Normative Data for Lung Function and Exhaled Nitric Oxide in Unsedated Healthy Infants

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ABSTRACT

Despite association with lung growth and long-term respiratory morbidity, there is a lack of normative lung function data for unsedated infants conforming to latest ERS/ATS standards.

Lung function was measured using an ultrasonic flowmeter in N=342 unsedated, healthy, term-born infants at a mean±SD age of 5.1±0.8 weeks during natural sleep according to latest standards. Tidal breathing flow-volume loops (TBFVL) and exhaled nitric oxide (eNO) measurements were obtained from 100 regular breaths. We aimed for three acceptable measurements for multiple-breath washout and five to ten acceptable interruptions for resistance (Rint) measurements.

Acceptable measurements were obtained in up to 285 infants with high variability. Mean (95% limits of agreement) values were 7.48 (4.95-10.0) mL·kg⁻¹ for tidal volume, 14.3 (2.6-26.1) ppb for eNO, 23.9 (16.0-31.8) mL·kg⁻¹ for functional residual capacity, 6.75 (5.63-7.87) for lung clearance index, and 3.78 (1.14-6.42) kPa·s·L⁻¹ for Rint. In boys, TBFVL outcomes were associated with anthropometric parameters, in girls with maternal smoking during pregnancy, maternal asthma, and caesarean section.

This large normative data set in unsedated infants offers reference values for future research and particularly for studies where sedation may put infants at risk. It furthermore highlights the impact of maternal and environmental risk factors on neonatal lung function.
**Definition of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BTPS</td>
<td>body temperature (37 °C or 310 K), ambient barometric pressure, and saturated with vapour (47 mmHg or 6.2 kPa)</td>
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<td>EIMM</td>
<td>end-inspiratory molar mass</td>
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<td>eNO</td>
<td>exhaled nitric oxide</td>
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<td>Fe</td>
<td>fraction tracer gas in multiple breath washout</td>
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<td>FRC</td>
<td>functional residual capacity</td>
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<tr>
<td>FRCao</td>
<td>FRC at airway opening</td>
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<td>FRCex</td>
<td>extrapolated FRC</td>
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<td>FRCmid</td>
<td>FRC at mid-sensor point</td>
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<td>LCI</td>
<td>lung clearance index</td>
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<td>MBW</td>
<td>multiple breath washout</td>
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<td>MM</td>
<td>molar mass</td>
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<td>NREM</td>
<td>non-rapid eye movement</td>
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<tr>
<td>Pao</td>
<td>pressure at airway opening</td>
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<tr>
<td>Rint</td>
<td>interrupter resistance</td>
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<tr>
<td>SF6</td>
<td>sulphur hexafluoride</td>
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<tr>
<td>TBFVL</td>
<td>tidal breathing flow-volume loop</td>
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<tr>
<td>T_PTEF/TE</td>
<td>ratio of time to peak tidal expiratory flow (PTEF) over expiratory time</td>
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<td>V’NO</td>
<td>NO output</td>
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<td>V_t</td>
<td>tidal volume</td>
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INTRODUCTION

Early lung development is important for long-term lung growth [1, 2]. So-called tracking of lung function has been found in retrospective chart and prospective cohort studies [3, 4]. Early lung function changes may affect long-term respiratory morbidity and even mortality [5-7], necessitating longitudinal studies. Although lung function in infants is frequently measured in various ongoing cohort studies, there is still a lack of contemporary and equipment-specific normative data to identify reduced lung function, to define disease states, and to assess lung growth [8].

Technical developments, improved understanding, and increased survival of preterm infants have facilitated a more widespread use of infant lung function measurements. However, due to the time-consuming and costly nature of rigorous lung function measurements, especially in non-sedated infants, reference values exist only for small cohorts of healthy infants. Moreover, especially for multiple breath washout (MBW) tests, most lung function equipment is custom-built, limiting comparability among centres despite the availability of standards by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) [9-11]. To further complicate matters, different centres tend to prefer and present data from different techniques.

Therefore, we aim to present normative data from tidal breathing flow-volume loops (TBFVL), exhaled nitric oxide (eNO), MBW, and interrupter resistance (Rint) measurements in unselected infants of a narrow age range using standardised equipment and collected without sedation. Subsets of the lung function data have been previously published [12-21]. The aim of this manuscript is to summarise cohort data and to present a comprehensive set of reference values for all measured lung function parameters as normative and reference values in a white Middle
European population of the given age. Furthermore, we investigated how lung function in early life is related to anthropometric, perinatal, and maternal as well as environmental factors.

