusion capacity limitations, impaired pulmonary function and reduced cardiorespiratory fitness demonstrated similar $\dot{V}_E$, P_{aO_2} and P_{aCO_2} at rest in hypoxia (F_{iO_2} = 0.12) relative to a term-born control group (Duke et al., 2014), highlighting that differences observed between studies cannot be explained solely by participant characteristics and/or methodological approaches.

With regard to hypercapnia, Davey et al. (1996) previously demonstrated a transient decrease in ventilatory CO_2 sensitivity in pre-term lambs which persisted for 2 weeks. Interestingly, the only study to have investigated this response in humans found exaggerated hypercapnic sensitivity in prematurely born adults breathing a 6% CO_2 gas mixture (Manferdelli et al., 2021). However, in line with the findings of this study, a 3% CO_2 stimulus did not reveal prematurity-specific differences. The present study further demonstrated that this held true in hypobaria, and with and without an additional hypoxic stimulus at sea level. Whether more severe hypercapnic stimuli do indeed induce differential chemosensitivity responses in pre-term individuals requires further investigation with various hypercapnic sensitivity protocols.

Barometric pressure

An important observation within this study was that a higher ventilation in hypoxia compared to normoxia was only observed under hypobaric (terrestrial altitude) conditions, and not in normobaria (sea level). This occurred despite approximately equivalent relative P_{iO_2} stimuli between the two barometric pressure conditions (ΔP_{iO_2}; Nb *vs.* Hb; 46.5 ± 6.3 *vs.* 46.3 ± 5.2 mmHg; P = 0.669), and similar decreases in S_{pO_2} (ΔS_{pO_2}; Nb *vs.* Hb; 6.8 ± 2.4% *vs.* 6.9 ± 1.9%; P = 0.742). It is also particularly surprising considering that P_{aCO_2} was substantially lower in the hypobaric conditions, and would therefore have independently stimulated ventilation to a lesser extent. However, the difference in P_{aO_2} between Nb_Nx (82.4 ± 6.7 mmHg) and Hb_Nx (87.7 ± 5.5 mmHg), while not considered to differ significantly (P = 0.055), could nevertheless be noteworthy due to the conservative *post hoc* P-value adjustment. Given that P_{aO_2} in Nb_Hx (52.3 ± 5.1 mmHg) and Hb_Hx (52.3 ± 2.5 mmHg) were certainly very similar ($P > 0.999$), ΔP_{aO_2} between the normoxic and hypoxic conditions was indeed greater in hypobaria as per an independent *post hoc* test of statistical significance (Nb *vs.* Hb; 30.3 ± 6.7 *vs.* 35.2 ± 4.4 mmHg; P = 0.007). From another perspective, the greater $\dot{V}_E$ observed in Hb_Hx relative to Nb_Hx implies that P_{aO_2} would have been reduced further, had it not been for the compensatory adjustment in ventilatory control. Ultimately, for reasons that can only be speculated on with the present data, a greater hypoxia-induced change in P_{aO_2}, particularly for a given $\dot{V}_E$, seemed to exist in hypobaria. This manifested as a higher absolute $\dot{V}_E$, and was also an effect that appeared consistent between the two groups.

Oscillatory parameters

The analyses of oscillations in the $\dot{V}_E$, S_{pO_2} and P_{ETCO_2} signals recorded under each condition allow further insight into the pattern of the respiratory responses. Indeed, oscillatory assessments of breath-by-breath $\dot{V}_E$ recordings have previously been used to identify and

quantify the periodic breathing phenomenon under metabolic and environmental stress (Hermand et al., 2015). In line with that investigation, the results of the present study suggest that there is no effect of moderate hypoxia on oscillatory periods in any of the three key outcome variables under resting conditions. In this study, that finding was extended to also include both barometric pressure and hypercapnia as independent and combined stimuli.

