Ventilatory Response to Nitrogen Multiple-Breath Washout in Infants

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Summary. Background: Nitrogen multiple-breath washout (N_2MBW) using 100% oxygen (O_2) has regained interest to assess efficiency of tracer gas clearance in, for example, children with Cystic Fibrosis (CF). However, the influence of hyperoxia on the infants' respiratory control is unclear. We assessed safety and impact on breathing pattern from hyperoxia, and if exposure to 40% O_2 first induces tolerance to subsequent 100% O_2 for N_2MBW. Methods: We prospectively enrolled 39 infants aged 3–57 weeks: 15 infants with CF (8 sedated for testing) and 24 healthy controls. Infants were consecutively allocated to the protocols comprising of 100% O_2 or 40/100% O_2 administered for 30 breaths. Lung function was measured using an ultrasonic flowmeter setup. Primary outcome was tidal volume (V_T). Results: None of the infants experienced apnea, desaturation, or bradycardia. Both protocols initially induced hypoventilation. V_T temporarily declined in 33/39 infants across 10–25 breaths. Hypoventilation occurred independent of age, disease, and sedation. In the new 40/100% O_2 protocol, V_T returned to baseline during 40% O_2 and remained stable during 100% O_2 exposure. End-tidal carbon dioxide monitored online did not change. Conclusion: The classical N_2MBW protocol with 100% O_2 may change breathing patterns of the infants. The new protocol with 40% O_2 induces hyperoxia-tolerance and does not lead to changes in breathing patterns during later N_2 washout using 100% O_2. Both protocols are safe, the new protocol seems an attractive option for N_2MBW in infants. **Pediatr Pulmonol.** © 2013 Wiley Periodicals, Inc.

BACKGROUND

Multiple-breath inert tracer gas washout (MBW) is an easy and sensitive lung function test assessing efficiency of inert tracer gas clearance across multiple tidal breaths.[1–4] In children with cystic fibrosis (CF), impaired tracer gas clearance quantified by the lung clearance index (LCI) correlates to other biomarkers of small airways disease,[5–8] and detects pulmonary function response upon short-term interventions.[9–11]

To overcome the need for expensive inert tracer gases such as sulfur hexafluoride, nitrogen (N_2) MBW using pure oxygen (O_2) has regained interest and seems attractive for MBW in infants. However, there is scanty evidence as to whether 100% O_2 for the purpose of MBW is applicable in infants.[12–14] A regular breathing pattern is a prerequisite for reliable MBW testing. By unloading peripheral chemoreceptors, hyperoxia may change tidal volume (V_T) if 100% O_2 is used.[15] We hypothesized that 40% O_2 may induce "tolerance" of the infants' respiratory control to subsequent 100% O_2 exposure N_2MBW. Breathing 40% O_2 would allow later washout of the lungs' remaining N_2 fraction using pure O_2. In the current study, we measured tidal breathing indices when infants

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were exposed to hyperoxia during the classical 1-step N_2MBW protocol with a switch from ambient to 100% O_2 gas, and the new 2-steps protocol introducing an intermediate 40% O_2 step, that is, 40/100% O_2. Our aim was to assess safety and impact of hyperoxia (40% or 100% O_2) on breathing patterns in infants with CF and healthy controls, and to compare tidal breathing parameters from both N_2MBW protocols. Primary outcome was change in V_T upon 40% or 100% O_2 exposure.

METHODS

We prospectively enrolled 40 infants (16 with CF) from two ongoing studies, the *Bern Infant Lung Development* (BILD) cohort and the *Swiss CF Newborn Screening Study*.[16–18] The following inclusion criteria applied: white ethnicity, term delivery (37 weeks), and no known other diseases likely to affect lung function. General exclusion criteria were clinical signs of lower respiratory tract infection. The population is described in Table 1. The study was approved by the Ethics Committee of the Canton of Bern, Bern, Switzerland. Parents provided written informed consent for this study. Parents from one child withdrew consent.

