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Intra-breath changes of respiratory mechanics in healthy infants by oscillometry: role of the upper airways

PhD Thesis

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List of papers underlying the PhD thesis:

I. **Radics, BL**., Makan, G., Coppens, T., André, N., Page, C., Dégrugilliers, L., Bayat, S., Gingl, Z., Gyurkovits, Z., M Tóth, T., Hantos, Z., & Bayat, S. (2020). Effect of nasal airway nonlinearities on oscillometric resistance measurements in infants. *J Appl Physiol* (1985), 129(3), 591-598. doi: 10.1152/japplphysiol.00128.2020.
IF: 3.531; SJR: Q1/Q2

II. **Radics BL**, Gyurkovits Z, Makan G, Gingl Z, Czövek D, Hantos Z. (2022) Respiratory oscillometry in newborn infants: conventional and intra-breath approaches. *Front Pediatr*. 4;10:867883. doi: 10.3389/fped.2022.867883.
IF: 2.6; SJR: Q1

List of papers related to the subject of the thesis:

III. Hantos, Z., Czövek, D., Gyurkovits, Z., Szabó, H., Maár, B., **Radics, B**., Virág, K., Makan, G., Orvos, H., Gingl, Z., & Sly, P. (2015). Assessment of respiratory mechanics with forced oscillations in healthy newborns. *Pediatr Pulmonol*, 50(4), 344-52.

IV. Czovek, D., Shackleton, C., Hantos, Z., Taylor, K., Kumar, A., Chacko, A., Ware, R., Makan, G., **Radics, B**., Gingl, Z., & Sly, P. (2016). Tidal changes in respiratory resistance are sensitive indicators of airway obstruction in children. *Thorax*, 71(10), 907-915.

V. Lorx, A., Czovek, D., Gingl, Z., Makan, G., **Radics, B.**, Bartusek, D., Szigeti, S., Gal, J., Losonczy, G., Sly, P., & Hantos, Z. (2017). Airway dynamics in COPD patients by within-breath impedance tracking: effects of continuous positive airway pressure. *Eur Respir J*, 49(2): 1601270.

VI. Shackleton, C., Czovek, D., Grimwood, K., Ware, R., **Radics, B**., Hantos, Z., Sly, P. (2018). Defining 'healthy' in preschool-aged children for forced oscillation technique reference equations. *Respirology*, 23(4):406-13.

VII. Gray, D., Czovek, D., McMillan, L., Turkovic, L., Stadler, J., Vanker, A., **Radics, B**., Gingl, Z., Hall, G., Sly, P., Zar, H., & Hantos, Z. (2019). Intra-breath measures of respiratory mechanics in healthy African infants detect risk of respiratory illness in early life. *Eur Respir J*, 53(2): 1800998.

INTRODUCTION

The burdens of infant pulmonary function testing (PFT) imposed by the lack of active cooperation, the requirement for natural (unsedated) sleep, the obligatory nasal breathing, the high mechanical impedance of the respiratory system (Z_{rs}) and several other factors have prevented the establishment of a gold standard in infant PFT. Most data on lung function are derived from studies focusing on preterms or infants with current or previous respiratory diseases and data on healthy term newborns or infants are scarce. The use of numerous PFTs and the insufficient standardisation resulted in low level of comparability of the data on infant lung function. The other major source of bias of infant PFTs originates from the high natural variability of spontaneous tidal breathing and lung mechanics in newborns and infants.

Tidal breathing and lung mechanics are highly variable in infants

Almost every infant PFT found great intra- and interindividual fluctuations of different measured variables. Unstable functional residual capacity (FRC) is a normal feature of the newborn lung. It originates from the imbalance of the low outward recoil of the soft chest wall and the high inward recoil of the immature lung. Newborns maintain their FRC with active respiratory control mechanisms that are all influenced by the different behavioural and sleep states.