METHODS

Subjects
Data were collected from an ongoing prospective birth cohort of unselected, healthy neonates recruited antenatally since 1999 in the region of Bern, Switzerland, the Bern Infant Lung Development (BILD) cohort [16]. The following inclusion criteria apply: white ethnicity, term delivery (≥ 37 weeks), no known major birth defects or perinatal disease of the newborn. Known and potential confounders of lung function (demographic data, sociodemographic status, smoke exposure and parental atopic disease, being defined as either atopic dermatitis, atopic rhinitis or atopic asthma in either parent) were assessed by interviews using standardized questionnaires [22, 23]. In addition, a skin prick test including six common allergens (Dog dander, cat dander, Dermatophagoides pteronyssinus, mixed tree pollens, mixed grass pollens, Alternaria tenuis, Allergomed AG, Therwil, Switzerland) was performed in a subgroup of mothers. We validated antenatal maternal smoking history by cotinine levels in the first urine of the newborn (gas-liquid chromatography, IST, Lausanne, Switzerland). The Bernese Cantonal Ethics Research Committee approved the study protocol and written parental consent was obtained at enrolment.

Lung function
Lung function was measured in unsedated neonates during behaviourally-defined quiet natural sleep [24]. Measurements followed regular feeding of the infants, usually resulting in natural sleep in this age group. They were performed supine with the head midline, via an infant mask (for TBFVL and MBW: Size 1; Homedica AG, Huenenberg, Switzerland), according to the ERS/ATS standards of infant lung function testing [9-11] and eNO measurement [25]. Flow was
measured using an ultrasonic flowmeter (Spiroson®; EcoMedics AG, Duernnten, Switzerland). Data were included, if no apparent volume drift was present, defined as a change of less than 2 mL·s⁻¹. Measurements were always performed in the same order: 10 minutes of tidal breathing, followed by three acceptable multiple breath wash-out measurements when possible, and interrupter measurements last if the child was still asleep. None of the infants had ever been given inhaled pulmonary medication.

**Tidal breathing**

For analysis, we used the first 100 regular breaths of tidal breathing during NREM sleep from the total recording over 10 min, and excluded the first 20 to 30 breaths after mask placement to allow for adjustment of breathing pattern. In addition, sighs together with 10 breaths before and after a sigh were excluded to reduce noise [13]. Mean tidal breathing parameters of flow, volume and flow-volume loop were then calculated according to ERS/ATS standards [11]. Main outcome parameters were tidal volume (Vₜ), minute ventilation (Vₜ multiplied by respiratory rate), mean tidal inspiratory as well as expiratory flow, and ratio of time to peak tidal expiratory flow over expiratory time (Tₚ/TE) to describe the shape of the TBFVL [11, 26].

**Nitric oxide**

Simultaneous to TBFVL recording, eNO was measured online with a rapid-response chemoluminescence analyzer (CLD 77 AM; EcoMedics AG, Duernnten, Switzerland). Contamination of eNO by ambient NO was avoided by using NO-free air for inspiration. We measured eNO breath-by-breath during the third quartile of expiration and calculated mean eNO over 100 breaths correcting for expiratory flow as described previously [16, 27]. Main outcome parameters were eNO and NO output (eNO concentration multiplied with corresponding expiratory flow, V'NO).
Multiple-breath washout

Lung volume and ventilation inhomogeneity (VI) were determined using MBW technique by ultrasonic flowmeter with 4% sulphur hexafluoride (SF₆) as described previously [18]. Main outcomes were functional residual capacity at airway opening (FRCao), FRCao per body weight, and lung clearance index (LCI, cumulative expired volume divided by FRCao). Data were excluded in case of REM sleep state or the infant waking up during the MBW measurement as well as occurrence of sighs during wash-in or within 10 breaths after start of wash-out and if extrapolated FRC was > 10% of FRC at mid-sensor point (FRCₘᵢᵩ), if breath baseline was > 10% FRCₘᵢᵩ, if the tracer gas fraction was > 1% at the end of washout, if the end-inspiratory molar mass in the respective MBW measurement trace was not constant, and if there was no quiet tidal breathing prior to wash-in.