We consecutively allocated 23 infants (7 with CF of which 4 were sedated) to protocol I and 16 (8 with CF of which 4 were sedated) to protocol II. The sequence of tidal breathing measurements in protocol I was (i) 1 baseline test during ambient air exposure (21% O_2) and (ii) 1 test during breathing 100% O_2. Protocol II implied (i) 1 baseline test during ambient air exposure, (ii) 1 test during breathing 40% O_2, and (iii) 1 test during breathing 100% O_2. Hyperoxia was administered for 30 breaths which reflect approximately twice the cumulative expired volume (CEV) of average N_2MBW in infants.[12] Oxygen saturation, heart rate, and the exhaled carbon dioxide (CO_2) fraction were monitored online.
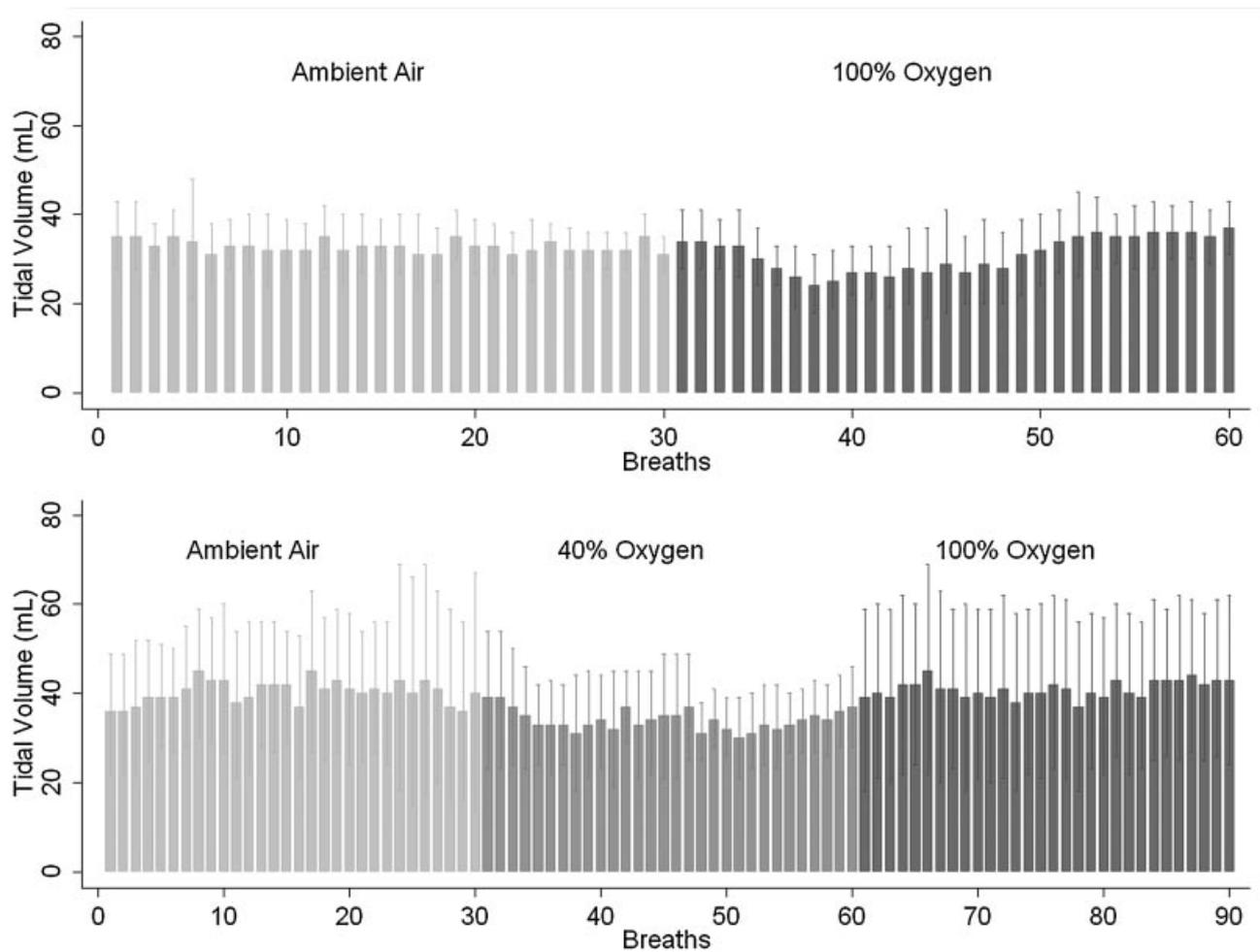
Lung function was measured in infants in supine position with the head midline via an infant mask (size 1; Homedica AG, Huenenberg, Switzerland), according to the ERS/ATS standards of infant lung function testing.[19–21] Thirty-one young infants, age range 3–13 weeks, 7 with CF, were studied during behaviorally defined natural sleep. Measurements followed regular feeding of the infants, usually resulting in quiet (non-rapid eye movement) sleep in this age group. Eight older infants with CF aged 13–14 months were studied during chloral–hydrate sedation. Flow was measured using a mainstream ultrasonic flow meter (Exhalyzer D; Eco Medics AG, Duernten, Switzerland).[17] Data were used if no apparent volume drift was present, defined as a change exceeding 2 ml s^{-1}. Tidal breathing indices derived from tidal flow were measured from all breaths. Main outcome was V_T which directly affects end-expiratory lung volume, the functional residual capacity (FRC). Secondary outcomes were respiratory rate (RR), minute ventilation (V_E), mean tidal expiratory flow (MTEF), and end-tidal CO_2 monitored online. Tidal breathing indices within children were compared by paired t-test. P-values < 0.05 were considered significant (STATA 11; College Station). Variability of V_T was estimated by the coefficient of variation (CV = SD/mean %) within each test and per child.