Upper airway rheology - a neglected source of variability in the measurement of respiratory system mechanics

The contribution of nasal resistance (R_n) to that of the respiratory system resistance (R_{rs}) in infants has been studied previously. Although some discrepancy in the estimates of the contribution of R_n to R_{rs} may be attributed to differences in methodology and population, measurement and interpretation of R_n is profoundly complicated by the anatomical irregularity and complex flow rheology of the nasal passages. The nonlinear pressure (P) - flow (V') relationship in the upper airways contributes to the dynamic changes in R_{rs}, which has been well-documented with intra-breath oscillometry in adults, children and infants. Measurement of R_{rs} at points of zero V' (namely at end expiration and end inspiration, R_{eE} and R_{eI}, respectively) minimizes the nonlinear contribution of the nasal pathways and the consequent bias in the estimation of R of the lower respiratory tract. Intra-breath oscillometry is a unique lung function technique that is able to identify these zero-flow values of R_{rs}.

Oscillometry as a promising PFT in infants

Recent work has demonstrated its feasibility in normally breathing unsedated infants with a high success rate [1]. Additionally, a new tracking modality of oscillometry has revealed disease-specific patterns of intra-breath changes in Z_{rs} and has proven unique in predicting lower respiratory tract illness during infancy. However, a comprehensive analysis is still needed to fully characterize the intra-breath dynamics of Z_{rs} in infants, with special regard to the substantial contribution of the upper airways. Confrontation of the novel intra-breath oscillometry with conventional spectral oscillometry is also lacking. While some data on the day-to-day Z_{rs} changes in newborn infants are available, the within-session reproducibility of oscillometry measures has not been studied.

AIMS

The current PhD work aimed to get a better insight in the confounding effects of upper airway rheology on the results of respiratory oscillometric measurements. Our basic assumption was that the V'-dependent nonlinear change in Z_{rs} originates primarily from the upper airways. We conducted two studies utilizing the V'-dependent changes in Z_{rs} to estimate the contribution of upper airways to total respiratory system mechanics.

Study 1

The aim of the first study was to use intra-breath oscillometry in anatomically faithful casts of the nasal pathways of infants from birth to 2 years, reconstructed from computed tomography (CT) images. We assessed the lowest oscillatory R without superimposed breathing (R_0), as well as the effect of varying V' on R_n by simulating breathing through the casts. We characterized the relationships of R_n to V' and volume acceleration (V"). Based on these relationships, we propose a graphical method to correct the error in intra-breath oscillometric R_{rs} measurements that results from V'-dependent nonlinearities. The relative contribution of these nonlinearities to R_{rs} was further assessed in a set of intra-breath oscillometric measurements in infants.

Study 2

The aims of the second study were a) to measure Z_{rs} in healthy term newborns to characterize the physiological (V')- and volume (V)-dependent changes via intra-breath oscillometry, b) to examine the potentially confounding effects of intra-breath changes on average Z_{rs} spectra obtained from conventional multi-frequency measurements and c) to

determine the within-session variability of conventional and intra-breath oscillometry variables.

METHODS

Study 1

Study population

Data of head CT examinations of infants from birth to 24 months, available in the Department of Radiology of the Amiens University Hospital database, performed between October 2011 and August 2014, were collected for analysis. Requirement for written informed consent was waived for this retrospective study by the Internal Review Board of the Amiens University Hospital. Cases not including the entire nasal passages from the nares to the tip of the epiglottis, and those with craniofacial abnormalities or upper airway mass based on the radiologist's report, were excluded. Forty-six image sets met these criteria.

Image analysis

All images were anonymized for analysis. The nasal airway structures were segmented from the nares to the epiglottis, using an active contour algorithm in itk-SNAP software. The accuracy of the segmentation was visually examined and manually edited when necessary by an Ear-Nose-Throat specialist. The resulting segmented image was converted to a mesh. A 3-dimensional model of the nasal airways was then produced with a 3D printer with polylactide.

The nasal airway passages were segmented and the volume, surface area, and various shape descriptors were measured with Imagej software, using the '3D Imagej Suite' plugins.

Experimental setup

Rheological measurements of the upper airway casts were performed with custom-made equipment. Baseline measurements were recorded with the oscillatory signal only, without simulated breathing (R_0). Tidal excursions were generated by a loudspeaker at three different respiratory rates (33, 45 and 57 cycles·min^{-1}). An estimated V_T (7 ml/kg) was applied at each rate. Measurements were repeated at half of the estimated V_T ($V_T/2$). Small-amplitude (0.5 hPa) oscillations at 16 Hz and the superimposed simulated breathing pattern were generated by the loudspeaker. The combined V' signal was delivered to the pharyngeal opening of the cast via a custom-made pneumotachograph and a modification of the wave-tube oscillometer for infants measuring the input impedance of the naso-pharyngeal casts.