Interrupter technique

Interrupter measurements were made with a rigid face mask (Size 1, Silkomed Model 852713; Jaeger Viasys, Hoechberg, Germany) lined with putty to ensure a leak-free seal and to reduce dead space. Following stable TBFVL recordings, a series of interruptions were made once every three to six breaths. No guidelines exist at present for interrupter measurements in infants. Thus, early in the study, infants were studied with an occlusion time of 500 ms, however during longer measurements it was noted that Rint tended to increase over time. Thus, the occlusion time was later shortened to 200 ms to attempt to ensure that normal breathing patterns were not altered. Otherwise measurements were performed as described previously [28]. Data were further excluded if less than five acceptable interruptions were recorded [29]. Summary Rint data were determined from the first five to ten acceptable interruptions per measurement. Rint was calculated using the linear back-extrapolation method, applied to the trace of pressure at airway
opening (Pao) between 30 and 70 ms post-interruption [28]. Repeatability was assessed in 22 infants with in whom more than one measurement was made.

**Statistical analysis**

Descriptive statistics and regression analyses were performed using STATA 10 for Windows (STATA Corporation, College Station, TX, USA). We assessed the influence of known (sex, postconceptual age, weight and length, as well as study weight and length, and maternal smoking during pregnancy) and potential (maternal asthma and atopy, positive maternal skin prick test, paternal asthma, caesarean section) confounders by performing uni- and multivariable regression analyses. For consistency of reporting, all confounders were included in univariable regression analyses of each outcome. Multivariable models were fitted with parameters that have been significantly associated with outcomes in the univariable models, and which remained significantly associated after a backward stepwise exclusion strategy of dropping the explanatory variable with the highest p-value until only significant associations were left in the final model. A p-value <0.05 was considered significant. Due to significant associations with sex, TBVL analyses were further stratified by sex. Final multiple regression models were used to build sex-specific (TBFVL outcomes) or unisex (for all other outcomes) regression equations containing predictors or determinants of each outcome and to calculate residual standard deviations (RSDs) if significant associations with analysed explanatory variables could be found.
RESULTS

Between 1999 and 2010, the study enrolled 365 eligible infants, of whom 342 presented for lung function measurements at approximately 5 weeks postnatal age. Of these, technically acceptable data with high variability were obtained from up to 286 infants, shown in Figure 1 together with numbers of infants not presented to lung function tests, and criteria for general and specific exclusion for each measurement technique. Table 1 presents anthropometric, demographic and socioeconomic characteristics and distribution of known and possible confounders among all study participants that presented for lung function and eNO measurement at the age of five weeks before application of general exclusion criteria prior to data analysis. We did not find any differences between infants for whom measurements were done compared to those that were not presented for measurements or generally excluded (data not shown), except for a trend towards a higher proportion of mothers who smoked during pregnancy among infants that did not present for measurements (25.0% vs. 10.8%, p=0.055). None of the presented outcomes showed significant deviation from the normal distribution.

Tidal breathing

As we found significant associations with sex for most of the studied TBFVL outcomes (see Tables E.3-4 in the online supplement, OLS), analyses were also stratified by sex. Normative TBFVL data for infants irrespective of sex are given in Table 2, data for girls and boys apart are shown in Tables E.1 and E.2 in the OLS, respectively. Median% (IQR) intrasubject CVs demonstrated variability for each outcome, such as 7.5% (6.1-10.1) for minute ventilation up to 23.8% (20.2-28.4) for $T_{PTEF/TE}$, irrespective of sex. Associations of known and possible confounders with tidal breathing parameters are shown in Tables E.3-4 in the OLS without stratification, and in Tables E.5-8 after stratification for sex. Whereas TBFVL outcomes were only significantly associated with anthropometric parameters in boys, in girls we found also
associations with maternal smoking during pregnancy, maternal asthma and caesarean section. Sex-specific regression equations containing predictors or determinants of TBFVL measurements are given in Table E.9 in the OLS.

Nitric oxide
Normative data for eNO measurements are shown in Table 3. Median% (IQR) intrasubject CV was relatively large both for eNO with 10.3% (6.4-15.7) and for V’NO with 12.4% (8.9-18.1). Associations of known and possible confounders with eNO and V’NO are shown in Table E.10 in the OLS. Only minute ventilation stayed significantly associated with both eNO and V’NO in the multivariable model, whereas eNO was also associated with body length at study date and V’NO with body weight at study date in the final models. Table E.13 in the OLS shows unisex regression equations containing predictors or determinants of eNO measurements.