RESULTS

Thirty-eight infants completed the study according to protocol (Table 1). None of the infants experienced apnea, desaturation ($\leq 92\%$ O_2), bradycardia (<60 min^{-1}) or change in end-tidal CO_2 levels. One non-sedated infant woke up during 100% O_2 exposure (protocol II). In total, V_T, RR, V_E, and MTEF were measured in 4,063 breaths from 132 tests.

In both protocols, initial hyperoxia (40% or 100% O_2) induced hypoventilation. In 33/39 infants, V_T declined after 2–4 breaths, remained low during 10–25 breaths, and then returned to baseline (Fig. 1). Similarly V_E and MTEF but not RR decreased and increased again (Table 2). V_T decreased irrespective of disease, age, or sedation. We stratified children according to disease, age, and sedation: Mean (95% CI) V_T decrease was 2.9 (1.4; 4.4) ml in the 24 controls, and 6.7 (2.9; 10.4) ml in the 15 infants with CF; 3.0 (1.7; 4.2) ml in the 31 non-sedated (younger) infants, and 9.7 (3.3; 16.2) ml in the eight sedated infants (older, all with CF). Intra-individual variability of V_T increased during initial hyperoxia (Table 2). In both protocols, mean (SD) CV rose from 8.8 (3.6)% at baseline to 15.1 (7.3)%, $P < 0.001$, during initial hyperoxia. Variability of V_T increased irrespective of age, sedation, or disease ($P \leq 0.001$ for all).

The infants' respiratory control apparently adapted to hyperoxia (Figs. 1 and 2). Here, we report ventilatory

TABLE 1—Anthropometric Characteristics of the Study Population (n = 39)

	Younger infants	Older infants
N	31 (7 with CF)	8 (8 with CF)
Protocol I/II	19/12	4/4
Males/females	16/15	3/5
Age	5 (5–6) weeks	13 (13–14) months
Weight	4,150 (3,600–4,960) g	9.3 (8.7–9.5) kg
Height	55 (52–58) cm	77 (73–78) cm

Anthropometric data from the 24 healthy infants and 15 infants with CF. Numeric data are described as median and interquartile range. Protocol I = classical 1-step nitrogen multiple-breath washout protocol using 100% oxygen; Protocol II = new 2-steps nitrogen multiple-breath washout protocol using 40–100% oxygen. Lung function was measured during natural quiet sleep in younger infants and during sedation in older infants.

Fig. 1. Tidal breathing pattern during the classical and new nitrogen MBW protocol. The course of tidal volumes (V_T) from 39 infants (16 with CF of which 8 were sedated) across both protocols illustrates transient hypoventilation induced by hyperoxia (40% or 100% O_2). The bars give median and inter-quartile ranges of V_T. V_T slowly returns to baseline during both 100% O_2 (protocol I: 23 infants, 4 sedated; upper panel) and 40% O_2 exposure (protocol II: 16 infants, 4 sedated; lower panel) and remains stable during subsequent 100% O_2 exposure in protocol II.

dynamics from infants in protocol (II). After initial V_T decrease during 40% O_2, V_T returned to baseline during 100% O_2 exposure. Mean (SD) baseline V_T was 51.4 (29.2) ml, V_T during 40% O_2 exposure was 46.4 (26.8) ml, $P = 0.007$ and 51.5 (29.2) ml, $P > 0.9$ during 100% O_2 exposure. Similarly V_E and MTEF returned to baseline while RR remained unchanged. Similarly end-tidal CO_2 levels monitored online during hyperoxia remained stable. V_T variability remained slightly elevated (Table 2).

