The impact of early life exposures on lung function in African preschool children

Dr Shaakira Chaya

CHYSHA002

Thesis presented for the degree of

Doctor of Philosophy

in the Department of Paediatrics and Child Health,

University of Cape Town

September 2025

Supervisors:

Professor Diane Gray

Professor Heather J. Zar

Abstract

Respiratory disease substantially impacts global health in children with the highest burden in low- and middle-income countries (LMIC). Pneumonia is a leading cause of death in children under five years, while chronic respiratory disease is a major contributor to non-communicable disease burden across the life-course. Early lung function is crucial for respiratory health, with low lung function in childhood linked to higher risks of respiratory and cardiovascular disease and premature mortality later in life. Lung growth occurs rapidly from birth to school age, yet most cohort studies have measured lung function later in childhood or adulthood. Until recently, tools for measuring preschool lung function have been limited. Additionally, much of the available data comes from cohorts in high-income countries. Many factors known to be associated with poor respiratory health such as indoor air pollution (IAP), environmental tobacco smoke (ETS) and preterm birth are prevalent in LMIC. However, data on the early life impact on lung health in these settings are lacking. Emergent pulmonary function tests, feasible in preschool children, have the potential to fill this gap.

This thesis aimed to investigate the determinants of preschool lung function in South African children enrolled in a birth cohort, the Drakenstein Child Health Study (DCHS). All 1143 children followed from birth who had infant lung function were included in the study, with lung function measured yearly between 3 to 5 years. Lung function measurements included tidal breathing, multiple breath washout and pre- and post-bronchodilator oscillometry and spirometry (from 5 years). At each visit, parents completed a questionnaire which assessed socioeconomic factors, smoking and exposure to passive cigarette smoke, current and past health history including lower respiratory tract infection (LRTI), HIV status and treatment history. Air pollutants were measured antenatally and at 4 to 6 months postnatally and included particulate matter 10, carbon monoxide, nitrogen dioxide, sulphur dioxide and volatile organic compounds.

The chapters of the thesis cover:

(1) A review of the need for and feasibility of lung function tools in preschool children, contributing to addressing the gap in our understanding of lung growth and development and its determinants during the preschool period. The results show that preschool lung function measurements are largely under-represented in LMIC, but feasible for clinical and epidemiological use, raising the potential of tracking lung function from early life through to adulthood and bridging the gap during the critical preschool period. The lack of suitable reference ranges for interpretation is highlighted, particularly for emergent preschool tests such as oscillometry.

(2) Deriving the first reference equation for oscillometry in African children using a cohort of 690 healthy children between 3 and 17 years.

(3) Investigation of the joint impact of antenatal and postnatal environmental exposures on lung development through to 3 years. These analyses show that both antenatal and postnatal IAP and ETS exposure impair lung function at 3 years. The effects of postnatal exposures on lung function were independent and additional to that of antenatal exposures, an important finding as these are modifiable factors.

(4) The impact of preterm birth on lung function through 5 years, providing the first longitudinal description of lung function in preterm infant survivors in LMIC. The findings

show that these children had impaired lung function trajectories over the first 5 years, notably in the moderate to late preterm group. LRTI and ETS were important modifiable factors contributing to reduced lung function in this vulnerable group. These finding have implications for clinical practice and public health, highlighting the need for improved maternal health initiatives to prevent preterm births, strengthen maternal pre-, peri- and postnatal health and to provide long-term follow-up of children born preterm. Additionally, the importance of educating healthcare professionals about the long-term effects of preterm birth is highlighted.

In conclusion, preschool lung function testing was found to be feasible in a LMIC setting. The reference range equation contributed to developing the usefulness of this tool for African children. Major determinants of lung function included LRTI, ETS, and preterm birth. Public health initiatives to enhance maternal health and peri- and postnatal care, and strategies to reduce ETS and early-life LRTI such as novel preventive therapies, should be prioritised in LMIC settings.

Declarations

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD; Paediatric Pulmonology) in the Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town.

I, Shaakira Chaya, hereby declare that the work included in this thesis is original research. No part of the thesis has been submitted in the past, or is being, or is to be submitted for a degree at this or any other University. This thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions by those acknowledged. I grant the University of Cape Town free licence to reproduce the thesis in whole or part, for the purpose of research.

Published manuscripts form part of this thesis. I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my thesis. My contribution to multi-authored papers is outlined at the beginning of each results chapter, and the assistance and contributions received from co-authors is described with each publication. I have obtained signed statements and agreement from all co-authors that I may include the following publications in my PhD dissertation:

1. Chaya S, Zar HJ, Gray DM. Lung function in preschool children in low and middle income countries: an under-represented potential tool to strengthen child health. Front Pediatr. 2022;10:908607. doi: 10.3389/fped.2022.908607.
2. Chaya S, MacGinty R, Jacobs C, Githinji L, Hlengwa S, Simpson SJ, Zar HJ, Hantos Z, Gray DM. Normal values of respiratory oscillometry in South African children and adolescents. ERJ Open Res. 2023;9(2):00371-2022. doi: 10.1183/23120541.00371-2022.
3. Chaya S, Vanker A, Brittain K, MacGinty R, Jacobs C, Hantos Z, Zar HJ, Gray DM. The impact of antenatal and postnatal indoor air pollution or tobacco smoke exposure on lung function at 3 years in an African birth cohort. Respirology. 2023;28(12):1154-1165. doi: 10.1111/resp.14576.
4. Chaya S, Simpson SJ, Marozva N, Jacobs C, Botha M, Workman L, Hantos Z, Zar HJ, Gray D. The effect of moderate-to-late preterm birth on lung function over the first 5 years of life in a South African birth cohort. ERJ Open Res. 2025;11(3):00733-2024. doi: 10.1183/23120541.00733-2024.

As per University guidelines, the text of each paper is presented verbatim in this thesis. As such, there are minor discrepancies in terminology between papers; these have not been changed in order to reflect the publications. Minor changes have been made to style and to figure and table numbers to ensure consistency throughout this thesis.

Signed

Shaakira Chaya

September 2025

Acknowledgements

This research was funded by the Harry Crossley Clinical Research Fellowship, SAMRC Researcher Development Award, Wellcome Trust (#098479/Z/12/Z, #204755/Z/16/Z). The Drakenstein Child Health Study (DCHS), within which this research was nested, was funded by grants from the Gates Foundation (#OPP 1017641, OPP1017579) to the Principal Investigator of DCHS, Prof Heather Zar.

I would like to thank the many individuals who made this research possible: Prof Zoltan Hantos, for his support with preschool oscillometry testing and data output management and interpretation. Thank you to Dr Shannon Simpson for her support in data analysis, interpretation as well as her optimism and encouragement. A special thank you to the DCHS statistical support team: Rae MacGinty, Kirsty Brittain and Nicola Marozva. Thank you for your patience, ongoing support and for going above and beyond.

A special thank you to Carvern Jacobs, for his help with data collection and analysis. No problem was ever too big to fix; and to the lung function team at Paarl hospital: Joavine Fourie, Frank Bantam, Maresa Botha and Simphiwe Erasmus, for their skill, hard work and dedication, which have contributed to the success of the study. Thank you to the participants and their families for their commitment to the study.