Multiple-breath washout
Normative data for MBW measurements are presented in Table 3, unisex regression equations containing predictors or determinants of MBW measurements are shown in Table E.13 in the OLS. MBW data showed relatively small intrasubject CVs with median% (IQR) of 6.3% (4.4-8.3) for FRCao and 5.8% (3.6-8.0) for LCI. Associations of known and possible confounders with FRCao and LCI are shown in Table E.11 in the OLS. FRCao was associated with age at study date and birth length in the final multivariable model. For LCI no associations were found.

Interrupter technique
With the mean difference between Rint measurements using the two occlusion times being significant (Table E.12, OLS) all analyses were corrected for occlusion time. Normative Rint data are presented in Table 3 and show high variability with a median% (IQR) intrasubject CV of 19.3% (14.7-25.3) irrespective of occlusion time. Rint was only significantly associated with
study age in the multivariable analysis after correction for occlusion time (Table E.12, OLS). During the assessment of median Rint repeatability, the mean difference between two successive measurements was 0.14 (95% CI -0.38–0.65) kPa·s·L⁻¹ (p=0.586), with the limits of agreement given by -2.12–2.39 kPa·s·L⁻¹ (2x SD), and without evidence of systematic bias. Table E.13 in the OLS shows unisex regression equations containing predictors or determinants of Rint measurements in infants.
DISCUSSION

Summary

To the authors’ knowledge, this is the first study in which reference values for TBFVL, eNO, MBW and Rint measurements, collected during quiet natural sleep without sedation in a prospective and homogenous cohort of healthy, unselected, and term-born infants at the postnatal age of 5 weeks, are presented. Since we assessed the data prospectively, it was possible to maintain the standards of measurements in a strictly consistent manner unchanged over this 10 years of work. We show in a large number of subjects that lung function tests without sedation are possible in the majority of infants in the first 2 months of postnatal life. The results obtained in healthy individuals display a high variability with implications for their use as normative data. In addition, we found sex-specific associations of TBFVL outcomes with gestational age, birth length, maternal smoking during pregnancy, maternal asthma but not atopy and also caesarean section in girls, whereas for boys significant associations were only found for anthropometric factors. The differences between sexes are scientifically interesting and may have their role on a population basis. But given the high within and between subject variability for most outcomes, absolute differences between girls and boys are unlikely to be of any clinical significance in individuals.

Significance and strengths of study

These equipment-specific normative data provide the basis to address questions for clinical and epidemiological research at the population level, such as lung function tracking or discrimination between disease groups. But before it may also be of use at the individual level in the future by overcoming the lack of reference values for clinical assessments, further work on interpretation of the large within-subject and intrasubject variability is clearly warranted and our work may in
addition help to prevent over interpretation of clinical findings and “abnormalities” on the individual level at this time point.

All data were collected in the same order and with the same equipment in a cohort that has a number of methodological strengths, especially with regard to the costly and time-consuming nature of lung function measurements in unsedated infants. We applied extensive quality control during data collection. Tidal breathing and eNO measurements adhere to latest standards by ERS and ATS [9-11]. Because we aimed at presenting reference values, to some extent an even more conservative approach was adopted, such as for the analysis of tidal breathing: for better accuracy we used 100 instead of 30 breaths as currently recommended [11] and excluded sighs together with 10 breaths before and after a sigh to reduce noise [13]. This approach does not affect the recommendations to use a total 30 breaths for analysis in daily practice, as long as these are not in vicinity, i.e. within 10 breaths before and after a sigh, because we have not found significant differences for TBFVL outcomes from measurements with 30 or 100 breaths but without sighs. MBW and Rint measurements were also performed according to the most recent validated recommendations [18, 29]. Because we aimed at presenting reference values, extensive quality control also included properly calibrated devices for anthropometric measures. Thus, similar careful approaches for calibration, measurement, and analyses for all measured outcomes are required if these normative data are to be applicable in other settings.

**Limitations and open questions**

As postnatal time represents a period of rapid lung growth, measuring lung function during such a narrow age range provides a snapshot of only a limited time period. However, it also enables the quantification of normative values for this specific age as well as their high variability.