Data analysis

Input impedance of the cast (Z_n) was calculated based on the auto- and cross-power spectra of the wave-tube's lateral pressures using the fast Fourier transform. Z_n is expressed in terms of resistance (R_n) and reactance (X_n). V was integrated from V' using the trapezoidal approach, while V" was calculated as the derivative of V' from individual datapoints, by simple one-sided difference quotients.

Estimation of Reynolds number (Re)

The airway volume (V_{aw}) of each cast was divided by the characteristic pathway length (l) resulting in a rough estimate of average cross-sectional area (A). Volumetric flow rate at breakpoint or at peak V' was divided by A, to obtain linear velocity (u). *Re* was calculated with the equation for smooth cylindrical tubes, taking kinematic viscosity (ν) of air as $1.849 \cdot 10^{-5}$ $m^2 \cdot s^{-1}$ (at 25 °C).

In vivo measurements

Oscillometric measurements of the respiratory system impedance (Z_{rs}) were performed in 15 healthy term newborns with the custom-made wave-tube setup. These subjects formed a subgroup of the newborns whose study protocol is specified below (Study 2). A 16-Hz sine wave was applied as the oscillatory signal, and Z_{rs} was estimated with the same signal processing technique as that used in the casts (see above) and expressed as total respiratory resistance (R_{rs}) and reactance (X_{rs}). The measurements were performed before the 4^{th} postpartum day, during quiet natural sleep. Exclusion criteria were: 1- poor cooperation; 2- lack of steady-state breathing; 3- nasal obstruction before or during measurement; 4- leakage around the face mask during measurement; 5- laryngeal breaking or inspiratory flow limitation detected during the measurements. A minimum of 5 steady-state, artefact-free breathing cycles was taken for analysis.

Statistical analysis

Data are expressed as median (interquartile range) unless stated otherwise. Relationships between R_n and V" and V' were examined by linear regression for expiratory and inspiratory data. To characterize the relation between R_n and V', two separate linear equations were fitted after logarithmic transformation below and above a characteristic breakpoint using the standard built-in and 'segmented' (1.0-0) packages in R 3.5.1 software. A p-value < 0.05 was considered as significant.

Study 2

The study protocol was approved by the Institutional Clinical Ethics Committee of the University of Szeged (91/2011, renewed in 2017). Written informed consent and assent were obtained from all mothers prior to the subject recruitment. The data collection period started in January 2017 and ended in May 2017. All measurements were performed in the Neonatal Unit, Department of Obstetrics and Gynaecology, University of Szeged.

Healthy term infants (>37th week of gestation, birthweight >2500 g, APGAR score at 5 minutes ≥8, uninterrupted early adaptation) were included in the study. Lung function was measured between the 2nd-5th postpartum day on a single occasion, during natural sleep. Exclusion criteria are detailed at the "*In vivo measurements"* section of Study 1.

Measurement setup

Oscillometric measurement of input Z_{rs} was made with a custom-made wave-tube setup, in a setting similar to that described previously [1]. Spectral oscillometric recordings were 30 s long, and a 8-48 Hz pseudorandom signal was applied. Intra-breath oscillometric recordings lasted for 90 s, and a single 16-Hz sinusoid was used. Multiple measurements were performed with both modalities in random order, without removing the face mask between recordings if the sleep stage was uninterrupted.

V' was measured with a custom-made pneumotachograph. Single-use bacterial filter and face mask were attached to the setup. The equipment's dead space was flushed with medical air at a rate of 2 L.min^{-1} to avoid hypercapnia.

Transcutaneous monitoring of peripheral haemoglobin oxygen-saturation was done during the recordings for safety reasons. No desaturation episode was detected during data collection.

Analysis of Z_{rs} spectra

An average spectrum was calculated from a minimum of 3 recordings of lowest R_{rs}. Recordings or segments thereof containing artefacts, such as glottis closure, vocalisation, body movements and leaks around the mask were discarded. No criteria relating to tidal volume (V_T) were set and sighs *per se* were not considered as artefacts. A simple resistance (R) - compliance (C) - inertance (L) model was fit to the average Z_{rs} data. Conventional spectral oscillometry measures, such as the lowest-frequency (8-Hz) values of Z_{rs} magnitude ($|Z_8|$), resonance frequency (f_{res}) and reactance area below f_{res} (Ax) were also calculated; the frequency dependence of R_{rs} was characterized by the difference in R_{rs} between 8 Hz and 32 Hz (R_{8-32}).