I would like to thank my University of Cape Town supervisor Prof Diane Gray for her encouragement, expert advice, patience, positive outlook, mentorship and guidance throughout the project; and for all the times you responded to my emails even while on a mountain with wildlife roaming around. To my second UCT supervisor, Prof Heather Zar for her wisdom, expert knowledge, invaluable insight and advice.

I thank my family and friends who have been patient, loving and supportive. A special thanks to my nieces Humairaa and Haaniyah for always being the light in my life. To my parents, Haroon and Yasmin, I dedicate this PhD to you. Thank you for everything!

List of Abbreviations

ART	antiretroviral therapy
ARV	antiretroviral
BDR	bronchodilator response
BMI	body mass index
BPD	bronchopulmonary dysplasia
C	Compliance
CF	cystic fibrosis
CMV	Cytomegalovirus
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
DCHS	Drakenstein Child Health Study
DHA	docosahexaenoic acid
ELBW	extremely low birth weight
ETS	environmental tobacco smoke
F_{res}	resonance frequency
FEF_{25-75}	forced expiratory flow at 25% and 75% of the forced vital capacity
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in the first second
FOT	forced oscillometry technique
FRC	functional residual capacity
FVC	forced vital capacity
GLI	Global Lung Initiative
HEU	HIV-exposed uninfected
HIC	high-income countries
I	Inertance
IAP	indoor air pollution
ICS	inhaled corticosteroid
IgG	immunoglobulin G
IUGR	intrauterine growth restriction
LBW	low birth weight
LCI	lung clearance index
LMIC	low- and middle-income countries
LRTI	lower respiratory tract infection

MBW	multiple breath washout technique
MLP	moderate to late preterm
NO_2	nitrogen dioxide
PCD	primary ciliary dyskinesia
PM	particulate matter
PMTCT	prevention of mother-to-child transmission
R	Resistance
R_6-R_{20}	difference between resistance at 6 Hz and resistance at 20 Hz
R_{eE}	R at end expiration
R_{eI}	R at end inspiration
R_{int}	interrupter resistance
RSV	respiratory syncytial virus
SES	socioeconomic status
SGA	small for gestational age
SO_2	sulphur dioxide
t_E	total expiratory time
t_{PTEF}	time to peak tidal expiratory flow
TB	Tuberculosis
TH2	T-helper cell type 2
V_E	expired tidal volume
V_{PTEF}	volume at peak tidal expiratory flow
VOC	volatile organic compound
X	Reactance
X_{eE}	X at end expiration
X_{eI}	X at end inspiration

Table of Contents

List of Tables

List of Figures

List of Supplemental Materials

Scope and layout of the thesis

This thesis represents the culmination of my research in the field of preschool lung function testing, focusing on the determinants and impacts of early life exposures on lung function.

The chapters of this thesis comprise the following:

Table 1.1 Overview of thesis chapters

Chapter	Overview
1	Background and aims.
2	A review of lung function in preschool children in low- and middle-income countries.
3	Deriving the first African data on normal reference equations for oscillometry in early childhood.
4	Investigating the impact of antenatal and postnatal indoor air pollution and tobacco smoke exposure on preschool lung function.
5	Investigating the impact of moderate late preterm birth on longitudinal lung function from birth to 5 years in African children.
6	Conclusion chapter which summarises findings, limitations and recommendations.

Chapter 1 provides a comprehensive and updated overview of the tools available for measuring and interpreting preschool lung function. The effects of antenatal and postnatal exposures on lung function are discussed, particularly within the context of low- and middle-income countries (LMICs). Background information on the need for and significance of preschool lung function is also included, and the rationale, aims and methodology of the thesis are described.

Chapter 2 / Publication 1: “Lung function in preschool children in low and middle income countries: an under-represented potential tool to strengthen child health”. This first paper describes the significant burden of respiratory disease in LMIC and highlights the importance of pulmonary function tests as objective measures of lung health and disease progression.

While spirometry is the most commonly used test, its application in preschool children is limited due to the complexity of the required manoeuvres. This review demonstrates that newer lung function techniques such as fractional exhaled nitric oxide (FeNO), oscillometry, the interrupter technique, and tidal breathing assessments have been successfully utilised in paediatric populations, but these tests are underrepresented in LMIC, yet they are both clinically feasible and applicable in epidemiological studies. Given the high burden of respiratory diseases in these regions, these tests hold the potential to enhance respiratory care. This paper has been published in Frontiers in Pediatrics (2022;10:908607).

Chapter 3 / Publication 2: "Normal values of respiratory oscillometry in South African children and adolescents". This paper includes the first derived oscillometry reference equations for African children and adolescents between the ages of 3 to 17 years. Oscillometry is a valuable tool for assessing respiratory function, particularly in young children from the age of 3 and in those who are unable to perform forced respiratory manoeuvres required for traditional tests such as spirometry. Oscillometry can identify healthy children and those with respiratory diseases. However, the clinical application of oscillometry in African populations has been limited due to the absence of a reference equation. To address this gap, we have established the first set of reference equations tailored to African children and adolescents, facilitating a more objective and clear understanding between health and disease within this population. Additionally, we also observed a broader and ongoing issue concerning the lack of global standardisation in oscillometry methodology and equipment. This is being addressed on a global platform. This paper has been published in ERJ Open Research (2023;9(2):00371-2022).

Chapter 4 / Publication 3: “The impact of antenatal and postnatal indoor air pollution or tobacco smoke exposure on lung function at 3 years in an African birth cohort”. IAP and ETS exposure contribute to the high burden of childhood respiratory illnesse, but research describing the impact of environmental exposures on preschool children in LMIC is limited. This paper addressed this gap by using comprehensive lung function testing and demonstrated that both antenatal and postnatal exposure to IAP and ETS are associated with impaired lung function at the age of three. These findings highlight the need for public health awareness and initiatives aimed at reducing these exposures. This paper has been published in Respirology (2023;28(12):1154-1165).

Chapter 5 / Publication 4: “The effect of moderate to late preterm birth on lung function over the first 5 years of life in a South African birth cohort.” Preterm birth is associated with increased mortality and morbidity, particularly due to lung disease. LMIC have the highest rates of preterm birth with the largest burden being MLP infants. The long-term impact on lung function in this group is poorly understood. This paper assessed the impact of MLP on lung function trajectories from birth to 5 years using oscillometry and MBW measurements. The results demonstrated that these children have impaired lung function trajectories over the first 5 years of life. LRTI and tobacco smoke exposure further impaired lung function, independent of being born MLP. These findings highlight the importance of strengthening maternal health, perinatal care and strategies to reduce tobacco smoke exposure and early life LRTI. This paper was recently published in ERJ Open Research (2025;11(3):00733-2024).

Chapter 6 / Research in context and concluding statements: This chapter summarises the key findings, broader implications, and impact of this research, as well as its relevance to South

Africa and other LMIC. The chapter discusses future directions and recommendations arising from this research.

Chapter 1: Background and aims

1.1 Background

1.1.1 Introduction

Childhood respiratory disease contributes to a high burden of morbidity and mortality globally with lower respiratory tract infections (LRTI) being a leading cause of death in children under 5 years [1]. Respiratory diseases such as asthma and wheeze are prevalent among children and adolescents, and this sets the trajectory for the development of chronic respiratory disease in adulthood [2-5].