For the analysis of associations of known and possible confounders with measured outcomes, we assumed linear relationships, which might be an oversimplification of their biological role. This
is especially true for the computation of regression equations, where we - given the narrow age range - have not applied further advanced methods widely used to construct reference charts over wide age ranges such as an extension of the LMS (lambda, mu, sigma) method [30]. Due to the narrow age range the equations should be used with caution and must not be extrapolated beyond measures in unsedated white infants in the first 8 weeks of life. As ethnicity has also been shown to play a role in tidal breathing patterns of young infants [31], we have applied stringent adherence to exclusion criteria including ethnicity. Thus, this set of normative data and regression equations is restricted to a white Middle-European ethnicity of the assessed age range.

In addition, we cannot exclude the possibility of bias towards a well-educated middle-class population. However, social class so far has neither been a risk factor nor a risk modifier in our past analyses, e.g. assessing the effect of traffic-related air pollution on lung function in infants [32].

**Comparison with other studies**

**Tidal breathing**

Data on lung function parameters in unsedated healthy infants using the same technique as our group are scarce. Comparable data are available from pneumotachography studies, such as a study by Lodrup-Carlsen and co-workers studying 802 awake healthy infants directly after birth (e.g. mean Vt 24.8 ml, mean \( \frac{T_{PTEF}}{T_E} \) ratio 32.0%) [33] and one by Stocks and co-workers assessing lung function in 23 full-term unsedated infants of equivalent age (mean Vt 36.1 ml, mean \( \frac{T_{PTEF}}{T_E} \) ratio 32.5%) [34]. Our data also compares favourably with studies assessing the \( \frac{T_{PTEF}}{T_E} \) ratio, supporting the idea of a slowly decreasing \( \frac{T_{PTEF}}{T_E} \) ratio during the first year of life although these studies use several techniques with and without sedation over a wide age range [35].
We found a significant association of maternal asthma on tidal inspiratory flow in girls, but otherwise no further effect of parental atopy or positive maternal skin prick test on lung function in our infants. Also here, comparable data are scarce, but our data are partly in contrast with other studies, mostly undertaken in high risk infants, where positive associations with parental atopy and subnormal lung function in infants could be found [36].

Several studies have reported reduced lung function in infants whose mothers smoked during pregnancy [37-39]. We were not able to replicate this finding without stratification for sex. This is most probably due to our low prevalence of approximately 10% of smoking mothers during pregnancy, validated by cotinine measurements in the urine. However, we found an effect of maternal smoking during pregnancy for several tidal breathing parameters in girls. This can be interpreted as worsened lung function with higher respiratory rate, lower tidal volume and in addition also a higher $T_{PTE}/T_e$ ratio demonstrating an adaptive response to altered lung mechanics in unsedated infants. Gender differences with regard to the effect of smoking on lung function are known in older children and in sedated infants [40, 41], but to the author’s knowledge not in unsedated infants to date. This is despite intense research, especially on the effect of genetic risk factors on the impact of prenatal ETS exposure on lung function in infants, for which these results would be highly relevant [42].

In addition, we present a small but significant influence of caesarean section on respiratory rate in our final model for girls. This is in contrast to a study where no effect was found in 24 awake healthy infants at the age of two to four days of life for the $T_{PTE}/T_e$ ratio after this being initially decreased on day one [43]. The differences may be due to different measurement techniques and numbers, and especially the different age at measurement. Current knowledge relates respiratory morbidity immediately after birth in children born per caesarean section partly to the failure to
clear fetal lung fluid. In contrast to immediate effects such as transient tachypnea of the newborn and surfactant deficiency, long-term physiological effects are so far unknown [44].

Nitric oxide

eNO plays an important role as a measure of airway inflammation. Past findings from subsets of this cohort have shown eNO to precede respiratory symptoms in newborns of atopic or smoking mothers [16] and to be elevated in infants exposed to traffic-related air pollution (0.98 ppb per μg·m⁻¹ nitrogen dioxide) [32]. So far, eNO has been measured using both single and multiple breath techniques, each with their own methodological issues [45]. There are only sparse eNO data available from unselected, healthy and unsedated infants collected using a common technique and adhering to standards. Our data are lower than those collected using a single breath method in older healthy infants (mean 18.8 ppb) [46], and with a median (IQR) of 14 (10.7-17.4) ppb also lower than those collected in a high-risk cohort of infants at comparable age using the offline method with a median (IQR) of 16 (12-22) ppb [47]. Further influencing factors such as ambient NO but also food, beverage or medication intake can be neglected in our study population of healthy and breast-fed infants. Breast-feeding has been shown to have no effect on eNO levels in infants [48]. As all infants with respiratory tract infection were excluded, this influence on eNO is also negligible. Except for anthropometric factors, we could not find further associations with eNO or V’NO levels, including family history of atopic disease in the whole cohort.

