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Intra-breath respiratory mechanics of prematurity-associated lung disease phenotypes in school-aged children

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**This publication is dedicated to our very dear, late friend Professor John Henderson.

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Authors Contributors Statement: SK and AJH conceived, designed and set-up the study; MC and KH recruited participants and performed all testing; BR and ZH provided equipment, training, and technical support; MC and SK were involved in the data analysis. BR, ZH and PS provided expertise for interpretation of data; MC, ZH and SK drafted the manuscript; all authors (with exception of AJH) were involved in revising the manuscript and approved the final submitted version.

Abstract (Word count: 230)

Intra-breath oscillometry potentially offers detailed information regarding airway function, with increasing magnitude of difference between resistance and reactance at end-expiration to end-inspiration potentially associated with obstructive airway disease, but less is known about specific respiratory mechanics in preterm-born children using this methodology. We investigated if different spirometry phenotypes of prematurity-associated lung disease (PLD) have specific intra-breath oscillometry features.

167 school-aged (7-12 years) children, 14 with prematurity-associated obstructive lung disease (POLD; FEV_1<LLN, FEV_1/FVC<LLN), 11 with prematurity-associated preserved ratio of impaired spirometry (pPRISm; FEV_1<LLN, FEV_1/FVC≥LLN), 90 preterm controls (FEV_1≥LLN) and 52 term controls, performed intra-breath oscillometry at baseline, following maximal cardiopulmonary exercise testing and following post-exercise bronchodilation.

Children with POLD showed greater resistance and more negative reactance throughout the respiratory cycle, including at zero-flow states of end-expiration and end-inspiration. The difference between end-expiration and end-inspiration did not show differences between groups, until corrected for tidal volume, whereby children with POLD and pPRISM both demonstrated approximately two-fold greater difference compared to both preterm and term controls for resistance (2.24 and 2.22 vs 1.28 and 1.11 hPa.s/L), and in particular a greater magnitude of difference for reactance for children with POLD versus preterm and term controls only (-1.58 vs -0.26 and 0.03 hPa.s/L).

Intra-breath respiratory mechanics for preterm-born children with obstructive lung phenotype have greater impedance throughout the respiratory cycle, features different to those observed in children with other wheeze phenotypes including preschool wheeze and asthma.

Introduction

Oscillometry (previously known as forced oscillation technique) is a useful tool for identifying differences in airway mechanics between populations. Changes occurring in respiratory impedance (Zrs) and its components resistance (Rrs) and reactance (Xrs) during tidal breathing can be beneficial for improving understanding of disease pathology, including the peripheral airway disease identified in preterm-born children [1]. Intra-breath oscillometry offers insight into dynamic changes that occur throughout the respiratory cycle [2, 3]. This information may be able to further differentiate between pathological entities [4]. This method superimposes a single frequency wave on tidal breathing and assesses changes in impedance at different points of the respiratory cycle, in particular those relating to zero-flow states, i.e., end-expiration and end-inspiration.

Intra-breath oscillometry has been used in a range of ages including in infancy [5], where the difference between end-expiration and end-inspiration respiratory system resistance (ΔR) and reactance (ΔX) was predictive for lower respiratory tract infection, potentially a result of airway flow abnormalities, which would not be clinically detectable [6]. Similarly, detection of airway obstruction presenting clinically as preschool wheeze or asthma is potentially identifiable with increasing magnitude of ΔR , with a ΔR of ≥1.42 able to distinguish between children with recurrent wheeze episodes and healthy controls [2] This suggests that in preschool wheeze/asthma phenotypes of obstructive lung disease that there is a predilection to the expiratory component of the respiratory cycle being affected. Intra-breath oscillometry has also been used in adults with obstructive airway disease including COPD [3] and asthma [7], with similar increasing magnitude for ΔR and ΔX in the latter, and flow limitation identified on volume versus reactance loops in the former.

Preterm-born children are known to have disrupted lung growth [8], and are at risk of long-term lung dysfunction. Preterm-born populations have increased respiratory symptoms including wheezing [9], spirometry abnormalities [10] (including similar dysfunction noted over time [11]), and exercise impairment [12, 13]. We have shown increasing evidence that prematurity-associated lung disease (PLD) does not fall into a single pathological entity, but more likely differing phenotypes within the preterm-born population [13-15], including prematurity-associated obstructive lung disease (POLD) and prematurity-associated preserved ratio of impaired spirometry (pPRISm). PRISm in adult populations has been shown to be associated with COPD and all-cause mortality [16, 17]; however, less is known about its significance when identified in childhood including in preterm populations.

In children, oscillometry has an advantage over other, effort-dependent, lung function tests such as spirometry, as it is only reliant on tidal breathing, and has been demonstrated to be feasible in infant [6] and pre-school [18] age groups. Additionally, with higher rates of neuro-disability in children born preterm [19], and the associated difficulty with performing spirometry in such patients [20], oscillometry is particularly suited for preterm-born children.

The use of intra-breath oscillometry in preterm populations has been relatively limited so far. A small sample of largely late preterm-born children identified small but significantly greater magnitudes of ΔR and ΔX compared to full-term controls [21]. Given the overlap of potential pathology, i.e., airway obstruction, between preterm-associated lung disease and wheeze or asthma, it would be reasonable to hypothesise that similar ΔR and ΔX changes may be identifiable in preterm-born children with an obstructive phenotype. Thus, we compared intra-breath

oscillometry data between three phenotypes of preterm children based on spirometric outcomes (POLD, pPRISm, and preterm controls (PT_c)) and a control group of term-born children (T_c), with additional measurements taken at post-exercise and post-exercise bronchodilator time points.

Material and methods

Population, spirometry and exercise testing

Preterm- and term-born children from birth years 2005 to 2011, identified during a previous questionnaire study [9, 22], were prospectively recruited for the Respiratory Health Outcomes in Neonates (RHiNO) study (EudraCT: 2015-003712-20) as previously described [13, 15, 23]. Following screening, spirometry was performed by trained research nurses, children meeting inclusion criteria (gestational age at birth ≤34 weeks' gestation for preterm-born children and at ≥37 weeks' gestation for term-born children; age 7-12 years; geographically accessible) were invited for in-depth lung function testing including spirometry, exercise testing and oscillometry at the local Children's Hospital, from January 2017 to August 2019. All preterm-born children with percent predicted forced expiratory volume in 1 second (%FEV_1) of ≤85% at screening were invited, so they could participate in the randomised control trial [23]; together with the first ten preterm-born children with %FEV_1 of >85% as controls during each calendar month. Term-born children with %FEV_1 >90% were randomly invited to participate. Children who could not perform acceptable spirometry did not complete the full visit. Children with significant congenital/cardiac/neurodevelopmental abnormalities were excluded, and testing was postponed in children with a recent (within the past 3 weeks) respiratory tract infection.

Spirometry and exercise testing have been described elsewhere in greater detail [13]. Briefly, spirometry was performed in line with ATS/ERS guidance [24] using the MasterScreen Body/PFT

systems with SentrySuite measurement software version 2.17 (Vyaire Medical, Germany). Global Lung Initiative (GLI) equations were used as reference standards for spirometry values [25].

Spirometry was used to classify children into the following phenotypes of interest as previously described [15]:

- **POLD** (Prematurity-associated obstructive lung disease): FEV_1 < LLN, FEV_1/FVC < LLN;
- **pPRISm** (Prematurity-associated preserved ratio of impaired spirometry): FEV_1 < LLN, FEV_1/FVC ≥ LLN;
- **PT_c** (Preterm controls): FEV_1 ≥ LLN.
- **T_c** (Term controls): percent predicted FEV_1 > 90%.

Cardiopulmonary exercise testing was performed on a Pediatric Cycle Ergometer (Lode, Netherlands) with a Masterscreen CPX system (Vyaire Medical, Germany). 'Maximal' testing was achieved if ≥2 of the following criteria were met: Respiratory Exchange Ratio >1.00; heart rate ≥80% predicted (220 bpm – age); ≥9/10 on OMNI scale (pictorial scale for rating of perceived exertion [26]); O_2 uptake plateau reached.

Oscillometry

Oscillometry was performed using a custom-built loudspeaker-in-box device, designed to operate during post-exercise rapid breathing, as previously described [1](see details in the online supplement).