Several early life exposures significantly impact lung health in adulthood. These include LRTI, environmental factors such as tobacco smoke exposure or air pollution, and poor living conditions. Additionally, malnutrition or premature birth also play a role [5, 6]. Maternal health conditions, including HIV infection and limited maternal education, further impact lung development [6]. However, the majority of data informing these findings come from high-income countries (HIC). In contrast, there is a scarcity of data examining the effects of these exposures on long-term lung health in low- and middle-income countries (LMICs), despite the high burden of respiratory illness in these regions which are home to the substantial majority of the global population [6].

Early lung development is key to lifelong lung health, with growing evidence suggesting that lung function established in early life is crucial for long-term respiratory health. Low lung function has been linked to increased risk of respiratory disease, morbidity, and mortality later in life [3, 7, 8]. Impaired lung function in childhood is associated with low lung function in later life [3]. Findings from the TUCSON study have shown that individuals with low

levels of forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) in the second or third decade of life are at increased risk for early cardiopulmonary mortality [8]. Significant lung growth occurs from birth to school age, making this period vital for determining future lung health [9, 10]. Lung development follows three phases: a growth phase until peak function (from birth to early adulthood), a brief plateau, and a decline phase [9]. Both genetic and environmental factors influence these trajectories. Since lung development continues through childhood, early postnatal exposures can disrupt this process. Many studies assess lung function in later childhood, with a notable gap in lung function measurements during the critical period of preschool years [2, 10-12]. Understanding how these exposures influence early and long-term lung health is essential for developing effective interventions and prevention strategies to promote optimal lung development.

A number of longitudinal birth cohort studies from HIC tracking lung development through childhood to adulthood have identified several important early life factors associated with chronic respiratory illness. These include early life LRTI, prematurity, antenatal and early life exposures such as indoor air pollution (IAP) and smoke exposure [4, 13-16]. Cohorts from LMICs have contributed additional evidence of the negative impact of early life factors on preschool lung function which include air pollution [7, 17, 18], early life LRTI [19, 20] and maternal HIV [7, 21]. However, there is a lack of comprehensive lung function data between infancy and preschool years, particularly in LMIC where there is a high burden of risk factors. While spirometry is the most commonly used and widely available test, its application is only feasible from 5 years and older. Available tools that are feasible in the preschool age group have limited availability in LMIC. Bridging this gap in lung function measurement is essential for understanding the impact of antenatal and early life factors on healthy lung development, as well as for identifying potential preventative measures.

1.1.2 Measuring lung function in preschool children

Pulmonary function tests evaluate the physiology and mechanics of the respiratory system. Spirometry is the most common pulmonary function test available in both the inpatient and outpatient setting, however its use is limited in preschool children due to the need for co-operation and specific respiratory manoeuvres. In recent years, non-invasive lung function tests feasible in young children have been developed and guidelines produced for preschool children [22, 23]. Other tests used in preschool children include tidal breathing flow and volume, multiple breath washout technique (MBW), oscillometry, fractional exhaled nitric oxide (FeNO) and other tests which measure airway resistance, include the interrupter technique and plethysmography [22]. These have been used to determine lung function and track lung growth in older children [2, 24, 25], summarised in Table 1.1. These tests are feasible and reproducible in preschool children and the advantage of being able to identify early lung disease makes them useful tools in the diagnosis and management of children with respiratory disease. They are also useful for assessing the impact of respiratory illness and risk factor exposure on lung health [26, 27], however access to lung function testing is limited in Africa due to technical reasons (poor access to training and skilled technicians, and poor technical support) and the cost of equipment and consumables [28].

Table 1.2 Summary of preschool lung function tests

Test	Use	Manoeuvre	Age	Advantages	Disadvantages	Variables	BDR / Bronchial challenge test
Spirometry [10, 22, 29-31]	Assess lung function impairment associated with chronic lung diseases such as cystic fibrosis, asthma, and chronic lung disease of prematurity. Provides information on medium to large airways. Can perform reversibility and hyperresponsive testing.	Forced manoeuvre- The procedure starts with normal tidal breathing. The child then takes a deep breath to reach total lung capacity, followed by a forceful exhalation, until all the air is expelled.	Reliable success from 6 years of age	Widely available and most used. Most childhood longitudinal lung function studies use spirometry. GLI race neutral reference available.	Complicated manoeuvre. Does not assess gas exchange or lung volumes comprehensively. Only feasible from around 5 years of age.	FEV_1, FVC, FEV_1/FVC%, FEF_{25-75}.	Yes
Oscillometry (previously known as FOT) [22, 32-37]	Sensitive for peripheral airway disease. Increased resistance in patients with bronchiectasis, CF, asthma, children with HIV lung disease. Can perform reversibility and hyperresponsive testing.	Performed during tidal breathing. Oscillations applied to the mouth in a frequency range of 4-50 Hz generated by a loudspeaker. Impedance calculated. Spectral (6-32 Hz) (multiple) and intrabreath measures (single) (10 Hz).	From 3 years	Non-invasive technique. Does not require cooperation. Does not require sedation. Intrabreath measured during zero flow- more sensitive measure.	Complex interpretation. Lack of standardisation across various commercial devices. Limited normative values and reference equations especially for underrepresented population groups.	Impedance comprises 2 components: R and X. R measures airway calibre, i.e. airway resistance. X measures the elastance or "stiffness" of the lungs. Other variables: F_{res}, frequency	Yes

					Unclear use in parenchymal disease.	dependence of R_{rs} (R_6-R_{20}) Intrabreath measurements: R_{eI}, R_{eE}, X_{eI}, and X_{eE}.	
Multiple breath washout [22, 32, 35, 38, 39]	Detects peripheral airway narrowing / obstruction. Monitoring of children with CF, asthma, bronchiolitis obliterans, chronic lung disease of prematurity, bronchiectasis and patients with HIV.	Tidal breathing	From 3 years	Performed during tidal breathing. GLI reference range established for *LCI* and *FRC* which include data from Africa.	Cost of equipment and tracer gases. Technical expertise required to perform. Mask resistance needs to be accounted for if child unable to use a mouthpiece.	Measures the *FRC* and calculating the *LCI* which is a measure of ventilation homogeneity.	No
Tidal breathing flow volume loops [22, 40]	Infants and children with wheeze- reflect airway obstruction. The shape of the tidal breathing flow– various airway disorders and can diagnose the level of obstruction.	Tidal breathing	From 3 years	Easy to perform	Variability in breathing patterns. No reference values for preschool children. Majority of studies focused on infancy-little data in preschool children.	t_{PTEF}, t_E, and the ratio of these (t_{PTEF}/t_E); V_{PTEF} (ml), V_E (ml), and the ratio of these (V_{PTEF}/V_E).	Yes
Interrupter technique [22, 41-43]	Resistance of the respiratory system-children with wheezing or asthma.	Tidal breathing-sudden interruption of flow during tidal breathing, this allows for alveolar and pressure at the	Preschool children- 6 years of age	Easily performed during tidal breathing. Reference ranges for children are available.	Different methodology used-comparison between tests difficult. The best algorithm to calculate pressure	R_{int}	Yes

		mouth to equilibrate so that alveolar pressure can be estimated.		Does not require sedation.	at the mouth during occlusion has not been determined The cut-off value for R_{int} post bronchodilator needs to be established.		
FeNO [44-46]	Non-invasive marker of TH2 eosinophilic airway inflammation causing asthma and guides ICS use. Assist in the differential diagnosis of other conditions such as cystic fibrosis, PCD, scleroderma, obstructive sleep apnoea syndrome and hepatopulmonary syndrome. Monitoring patients with pulmonary hypertension.	Deep inhalation through the mouth; slow exhalation, keeping a constant flow rate with feedback given to the patient during the test to maintain a constant flow.	Children from 5 years of age	Normal values from 4 years of age. Adjunct to the diagnosis of TH2 type asthma and guiding the use of ICS treatment.	Several techniques leading to variability of results. Requires co-operation. Cost of the equipment and running cost- especially within a LMIC setting.	FeNO	No