In addition to the period of the oscillatory cycles, the power around ±0.02 Hz of the peak in the frequency domain was computed. Of particular note was the higher $\dot{V}_E$ peak power with both hypoxia and hypercapnia at sea level, compared to ambient sea level conditions, only in the pre-term group. Put simply, this means that more accentuated periodic fluctuations in $\dot{V}_E$ were observed when both ventilatory stimuli were applied only in the pre-term participants, compared to their (lack of) regular ventilatory oscillations under normobaric normoxic normocapnia. This finding implies that, while pre-term individuals were able to upregulate their breathing to compensate for the combined O_2 decrease and CO_2 increase induced experimentally, there was an irregularity in the feedback mechanisms that modulated this response. Given previous observations in which pre-term individuals have demonstrated reduced hypoxic ventilatory responsiveness (Bates et al., 2014) and increased hypercapnic ventilatory responsiveness (Manferdelli et al., 2021), it is possible that the integration of central and peripheral chemoreceptor activity may lead to such an irregularity. That being said, the pre-term individuals did not respond differently from their term-born counterparts when hypoxia and hypercapnia were administered independently in this study, and the use of a hypobaric hypoxic hypercapnic condition to further corroborate this observation was not feasible within the study design. At present, then, this observation primarily highlights the unique insight that such analyses can provide, over and above a simple quantification of ventilatory magnitude.

Regarding S_{pO_2}, there appeared to be a clear increase in peak power under hypoxic conditions relative to their normoxic comparators, although this is likely in part due to hypoxaemia providing more 'room' for fluctuations in the signal to occur. More specifically, under normoxic conditions, S_{pO_2} remains close to 100%, and may therefore fluctuate to a lesser extent, if at all. Interestingly, however, when comparing sea level conditions to those at terrestrial altitude, S_{pO_2} peak power was similar between hypobaric normoxia and normobaric hypoxia. This occurred despite substantial differences between the two conditions in absolute S_{pO_2} (99.0 ± 0.6% *vs.* 92.3 ± 2.4%; $P < 0.001$), and suggests once more that in this study, barometric pressure *per se* played a role within the integration of cardiorespiratory regulation. Lastly, with regards to P_{ETCO_2} regulation, the addition of a 3% CO_2 stimulus appeared to stabilize apparent fluctuations in P_{ETCO_2}, most likely due to CO_2 'saturation'. This occurred with and without both hypoxia and hypobaria, and reveals that experimentally induced hypercapnia, despite not influencing the peak power of oscillations in $\dot{V}_E$, decreases these fluctuations in P_{ETCO_2}. In addition, it was interesting to observe that P_{ETCO_2} peak power was generally lower, indicating greater stability in this parameter, under the conditions in which tidal volume was greater. Therefore, regulation of $\dot{V}_E$ through deeper (as opposed to faster) breathing could stabilize end-tidal (and theoretically then also arterial) CO_2.

Methodological considerations

Several important factors should be noted when generalizing these results. Firstly, participants were exposed to the ambient high-altitude condition for ∼20 h prior to testing, which may have impacted their responses to the hypobaric conditions. However, given the similar P_{aO_2} and S_{pO_2} values observed between the normobaric and hypobaric hypoxic conditions, and the known time course of hypoxic ventilatory acclimatization (Pamenter & Powell, 2016), this did not appear to influence the effects that we observed. Secondly, the pre-term participants recruited for this research might not precisely reflect the wider prematurely born population. Not only were they free from cardiometabolic and respiratory diseases, they were specifically recruited to relatively closely match the control group in lung function and cardiorespiratory fitness. Indeed, cardiorespiratory fitness also tends to be impaired in healthy individuals born pre-term (Duke et al., 2022), and these findings may therefore not apply to pre-term individuals with cardiorespiratory impairments. Thirdly, emerging evidence suggests that advancements in neonatal care across the last ∼50 years are associated with ever-reducing lung function deficits in pre-term individuals (Bårdsen et al., 2022; Vollsæter et al., 2015). This suggests that prematurely born adults represent a dynamic population, where the specific location and decade within which such a birth occurred, and the consequent medical treatment(s) provided, are key determinants of the acute and chronic physiological outcomes (Debevec et al., 2022). The participants in the present study were born between 1991 and 2003, and were 21 ± 4 years of age at the time of testing. They could therefore be considered reflective of a more modern pre-term population, where lung function and physical fitness deficits are gradually being attenuated or even abolished. Lastly, due to the logistical constraints of scheduling and executing high-altitude research, and the potential for menstrual cycle status to influence important outcome measures across the broader research expedition