A nose-clip was worn and cheeks firmly held during testing. The loudspeaker superimposed a 10 Hz soundwave at 0.1 second intervals onto tidal breathing, with respiratory impedance measured

at the mouth using pressure and flow sensors. A minimum of 3 recordings lasting 23.5 seconds were obtained, and analysis performed on the recording with most regular artefact-free breaths (i.e. no coughs, glottic closure, breath holds). Average measures across breaths for key parameters were calculated, for mean impedance (reactance (R) and reactance (X)) measured at end expiration (eE)/ inspiration (eI), and mean impedance during expiration (meanE) and inspiration (meanI) calculated.

Intra-breath oscillometry measures were obtained at baseline, 20 minutes post-maximal exercise testing, and following administration of post-exercise bronchodilator (400 micrograms of salbutamol (Salamol®, TEVA UK Limited) administered with an MDI using a Volumatic spacer (GSK, UK)).

Ethical approval

Parents and children provided informed written consent/assent respectively, with ethical approval granted by the Southwest Central Bristol Ethics Committee (Ref 15/SW/0289).

Statistical analysis

One-way ANOVA with Bonferroni correction was used for multi-group comparisons for continuous data. Categorical data were assessed using Pearson's χ^2 tests. Two-way repeated measures ANOVA with Bonferroni correction was used for within-group and between group comparisons across time points. p-value <0.05 was considered statistically significant. Where there was missing data at one or more time points (recording issue, time constraint, test quality, or participant declining test), all data for these participants were excluded from the repeated measures analysis. Statistical analysis was performed using SPSS version 26 (IBM, USA).

Results

Participant details

Of 241 original invited participants, 20 were excluded due to inadequate spirometry (see *Figure 1*). 3 children did not perform exercise testing and 15 children did not achieve maximal exercise testing, thus were excluded from full analysis. Of the remaining 203 children, 36 had one or more time points missing from their oscillometry testing (missed or declined test, suboptimal quality of recording, recording issue). 167 children were included in repeated measures analysis of oscillometry data and were phenotyped based on their spirometry into the following groups:

- 14 POLD;
- 11 pPRISm;
- 90 PT_c;
- 52 T_c.

Participant demographics are summarised in *Table 1*. Anthropometric measurements were similar between groups with the exception of lower weight z-scores in children with pPRISm compared to both control groups. There were no differences in raw or z-score heights between the groups. The PT_c group were slightly older than the T_c group. Children with POLD were born at an earlier gestation compared to PT_c group (29.3 vs 31.1 weeks' gestation). There were no differences for birth weight, invasive ventilation or for CLD rates between the preterm groups. Children with POLD had higher rates compared to T_c children for wheeze ever (86% vs 25%; and vs 46% in PT_c), recent (last 12 months) wheeze (50% vs 15%), asthma diagnosis (43% vs 10%), and salbutamol use (36% vs 8%). There was no difference in rates of exposure to maternal smoking.

Respiratory parameters

Table 2 summarises the participants' respiratory parameters. Expiratory time (T_E) between groups showed no differences at any time point. However, on repeated measures across time points, there was a small decrease in expiratory time for preterm and term controls from baseline to post-exercise (both 1.7 secs to 1.5 seconds), and for children with POLD from post-exercise to post-exercise bronchodilator time points (1.6 to 1.3 seconds). The POLD group had a higher baseline proportion of expiratory time to total respiratory time (T_E /T_{Tot}) for the POLD group compared to both preterm and term controls (0.55 vs 0.52 for both controls). This difference persisted to the post-exercise time point but not to post-exercise bronchodilation. Respiratory rate (F_{br}) increased in preterm and term controls post-exercise (20.5 to 22.6 and 20.2 to 22.9 breaths per minute from baseline to post-exercise respectively). Repeated measures showed no significant changes at post-exercise bronchodilation time point.

End-respiratory impedance (Table 3)

Evaluation of resistance at end-expiration (R_{eE}) and end-inspiration (R_{eI}) at baseline revealed higher resistance in the POLD group compared to both preterm and term control groups (R_{eE}: 6.7 vs 5.2 vs 5.1 hPa.s/L; R_{eI} 5.8 vs 4.5 vs 4.4 hPa.s/L respectively); however, no difference was observed in the difference between these two values between any groups at baseline (RΔR) (0.9 vs 1.0 vs 0.7 vs 0.7 hPa.s/L). When standardised against the change in tidal volume (ΔR/VT), there was a non-significant trend towards higher values in POLD and pPRISm groups (2.24 and 2.22 versus 1.28 and 1.11 hPa.s/L^2 greater than PT_c and T_c respectively).

Repeated measures analysis showed no difference for end expiratory or inspiratory resistance for any group from baseline to post-exercise. All groups demonstrated a reduction in end-expiratory and end-inspiratory resistance from post-exercise to post-exercise bronchodilator. At post-

exercise bronchodilator time-point, no difference between children with POLD and controls remained, suggesting a greater improvement for children with POLD to bronchodilator compared to controls.

ΔR reduced in the pPRISm group from baseline to post-exercise (1.0 to 0.4 hPa.s/L). ΔR reduced in the POLD group from post-exercise to post-bronchodilation (0.9 to 0.3 hPa.s/L), and children with POLD had a reduction in the ΔR/VT (1.90 to 0.96 hPa.s/L^2) from post-exercise to post-exercise bronchodilation, a reduction not seen in any of the other groups.

End expiratory and end inspiratory reactances (X_{eE} and X_{eI}) were significantly more negative (worse) for children with POLD when compared to both preterm and term control groups (X_{eE}: -3.2 vs -1.2 vs -0.9 hPa.s/L; X_{eI} -2.5 vs -1.1 vs -0.9 hPa.s/L respectively). Similar to resistance, ΔX did not show any between group differences, until standardised against change in tidal volume (ΔX/VT: -1.58 vs -0.26 vs 0.03 for POLD, PT_c and T_c groups respectively).

Following exercise, there was no change for any group in X_{eE}, ΔX and ΔX/VT, but there was a more negative X_{eI} for pPRISm children (-1.5 to -2.2 hPa.s/L).

All 4 groups had improved (less negative) reactance for X_{eE} and X_{eI} following post-exercise bronchodilator, but a statistically significant improvement for ΔX (-0.6 to 0.0 hPa.s/L) and ΔX/VT (-1.14 to 0.03 hPa.s/L^2) was only observed for children with POLD.

Figure 2 displays the end-respiratory and mean impedances at various parts of the respiratory cycle.

Impedance loops (Table 4, Figure 3)

There are no baseline differences between groups for area within either Resistance-Volume (ARV) or Resistance-Flow (ARV') loops. Following exercise there is a significant increase in ARV for children with POLD (-0.26 to -0.70 hPa.s) which persists after bronchodilator therapy. The preterm control children were the only group showing an increase in ARV after post-exercise bronchodilation (-0.48 to -0.61 hPa.s).

Similarly, there are no between-group differences within either Reactance-Volume (AXV) or Reactance-Flow (ARV') loops at baseline. In the POLD group, following exercise, there is an increase in AXV 0.49 to 1.02 hPa.s (statistically significant) and in AXV' -1.55 to -1.72 hPa (non-statistically significant), resulting in POLD having significantly greater post-exercise AXV compared to all 3 groups (1.02 vs -0.01 vs 0.34 vs 0.37 hPa.s) and AXV' against PT_c and T_c (-1.72 vs -0.55 vs -0.66 hPa). POLD and PT_c groups show decrease in AXV' following post-exercise bronchodilator (-1.72 to -0.81 and -0.55 to -0.29 hPa respectively).

Mean impedance (Table 5)

Mean resistance at baseline during expiration (R_{meanE}) was not statistically significantly higher in the POLD group than the other groups; however, during inspiration (R_{meanI}) it was higher compared to PT_c and T_c groups (6.4 vs 5.1 vs 5.0 respectively). Following exercise, the difference between resistance during expiration and inspiration (ΔR_{mean}) in the POLD group doubled, i.e. increased disproportionally in expiration compared to inspiration. Almost all groups showed improvement following bronchodilator for both R_{meanE} and R_{meanI}.

X_{meanE} and X_{meanI} were both significantly more negative in the POLD group when compared to the PT_c and T_c groups. For the POLD group, there was a more negative ΔX_{mean} following exercise (-1.0 to -1.6 hPa.s/L), while the pPRISm group had more negative reactance (-2.0 to -3.1) following exercise. Almost all groups showed improvement following bronchodilator for both X_{meanE} and X_{meanI}.