Intra-breath measures

All regular artefact-free breaths except sighs were included in the analysis. Specific points of the respiratory cycle were selected to characterize the intra-breath dynamics of Z_{rs}. R_{eE} and R_{eI} were calculated from the closest data points to zero V' obtained with linear interpolation. Peak-to-peak changes in R_{rs} during inspiration (R_{ppI}) and expiration (R_{ppE}) were determined. The corresponding parameters of X_{rs} (X_{eE}, X_{eI}, X_{ppE} and X_{ppI}) and the average zero-flow impedance magnitude, $|Z_0|=|½(Z_{eE}+Z_{eI})|$ were also calculated.

Tidal breathing parameters

Simple tidal breath descriptors, such as V_T, respiratory rate (f_{br}), ratio of expiratory time over cycle time (T_E/T_{tot}), and the ratio of time to peak expiratory flow (V'_{maxE}) and T_E (T_{PTEF}/T_E) were obtained from the spirogram. Volume acceleration at end-expiration and end-inspiration (V''_{eE} and V''_{eI}, respectively) were determined from pairs of V'' data adjacent to the zero crossing.

Statistical analysis and graphics

Data are presented as mean ± standard deviation (SD). Two sample t-tests, ANOVA and correlation analysis with Pearson's correlation coefficients were performed with the open-source RStudio software based on R language (R.4.1). Graphs were made in SigmaPlot 12.5.

RESULTS AND DISCUSSION

Study 1

A total of 45 of the 46 casts were measured successfully (37 females, 9 males). The median *post partum* age of the patients was 25 weeks (9 – 46); their weight was 6.5 kg (5.4 – 8.9) and their height was 59 cm (57-62).

One cast was excluded because of apparent bilateral nasal obstruction, confirmed by re-assessment of the CT images, which resulted in extremely high R_n values.

1.1 R_{eE} is systematically higher than R_0 in the upper airway casts during simulated breathing. V'' is responsible for the increase in R_n at V'=0.

Intra-breath changes in R_n and X_n as functions of V' and V'' are illustrated in Figure 1. R_{eE} was shifted to higher values from R_0 even at the smallest V''. For all casts, a median value of shift in R_{eE} of 47% (41-52%) was observed at the lowest and 266 % (114-669%) at the highest respiratory rate and V_T, respectively. A very strong linear relationship was observed between R_{eE} and V''_{eE} in each set of cast measurements (r^2=0.99 (0.98-1.00) , $p<0.001$) with

considerable inter-individual variability in the coefficients of regression. The intercept of the linear regression was found to be a close estimate of R_0, characterized by a median of relative bias of -4.5% (-12 – 6%).

If the nasal pathway acted as a pure linear resistor, the R_{eE} would be independent from respiratory rate and V' and would thus be equal to R_0. Direct measurement of R_{eE} as well as R_{eI}, are biased by the extra dissipation due to unstable V' arising in the nasal pathway at phase transitions. This phenomenon was described previously in tracheal models by Isabey and coworkers [2].