DISCUSSION

Summary

This is the first study to report on a new infant N_2MBW protocol inducing tolerance to hyperoxia. Infant N_2MBW protocols using 100% O_2 or both 40% and 100% O_2 applied sequentially are safe. Both protocols initially lead to hypoventilation and increased variability of V_T. These V_T dynamics were apparently independent of age, disease, and natural or induced sleep in infants younger than 14 months. Of importance, the infants' hypoventilation is temporary. The new protocol comprising 40% O_2 exposure prior to N_2MBW using 100% O_2 induces tolerance to hyperoxia. Breathing 40% O_2 allows later washout of the lungs' remaining N_2 fraction using pure O_2. The new protocol seems applicable for future N_2MBW studies in healthy infants and infants with CF.

Physiological Aspects of Nitrogen Multiple-Breath Washout in Infants

The principle of N_2MBW is based on lung inherent N_2 washout via breathing pure O_2. It appears that temporary

TABLE 2—Impact of the Classical and New Nitrogen MBW Protocol on Tidal Breathing

	Baseline	Protocol I	Protocol II	
	Ambient air	100% O_2	40% O_2	100% O_2
N	39	23	16	15
	Mean [range]	Mean diff. (95% CI)	Mean diff. (95% CI)	Mean diff. (95% CI)
V_T (ml)	44.7 [23.4–121.0]	−3.9 (−6.1; −1.7)[#]	−4.9 (−8.0; −1.9)[#]	0.1 (−2.2; 2.4)[*]
CV_{VT} (%)	8.8 [4.3–23.1]	8.7 (5.9; 11.5)[#]	2.2 (−0.8; 5.1)[*]	2.1 (−0.8; 5.1)[*]
RR (min^{-1})	39 [24–59]	−1 (−3; 1)[*]	1 (−2; 4)[*]	0 (−3; 3)[*]
V_E (ml min^{-1})	1,575 [980–2,993]	−177 (−246; −108)[#]	−141 (−251; −31)[##]	−9 (−102; 119)[*]
MTEF (ml s^{-1})	46.1 [27.1–80.5]	−6.5 (−8.9; −4.0)[#]	−3.4 (−7.1; 0.3)[*]	0.9 (−2.7; 4.5)[*]

Tidal breathing indices from baseline are given as mean [range], and as mean difference (95% confidence intervals) between (...) baseline and later tests. One infant woke up during breathing 100% O_2. V_T, tidal volume; CV_{VT}, intra-subject coefficient of variation from tidal volume; RR, respiratory rate; V_E, minute ventilation; MTEF, mean tidal expiratory flow. Paired t-tests:
[#]$P \leq 0.003$;
[##]$P = 0.015$;
[*]$P \geq 0.100$.

hyperoxia does not influence respiratory control in children older than 4 years,[22] but may trigger the infants' peripheral chemoreceptors which then activate the brainstem respiratory pattern generator network.[15] Hyperoxia may also induce temporary vasoconstriction of cerebral circulation, an effect that theoretically could have contributed to the observed change in ventilation by the induced local increase in tissue CO_2. Alternative pathways mediated by systemic hypercapnia seem less likely as end-tidal CO_2 levels monitored throughout the studies remained stable. During hyperoxia, the respiratory control adapted. Administration of 40% O_2 induced tolerance to hyperoxia leading to normal but slightly more variable V_T during 100% O_2 exposure. Of note, breathing pattern changed in some but not all infants (Fig. 2). Respiratory responses were heterogeneous and apparently independent of age, disease, and sedation.