Discussion

We have used intra-breath oscillometry to assess potential differences between phenotypes of PLD, with regards to changes occurring throughout the respiratory cycle, something that cannot be identified with standard oscillometry.

We have demonstrated that children with POLD have impaired impedance throughout the respiratory cycle, particularly in comparison to preterm-born children without any current lung dysfunction and term-born children. This includes increased resistance and more negative reactance during inspiration and expiration, as well as at the end of each phase of the respiratory cycle. Of interest, in children with POLD, mean expiratory impedance (both resistance and reactance) increased in greater magnitude compared to mean inspiratory impedance following exercise. During standard oscillometry there were few differences noted in resistance parameters following exercise [1]. This suggests that exercise affects expiratory flow to a greater degree than inspiratory flow in peripheral airway obstruction in preterm-born children, and that intra-breath oscillometry is sensitive for detecting such changes that may not be seen overall using standard oscillometry.

Of specific interest is what happens to impedance at the zero-flow states of end-expiration and end-inspiration, removing potential dynamic factors such as upper airway obstruction [24]. The ΔR and ΔX at baseline showed no significant difference between groups, perhaps unexpectedly for the children with POLD as obstructive lung disease potentially shows increased ΔR in particular, such as in the case of preschool wheeze [2] and adult asthma [7], the latter showing that intra-breath oscillometry has greater sensitivity for detecting differences compared to traditional oscillometry. It also differs from the expiratory versus inspiratory difference in reactance seen in patients with COPD, potentially associated with flow imitation and linked to dyspnoea [25]. Instead, a pan-respiratory cycle difference was noted in the POLD children suggesting a different pathology to preschool wheeze/asthma phenotypes where the expiratory rather the inspiratory components are most affected.

One possibility is that the nature of the obstructive lung disease is different to these other conditions. Resistance is volume dependant, and children with POLD demonstrate higher functional residual capacity (FRC) compared to controls and children with pPRISm [13]. The combination of higher baseline FRC from air trapping, negating any changes in end expiratory constrictor tone as seen in other obstructive airway disease [2], plus smaller tidal volumes, thus results in smaller ΔR and ΔX compared to other obstructive lung disease. Interestingly, when accounting for change in tidal volume in oscillometry, the children with POLD then demonstrated approximately twice the magnitude of ΔR compared to both control groups, which suggests that this diminished tidal volume is a significant factor. Furthermore, standard oscillometry has shown that resistance in children with POLD is frequency dependent, with lower frequencies demonstrating higher resistance, suggestive of peripheral airways being affected to the greatest extent. It may be that intra-breath oscillometry performed at 6 Hz would theoretically detect

greater differences compared to the higher frequency of 10 Hz we used in this study. However, lower oscillation frequencies are likely to provide worse temporal resolution and are more likely to be contaminated by breathing harmonics, especially given the higher breathing rates of children, which may confound any benefits of using a lower frequency.

Similarly, reactance demonstrated an overall greater negative magnitude at both zero-flow states (X_{eE} and X_{eI}), and throughout the respiratory cycles (X_{meanE} and X_{meanI}) in children with POLD. This is again in keeping with the findings from standard oscillometry where reactance in an obstructive phenotype was more negative overall. Expiratory reactance, including at end-expiration, was more negative than during inspiration. The ΔX was not significantly different in obstructive or pPRISm phenotypes. However, when normalised against tidal volume, the baseline ΔX in children with POLD was significantly different compared to both control groups, with a greater difference observed. At 10Hz, with a negative reactance, compliance is the dominant force. As children with obstructive airway disease most likely have reduced compliance as a result of possible fixed structural defects either within, or more likely, outside of the airways, reactance is disproportionately affected over resistance when tidal volume changes occur, thus greater differences were observed.

Exercise showed little difference for intra-breath values of resistance. This could be for at least two reasons. One relates to the timing of exercise-induced bronchoconstriction (EIB), and whether timings of EIB detectable with oscillometry, either standard or intra-breath format, are at their peak at 20 minutes following exercise as noted with spirometry [12]. The other factor as discussed previously is the peripheral location of lung pathology. Potentially there are some differences in how airways in children with POLD and pPRISm respond to exercise, with

obstructive phenotypes having typical expiratory changes compared to children with pPRISm, with the latter likely to reflect a degree of restrictive airway, where in inspiration there is perhaps a difference in dynamic compliance of their airways, as demonstrated by the trend towards post-exercise reactance changes seen in inspiration for the pPRISm group, and expiration for the POLD group.

Post-exercise bronchodilator generally showed improvements in all groups during all aspects of the respiratory cycle. The greatest responses in ΔR and ΔX (both in isolation and when accounting for tidal volume changes) were noted following post-exercise bronchodilator in children with POLD. This suggests that while there may not be as clear a distinction as seen in other obstructive lung disease [2], there is still reversible airway obstruction that can be treated with beta-2 agonists which have detectable oscillometry changes. Post-exercise increases in areas within resistance/reactance-volume and reactance-flow curves are seen in children with POLD, which potentially suggests some gas trapping and lung hyperinflation. The influence of subsequent bronchodilation may be to reduce this dynamic hyperinflation, represented by the above improvements in ΔR/VT and ΔX/VT.

Children with pPRISm are another phenotype of PLD which has been underexplored until recently [13, 15]. While this phenotype is likely of interest due to its potential for morbidity, particularly if the changes persist into adulthood, the oscillometry findings are relatively unremarkable, albeit with a trend towards greater impedance compared to controls; however, there is not an obvious picture within intra-breath oscillometry that distinguishes them, unlike the children with POLD. Given this phenotype likely represents a number of children with restrictive pattern of lung disease [13], then similarities may be expected with other restrictive lung diseases, where

abnormal end-inspiratory reactances can be seen due to increased distension at end-inspiration [26]. Indeed, post-exercise, a more negative X_{el} was found in children with pPRISm, suggesting that there is some tendency towards this pattern.

By identifying specific phenotypes of PLD, our study suggests several avenues to pursue in the future. Since the trajectories of intra-breath oscillometry especially for the different phenotypes we have described are largely unknown, longitudinal studies would aid understanding of these phenotypes [27]. In addition, the technique can be used in the clinic, given its ease of use. However, robust standardisation and generation of accurate reference values for both sexes, at different ages, heights and of different ethnicity are required. The method has potential to assess response to treatment.

The main strength of this study is the assessment of changes in intra-breath oscillometry measures of recently described phenotypes of PLD, as well as assessment after exercise and after post-exercise bronchodilator administration. The main limitation is the small numbers in the POLD and pPRISm groups, although we had sufficient numbers of controls to compare with. Our findings need to be replicated in larger cohorts of children with PLD. We used local relevant preterm- and term-born controls but there is a need for standardised reference values.

In conclusion, there are limited differences between the zero-flow state in preterm-born children with obstructive airway phenotype, suggesting alternative pathology to that seen in other obstructive airway disease, although differences, particularly in reactance, become apparent once changes in tidal volume were taken into consideration. Detection of exercise-induced changes may be more sensitive. Given peripheral airway disease is likely to be the predominant

pathology, investigation of intra-breath changes at lower frequency may distinguish POLD from other obstructive diseases such as asthma and COPD.

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Table 1: Baseline characteristics of participants including anthropometric, perinatal and respiratory details for prematurity-associated obstructive lung disease (POLD), prematurity-associated preserved ratio of impaired spirometry (pPRISm), preterm (PT_c) and term (T_c) control groups.