BDR: bronchodilator response; CF: cystic fibrosis; F_{res}: resonance frequency; FEF_{25-75}: forced expiratory flow at 25% and 75% of the forced vital capacity; FEV_1: forced expiratory volume in the first second; FeNO: fractional exhaled nitric oxide; FOT: forced oscillometry technique; *FRC*: functional residual capacity; *FVC*: forced vital capacity; GLI: Global Lung Initiative; ICS: inhaled corticosteroid; *LCI*: lung clearance index; PCD: primary ciliary dyskinesia; *R*: resistance; R_6-R_{20}: difference between resistance at 6 Hz and resistance at 20 Hz; R_{eE}: *R* at end expiration; R_{eI}: *R* at end inspiration; R_{int}: interruptor resistance; R_{rs}: respiratory resistance; t_E: total expiratory time; TH2: T-helper cell type 2; t_{PTEF}: time to peak tidal expiratory flow; V_E: expired tidal volume; V_{PTEF}: volume at peak tidal expiratory flow; *X*: reactance; X_{eE}: *X* at end expiration; X_{eI}: *X* at end inspiration.

1.1.3 Tools needed for lung function interpretation

Interpretation of pulmonary function tests requires appropriate reference equations to differentiate between health and disease [47], and for understanding of normal growth and development [47-49]. Since most lung function measures are age and somatic size dependent, a reference range equation needs to include age and height [47]. Furthermore, reference data needs to include a large number of healthy participants to account for biological difference and population variation [50]. Additionally, population based determinants need to be taken into account during interpretation [51].

Over the past decade, global collaborative efforts have attempted to validate all age international reference standards for use globally [52]. These initially included an ancestry correction, or an averaged equation for un-represented groups. However, the reference equations for lung function have recently come under scrutiny especially with regards to the historical simplistic use of ancestry in interpretation and have moved towards a proposed global race neutral lung function equation [52]. Differences due to race or ethnicity may reflect socioeconomic status (SES) and exposures rather than inherent biological differences [51, 52]. Therefore the Global Lung Initiative (GLI) have now developed a race neutral equation, acknowledging that more research is needed on ways to include the social and environmental determinants of pulmonary function in interpretation [51]. Global reference equations are being developed for other lung function tests, though the majority of data currently remains from Caucasian population groups [53, 54]. A recent MBW reference equation was published including two sites from South Africa, one in Cape Town and the other in Johannesburg [39].

With respect to oscillometry, there are numerous population reference equations, with the majority representing Caucasian populations [55]. These reference equations were derived from small population groups with few people without uniform standardisation of equipment [36, 55]. Validation of these equations to other populations is rarely performed [55]. Currently there are no oscillometry equations for the African continent despite the high burden of respiratory illnesses. GLI reference equations are underway. Healthy data are needed for Africa and similar representative population groups to strengthen interpretation of these measures.

1.1.4 Exposures affecting lung development

Antenatal and postnatal exposures influence foetal lung development and have long-term effects on respiratory health throughout life [3]. Various antenatal and postnatal exposures such as maternal smoking, air pollution, maternal nutrition, HIV and early life exposures such as preterm birth, nutrition and LRTI are critical during pregnancy and can adversely affect the developing foetal lungs [21, 56-66].

1.1.4.1 Nutrition

Maternal nutrition

Maternal nutrition plays an important role in foetal lung development by directly affecting lung growth as well as through epigenetic changes. Most of our understanding of prenatal nutrition is derived from animal models [59, 67]. One of the common effects of chronic prenatal malnutrition is intrauterine growth restriction (IUGR). This is as a result of fewer nutrients and oxygen delivery to the foetus via the placenta either due to maternal dietary deficiency or placental insufficiency [59, 68]. Low birth weight (LBW) is long recognised as one of the predictors of poor health outcomes, including lung function in children and adults

[58]. This was first described by Barker who hypothesised that suboptimal in-utero environment resulting in IUGR, LBW, and premature birth leads to the development of insulin resistance, hypertension, coronary heart disease and chronic obstructive pulmonary disease (COPD) in adulthood, all factors associated with increased mortality [69, 70].

Antenatal micronutrient deficiencies may also be important in foetal lung development. Maternal micronutrients such as vitamin A regulate expression of the extracellular matrix proteins that are essential for airway development and alveolarization. Vitamin D plays a role in surfactant metabolism and in epithelial mesenchymal interactions that is needed for lung maturation. Maternal deficiency in vitamin E and selenium have been linked to increased wheezing in children. Docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid, found in fish oil and fatty fish plays a role in lung maturation and decreases hyperoxic lung injury. A deficiency in infants born less than 30 weeks may increase the risk of bronchopulmonary dysplasia (BPD). Maternal supplementation with fish oil in pregnancy has been associated with a reduced likelihood of persistent wheezing at 3 years and asthma at 5 years [59, 71, 72].

Women living in LMICs are more often exposed to food insecurity and malnutrition [73]. South Africa, like other LMIC, faces a double burden of malnutrition which includes both undernutrition (micronutrient deficiency, underweight, and childhood stunting and wasting) and overnutrition [74]. Overweight/obese mothers are more likely to have stunted children and an increase in maternal body mass index (BMI) is associated with increased wheezing in the first year of life [75]. Maternal malnutrition can affect the immune system as well as alter inflammatory processes. This has an impact on foetal immune system and increases the risk of infections in infancy [76].

These findings suggest that the effects of maternal malnutrition are both immediate and long term, and are associated with respiratory morbidity in infancy and later lung function.

Postnatal nutrition

Postnatal nutrition plays a crucial role in lung function development [59, 77], and should be one of the key areas of focus in newborn infants especially those born preterm. Studies have shown that there is a higher risk of asthma and wheeze in infants and children with LBW (or late preterm), and in extremely low birth weight (ELBW) infants [78, 79]. Furthermore, growth failure in the first weeks of life was associated with a high rate of BPD [59, 80]. On the other hand, a review of 24 birth cohort studies showed that greater infant weight gain in those born LBW was associated with an increased risk of childhood asthma and lower FEV_1 and FEV_1/FVC z-scores [59, 81]. This may be a result of dysnaptic growth, a disproportionate growth between the airways and lung parenchyma which may result in in airflow limitation [59].

Breastfeeding has been shown to be protective against viral infections, likely due to the immune properties of breastmilk [59, 82]. Children and adolescents who were breastfed for more than 4 months had improved lung function volumes as measured by spirometry compared to formula-fed children [5, 83].