Methodological Aspects of Nitrogen Multiple-Breath Washout in Infants

Previous MBW techniques based on sulfur hexafluoride or helium are safe and have been frequently used in children. However, sulfur hexafluoride is a potent greenhouse gas and not available in many countries.[1] Helium is highly volatile which increases the risk of gas
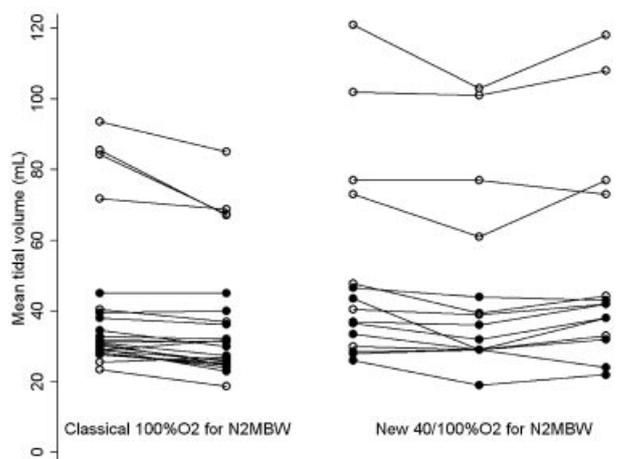
Fig. 2. Impact of the classical and new nitrogen MBW protocol on tidal volumes. Mean tidal volumes during nitrogen multiple-breath washout (N2MBW) in 23 healthy infants (closed circles) and 16 infants with CF (hollow circles) of which 8 were sedated. In the classical 1-step protocol, infants were exposed to ambient air and 100% oxygen (O_2). In the new 2-steps protocol, infants were exposed to ambient air, 40% O_2, and 100% O_2. Classical protocol on the left panel: 23 infants, 4 sedated; new protocol on the right panel: 16 infants, 4 sedated.

leakage during infant MBW. N_2MBW has regained interest for lung function testing in infants and young children as N_2MBW seems appropriate for widespread use. N_2MBW setups are available and O_2 supply is easily accessible. Modern N_2MBW setups provide online tracing of expired N_2 and flow-volume loops.[22,23] These technical features are also recommended by the new ERS/ATS consensus statement on inert gas washout testing[1] and could make the new N_2MBW protocol attractive for early CF lung disease assessment.[12,22,24,25] Previous studies[12,13] reported initial hypoventilation during classical N_2MBW (100% O_2) in healthy infants and infants with CF, bronchopulmonary dysplasia, or wheezy bronchitis. However analysis of the breathing pattern was presumably done post hoc as online V_T control in previous N_2MBW setups was not available.

Strengths and Limitations of the Study

The ventilatory response to hyperoxia was similar in older sedated sleeping CF infants and younger non-sedated sleeping CF infants. However, carotid chemoreceptor and ventilatory response to hypoxia are known to undergo significant changes early in life. Due to ethical considerations, we did not sedate younger infants as MBW success rate is excellent without sedation in this age group.[26] We acknowledge that consecutive allocation hampered the assessment of the magnitude of the ventilatory chemoreflex and possible interactions between ventilatory response and age and sedation. Allocation ensured equal distribution of healthy and diseased infants and older infants requiring sedation in both protocols.

The current N_2MBW setup is accurate for measuring infant lung volumes as recently shown in a plastic lung model.[23] Due to expected software adaptations for 40% O_2, we could not measure classical in vivo N_2MBW outcomes such as the LCI which may limit the interpretation of the current data. However, because of the complex dynamic respiratory control and the likely change in FRC, changes in, for example, LCI seem also likely (LCI = CEV/FRC).[13] Furthermore, the observed initial depression of the first few breaths when hyperoxia is administered is of importance. The depression in ventilation affects our potential to derive alternative indices of gas mixing efficiency based on phase III slopes (Sacin and Scond). The latter assumptions are supported by recent preliminary data from school-aged children suggesting a significant decrease in gas mixing efficiency upon change in breathing pattern.[27] The impact from both N_2MBW protocols on FRC and LCI in infants nevertheless remains to be determined. Before this is done, MBW tests using foreign inert tracer gases seem preferable in infants.

CONCLUSION

Taken together, we provide new perspectives for future N_2MBW infant studies. Hyperoxia for the purpose of pulmonary function testing is safe but influences ventilatory control in infants. The new 40/100% O_2 protocol comprising 40% O_2 exposure prior to N_2MBW using 100% O_2 may induce tolerance to hyperoxia. The current technique requires adaption for gradual O_2 administration in infants. We assume that continued 100% O_2 exposure, for example for 60 breaths, would similarly induce tolerance to hyperoxia but would not allow subsequent N_2MBW. The new 40/100% O_2 protocol seems an attractive option for N_2MBW in infants.

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