(Cornelli et al., 2013; Dunne et al., 1991; Peltonen et al., 2016), only male participants were recruited for this study. There are, however, suspected sex differences within the context of premature birth. Specifically, in the final 2 months of pregnancy, the female fetus presents with a higher degree of lung maturity at a given gestational age (Fleisher et al., 1985). Therefore, together with evidence for increased rates of spontaneous pre-term birth in male fetuses (Peelen et al., 2016), male sex is considered a key risk factor for poor neonatal outcomes (Peacock et al., 2012). It may therefore be speculated that female participants recruited using the present criteria would also demonstrate similar responses to a well-matched control group, under the assumption that the potential for blunted physiological responsiveness is reduced in relation to prematurely born males. However, evidence of long-term cardiovascular sequelae specific to prematurely born girls also exists (Bonamy et al., 2005), so further work to elucidate potential sex-related different consequences of prematurity is undoubtedly warranted.

Conclusions

In conclusion, 6 min exposures to hypoxic, hypercapnic and hypobaric conditions induced similar pulmonary ventilation in prematurely born adults, and in a term-born control group matched for age, aerobic capacity and health status. Arterial blood gases were also similar between groups under each environmental condition. Under hypoxic conditions equivalent to ∼3375 m, changes in ventilation only occurred in hypobaria (terrestrial altitude) and not in normobaria (simulated altitude). Lastly, oscillatory analyses have been further demonstrated to provide additional insight into the pattern of breathing under various steady-state experimental conditions, and elude to evidence of fluctuations in ventilation in prematurely born adults under hypoxic hypercapnic conditions. Together with previous research, the findings of this study imply a dynamic nature to pre-term birth status in relation to the manifestation of previously observed long-term sequelae. The findings also highlight the potential relevance of barometric pressure in the ventilatory responses to reduced environmental oxygen pressure.

References

Bårdsen, T., Røksund, O. D., Benestad, M. R., Hufthammer, K. O., Clemm, H. H., Mikalsen, I. B., Øymar, K., Markestad, T., Halvorsen, T., & Vollsæter, M. (2022). Tracking of lung function from 10 to 35 years after being born extremely preterm or with extremely low birth weight. *Thorax*, **77**(8), 790–798.

Barnard, C. R., Peters, M., Sindler, A. L., Farrell, E. T., Baker, K. R., Palta, M., Stauss, H. M., Dagle, J. M., Segar, J., Pierce, G. L., Eldridge, M. W., & Bates, M. L. (2020). Increased aortic stiffness and elevated blood pressure in response to exercise in adult survivors of prematurity. *Physiological Reports*, **8**(12), e14462.

Bärtsch, P., & Saltin, B. (2008). General introduction to altitude adaptation and mountain sickness. *Scandinavian Journal of Medicine & Science in Sports*, **18**(Suppl s1), 1–10.

Bates, M. L., Farrell, E. T., & Eldridge, M. W. (2014). Abnormal ventilatory responses in adults born prematurely. *New England Journal of Medicine*, **370**(6), 584–585.

Bates, M. L., Pillers, D.-A. M., Palta, M., Farrell, E. T., & Eldridge, M. W. (2013). Ventilatory control in infants, children, and adults with bronchopulmonary dysplasia. *Respiratory Physiology & Neurobiology*, **189**(2), 329–337.

Bisgard, G. E., Olson Jr E. B., Wang Z. Y., Bavis R. W., Fuller D. D., & Mitchell G. S. (2003). Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. *Journal of Applied Physiology*, **95**(3), 946–952.

Bonamy, A.-K. E., Bendito, A., Martin, H., Andolf, E., Sedin, G., & Norman, M. (2005). Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. *Pediatric Research*, **58**(5), 845–849.