There are limited data on the effect of childhood malnutrition on lung development. Alveolarisation, a critical phase of lung growth, continues into early childhood. Malnutrition in early life can potentially affect lung growth and development, however one study showed that children who suffered from malnutrition before 2 years of age had similar lung function to those that were not malnourished [84]. Notably, 46% of patients in this study died before

follow up raising concerns of the potential for lower lung function that was not monitored [84, 85]. Furthermore, lower spirometry values across all participants in the study indicated a generally poorer nutritional status, complicating the comparisons between cases and controls [85]. Another cross-sectional study also found that children with a BMI < -2SD had a lower FEV_1 and FVC, yet maintained a normal FEV_1/FVC [86]. Both studies report smaller lung sizes in malnourished children but preserved lung function [87] as well as shorter leg lengths while sitting heights remained similar concluding that these findings were in keeping with "lung sparing growth" [59, 84, 86]. Since spirometry assesses large and medium airways, other lung function tools like oscillometry or MBW may be more sensitive in detecting subtle or earlier differences. These finding also demonstrate the challenges in identifying correlations between severe acute malnutrition and lung growth [85].

Overnutrition resulting in obesity is a major problem globally and also in lower income countries especially sub-Saharan Africa [88]. Obesity impacts on lung mechanics resulting in decreased diaphragm movement, abnormal gas exchange and increased work of breathing [89]. Obesity is associated with increased inflammation resulting in airway hyperactivity and asthma [89, 90]. Being overweight and obese is associated with airway dysynapsis which is linked to increased asthma morbidity [91]. Lung function measurements show that children have lower lung volume and capacity (reducing functional residual capacity, expiratory reserve volume and residual volume) and a decrease in FEV_1/FVC ratio [92-94].

A longitudinal study assessing the effect of BMI on lung function in children aged 6-18 years demonstrated that higher appendicular lean body mass positively correlated with a higher FEV_1, FVC and total lung capacity. An increase in muscle mass was associated with a higher FVC and total lung capacity. However, a higher fat mass was linked to a lower functional

residual capacity (*FRC*) [95]. This is further highlighted by a birth cohort study identifying six distinct BMI trajectories from birth to early adulthood, all diverging from about 2 years. Of these, the persistently low BMI group had decreased FEV_1 and *FVC* growth during 8 to 16 years. The accelerated increasing BMI group had lower pre and post bronchodilator FEV_1/FVC ratio z-scores and higher lung clearance index (*LCI*) at 24 years compared to the stable normal BMI group. The persistent high BMI group showed reduced FEV_1 and *FVC* growth from 16 to 24 years, along with elevated *LCI*. BMI trajectories from 5 to 43 years showed that patients with high and low BMI trajectories were at risk of a restrictive and obstructive lung function pattern respectively [96]. These longitudinal studies emphasise the importance of healthy nutrition, the lack of preschool lung function data and the need for utilising alternative lung function tools feasible in preschool children to track lung function during this period. This will help identify divergent lung function earlier and potential intervention to improve long-term lung health [97].

These findings suggest that postnatal nutrition both in early life and childhood are important for optimal lung health. Both under and overnutrition impact lung function negatively and are important targets for intervention.

1.1.4.2 Tobacco smoke exposure

The incidence of smoking and second hand smoke exposure remains high in LMICs, notably among pregnant women who are at home living in household where individuals smoke [98].

Antenatal tobacco exposure

Antenatal smoking during pregnancy can impact lung growth and development. Studies have shown that exposure to tobacco smoke in utero can alter lung development, resulting in reduced lung function in childhood and beyond [7, 99], and a higher risk of respiratory issues

such as asthma, bronchitis, and decreased lung function especially involving the small airways later in childhood [3, 60, 100].

Tobacco exposure in the pseudoglandular phase interferes with structural development of the airways. This disruption affects the conducting airways causing dysynaptic growth [63, 101, 102] which results in decreased airflow and increased resistance. Nicotine exposure results in an increase in collagen deposition in the airways and parenchyma [63, 102].

Children exposed to tobacco smoke in utero are more likely to experience complications such as preterm birth and LBW, both of which are associated with increased respiratory morbidity. The effects are compounded by postnatal smoke exposure [3, 63, 100]. Smoke exposure within the Drakenstein Child Health Study (DCHS), a LMIC setting in South Africa, was associated with abnormal lung function at 6 weeks and 1 year of age [7, 19, 103, 104].

Postnatal tobacco exposure

It is difficult to dissect out the respiratory effects of antenatal smoke and additional postnatal smoke exposure. Postnatal tobacco smoke exposure is also associated with impaired pulmonary function in infants and children, with these effects persisting into adulthood, the major effect occurs in utero [60, 105].

A study found three key environmental factors associated with impaired lung growth and the progression of preschool wheezing to school-age asthma: aeroallergen sensitization, severe wheezing episodes, and tobacco smoke exposure. Of these, tobacco smoke exposure was the only modifiable factor, highlighting the importance of addressing this [2]. Children exposed to environmental tobacco smoke (ETS) are more likely to experience cough and wheeze

[100], as well as lower lung function in childhood and early adulthood. Lower FEV_1 has been demonstrated in previous studies [60, 105, 106], which is an indicator for early mortality. Additionally, the interaction of genetic and environmental factors contributes to the long-term effects of tobacco smoke exposure on lung health, with research focussed on the association among genes, smoke exposure, and the development of asthma [107].

Understanding these interactions is crucial for developing targeted interventions aimed at mitigating the impact of tobacco smoke on respiratory health in children.

1.1.4.3 Air pollution

Air pollution, both indoor and outdoor, impacts lung function in infants and children. Alternative fuels such as coal, wood, dung, and crop residues, paraffin and liquefied petroleum gas are used for indoor heating and cooking which releases toxic by-products [108]. Pregnant individuals are frequently exposed to high levels of air pollutants, such as particulate matter (PM), nitrogen dioxide (NO_2), and sulphur dioxide (SO_2) which may impact on foetal development [62, 63, 109].

IAP is especially prevalent in LMIC, and in South Africa, despite advancements in electrification, approximately 40% of the population continues to rely on alternative fuel sources in the household [110, 111] and this increases to about 90% in rural communities [111, 112].

Antenatal exposure

Air pollutants can trigger inflammatory responses and oxidative stress in the maternal body, which in turn can affect placental function and foetal oxygenation [113]. Additionally, the

developing lungs of the foetus are particularly vulnerable to environmental toxins, which may interfere with normal developmental processes [64]. This results in the disruption of lung growth and development leading to reduced lung function, increased susceptibility to respiratory diseases, and a higher risk of developing conditions such as asthma later in life [63, 109].

Like tobacco smoke exposure, air pollution exposure is increasingly being recognised as a public health concern due to its potential adverse effects on foetal development and long-term health outcomes.

Postnatal air pollution

Children exposed to air pollution exposure (indoor and outdoor), are likely to have associated antenatal exposures as well [114]. Children are vulnerable to environmental pollution as they receive a proportionally higher dose than adults within the same environment due to the larger body surface area [115], lower capacity to metabolise and excrete the toxic substance and, specifically to younger children, they have a higher respiratory rate resulting in a proportionally higher dose of pollutant exposure [63]. Environmental exposures can adversely affect lung growth and function, predisposing children to chronic respiratory conditions that can persist into adulthood. For example, long-term exposure to ambient air pollution between the ages of 10 and 18 years was associated with decreased FEV_1 [116], while similar exposures in younger children aged 8 to 11 years resulted in reductions in both FEV_1 and FVC [117]. IAP poses significant risks to the respiratory system. Indoor biomass fuel exposure was associated with an increased risk of pneumonia [112, 118]. Tracking early life lung function in high air pollution areas is crucial for understanding long-term respiratory health consequences and informing public health policies that promote cleaner air. By

identifying early respiratory decline, targeted interventions can be implemented to protect vulnerable populations and mitigate the risk of chronic conditions such as asthma.