	POLD (n=14)	**pPRISm (n=11)**	**PT_c (n=90)**	**T_c (n=52)**
Current demographics				
Age, years	11 (10.2 to 11.7)	11.1 (10.2 to 12.1)	**11.2 (11 to 11.4) ₴₴**	10.5 (10.2 to 10.8)
Male, n (%)	9 (64%)	2 (18%)	46 (51%)	27 (52%)
Height, cm	142.5 (136.8 to 148.2)	142.8 (133.3 to 152.3)	146.8 (144.9 to 148.6)	143.4 (140.8 to 145.9)
Height, Z-score	-0.13 (-0.7 to 0.43)	-0.32 (-1.35 to 0.71)	0.26 (0.09 to 0.43)	0.4 (0.11 to 0.69)
Weight, kg	37.9 (32.5 to 43.3)	35.5 (26.5 to 44.6)	40 (37.9 to 42.1)	36.9 (34.7 to 39)
Weight, Z-score	0.21 (-0.43 to 0.85)	**-0.63 (-1.84 to 0.58) ¥ ¶**	0.32 (0.11 to 0.54)	0.39 (0.12 to 0.66)
BMI, kg/m^2	18.5 (16.6 to 20.3)	16.8 (14.4 to 19.2)	18.4 (17.6 to 19.1)	17.8 (17.1 to 18.5)
BMI, Z-score	0.36 (-0.37 to 1.08)	-0.67 (-1.74 to 0.39)	0.18 (-0.08 to 0.45)	0.26 (-0.02 to 0.54)
Perinatal demographics				
Gestation, decimal weeks	**29.3 (27.6 to 31.0) † ‡‡‡**	**30.0 (28.0 to 32.0) ¶¶¶**	**31.1 (30.5 to 31.7) ₴₴₴**	40.0 (39.7 to 40.3)
Birth weight, grams	**1361 (1063 to 1660) ‡‡‡**	**1487 (1077 to 1898) ¶¶¶**	**1721 (1602 to 1840) ₴₴₴**	3490 (3363 to 3617)
Birth weight, Z-score	-0.07 (-0.63 to 0.48)	0.03 (-0.73 to 0.78)	0.22 (-0.07 to 0.52)	0.03 (-0.21 to 0.27)
IUGR, n (%)	1 (7%)	2 (18%)	14 (16%)	2 (4%)
Antenatal steroids, n (%)	**12 (86%) ‡‡‡**	**10 (91%) ¶¶¶**	**74 (82%) ₴₴₴**	0 (0%)
Invasive ventilation, n (%)	**9 (64%) ‡‡‡**	**4 (36%) ¶¶¶**	**33 (37%) ₴₴₴**	0 (0%)
CLD, n (%)	**5 (36%) ‡‡‡**	**3 (27%) ¶¶¶**	**19 (21%) ₴₴₴**	0 (0%)
Respiratory history				
Doctor-diagnosed asthma, n (%)	**6 (43%) ‡**	2 (18%)	20 (22%)	5 (10%)
Wheeze ever, n (%)	**12 (86%) † ‡‡‡**	6 (55%)	41 (46%)	13 (25%)
Recent wheeze, n (%)	**7 (50%) ‡**	2 (18%)	19 (21%)	8 (15%)
Current salbutamol use, n (%)	**5 (36%) ‡**	1 (9%)	16 (18%)	4 (8%)
Current maternal smoking, n (%)	1 (7%)	1 (9%)	6 (7%)	0 (0%)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified.
Abbreviations: BMI – Body Mass Index; **IUGR** – Intrauterine Growth Restriction; **CLD** – Chronic Lung Disease of prematurity.
Significance symbols: * POLD vs pPRISm, **†** POLD vs PT_c, **‡** POLD vs T_c, **¥** pPRISm vs PT_c, **¶** PRISm vs T_c, **₴** PT_c vs T_c.
(Single symbol denotes significance level <0.05, double symbol <0.01, triple symbol <0.001).

Table 2: Respiratory parameters for prematurity-associated obstructive lung disease (POLD), prematurity-associated preserved ratio of impaired spirometry (pPRISm), preterm (PT_c) and term (T_c) control groups, at baseline, at post-exercise, and at post-exercise bronchodilation timepoints.

		POLD (n=14)	**pPRISm (n=11)**	**PT_c (n=90)**	**T_c (n=52)**
T_E (seconds)	Baseline	1.5 (1.3 to 1.8)	1.6 (1.4 to 1.9)	1.7 (1.6 to 1.8)	1.7 (1.5 to 1.9)
	Post-exercise	1.6 (1.2 to 2)	1.5 (1.1 to 1.9)	∂ 1.5 (1.4 to 1.6)	∂∂ 1.5 (1.4 to 1.6)
	Post-exercise BD	ᵀ1.3 (1.0 to 1.6)	1.4 (1.1 to 1.7)	1.5 (1.4 to 1.6)	1.5 (1.4 to 1.6)
T_E/T_{Tot}	Baseline	**0.55 (0.52 to 0.57) †**	0.53 (0.51 to 0.56)	0.52 (0.51 to 0.53)	0.52 (0.51 to 0.53)
	Post-exercise	**0.55 (0.52 to 0.58) † ‡**	0.51 (0.49 to 0.53)	0.52 (0.51 to 0.53)	0.52 (0.51 to 0.53)
	Post-exercise BD	0.53 (0.49 to 0.57)	0.52 (0.50 to 0.54)	0.53 (0.52 to 0.54)	0.53 (0.52 to 0.54)
T_{tpef}/T_E	Baseline	0.28 (0.24 to 0.32)	0.37 (0.28 to 0.47)	0.33 (0.31 to 0.35)	0.34 (0.31 to 0.36)
	Post-exercise	**0.26 (0.22 to 0.31) † ‡**	0.34 (0.26 to 0.42)	0.34 (0.32 to 0.36)	0.34 (0.32 to 0.37)
	Post-exercise BD	0.35 (0.25 to 0.44)	0.39 (0.32 to 0.47)	0.35 (0.33 to 0.38)	0.35 (0.32 to 0.38)
VT (L)	Baseline	0.48 (0.37 to 0.59)	0.44 (0.32 to 0.55)	0.58 (0.53 to 0.63)	0.60 (0.52 to 0.68)
	Post-exercise	0.53 (0.41 to 0.66)	0.38 (0.33 to 0.43)	0.55 (0.51 to 0.59)	0.57 (0.49 to 0.66)
	Post-exercise BD	0.55 (0.41 to 0.68)	0.44 (0.36 to 0.51)	0.58 (0.53 to 0.63)	0.59 (0.53 to 0.66)
F_{br} (bpm)	Baseline	23.4 (19.6 to 27.3)	20.9 (17 to 24.8)	20.5 (19.3 to 21.7)	20.2 (18.4 to 22.0)
	Post-exercise	23.8 (19.3 to 28.3)	24.5 (18.4 to 30.5)	∂ 22.6 (21.2 to 24.0)	∂ 22.9 (20.9 to 24.8)
	Post-exercise BD	26.5 (22.4 to 30.6)	25.8 (20.0 to 31.6)	23.7 (21.9 to 25.4)	22.5 (20.9 to 24.1)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction).

Abbreviations: **T_E** – Expiratory time; **T_{Tot}** – Total expiratory time; **$T_{V'maxE}$** – Time to maximal expiratory flow; **VT** – Tidal volume; **Fbr** – Breathing frequency.

Significance symbols: ***POLD vs pPRISm, †POLD vs PT_c, ‡POLD vs T_c, ¥pPRISm vs PT_c, ¶PRISm vs T_c, ₴PT_c vs T_c;** ∂Baseline vs Post-exercise; ᵀPost-exercise vs Post-bronchodilator

(Single symbol denotes significance level <0.05, double symbol <0.01, triple symbol <0.001).

Table 3: Zero-flow impedance for prematurity-associated obstructive lung disease (POLD), prematurity-associated preserved ratio of impaired spirometry (pPRISm), preterm (PT_c) and term (T_c) control groups, at baseline, at post-exercise, and at post-exercise bronchodilation timepoints.