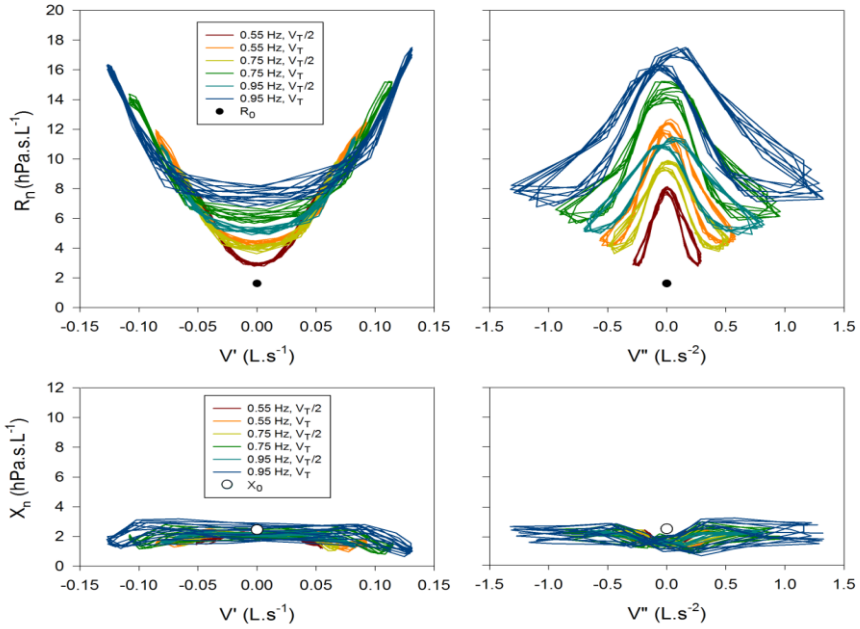

Figure 1. *Resistance (R_n) and reactance (X_n) plotted against flow (V') (left) and volume accelaration (V") (right) from a representative cast. Each set of coloured loops (see insets) corresponds to preset breathing frequencies and tidal volumes (V_T). Filled and empty circles indicate the lowest oscillatory R (R_0) and X (X_0), respectively, determined without tidal flow (V'=0).*

1.2 The intra-breath relationship of R_n and V' is nonlinear and can be described with a segmental power-law model. V" can alter the shape of R_n – V' loops.

A characteristic nonlinear relationship was found between R_n and V', exhibiting segmental linearity and a prominent breakpoint after logarithmic transformation (Figure 2). Similar piecewise linear R_{rs} *vs* V' relationships were observed in the *in vivo* measurements.

The adjusted r^2 values for the fitted piecewise linear model were 0.98 (0.97-0.99) for the expiratory and inspiratory phase (all frequencies and volumes are included). V' at the breakpoint (V'_{bp}) during the expiratory phase had a median value of 41 $mL \cdot s^{-1}$ (28-58) for the entire dataset. V'_{bp} was found to be linearly related to V" (r^2=0.96, p<0.001). The *in vivo* data exhibited a similar V'_{bp} *vs* V" relationship at slightly lower values of V'_{bp}.

This non-linear behaviour of the P-V' relationship in the nasal airways may be related to a developing non-laminar flow regime [2], [3] and to singular pressure losses at abrupt narrowings, i.e. the so-called orifice effect. Increasing V'' may affect flow rheology by the re-laminarisation of turbulent flow [4].

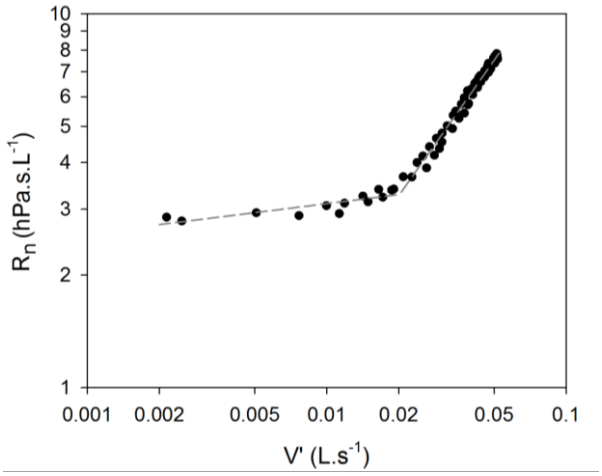

Figure 2. *Log-log plot of resistance (R_n) vs absolute value of flow (V') during expiration from a representative measurement. A segmented linear model with a breakpoint is fitted to the data, indicating power-law relationship with two exponents: a lower exponent for the first segment and a higher exponent for the second segment (below and above breakpoint, respectively).*

1.3 There is a moderate correlation between structure and oscillatory mechanics of upper airway casts.

The estimated *Re* at maximal V' was 118 (91-155), while *Re* at V'_{bp} was 61 (47-81). The *Re* at V'_{bp} (*Re*(V'_{bp})) was found to be inversely proportional to the sparseness of the airway ($r^2 = 0.43$, $p<0.001$) and directly proportional to its flatness ($r^2 = 0.31$, $p<0.001$).

Upper airway casts with higher sparseness had a relatively spacious cavity with less wall irregularities and lower surface/volume ratio. Flatness on the other hand is an indicator of relative narrowness of the nasal passage, mimicking a slit between two infinite parallel plates. Sparse but not flat nasal cavities, acting as wide cylinders, had low *Re*(V'_{bp}) values, suggesting that this geometry is advantageous for the development of turbulent flow.