1.1.4.4 HIV exposure

The prevalence of HIV among pregnant women in South Africa is 27.5% [119]. With the implementation of prevention of mother-to-child transmission (PMTCT) program and the introduction of antiretroviral therapy (ART) the rates of perinatal HIV transmission have declined, however there are still a number of women living with HIV giving birth each year. This results in a growing population of HIV-exposed uninfected (HEU) children who have been exposed not only to HIV but also to maternal antiretrovirals (ARVs) during pregnancy [120, 121]. Exposure to ART in-utero can affect lung growth and function by dysregulating metabolic pathways potentially affecting lung growth and development [122].

Maternal HIV has shown to be associated with increased infectious morbidity and mortality, and poor birth outcomes such LBW, preterm birth and small for gestational age (SGA) [56, 57, 122, 123-125]. In the DCHS study, HEU infants had higher size-adjusted tidal volumes at 6 weeks, likely caused by a combination of antenatal exposure to a lower maternal immunity, ARVs, and the HIV virus [21]. Initiating ART in the mother during pregnancy was associated with a lower tidal volume at 6 weeks compared to initiating ART before pregnancy, highlighting the importance of prioritising maternal health by early ART initiation as this is beneficial to infant lung growth. At 2 years of age, *LCI* was higher in vulnerable HEU children, particularly in children whose mothers had uncontrolled disease, compared to HIV uninfected infants [21].

In addition to the direct impact of HIV, factors such as maternal nutrition, maternal viral load, maternal inflammation and ART have also been associated with impaired infant immune status and clinical outcomes [56, 126]. This is as a result of higher maternal viral load and lower CD4 counts associated leading to increased susceptibility to infections in early life. In addition, lower placental immunoglobulin G (IgG) transfer is associated with increased infectious morbidity in infants [56, 127]. While antenatal exposures to HIV is linked to potential adverse effects on lung function and development, these effects are influenced by multiple factors including social and environmental determinants. Understanding these factors is important to support the health and respiratory development of HEU children. HEU children have a high rate of respiratory diseases which include invasive pneumococcal infections, tuberculosis (TB), cytomegalovirus (CMV) and respiratory syncytial virus (RSV). The risk is highest in the first year of life. It has been shown that HEU children are 40% more likely to be hospitalised with LRTI, have longer hospital admissions and more likely to require mechanical ventilations compared to unexposed children [123, 128, 129]. Additional risk factors such as poverty and exposure to ETS are associated with a higher risk for infections by family members who have a compromised immunity [56, 57]. This highlights the multifactorial burden of HIV exposure on the developing lung, and the need to track lung function from early life to inform optimal management strategies.

1.1.4.5 Prematurity and LBW

Being born prematurely and having a low birth weight for gestational age are risk factors for low lung function which extends to school aged children and adolescents [130]. LBW and premature delivery leads to an increased prevalence of respiratory symptoms including frequent LRTI especially within a LMIC setting [131].

The majority of studies on the impact of preterm birth have focused predominantly on early preterm (<32 weeks) infants requiring ventilation, prolonged oxygen requirements and predisposition to sepsis and with the subsequent complication of BPD resulting in ongoing respiratory symptoms, airway obstruction in childhood and increased hospitalisations for infections in the first year of life [10, 132].

Studies in early preterm infants demonstrated significant impairment in lung function at 9 to 11 years, moreover if prematurity is associated with BPD, it may lead to irreversible airway obstruction in adulthood [133, 134]. BPD results in altered lung development and children develop chronic respiratory symptoms including wheeze and cough. These symptoms may decrease over time, but overall lung function remains low during late adolescence [132, 135]. These conditions, in addition to alveolar dysfunction, reflect small airway disease therefore a reliable measure of small airway function is needed for early diagnosis, to monitor the disease and to track respiratory function overtime [10].

Approximately 85% of global prematurity is late preterm, with 80% occurring in Asia and sub-Saharan Africa. There is however the belief that moderate to late preterm (MLP) infants (32-37 weeks) are physiologically and metabolically similar to term infants with a low risk of morbidity and mortality [66]. Late preterm and early term infants have increased respiratory morbidity during the neonatal period [65], and are more likely to require mechanical ventilation at birth compared to term infants. MLP birth has also been associated with an increased risk of LRTI in infancy from respiratory viruses such as RSV [65, 136], more wheezing episodes and risk of asthma in childhood [136]. Studies reporting the long-term respiratory outcomes of MLP in childhood are limited; however the finding from a recent systematic review and meta-analysis concluded that MLP had lower spirometry expiratory

airflows than those born at term, though the reductions were modest [137]. This data is particularly relevant to LMIC regions where the burden of preterm birth lies and where early preterm survival rates are significantly lower, reflecting limited access to neonatal intensive resources [138, 139]. The majority of current studies are focused on ELBW infants and are limited to HIC. Studies on the long-term respiratory effects in the MLP group, especially within LMIC settings, are limited, and data is needed to assess the long-term impact of lung growth and development in this vulnerable group.

1.1.4.6 LRTI

LRTI is among the leading causes of morbidity in LMIC, and is associated with chronic respiratory illness and impaired lung function in later childhood and adulthood [4, 7, 19, 140]. Impaired lung function in early childhood persists and is associated with increased respiratory morbidity in adulthood [2, 141]. Children with a LRTI by 2 years of age are more likely to die prematurely from respiratory disease as adults compared to those that had no LRTI [141, 142]. Pneumonia in early childhood has been associated with obstructive and restricted lung disease in later life [4, 141]. However, there is a gap in the data on the impact of early LRTI and its effect on long-term lung growth and development in preschool years, a crucial time for lung growth and development, particularly from LMIC with a high burden of LRTI such as poverty. Poverty is often associated with increased exposure to tobacco smoke, air pollution, poor living conditions, overcrowding and lack of ventilation all of which contribute to the transmission of respiratory infections [6].

Understanding the timing of these infections and their impact on lung function allows for the implementation of targeted early interventions aimed at preventing and mitigating the adverse effects of LRTI. Therefore, having tools to measure early life lung function is crucial for

assessing the impact of these infections on lung growth and for monitoring the trajectories of affected individuals. Furthermore, it is important to assess the relative impact of additional exposures that may either exacerbate or protect against lung function decline.

1.2 Rationale

Respiratory illnesses pose a significant risk to lifelong health and are a particular challenge in LMIC, leading to a high morbidity and mortality. Social disadvantage, with increased exposure to environmental pollutants, food insecurity, and crowded living conditions are some of the factors that may contribute to worse outcomes. Both antenatal and postnatal exposures impact maternal and foetal health, leading to complications such as IUGR and preterm birth. These effects are compounded by ongoing exposures. Identifying and quantifying the determinants of respiratory illness is key in understanding their role in early lung development and longer-term respiratory health outcomes. Lung function measurements in early life, which can measure the impact of harmful exposures and track respiratory health to detect early declines in lung function and which are useful to children living in LMIC are needed. A better understanding of the determinants and impacts of early life exposures during this period of substantial lung growth will contribute to informing management, public health interventions and policies to strengthen lifelong respiratory health.