		POLD (n=14)	pPRISm (n=11)	PT_c (n=90)	T_c (n=52)
End-respiration					
R_{eE} (hPa.s/L)	Baseline	**6.7 (5.6 to 7.9) † ‡‡**	6.3 (5.2 to 7.3)	5.2 (4.9 to 5.6)	5.1 (4.7 to 5.5)
	Post-exercise	**7.2 (6.1 to 8.3) ††† ‡‡**	6 (4.6 to 7.4)	5.2 (4.9 to 5.6)	5.3 (4.9 to 5.8)
	Post-exercise BD	ᵀᵀᵀ 4.7 (4.0 to 5.4)	ᵀᵀ 5.1 (3.8 to 6.4)	ᵀᵀᵀ 4.3 (4 to 4.7)	ᵀᵀᵀ 4.5 (4.1 to 4.9)
R_{eI} (hPa.s/L)	Baseline	**5.8 (4.9 to 6.7) †† ‡‡**	5.3 (4.5 to 6)	4.5 (4.2 to 4.8)	4.4 (4.1 to 4.8)
	Post-exercise	**6.2 (5.4 to 7.1) ††† ‡‡**	5.6 (4.6 to 6.6)	4.6 (4.4 to 4.9)	4.7 (4.3 to 5.1)
	Post-exercise BD	ᵀᵀᵀ 4.4 (3.8 to 4.9)	ᵀᵀᵀ 4.6 (3.6 to 5.5)	ᵀᵀᵀ 3.8 (3.5 to 4)	ᵀᵀᵀ 4 (3.7 to 4.3)
ΔR (hPa.s/L)	Baseline	0.9 (0.4 to 1.4)	1.0 (0.4 to 1.6)	0.7 (0.6 to 0.9)	0.7 (0.5 to 0.9)
	Post-exercise	0.9 (0.3 to 1.5)	∂ 0.4 (-0.2 to 1)	0.6 (0.5 to 0.8)	0.6 (0.4 to 0.9)
	Post-exercise BD	ᵀ 0.3 (0.0 to 0.7)	0.5 (-0.1 to 1.1)	0.6 (0.4 to 0.8)	0.5 (0.3 to 0.8)
ΔR/VT (hPa.s/L^2)	Baseline	2.24 (1.12 to 3.36)	2.22 (0.94 to 3.51)	1.28 (1 to 1.55)	1.11 (0.68 to 1.55)
	Post-exercise	1.90 (0.63 to 3.18)	1.08 (-0.55 to 2.71)	1.08 (0.82 to 1.35)	0.96 (0.55 to 1.37)
	Post-exercise BD	ᵀ 0.96 (0.10 to 1.81)	1.2 (-0.19 to 2.59)	1.00 (0.67 to 1.32)	0.84 (0.46 to 1.23)
X_{eE} (hPa.s/L)	Baseline	**-3.2 (-4.2 to -2.1) ††† ‡‡‡**	-1.7 (-2.5 to -0.8)	-1.2 (-1.6 to -0.9)	-0.9 (-1.1 to -0.7)
	Post-exercise	**-3.4 (-4.0 to -2.7) ††† ‡‡‡**	-2.1 (-3.5 to -0.8)	-1.3 (-1.6 to -1)	-1.2 (-1.5 to -0.9)
	Post-exercise BD	ᵀᵀᵀ -1.1 (-1.6 to -0.7)	ᵀᵀ -1.2 (-2.1 to -0.3)	ᵀᵀᵀ -0.7 (-0.9 to -0.5)	ᵀᵀ -0.9 (-1.1 to -0.6)
X_{eI} (hPa.s/L)	Baseline	**-2.5 (-3.2 to -1.8) * ††† ‡‡‡**	-1.5 (-2.1 to -1)	-1.1 (-1.3 to -0.9)	-0.9 (-1 to -0.7)
	Post-exercise	**-2.8 (-3.2 to -2.4) ††† ‡‡‡**	**∂∂ -2.2 (-3 to -1.3) ¥¥ ¶¶**	-1.2 (-1.4 to -1)	∂ -1.1 (-1.4 to -0.9)
	Post-exercise BD	ᵀᵀᵀ -1.2 (-1.5 to -0.8)	ᵀᵀᵀ -1.4 (-2.1 to -0.6)	ᵀᵀᵀ -0.7 (-0.9 to -0.6)	ᵀᵀ -0.9 (-1.1 to -0.7)
ΔX (hPa.s/L)	Baseline	-0.7 (-1.1 to -0.2)	-0.1 (-0.7 to 0.4)	-0.2 (-0.4 to 0.0)	0.0 (-0.2 to 0.1)
	Post-exercise	-0.6 (-1.0 to -0.2)	0.0 (-0.8 to 0.8)	-0.1 (-0.2 to 0.1)	-0.1 (-0.3 to 0.2)
	Post-exercise BD	ᵀᵀ 0.0 (-0.3 to 0.4)	0.2 (-0.1 to 0.5)	0.0 (-0.1 to 0.2)	0.0 (-0.1 to 0.2)
ΔX/VT (hPa.s/L^2)	Baseline	**-1.58 (-2.63 to -0.52) † ‡‡**	0.02 (-1.17 to 1.2)	-0.26 (-0.61 to 0.1)	0.03 (-0.24 to 0.31)
	Post-exercise	**-1.14 (-1.89 to -0.40) ‡**	0.20 (-1.73 to 2.12)	-0.05 (-0.34 to 0.24)	0.14 (-0.28 to 0.56)
	Post-exercise BD	ᵀᵀ 0.03 (-0.54 to 0.61)	0.57 (-0.23 to 1.37)	0.09 (-0.17 to 0.34)	0.17 (-0.11 to 0.44)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction).

Abbreviations: **R** – Resistance; **X** – Reactance; **e** – End respiratory; **E** – Expiration; **I** – Inspiration; **Δ** – Difference; **VT** – Tidal volume; **BD** – bronchodilator.

Significance symbols: *** POLD vs pPRISm, † POLD vs PT_c, ‡ POLD vs T_c, ¥ pPRISm vs PT_c, ¶ PRISm vs T_c, ₴ PT_c vs T_c.**

∂Baseline vs Post-exercise; ᵀPost-exercise vs Post-bronchodilator

(Single symbol denotes significance level <0.05, double symbol <0.01, triple symbol <0.001).

Table 4: Area within the impedance:volume (V) and impedance:flow (V') loops for prematurity-associated obstructive lung disease (POLD), prematurity-associated preserved ratio of impaired spirometry (pPRISm), preterm (PT_c) and term (T_c) control groups, at baseline, at post-exercise, and at post-exercise bronchodilation timepoints.

		POLD (n=14)	**pPRISm (n=11)**	**PT_c (n=90)**	**T_c (n=52)**
ARV (hPa.s)	Baseline	-0.26 (-0.44 to -0.07)	-0.34 (-0.47 to -0.20)	-0.42 (-0.49 to -0.35)	-0.55 (-0.72 to -0.39)
	Post-exercise	ð -0.70 (-1.10 to -0.31)	-0.17 (-0.32 to -0.02)	-0.48 (-0.58 to -0.37)	-0.56 (-0.74 to -0.38)
	Post-exercise BD	-0.77 (-1.23 to -0.31)	-0.38 (-0.65 to -0.12)	ŦŦ -0.61 (-0.76 to -0.45)	-0.69 (-0.89 to -0.50)
ARV' (hPa)	Baseline	2.12 (1.47 to 2.76)	1.81 (0.86 to 2.75)	1.85 (1.54 to 2.16)	1.76 (1.34 to 2.19)
	Post-exercise	2.42 (1.78 to 3.07)	1.50 (0.33 to 2.67)	1.82 (1.57 to 2.07)	2.09 (1.57 to 2.62)
	Post-exercise BD	1.93 (1.29 to 2.56)	1.84 (0.46 to 3.23)	1.80 (1.47 to 2.12)	1.90 (1.42 to 2.38)
AXV (hPa.s)	Baseline	0.49 (0.19 to 0.78)	0.23 (0.08 to 0.38)	0.28 (0.18 to 0.37)	0.34 (0.19 to 0.49)
	Post-exercise	**ðð 1.02 (0.42 to 1.61) ** †† ‡**	-0.01 (-0.34 to 0.32)	0.34 (0.20 to 0.49)	0.37 (0.20 to 0.54)
	Post-exercise BD	0.76 (0.27 to 1.26)	0.23 (0.00 to 0.47)	0.36 (0.22 to 0.49)	0.44 (0.29 to 0.58)
AXV' (hPa)	Baseline	-1.55 (-2.36 to -0.75)	-0.64 (-1.29 to 0.02)	-0.61 (-0.97 to -0.26)	-0.44 (-0.67 to -0.21)
	Post-exercise	**-1.72 (-2.40 to -1.04) † ‡**	-0.66 (-1.92 to 0.60)	-0.55 (-0.79 to -0.31)	-0.66 (-1.05 to -0.26)
	Post-exercise BD	ŦŦ -0.81 (-1.45 to -0.16)	-0.26 (-0.82 to 0.29)	ŦŦ -0.29 (-0.48 to -0.09)	-0.41 (-0.70 to -0.12)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction).

Abbreviations: ARV – Area within resistance-volume loop; **ARV'** – Area within resistance-flow loop, **AXV** – Area within reactance-volume loop; **AXV'** – Area within reactance-flow loop.