In older children, oral eNO has been shown to be significantly lower than mixed (oral and nasal) eNO (geometric means 4.5 and 10.5 ppb, respectively) [48]. The same is true for lower airway and nasal levels of eNO [49]. However, at the age of our study participants, paranasal sinuses have if at all only rarely developed. For infants slightly older than our study participants, there has not been any difference between strictly oral or nasal eNO [50]. Although mixed eNO has
been shown in infants to differentiate health from a variety of airway diseases [27, 50, 51], this must be considered before using our normative values as reference.

Multiple-breath washout

Gas mixing techniques such as MBW primarily reflect small airways and effect of disease or other factors on changes in lung size and the degree of VI [52]. Also here normative data for healthy unsedated infants are scarce. In addition to previous data from subsets of our cohort [17, 18, 20], Hulskamp and co-workers published normative values from a multicenter study also including 64 healthy infants of mixed ethnicity and with a mean post gestational age of 4 weeks, thus slightly younger than our study participants [53], but collected with the same technique in unsedated infants during quiet natural sleep, using similar quality control measures [18]. Compared to our data, they found slightly higher mean(SD) values for LCI with 7.17 (0.45) and lower ones for FRCao per body weight with 18.4 (3.6) mL·kg⁻¹. As Hulskamp and co-workers performed measurements in younger infants from a multi-centre study with mixed ethnicity and appreciated inter-center differences [53], this is most probably due to the different populations measured. Also, although the same technique and identical quality control and acceptability criteria were used, small differences due to different hard- and software settings cannot be excluded.

Interrupter technique

Comparable data from infants are again scarce. The median Rint value from this study was higher than that obtained by Chavasse and co-workers in healthy sedated infants with a mean(SD) of 2.94 (0.68) kPa·L·s⁻¹ [54], in whom upper airway mechanics are likely more relaxed and control of breathing different. Furthermore, Chavasse and co-workers used a shorter occlusion time of 100 ms. Our study compares favourably with the calculated Rint from reference equations obtained from infants and children from 3 weeks to 15 years of age [55].
It is difficult to determine the reason for the difference we saw between occlusion times. While the effect of various occlusion parameters on interrupter resistance has been investigated, there have been no studies examining the effect of interruption time. One possible explanation is the alteration of breathing mechanics with longer interruption times, affecting control of breathing, and slightly increased humidity or hypercapnic conditions in the airways due to increased extent of flow occlusion. In addition to the increased Rint with increased interruption time, we observed that Rint also increased slowly with time during longer interrupter measurements (data not shown). Alternatively, the longer interruption time allowed for more muscle relaxation and pressure equilibration, although this can be discounted since evidence of steady state in airway opening pressure was seen in all included measurements. Recommendations for infant Rint measurement also covering interruption time are clearly warranted, and in the paucity of studies examining these issues [28], it appears that a shorter interruption time would reduce the risk of altered breathing mechanics and increased resistance.

**Conclusions**

Despite their time-consuming and costly nature, lung function measurements in unsedated infants shortly after birth are feasible. This enables determination of lung growth and influencing factors during the vulnerable phase of lung development before birth and the first few weeks of life. These have been shown to be crucial, also with regard to later pulmonary morbidity. The presented normative data adhere to latest standards and were collected under extensive quality control. The sex-specific associations of covariates with lung function provide the basis to address research questions at the population level, such as the tracking of lung function, discrimination between disease groups, and the effect of influencing factors on lung function and airway inflammation without the influence of sedation.
At the individual level, our data also address the lack of reference values for the clinical assessment of lung function, one of the major barriers to its routine implementation. Collecting reference values in unsedated infants is particularly needed for studies in infants suffering from lung disease in whom sedation may put the individual infant at risk. The high within and between subject variability of normative values in healthy term infants may help explain why there is still no convincing evidence for the clinical utility of infant lung function techniques in the individual clinical decision making process. However, our data may provide the basis for future studies addressing the issue of interpreting the large within-subject and intrasubject variability. They may in addition prevent current mis- or over interpretation of clinical findings on the individual level and may help to prepare ground for using infant lung function in clinical routine such as to assess effects of clinical interventions.
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COMPETING INTERESTS

None

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TABLES

Table 1. Anthropometric, demographic and socioeconomic characteristics and distribution of known and possible confounders among study participants presented for lung function (n=342).