1.4 The confounding effect of V'' can be eliminated from R_{eE} with a simple geometrical method *in vitro*.

To estimate R_0 based on the intra-breath R-V' loops, the slope of a fitted line was determined between R_{eE} and R_n at maximum inspiratory flow ($R_{V'maxI}$). The slope of this linear fit remained constant across the different respiratory rates and V_T's. When this linearly approximated V'-dependent increase in R_n was compared to the V''-dependent shift in R_{eE}

strong linear relationship (r^2=0.94, p<0.001) was found. The same correlation was found for the expiratory phase. By utilizing this relationship, R_0 can be estimated from a single intra-breath impedance measurement:

$$R_0 = R_{eI} - V''_{eI} \times c \times \frac{R_{V'maxE} - R_{eI}}{V'_{maxE}} \quad .$$

where *c* is a correction factor with a value of 0.0646 s^{-1} (determined from the expiratory limb). The estimated R_0 had a relative error of 5.4 % ± 27.8 % using the simple geometrical method described above.

This estimation can be applied to a single set of intra-breath oscillometric measurements, without the need for changing respiratory rate or V_T, as performed in our simulations.

1.5 The V'- and V"-dependent intra-breath changes in X_n are relatively small in the rigid upper airway casts.

X_0 had positive values in all casts, indicating the presence of inertance in the rigid upper airway casts - an expected significant property of the upper airways. The overall shape of X_n-V' loops mirrored the pattern of R_n-V' loops (Figure 1); however, the changes of X_n were smaller and not as regular as seen for R_n. End-expiratory X_n (X_{eE}) was found to be somewhat lower than X_0, indicating the influence of V" on X_n. X_n was found to be V'-dependent: the lowest values of X_n were measured at peak inspiratory and expiratory flows ($X_{V'maxI}$ and $X_{V'maxE}$).

The V'-dependent decrease in X_n can be explained by the decrease in apparent inertance caused by the vortex formation at orifices [5].

Study 2

A total of 109 newborns were enrolled in the study. Six subjects were excluded due to technical reasons (see pre-defined exclusion criteria in the Methods section). Although the measurements were technically acceptable, 17 of the remaining 103 subjects were excluded on the basis of physiologically unrealistic values of Z_{rs} parameters, such as negative L (n=4), low C (n=6) or high RL product suggestive for nasal obstruction (n=7); in 4 of these 17 subjects, two exclusion criteria applied.

Statistical analysis was performed on the data of the remaining 86 newborns (41 females, 45 males; spontaneous delivery: 41, caesarean section: 45). The birth weight was (mean ± sd) 3269 ± 546 g, the body length was 49 ± 2 cm. The gestational age at birth was 38.7 ± 1.3 weeks.

On the average, 48 (range: 15-105) respiratory cycles were analysed from the intra-breath oscillometry in each newborn- The average values of spectral outcomes were calculated from 6 (3-11) recordings of a mean length of 26 s (12-30 s).

The 94% success rate in the present study confirms earlier observations on the feasibility of oscillometry in unsedated newborns and infants.

2.1 The intra-breath changes in Z_{rs} are dominated by V' dependence in healthy newborns. R_{rs} shows a nonlinear V'-dependent increase which is a uniform feature among healthy newborns.

Overall, the intra-breath changes in Z_{rs}, dominated by the V' dependence, were remarkably large. The maximum R_{rs} was usually located near the peak V', while the minimum was found around V'=0. R_{ppE} and R_{ppI} amounted to 91.4 ± 33.3 % and 55.9 ± 27.6 %, respectively, of $|Z_0|$. The corresponding changes in X_{rs} (X_{ppE} and X_{ppI}) were roughly half as large.

Previous observations ([2] and our Study 1) suggest that the nonlinear, V'-dependent increase in R_{rs} originates from the upper airways obeying the classical empirical description by Rohrer [6].