Significance symbols: ***POLD vs pPRISm, †POLD vs PT_c, ‡POLD vs T_c, ¥pPRISm vs PT_c, ¶PRISm vs T_c, ₴PT_c vs T_c;**
ðBaseline vs Post-exercise; ŦPost-exercise vs Post-bronchodilator

(Single symbol denotes significance level <0.05, double symbol <0.01, triple symbol <0.001).

Table 5: Mean impedance in expiration and inspiration for prematurity-associated obstructive lung disease (POLD), prematurity-associated preserved ratio of impaired spirometry (pPRISm), preterm (PT_c) and term (T_c) control groups, at baseline, at post-exercise, and at post-exercise bronchodilation timepoints.

		POLD (n=14)	pPRISm (n=11)	PT_c (n=90)	T_c (n=52)
Mean-respiratory parameters					
R_{meanE} (hPa.s/L)	Baseline	7.1 (5.8 to 8.3)	6.9 (5.9 to 7.9)	5.7 (5.4 to 6.1)	5.8 (5.3 to 6.3)
	Post-exercise	**7.8 (6.4 to 9.2) †† ‡**	6.6 (5.3 to 7.9)	5.8 (5.5 to 6.2)	6.1 (5.6 to 6.6)
	Post-exercise BD	ᵀᵀᵀ 5.7 (4.6 to 6.8)	5.8 (4.7 to 7.0)	ᵀᵀᵀ 5.0 (4.7 to 5.4)	ᵀᵀᵀ 5.4 (4.9 to 5.9)
R_{meanI} (hPa.s/L)	Baseline	**6.4 (5.5 to 7.3) † ‡**	6.1 (5.2 to 7)	5.1 (4.8 to 5.4)	5.0 (4.6 to 5.4)
	Post-exercise	**6.6 (5.7 to 7.6) †† ‡‡**	6.2 (5.0 to 7.5)	5.1 (4.8 to 5.4)	5.2 (4.8 to 5.6)
	Post-exercise BD	ᵀᵀᵀ 4.4 (3.9 to 5.0)	ᵀ5.1 (3.9 to 6.4)	ᵀᵀᵀ 4.1 (3.9 to 4.4)	ᵀᵀᵀ 4.4 (4.0 to 4.9)
ΔR_{mean} (hPa.s/L)	Baseline	0.6 (0.2 to 1.1)	0.8 (0.5 to 1.1)	0.7 (0.5 to 0.8)	0.8 (0.6 to 1.0)
	Post-exercise	∂∂ 1.2 (0.5 to 1.9)	0.4 (0.0 to 0.8)	0.8 (0.6 to 0.9)	0.9 (0.6 to 1.1)
	Post-exercise BD	1.2 (0.5 to 2.0)	0.7 (0.3 to 1.1)	0.9 (0.7 to 1.1)	1.0 (0.7 to 1.2)
X_{meanE} (hPa.s/L)	Baseline	**-4.0 (-5.3 to -2.8) ††† ‡‡‡**	-2.4 (-3.2 to -1.6)	-1.9 (-2.2 to -1.5)	-1.7 (-2.0 to -1.3)
	Post-exercise	**-4.9 (-6.0 to -3.8) * ††† ‡‡‡**	-3.1 (-4.6 to -1.6)	-2 (-2.4 to -1.7)	-2.1 (-2.5 to -1.7)
	Post-exercise BD	ᵀᵀᵀ -2.4 (-3.4 to -1.4)	ᵀᵀ -2.1 (-3.1 to -1)	ᵀᵀᵀ -1.4 (-1.7 to -1.1)	-1.8 (-2.2 to -1.4)
X_{meanI} (hPa.s/L)	Baseline	**-3.1 (-3.7 to -2.4) ††† ‡‡‡**	-2.0 (-2.7 to -1.3)	-1.4 (-1.7 to -1.2)	-1.2 (-1.5 to -1)
	Post-exercise	**-3.3 (-3.8 to -2.7) ††† ‡‡**	∂∂ **-3.1 (-4.7 to -1.4) ¥¥ ¶¶**	-1.5 (-1.8 to -1.3)	-1.6 (-2.0 to -1.2)
	Post-exercise BD	ᵀᵀᵀ -1.2 (-1.5 to -0.9)	ᵀᵀᵀ -1.7 (-2.7 to -0.7)	ᵀᵀᵀ -0.9 (-1.1 to -0.7)	ᵀᵀ -1.2 (-1.5 to -0.9)
ΔX_{mean} (hPa.s/L)	Baseline	-1.0 (-1.6 to -0.4)	-0.4 (-0.7 to -0.2)	-0.4 (-0.6 to -0.3)	-0.5 (-0.7 to -0.2)
	Post-exercise	∂∂ **-1.6 (-2.6 to -0.7) ** †† ‡‡**	0.0 (-0.7 to 0.6)	-0.5 (-0.7 to -0.3)	-0.5 (-0.7 to -0.2)
	Post-exercise BD	-1.2 (-2.0 to -0.4)	-0.4 (-0.8 to 0.0)	-0.5 (-0.7 to -0.3)	-0.6 (-0.8 to -0.4)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction).
Abbreviations: R – Resistance; **X** – Reactance; **E** – Expiration; **I** – Inspiration; **Δ** – Difference; **BD** – Bronchodilator.
Significance symbols: *** POLD vs pPRISm, † POLD vs PT_c, ‡ POLD vs T_c, ¥ pPRISm vs PT_c, ¶ PRISm vs T_c, ₴ PT_c vs T_c.**
∂Baseline vs Post-exercise; ᵀPost-exercise vs Post-bronchodilator
(Single symbol denotes significance level <0.05, double symbol <0.01, triple symbol <0.001).