Calder, N. A., Williams, B. A., Smyth, J., Boon, A. W., Kumar, P., & Hanson, M. A. (1994). Absence of ventilatory responses to alternating breaths of mild hypoxia and air in infants who have had bronchopulmonary dysplasia: Implications for the risk of sudden infant death. *Pediatric Research*, **35**(6), 677–681.

Cornelli, U., Belcaro, G., Cesarone, M. R., & Finco, A. (2013). Analysis of oxidative stress during the menstrual cycle. *Reproductive Biology and Endocrinology*, **11**(1), 74.

Crump, C. (2020). An overview of adult health outcomes after preterm birth. *Early Human Development*, **150**, e105187.

Davey, M. G., Moss, T. J., Mccrabb, G. J., & Harding, R. (1996). Prematurity alters hypoxic and hypercapnic ventilatory responses in developing lambs. *Respiration physiology*, **105**(1–2), 57–67.

Debevec, T., Narang, B. J., Manferdelli, G., & Millet, G. P. (2022). Premature birth: A neglected consideration for altitude adaptation. *Journal of Applied Physiology*, **133**(4), 975–978.

Debevec, T., Pialoux, V., Millet, G. P., Martin, A., Mramor, M., & Osredkar, D. (2019). Exercise overrides blunted hypoxic ventilatory response in prematurely born men. *Frontiers in Physiology*, **10**, 437.

Duffin, J. (2007). Measuring the ventilatory response to hypoxia. *The Journal of physiology*, **584**(1), 285–293.

Duffin, J. (2011). Measuring the respiratory chemoreflexes in humans. *Respiratory Physiology & Neurobiology*, **177**(2), 71–79.

Duke, J. W., Elliott, J. E., Laurie, S. S., Beasley, K. M., Mangum, T. S., Hawn, J. A., Gladstone, I. M., & Lovering, A. T. (2014). Pulmonary gas exchange efficiency during exercise breathing normoxic and hypoxic gas in adults born very preterm with low diffusion capacity. *Journal of Applied Physiology*, **117**(5), 473–481.

Duke, J. W., Lewandowski, A. J., Abman, S. H., & Lovering, A. T. (2022). Physiological aspects of cardiopulmonary dysanapsis on exercise in adults born preterm. *The Journal of Physiology*, **600**(3), 463–482.

Dunne, F. P., Barry, D. G., Ferriss, J. B., Grealy, G., & Murphy, D. (1991). Changes in blood pressure during the normal menstrual cycle. *Clinical Science*, **81**(s25), 515–518.

Erickson, J. T., Mayer, C., Jawa, A., Ling, L., Olson Jr E. B., Vidruk E. H., Mitchell G. S., & Katz D. M. (1998). Chemoafferent degeneration and carotid body hypoplasia following chronic hyperoxia in newborn rats. *The Journal of Physiology*, **509**(2), 519–526.

Fleisher, B., Kulovich, M. V., Hallman, M., & Gluck, L. (1985). Lung profile: Sex differences in normal pregnancy. *Obstetrics and Gynecology*, **66**(3), 327–330.

Fulco, C. S., Beidleman, B. A., & Muza, S. R. (2013). Effectiveness of preacclimatization strategies for high-altitude exposure. *Exercise and Sport Sciences Reviews*, **41**(1), 55–63.

Hermand, E., Pichon, A., Lhuissier, F. J., & Richalet, J.-P. (2015). Periodic breathing in healthy humans at exercise in hypoxia. *Journal of Applied Physiology*, **118**(1), 115–123.

Hermand, E., Pichon, A., Lhuissier, F. J., & Richalet, J.-P. (2019). Low-frequency ventilatory oscillations in hypoxia are a major contributor to the low-frequency component of heart rate variability. *European Journal of Applied Physiology*, **119**(8), 1769–1777.

Hohenhaus, E., Paul, A., McCullough, R., Kucherer, H., & Bartsch, P. (1995). Ventilatory and pulmonary vascular response to hypoxia and susceptibility to high altitude pulmonary oedema. *European Respiratory Journal*, **8**(11), 1825–1833.