1.3 Aims

The overall aim was to investigate the determinants of lung function from birth through early childhood in South African children. Specific objectives were:

1. To review the current state of preschool lung function measurement tools and data in African children.

2. To describe normal lung function from birth to 5 years of age in South African children and develop oscillometry reference equations for children.
3. To investigate the impact of antenatal and postnatal IAP or tobacco smoke exposure at 3 years of age.
4. To investigate the effect of MLP birth on lung function over the first 5 years of life.

1.4 Methodology

1.4.1 Study design and population

This was a longitudinal observational study nested within the DCHS, an African birth cohort in Paarl, a peri-urban area approximately 60km outside of Cape Town [140].

1.4.2 Recruitment, enrolment and consent

Pregnant mothers were enrolled during the second trimester. There were 1143 live births with a high cohort retention rate. All children enrolled in the DCHS study were eligible for inclusion in the lung function study. Inclusion criteria were broad to maximize generalisability: age older than 18 years and intention to remain in the area for at least one year. Infants were followed from birth and lung functions measured at 6 weeks, 1 year and 2 years. All children were subsequently eligible for inclusion in this preschool study and lung function was measured annually from 3 to 5 years of age.

Mothers provided informed consent to participate in the study which initially included infant lung function testing. Consent for participation in preschool lung function study was taken at birth and again at 3 years to include annual testing from 3 years to 6 years.

1.4.3 Investigations and data collection

Mothers and fathers were interviewed at enrolment antenatally as per DCHS protocol (401/2009). Other investigations done at annual visit included blood samples, nasopharyngeal swabs in children less than 5 years, TB tuberculin skin test (Mantoux), stool sample to assess the microbiome and a urine sample to test for cotinine. At each annual visit, parents were asked to complete a validated questionnaire assessing socioeconomic factors, smoking and exposure to passive smoke, current and past health history, HIV status and treatment history. In addition, infants had air pollutants measured in their homes antenatally and postnatally. The postnatal measurement was conducted between 4 to 6 months of life and indoor air pollutants and by-products of combustion were measured. Exposures measured included particulate matter (PM_{10}), carbon monoxide (CO), NO_2, SO_2 and volatile organic compounds (VOCs) [104].

1.4.4 Respiratory symptoms

Information about respiratory symptoms (cough, wheeze, difficulty breathing), exposure to risk factors for respiratory disease (including tobacco smoke exposure, infectious contacts) and acute respiratory events (ambulatory and inpatient) were collected. Parents had access by telephone to a study worker 24 hours a day to report any events.

1.4.5 Preschool lung function

Children were tested at 36 months (range 36 months to 42 months), 48 months (range 46 to 52 months), 60 months (range 58-64 months) and 72 months (range 68-76) at the Paarl Hospital study site. The site has a dedicated room in a quiet section of the hospital in close proximity to the paediatric ward. The room has piped oxygen and wall suction. The respiratory function testing was completed by 2 of a team of 3 trained professionals: Dr

Shaakira Chaya (paediatric pulmonologist), Professor Diane Gray (paediatric pulmonologist) and Mr Carvern Jacobs (respiratory scientist and technologist).

Preschool lung function assessment was done in accordance with American/European Thoracic Society Statements on pulmonary function testing in preschool children [22, 23].

1.4.5.1 Tidal breathing measures

Measurements were taken with children sitting in a chair while breathing normally. A 100 consecutive breath epoch is measured with an ultrasonic flow-meter (ExhalyzerD, EcoMedics, Switzerland) via a face mask and filter. Measurements include respiratory rate, tidal volume and ratio of time to peak expiratory flow over total expiratory time (t_{PTEF}/t_E).

1.4.5.2 MBW testing

This was measured with the children sitting upright in a chair during normal tidal breathing. The MBW uses endogenous nitrogen as the inert gas and equilibrium of the inert gas occurs at baseline by breathing room air. Then a switch from room air to 100% O_2 at the start of the washout phase begins after 5 stable tidal breathing breaths. Resident N_2 is then washed out of the lungs by 100% O_2 and continues until the tracer gas has washed out to beyond 1/40th of its starting end-tidal concentration. Each measurement takes approximately 3-5 minutes. Three good quality measures (minimum of 2) are taken with a maximum of 5 attempts. Measurements include lung volumes (*FRC*) and measures of ventilation homogeneity (*LCI* and moment ratios).

1.4.5.3 Respiratory function using oscillometry

This measure was taken with a child sitting comfortably and breathing through a mouth piece with cheeks supported. The mouth piece and filter attached to a wave-tube and pneumotachograph with 4L per min bias flow of medical air. This was attached to a speaker which generated composite signals of 6-32 hertz at the mouth (FOT system, Z Hantos, University of Szeged, Hungary). Measurements were done with a maximum of five 16 second epochs of composite signals to yield a minimum of 3 acceptable measurements and one 16 sec epoch of single 10Hz frequency. Testing measures include mean respiratory resistance (R) and reactance (X), resonant frequency as well as R and X at specific frequencies. The single frequency measure allows for intra-breath analysis of resistance and reactance, a more comprehensive assessment of respiratory mechanics.

1.4.5.4 Spirometry

Spirometry was done at 5 years and 6 years. The test was performed with the child standing and testing adhered to ATS/ERS testing standards [143]. A minimum of 3 repeatable efforts were obtained. Measurements included FEV_1, FVC, and the ratio between FEV_1/FVC.

1.4.5.5 Bronchodilator response test

Following spirometry 4 puffs of bronchodilator (salbutamol, 400ug) was administered via spacer and oscillometry and spirometry was repeated after 10-15 minutes. Bronchodilator response testing was performed from infancy through to 5 years.

1.5 Significance of this thesis

In LMIC, antenatal and postnatal risk factors for low lung function in early life are prevalent, leading to poor life-long respiratory health. However, most data are from HIC, and a better

understanding of the determinants and impact of early life exposures on later lung function is needed to inform interventions. This thesis will show the feasibility and value of preschool lung function, and describe various factors impacing on lung health. Taken together, this thesis will show that interventions to mitigate these exposures and promote early life respiratory health are urgently needed.