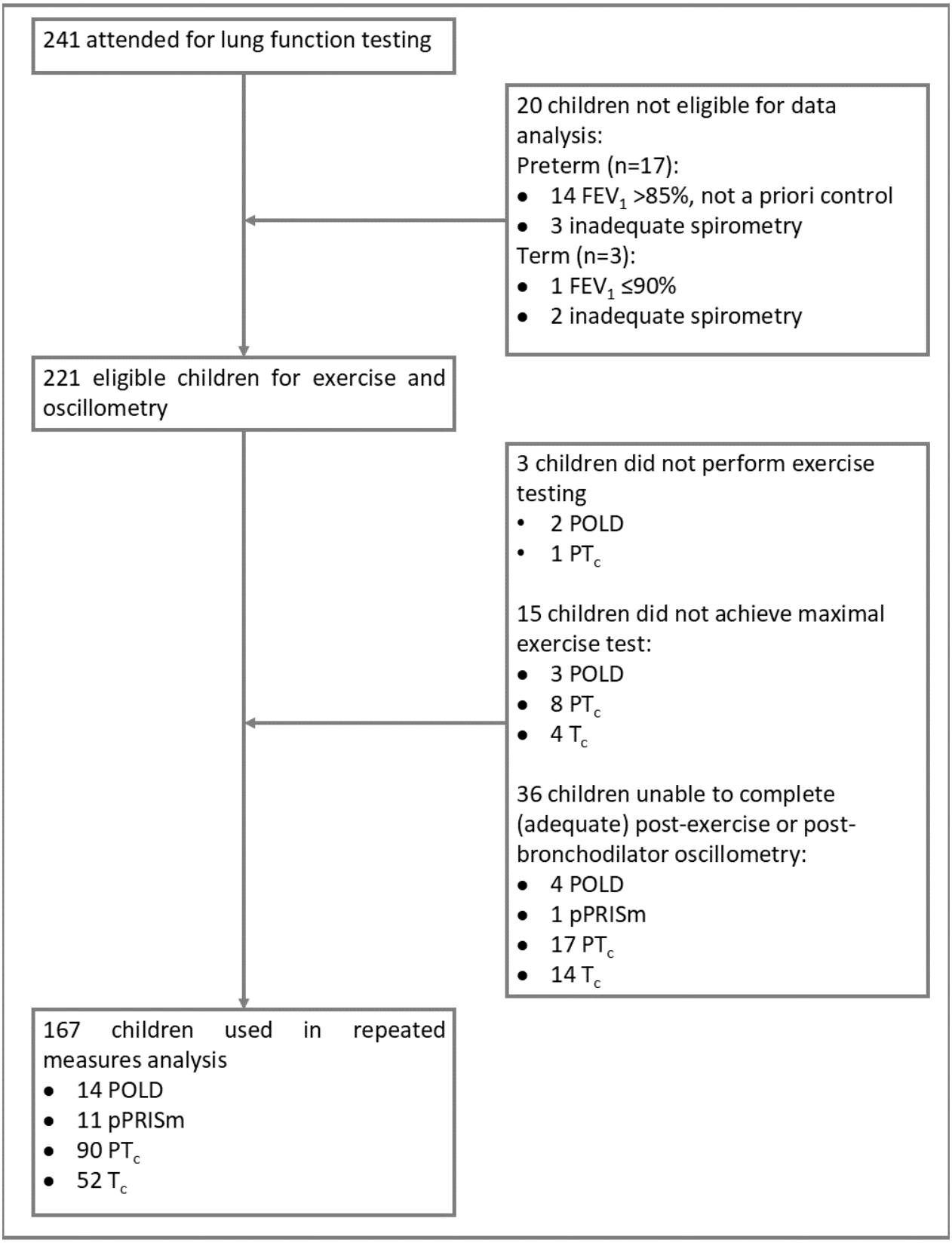

Figure 1

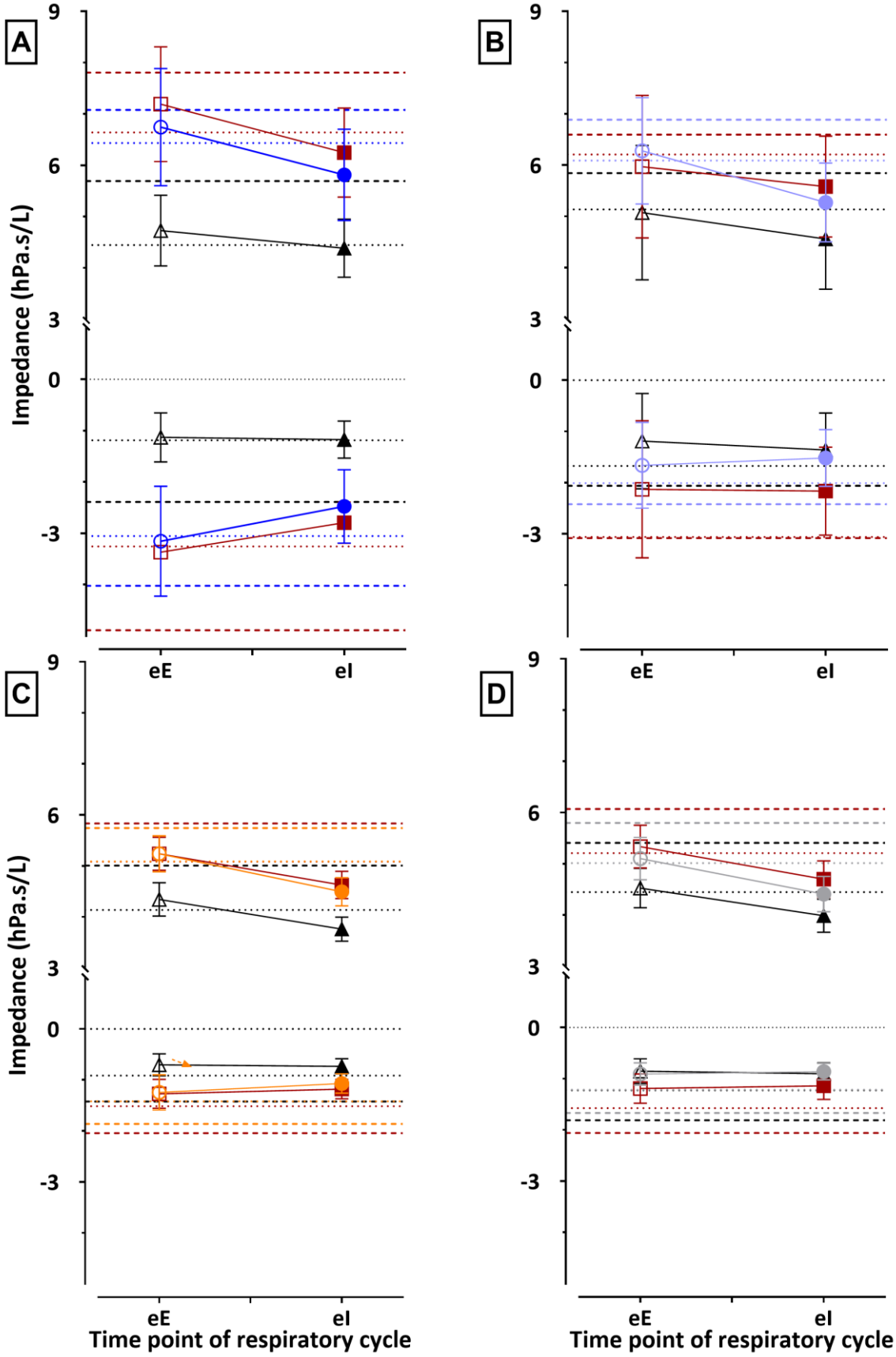

Figure 2

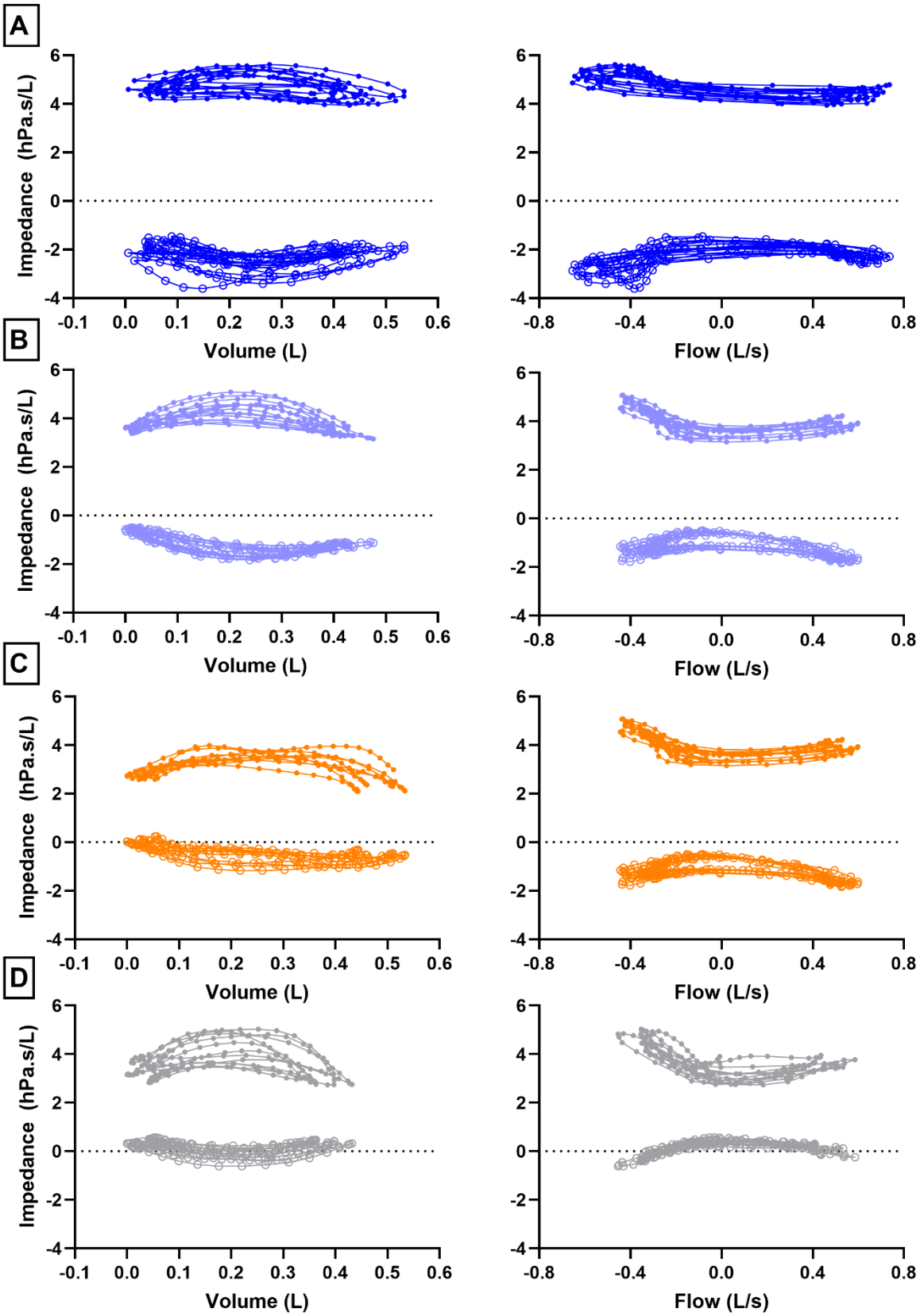

Figure 3

Intra-breath respiratory mechanics of prematurity-associated lung disease phenotypes in school-aged children

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**This publication is dedicated to our very dear, late friend Professor John Henderson.