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<td>54.8±2.3</td>
<td>54.6</td>
<td>53.0–56.5</td>
<td>48.0–61.5</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>39.7±1.3</td>
<td>39.9</td>
<td>38.9–40.6</td>
<td>35.3–41.1</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.4±0.4</td>
<td>3.4</td>
<td>3.1–3.6</td>
<td>2.2–4.9</td>
</tr>
<tr>
<td><strong>Demographic and socioeconomic characteristics, distribution of known and possible confounders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male Sex</td>
<td>188 (55.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One older sibling</td>
<td>104 (30.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two or more older siblings</td>
<td>57 (16.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal asthma</td>
<td>38 (11.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal atopy</td>
<td>122 (35.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive maternal skin prick test(^2)</td>
<td>123 (39.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal smoking in pregnancy</td>
<td>37 (10.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High maternal education(^3)</td>
<td>229 (68.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High paternal education(^4)</td>
<td>265 (79.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td>55 (16.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)all data presented here refer to study participants presented for lung function tests before application of general exclusion criteria prior to data analysis (see Figure 1).
\(^2\) missing data on n=34, data available for n=308 mothers
\(^3\) missing data on n=7, data available for n=335 mothers
\(^4\) missing data on n=10, data available for n=332 fathers
Table 2. Normative lung function values from acceptable tidal breathing measurements for n=285 infants at 5 weeks of age without stratification for sex.

<table>
<thead>
<tr>
<th>Tidal breathing parameter (n=285 infants)</th>
<th>Mean±SD</th>
<th>95% Limits of Agreement(^1)</th>
<th>Range</th>
<th>Intrasubject CV(^2) Median (IQR) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minute ventilation mL·min(^{-1})</td>
<td>1420±277</td>
<td>876–1960</td>
<td>733–2370</td>
<td>7.5 (6.1–10.1)</td>
</tr>
<tr>
<td>Minute ventilation per body weight mL·min(^{-1})·kg(^{-1})</td>
<td>328±65</td>
<td>201–455</td>
<td>174–613</td>
<td></td>
</tr>
<tr>
<td>Respiratory rate min(^{-1})</td>
<td>45.2±10.5</td>
<td>24.6–65.7</td>
<td>24.7–78.7</td>
<td>9.1 (7.4–11.3)</td>
</tr>
<tr>
<td>Tidal volume mL</td>
<td>32.4±5.5</td>
<td>21.7–43.1</td>
<td>21.0–51.1</td>
<td>8.6 (7.1–10.8)</td>
</tr>
<tr>
<td>Tidal volume per body weight mL·kg(^{-1})</td>
<td>7.48±1.29</td>
<td>4.95–10.0</td>
<td>4.28–11.8</td>
<td></td>
</tr>
<tr>
<td>Tidal expiratory flow mL·sec(^{-1})</td>
<td>42.7±10.2</td>
<td>22.8–62.7</td>
<td>20.0–74.9</td>
<td>9.4 (7.4–12.5)</td>
</tr>
<tr>
<td>Tidal inspiratory flow mL·sec(^{-1})</td>
<td>54.4±9.7</td>
<td>35.4–73.3</td>
<td>30.0–84.0</td>
<td>8.1 (6.8–10.5)</td>
</tr>
<tr>
<td>Ratio TPTEF/TE %</td>
<td>34.8±10.7</td>
<td>13.8–55.9</td>
<td>14.2–73.3</td>
<td>23.8 (20.2–28.4)</td>
</tr>
</tbody>
</table>