Katz-Salamon, M., Jónsson, B., & Lagercrantz, H. (1995). Blunted peripheral chemoreceptor response to hyperoxia in a group of infants with bronchopulmonary dysplasia. *Pediatric Pulmonology*, **20**(2), 101–106.

Loeppky, J. A., Icenogle, M., Scotto, P., Robergs, R., Hinghofer-Szalkay, H., & Roach, R. C. (1997). Ventilation during simulated altitude, normobaric hypoxia and normoxic hypobaria. *Respiration Physiology*, **107**(3), 231–239.

Lofaso, F., Dauger, S., Matrot, B., Vardon, G., Gaultier, C., & Gallego, J. (2007). Inhibitory effects of repeated hyperoxia on breathing in newborn mice. *European Respiratory Journal*, **29**(1), 18–24.

MacIntyre, N., Crapo, R. O., Viegi, G., Johnson, D. C., van der Grinten, C. P. M., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Enright, P., Gustafsson, P., Hankinson, J., Jensen, R., McKay, R., Miller, M. R., Navajas, D., Pedersen, O. F., Pellegrino, R., & Wanger, J. (2005). Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *European Respiratory Journal*, **26**(4), 720–735.

Malassiné, A., Frendo, J. L., & Evain-Brion, D. (2003). A comparison of placental development and endocrine functions between the human and mouse model. *Human Reproduction Update*, **9**(6), 531–539.

Manferdelli, G., Narang, B. J., Bourdillon, N., Debevec, T., & Millet, G. P. (2023). Physiological responses to exercise in hypoxia in preterm adults: Convective and diffusive limitations in the O2 transport. *Medicine & Science in Sports & Exercise*, **55**(3), 482–496.

Manferdelli, G., Narang, B. J., Poussel, M., Osredkar, D., Millet, G. P., & Debevec, T. (2021). Long-term effects of prematurity on resting ventilatory response to hypercapnia. *High Altitude Medicine & Biology*, **22**(4), 420–425.

Maxwell, S. E., & Delaney, H. D. (1990). *Designing Experiments and Analyzing Data: A Model Comparison Perspective*. Wadsworth, Belmont, CA, USA.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C. P. M., Gustafsson, P., Jensen, R., Johnson, D. C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O. F., Pellegrino, R., Viegi, G., & Wanger, J. (2005). Standardisation of spirometry. *European Respiratory Journal*, **26**(2), 319–338.

Millet, G. P., & Debevec, T. (2020). CrossTalk proposal: Barometric pressure, independent of PO2, is the forgotten parameter in altitude physiology and mountain medicine. *The Journal of Physiology*, **598**(5), 893–896.

Millet, G. P., Faiss, R., & Pialoux, V. (2013). Evidence for differences between hypobaric and normobaric hypoxia is conclusive. *Exercise and Sport Sciences Reviews*, **41**(2), 133.

Mortara, A., Sleight, P., Pinna, G. D., Maestri, R., Prpa, A., Rovere, M. T. L., Cobelli, F., & Tavazzi, L. (1997). Abnormal awake respiratory patterns are common in chronic heart failure and may prevent evaluation of autonomic tone by measures of heart rate variability. *Circulation*, **96**(1), 246–252.

Narang, B. J., Manferdelli, G., Millet, G. P., & Debevec, T. (2022). Respiratory responses to hypoxia during rest and exercise in individuals born pre-term: A state-of-the-art review. *European Journal of Applied Physiology*, **122**(9), 1991–2003.

Näsänen-Gilmore, P., Sipola-Leppänen, M., Tikanmäki, M., Matinolli, H.-M., Eriksson, J. G., Järvelin, M.-R., Vääräsmäki, M., Hovi, P., & Kajantie, E. (2018). Lung function in adults born preterm. *PLoS ONE*, **13**(10), e0205979.