2.2 Different patterns can be identified according to the shape of intra-breath X_{rs} – V' loops *in vivo*.

Whereas Rrs exhibited positive V' dependences during inspiration and expiration, the intra-breath changes in X_{rs} were more diverse. Four typical patterns were determined qualitatively and are exemplified in Fig. 3. These patterns are characterised as minimal dependence of X_{rs} on V' (Pattern A), marked V'-dependent decrease in X_{rs} during expiration (Pattern B), marked V'-dependent decrease during inspiration (Pattern C) and marked V'-dependent increases in X_{rs} (Pattern D). Each newborn was classified into one group (A-D) according to the V'-dependence of X_{rs} by cluster analysis. The classification resulted in groups with a size of n= 47, 27, 5 and 7 for group A, B, C and D, respectively.

Inference to the underlying mechanisms of each X_{rs} pattern is burdened by the lack of additional signals (e.g. nasopharyngeal pressure) unavailable in the non-invasive setting of the current study. A decrease in X_{rs} during expiration (Pattern B) is most likely caused by glottal braking that help maintain the end-expiratory lung volume during the postnatal period. The significant ($p<0.01$) decrease in V'_{maxE} (81 ± 15 *vs* 93 ± 17 $ml.s^{-1}$, Pattern B *vs* Pattern A, respectively) supports the above argument. Intuitively, a similar change in X_{rs} but in inspiration (Pattern C) can be attributed to the negative pressure swings in the

glossopharyngeal area, which lead via deformation of soft tissues to inspiratory V' limitation. Pattern D is likely to be associated with the increased impedance of the nasal pathway, as reflected by the higher values of R and L in this group (see Table 1).

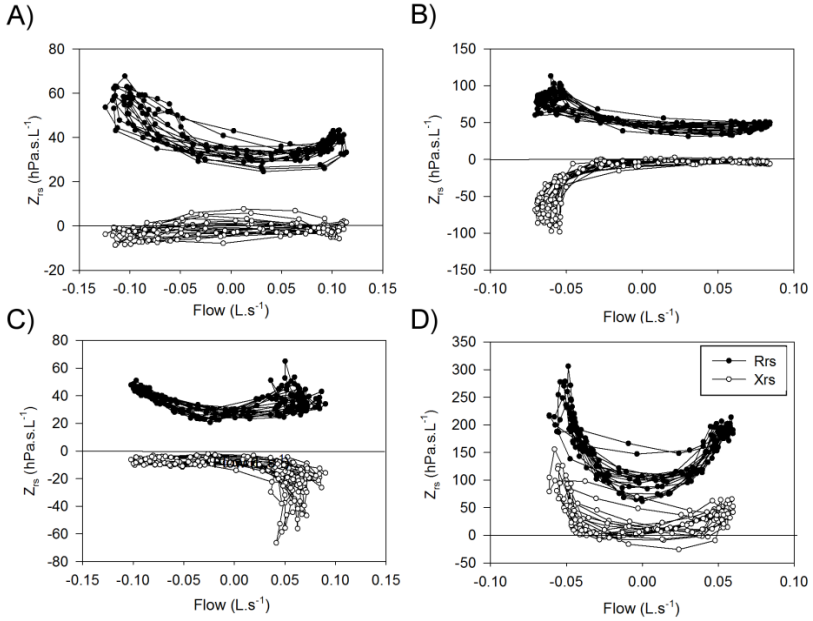

Figure 3. *Typical patterns of intra-breath changes in respiratory impedance (Z_{rs})vs flow (V'). The definition of each pattern (A-D) are detailed in the text above.*

2.3 Certain differences in tidal breathing and spectral oscillometry can be explained by the different patterns of X_{rs}-V' loops in healthy newborns.

Table 1. *Comparison of spectral oscillometry data between subject groups of different patterns of flow dependence of respiratory reactance. Mean ± SD values.*

	All (n=86)	Pattern A (n=47)	Pattern B (n=27)	Pattern C (n=5)	Pattern D (n=7)
R (hPa.s.L^{-1})	48.7 ± 12.9	46.0 ± 12.6	48.7 ± 11.5	59.9 ± 17.4	58.3 ± 9.4*
C (mL.hPa^{-1})	1.08 ± 0.30	1.13 ± 0. 32	1.01 ± 0. 29	1.05 ± 0.28	1.06 ± 0. 15
L (hPa.s^2.L^{-1})	0.068 ± 0.028	0.071 ± 0.027	0.057 ± 0.023*	0.047 ± 0.031	0.102 ± 0.020**
R_{8-32} (hPa.s.L^{-1})	18.6 ± 7.3	16.9 ± 6.4	20.6 ± 8.5	24.7 ± 5.9*	17.6 ± 5.0
f_{res} (Hz)	21.4 ±5.9	20.1 ± 5.2	23.7 ± 4.7**	29.2 ± 11.9	16.3 ± 1.9**
Ax (hPa.L^{-1})	103.1 ± 59.6	90.7 ± 55.1	124.8 ± 58.5*	157.0 ± 80.3	64.0 ± 20.8*

The definition of each pattern (A-D) are detailed in the text above.