\(^1\)95% Limits of Agreement calculated as mean±1.96*SD

\(^2\)Intrasubject coefficients of variation (CV) were calculated as the ratio of standard deviation for each outcome parameter over each mean value of outcome parameter per study participant. Due to positively skewed distributions, the table presents the overall median and interquartile range for all intrasubject CVs.
Table 3. Normative lung function values from MBW, eNO and Rint measurements at 5 weeks of age without stratification for sex, numbers of study participants with valid measurements given in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>95% Limits of Agreement</th>
<th>Range</th>
<th>Intrasubject CV&lt;sup&gt;6&lt;/sup&gt; Median (IQR) %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory markers (n=261)&lt;sup&gt;2&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eNO ppb</td>
<td>14.3±6.0</td>
<td>2.57–26.1</td>
<td>0.9–35.0</td>
<td>10.3 (6.4–15.7)</td>
</tr>
<tr>
<td>NO output nL·sec&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.63±0.25</td>
<td>0.14–1.12</td>
<td>0.03–1.57</td>
<td>12.4 (8.9–18.1)</td>
</tr>
<tr>
<td><strong>Multiple-breath washout (n=201)&lt;sup&gt;3&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRCao mL</td>
<td>102±16</td>
<td>70.0–134</td>
<td>65.8–145</td>
<td>6.3 (4.4–8.3)</td>
</tr>
<tr>
<td>FRCao per body weight mL·kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>23.9±4.0</td>
<td>16.0–31.8</td>
<td>13.0–35.8</td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>6.75±0.57</td>
<td>5.63–7.87</td>
<td>5.51–8.62</td>
<td>5.8 (3.6–8.0)</td>
</tr>
<tr>
<td><strong>Interrupter resistance kPa·s·L&lt;sup&gt;-1&lt;/sup&gt; (n=102)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occlusion time = 500 ms (n = 21)</td>
<td>4.31±1.34</td>
<td>1.68–6.94</td>
<td>2.69–7.56</td>
<td>16.6 (10.4–20.2)</td>
</tr>
<tr>
<td>Occlusion time = 200 ms (n = 81)</td>
<td>3.64±1.32</td>
<td>1.05–6.23</td>
<td>1.43–7.72</td>
<td>20.5 (15.9–25.9)</td>
</tr>
<tr>
<td>All</td>
<td>3.78±1.35</td>
<td>1.14–6.42</td>
<td>1.43–7.72</td>
<td>19.3 (14.7–25.3)</td>
</tr>
</tbody>
</table>

1<sup>95% Limits of Agreement calculated as mean±1.96*SD</sup>
2<sup>proportion of male infants for all 261 measurements 55.9%.</sup>
3<sup>n=105 acceptable MBW measurements with at least 3 tests each used for calculation of intra-subject variability of FRCao and LCI, proportion of male infants for all 201 measurements 48.8%.</sup>
4<sup>proportion of male infants for all 103 measurements 56.9%.</sup>
5<sup>The mean±SD number of interruptions used to calculate these summary data was 9.6 ± 1.2 interruptions.</sup>
6<sup>Intrusubject coefficients of variation (CV) were calculated as the ratio of standard deviation for each outcome parameter over each mean value of outcome parameter per study participant. As all presented outcomes except for LCI, for which there was a negatively skewed distribution, showed positively skewed distributions for intrasubject CVs, the table presents the overall median and interquartile range for all intrasubject CVs.</sup>
FIGURES

Figure 1. Numbers of valid tests of lung function and eNO measurements and of datasets excluded from analyses.
FIGURE LEGENDS

Figure 1.

1 n=11 infants, whose parents were not interested in lung function tests, and n=3 infants, who were sick and for whom no auxiliary data could be found, still took part in other sections of the cohort study.

2 for n=4 infants with gestational age < 37 weeks, lung function and eNO were measured despite non-eligibility but data were excluded for analyses.

3 for n=18 infants with either one or both parents of non-white ethnicity, lung function and eNO were measured despite non-eligibility but data were excluded for analyses.

4 at time-point of Rint measurements, only n=140 were still asleep and data were not excluded due to general exclusion criteria.

5 one infant had a very high median Rint value of 14.37 ± 3.61 kPa·s·L⁻¹ and was excluded as outlier.

Abbreviations: Fe: fraction tracer gas, FRC_{extrapol}: extrapolated FRC, FRC_{mid}: FRC at mid-sensor point, EIMM: end-inspiratory molar mass (in molar mass trace of MBW measurement).
REFERENCES