Pamenter, M. E., & Powell, F. L. (2016). Time domains of the hypoxic ventilatory response and their molecular basis. *Comprehensive Physiology*, **6**(3), 1345–1385.

Peacock, J. L., Marston, L., Marlow, N., Calvert, S. A., & Greenough, A. (2012). Neonatal and infant outcome in boys and girls born very prematurely. *Pediatric Research*, **71**(3), 305–310.

Peelen, M. J., Kazemier, B. M., Ravelli, A. C., De Groot, C. J., Van Der Post, J. A., Mol, B. W., Hajenius, P. J., & Kok, M. (2016). Impact of fetal gender on the risk of preterm birth, a national cohort study. *Acta obstetricia et Gynecologica Scandinavica*, **95**(9), 1034–1041.

Peltonen, G. L., Harrell, J. W., Aleckson, B. P., LaPlante, K. M., Crain, M. K., & Schrage, W. G. (2016). Cerebral blood flow regulation in women across menstrual phase: Differential contribution of cyclooxygenase to basal, hypoxic, and hypercapnic vascular tone. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, **311**(2), R222–R231.

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., Enright, P. L., Hankinson, J. L., Ip, M. S. M., Zheng, J., & Stocks, J. (2012). Multi-ethnic reference values for spirometry for the 3–95-yr age range: The global lung function 2012 equations. *European Respiratory Journal*, **40**(6), 1324–1343.

Richalet, J.-P., Larmignat, P., Poitrine, E., Letournel, M., & Canouï-Poitrine, F. (2012). Physiological risk factors for severe high-altitude illness: A prospective cohort study. *American Journal of Respiratory and Critical Care Medicine*, **185**(2), 192–198.

Savourey, G., Launay, J.-C., Besnard, Y., Guinet, A., & Travers, S. (2003). Normo- and hypobaric hypoxia: Are there any physiological differences? *European Journal of Applied Physiology*, **89**(2), 122–126.

Schuermans, A., & Lewandowski, A. J. (2022). Understanding the preterm human heart: What do we know so far? *The Anatomical Record*, **305**(9), 2099–2112.

Stanojevic, S., Graham, B. L., Cooper, B. G., Thompson Bruce, R., Carter, K. W., Francis, R. W., & Hall Graham, L.(2017). Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. *European Respiratory Journal*, **50**(3), e1700010.

Teppema, L. J., & Dahan, A. (2010). The ventilatory response to hypoxia in mammals: Mechanisms, measurement, and analysis. *Physiological Reviews*, **90**(2), 675–754.

Vollsæter, M., Skromme, K., Satrell, E., Clemm, H., Røksund, O., Øymar, K., Markestad, T., & Halvorsen, T. (2015). Children born preterm at the turn of the millennium had better lung function than children born similarly preterm in the early 1990s. *PLoS ONE*, **10**(12), e0144243.

Additional information

Data availability statement

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflicts of interest in relation to the data presented in this manuscript.

Author contributions

Conceptualization and design: B.J.N., G.M., G.P.M, T.D.; funding acquisition: G.P.M., T.D.; data collection: B.J.N., G.M.; data analysis: B.J.N., N.B.; writing – original draft, B.J.N; writing – review and editing: B.J.N., G.M., N.B., G.P.M, T.D. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This study was financially supported by the Slovenian National Research Agency (ARRS grant no. N5-0152) and the Swiss National Science Foundation (SNSF grant no. 320030L_192 073).

Acknowledgements

The authors would like to express their gratitude to all of the volunteers who participated in the project. The authors are also grateful for the work of Dr Aleš Koščak, Dr Matevž Harlander and Ms Ksenija Mežnarič in facilitating some of the baseline testing procedures. Additionally, the authors would like to thank Mr Miro Vrhovec for his technical assistance. Finally, the authors would like to thank Dr Guidio Giardini from the Aosta Hospital, and the teams at SkyWay Mont Blanc and the Rifugio Torino mountain hut, for their logistical support in setting up a high-altitude research laboratory.

Keywords

altitude, chemosensitivity, hypercapnia, hypobaria, hypoxia, prematurity, ventilation

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History