R: resistance (model fitting), C: compliance (model fitting), L: inertance (model fitting), R_{8-32}: resistance difference between 8 and 32 Hz, f_{res}: resonance frequency, Ax: reactance area below f_{res}.

**p<0.05 vs Pattern A; **p<0.01 vs Pattern A*

Subjects with the lowest V'-dependence in X_{rs} (Pattern A) were considered the control group. No difference was found in V_T between the different groups. f_{br} was slightly lower in group B (58 ± 10 min^{-1}) and group C (58 ± 5) when compared to group A (65 ± 12, $p<0.05$).

Table 1 contains spectral oscillometry variables in the 4 groups. L was the highest while f_{res} and Ax were the lowest in the positive V' dependence (Pattern D) group. L was significantly ($p<0.05$) lower and Ax was higher in subjects with negative expiratory swings in X_{rs} (Pattern B) compared to Pattern A. Unlike the values of X_8, parameter C was found to be not different between groups.

The highest variability among spectral measures was observed in Ax, which is widely considered as a robust measure of elastic properties of the respiratory system. The intra-breath analysis revealing the patterns of V' dependence indicated that Ax was biased by changes in X_{rs} (Table 1), whereas the model fitting of Z_{rs} spectra showed that C is less influenced by the upper airway compartment and more specific to the elastic properties of the lungs.

2.4 Airway obstruction index T_{PTEF}/T_E is determined by respiratory control and not expiratory flow limitation.

T_{PTEF}/T_E was not correlated with any of the spectral and intra-breath R_{rs} or X_{rs} outcomes, but exhibited a very strong linear relationship ($r=0.84$, $p<0.001$) with $|V''_{eE}/V''_{eI}|$, apparently unrelated to the pattern of V' dependence of X_{rs}.

This suggests that in healthy term newborns, such as those in the present study, marked differences in the activity of the respiratory control mechanisms rather than airway obstruction exist and determine the values of T_{PTEF}/T_E.

SUMMARY AND CONCLUSIONS

Study 1 investigated R-V' relationship in anatomically faithful rigid nasal airway casts during simulated respiration. We found a characteristic nonlinear relationship between R and V', exhibiting segmental power-law behaviour with a prominent breakpoint. This model was reproducible in the *in vivo* study on a small group of infants. We observed a linear shift in R_{eE} which was attributable to increasing values of V''. We developed a geometrical approach to quantify intra-breath nonlinearities in R_n, allowing to estimate R_0 from a single oscillometric measurement. Using this correction may reduce the masking effect of the nonlinear upper airways on the changes in the intrathoracic R in future studies.

The impedance tracking employed in Study 2 revealed marked intra-breath changes in R_{rs} and X_{rs} in healthy term neonates during natural sleep in the first few days of life. These changes were dominated by the increases in R_{rs} with V' in both inspiration and expiration, whereas X_{rs} exhibited different patterns of change, including inspiratory and expiratory flow limitations. It is suggested that these intra-breath nonlinearities are of upper airway origin, with fundamental contributions from the nasal pathways. Intra-breath changes exert a biasing effect on the conventional measures of the multi-frequency oscillometry that are intended to characterise pulmonary mechanics. Use of intra-breath oscillometry is proposed to gain more insight into the mechanisms determining Z_{rs} and to properly interpret the results of conventional spectral oscillometry in infants.

Our studies demonstrated that oscillometry is a promising, noninvasive pulmonary function test achieving high success rate in infants. The single frequency (tracking) mode of oscillometry revealed considerable intra-breath changes in Z_{rs} in healthy newborns. Our results verified the basic assumption that V'-and V''-dependent changes in Z_{rs} develop in the

upper airways. We conclude that upper airway rheology can cause non-negligible bias and can contribute to the high natural variability of respiratory oscillometry results.

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