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Methods

Population

Children were recruited from the Respiratory Health outcomes in NeOnates (RHiNO) study (EudraCT: 2015-003712-20). Inclusion criteria for preterm-born children into the study were birth at ≤34 weeks' gestation, aged 7-12 years, and being geographically accessible. Exclusion criteria were congenital or cardiorespiratory abnormalities, or significant neurodevelopmental impairment. The main RHiNO trial was a randomised control trial comparing a treatment course of inhaled corticosteroids alone or in combination with long-acting beta-2 agonist vs placebo in preterm-born children with percent predicted forced expiratory volume in 1 second (%FEV_1) ≤85%, to assess potential improvement in lung function. The aims of the wider study was to characterise children with lung function decrements based on perinatal, lung function and mechanistic influences, by comparison with preterm- and term-born controls.

Initial screening took place where baseline spirometry (Microloop Spirometer, Vyaire, Germany), along with history exploration and exhaled nitric oxide testing (NiOX VERO, Circassia, UK), was performed [1]. All preterm-born children with %FEV_1 ≤85% at screening were invited for participation in the randomised control trial [2], along with randomly selected preterm-born children with %FEV_1 >85% (within the first 10 screening visits of each calendar month) and all term children with %FEV_1 >90%, for control purposes. In depth lung function testing was performed at the Children and Young Adults' Research Unit at the Noah's Ark Children's Hospital for Wales in Cardiff. Term-born children were only recruited if their %FEV_1 was >90%, therefore no data were obtained from term-born children with lower %FEV_1.

Children were excluded if they were unable to perform adequate spirometry. Children prescribed medication that could potentially affect results were asked to withhold prior to testing for specified time periods [2], and testing delayed in the context of recent respiratory tract infections.

Oscillometry testing

Oscillometry testing was performed using a custom-built set-up and computer programme (NDAQ) developed by a team at University of Szeged in Hungary (Figure E1). A loudspeaker was connected to pressure and flow sensors within a measurement head, at the airway opening. The loudspeaker was further encased within a larger, sealed cylinder connected to above the loudspeaker via a shunt tube for pressure equalisation due to the potential of increased breathing frequency and pressure following exercise.

For testing, children sat upright on a chair, and breathed via a Microgard II microbial filter (Vyaire, Germany). A nose clip was worn and cheeks were held by the child or parent/researcher during testing. The loudspeaker generated a signal at 10Hz with impedance measured at the mouth using the pressure and flow sensors at 100 millisecond intervals. A minimum of 3 recordings over 24 seconds were taken to obtain adequate, artifact-free, sections for analysis.

Intra-breath oscillometry was performed at 3 separate (baseline; 20 minutes following maximal exercise testing; following post-exercise bronchodilation with 400 micrograms of salbutamol (Salamol, TEVA UK Limited) given via MDI using a Volumatic spacer (GSK, UK)).

Raw oscillometry data was analysed post-acquisition to obtain the results. Each of the recordings was assessed, and the trace with most regular, artefact-free respiration was used for analysis, with resistance (Rrs) and reactance (Xrs) measurements at various time points calculated by the software (i.e. at end-inspiration/expiration, maximal flow).

Table E2 displays the parameters analysed.

Spirometry and cardiopulmonary exercise testing (CPET)

Spirometry and cardiopulmonary exercise testing has been described in detail elsewhere [3]. Spirometry was performed using the MasterScreen Body and PFT systems with SentrySuite measurement software version 2.17 (Vyaire Medical, Germany) as per ERS/ATS guidance [4], with a

minimum of 3 tests performed, and QC to ensure the appropriate results from all the measurements were used. Calibration was performed as recommended. Results were measured at BTSP and Global Lung Initiative predicted values were used to ensure results comparable [5].

Cardiopulmonary exercise testing was performed on a Pediatric Cycle Ergometer (Lode, Netherlands) linked to a Masterscreen CPX system (Vyaire Medical, Germany). A ramp protocol of increasing Wattage (1 Watt every 6 seconds) following baseline measurements, was used, with testing ending when cadence was no longer consistently maintained. A 'maximal' test was defined by meeting ≥2/4 of the following criteria: Respiratory Exchange Ratio >1.00; heart rate ≥80% predicted (220 bpm – age); ≥9/10 on OMNI scale (pictorial scale for rating of perceived exertion [6]); VO_2 plateau based on visual analysis.

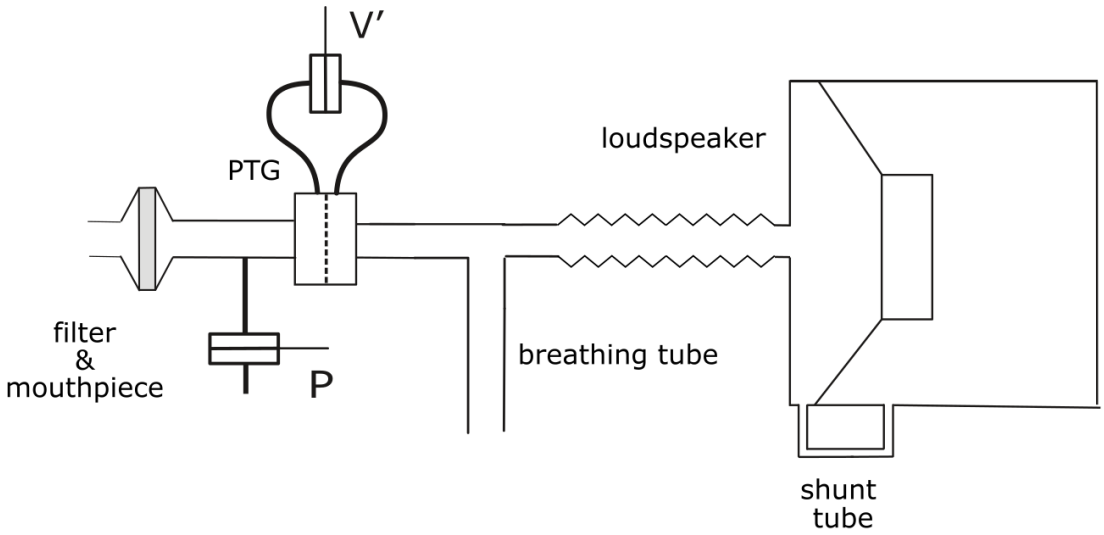

Figure E1. Schematic arrangement of the oscillometry device. PTG: pneumotachograph; pressure (P) and flow (V') sensors: Honeywell model 26PCAFA6D (Golden Valley, MN, USA). Antibacterial filter with mouthpiece (Microgard-II microbial filter, Vyaire, Germany).

Group	Abbreviation	Definition
Prematurity-associated obstructive lung disease	POLD	FEV_1 <LLN; FEV_1/FVC ratio <LLN)
Prematurity-associated preserved ratio of impaired spirometry	pPRISm	(FEV_1 <LLN; FEV_1/FVC ratio ≥LLN)
Preterm Controls	PT_c	FEV_1 ≥LLN
Term Controls	T_c	%FEV_1 >90%

Table E1. Abbreviations and definitions for grouping participants based on lung function.

Parameter	Definition
R	Resistance
X	Reactance
eE eI	Impedance at end expiration/inspiration
ΔR ΔX	Difference in resistance/reactance between end expiration and end inspiration
ΔR/VT ΔX/VT	Difference in resistance/reactance between end expiration and end inspiration, adjusted for tidal volume
meanE/I	Mean impedance in expiration/inspiration
ARV AXV	Area within the resistance/reactance-volume loops
ARV' AXV'	Area within the resistance/reactance-flow loops

Table E2. Intra-breath parameters used in analysis with abbreviations and explanations.

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