References

1. GBD 2021 Lower Respiratory Infections and Antimicrobial Resistance Collaborators. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990-2021: a systematic analysis from the Global Burden of Disease Study 2021. Lancet Infect Dis. 2024;24(9):974-1002. doi: 10.1016/S1473-3099(24)00176-2.
2. Belgrave DCM, Granell R, Turner SW, Curtin JA, Buchan IE, Le Souëf PN, et al. Lung function trajectories from pre-school age to adulthood and their associations with early life factors: a retrospective analysis of three population-based birth cohort studies. Lancet Respir Med. 2018;6(7):526-534. doi: 10.1016/S2213-2600(18)30099-7.
3. Bush A. Impact of early life exposures on respiratory disease. Paediatr Respir Rev. 2021;40:24-32. doi: 10.1016/j.prrv.2021.05.006.
4. Chan JY, Stern DA, Guerra S, Wright AL, Morgan WJ, Martinez FD. Pneumonia in childhood and impaired lung function in adults: a longitudinal study. Pediatrics. 2015;135(4):607-616. doi: 10.1542/peds.2014-3060.
5. Grant T, Brigham EP, McCormack MC. Childhood origins of adult lung disease as opportunities for prevention. J Allergy Clin Immunol Pract. 2020;8(3):849-858. doi: 10.1016/j.jaip.2020.01.015.
6. Bush A, Byrnes CA, Chan KC, Chang AB, Ferreira JC, Holden KA, et al. Social determinants of respiratory health from birth: still of concern in the 21st century? Eur Respir Rev. 2024;33(172):230222. doi: 10.1183/16000617.0222-2023.
7. Gray D, Willemse L, Visagie A, Czövek D, Nduru P, Vanker A, et al. Determinants of early-life lung function in African infants. Thorax. 2017;72(5):445-450. doi: 10.1136/thoraxjnl-2015-207401.
8. Vasquez MM, Zhou M, Hu C, Martinez FD, Guerra S. Low lung function in young adult life is associated with early mortality. Am J Respir Crit Care Med. 2017;195(10):1399-1401. doi: 10.1164/rccm.201608-1561LE.
9. Agusti A, Faner R. Lung function trajectories in health and disease. Lancet Respir Med. 2019;7(4):358-364. doi: 10.1016/S2213-2600(18)30529-0.
10. Jordan BK, McEvoy CT. Trajectories of lung function in infants and children: setting a course for lifelong lung health. Pediatrics. 2020;146(4):e20200417. doi: 10.1542/peds.2020-0417.
11. Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet. 2007;370(9589):758-764. doi: 10.1016/S0140-6736(07)61379-8.
12. Turner S, Fielding S, Mullane D, Cox DW, Goldblatt J, Landau L, et al. A longitudinal study of lung function from 1 month to 18 years of age. Thorax. 2014;69(11):1015-1020. doi: 10.1136/thoraxjnl-2013-204931.
13. Usemann J, Decrue F, Korten I, Proietti E, Gorlanova O, Vienneau D, et al. Exposure to moderate air pollution and associations with lung function at school-age: a birth cohort study. Environ Int. 2019;126:682-689. doi: 10.1016/j.envint.2018.12.019.
14. Salem Y, Jakob J, Steinberg R, Gorlanova O, Fuchs O, Müller L, et al. Cohort profile update: the Bern Basel infant lung development cohort. Int J Epidemiol. 2024;53(1):dyad164. doi: 10.1093/ije/dyad164.

15. Thunqvist P, Gustafsson PM, Schultz ES, Bellander T, Berggren-Broström E, Norman M, et al. Lung function at 8 and 16 years after moderate-to-late preterm birth: a prospective cohort study. Pediatrics. 2016;137(4):e20152056. doi: 10.1542/peds.2015-2056.
16. Hallberg J, Anderson M, Wickman M, Svartengren M. Factors in infancy and childhood related to reduced lung function in asthmatic children: a birth cohort study (BAMSE). Pediatr Pulmonol. 2010;45(4):341-348. doi: 10.1002/ppul.21190.
17. Lee AG, Kaali S, Quinn A, Delimini R, Burkart K, Opoku-Mensah J, et al. Prenatal household air pollution is associated with impaired infant lung function with sex-specific effects. Evidence from GRAPHS, a cluster randomized cookstove intervention trial. Am J Respir Crit Care Med. 2019;199(6):738-746. doi: 10.1164/rccm.201804-0694OC.
18. Muttoo S, Naidoo R, Jeena P, Asharam K. Air pollution exposure and infant lung function in the MACE cohort, South Africa. Environmental Epidemiology. 2019;3:280. doi: 10.1097/01.EE9.0000609000.48206.68.
19. Gray DM, Turkovic L, Willemse L, Visagie A, Vanker A, Stein DJ, et al. Lung function in African infants in the Drakenstein Child Health Study. Impact of lower respiratory tract illness. Am J Respir Crit Care Med. 2017;195(2):212-220. doi: 10.1164/rccm.201601-0188OC.
20. Verwey C, Ramocha L, Laubscher M, Baillie V, Nunes M, Gray D, et al. Pulmonary sequelae in 2-year-old children after hospitalisation for respiratory syncytial virus lower respiratory tract infection during infancy: an observational study. BMJ Open Respir Res. 2023;10(1):e001618. doi: 10.1136/bmjresp-2023-001618.
21. Gray DM, Wedderburn CJ, MacGinty RP, McMillan L, Jacobs C, Stadler JAM, et al. Impact of HIV and antiretroviral drug exposure on lung growth and function over 2 years in an African Birth Cohort. AIDS. 2020;34(4):549-558. doi: 10.1097/QAD.0000000000002444.
22. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 2007;175(12):1304-1345. doi: 10.1164/rccm.200605-642ST.
23. Robinson PD, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, et al. Preschool multiple-breath washout testing. An official American Thoracic Society Technical Statement. Am J Respir Crit Care Med. 2018;197(5):e1-e19. doi: 10.1164/rccm.201801-0074ST.
24. Bui DS, Lodge CJ, Burgess JA, Lowe AJ, Perret J, Bui MQ, et al. Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. Lancet Respir Med. 2018;6(7):535-544. doi: 10.1016/S2213-2600(18)30100-0.
25. Okyere DO, Bui DS, Washko GR, Lodge CJ, Lowe AJ, Cassim R, et al. Predictors of lung function trajectories in population-based studies: a systematic review. Respirology. 2021;26(10):938-959. doi: 10.1111/resp.14142.
26. McCready C, Zar HJ, Chaya S, Jacobs C, Workman L, Hantos Z, et al. Determinants of lung function development from birth to age 5 years: an interrupted time series analysis of a South African birth cohort. Lancet Child Adolesc Health. 2024;8(6):400-412. doi: 10.1016/S2352-4642(24)00072-5.

27. Martinez L, Gray DM, Botha M, Nel M, Chaya S, Jacobs C, et al. The long-term impact of early-life tuberculosis disease on child health: a prospective birth cohort study. Am J Respir Crit Care Med. 2023;207(8):1080-1088. doi: 10.1164/rccm.202208-1543OC.
28. Masekela R, Zurba L, Gray D. Dealing with access to spirometry in Africa: a commentary on challenges and solutions. Int J Environ Res Public Health. 2018;16(1):62. doi: 10.3390/ijerph16010062.
29. Maree DM, Swanepoel RA, Swart F, Gray DM, Masekela R, Allwood BW, et al. Position statement for adult and paediatric spirometry in South Africa: 2022 update. Afr J Thorac Crit Care Med. 2022;28(4):10.7196/AJTCCM.2022.v28i4.287. doi: 10.7196/AJTCCM.2022.v28i4.287.
30. Masekela R, Gray D, Verwey C, Halkas A, Jeena PM. Paediatric spirometry guideline of the South African Thoracic Society: Part 1. S Afr Med J. 2013;103(12 Suppl 2):1036-1041. doi: 10.7196/samj.7239.
31. Nystad W, Samuelsen SO, Nafstad P, Edvardsen E, Stensrud T, Jaakkola JJ. Feasibility of measuring lung function in preschool children. Thorax. 2002;57(12):1021-1027. doi: 10.1136/thorax.57.12.1021.
32. Rosenfeld M, Allen J, Arets BH, Aurora P, Beydon N, Calogero C, et al. An official American Thoracic Society workshop report: optimal lung function tests for monitoring cystic fibrosis, bronchopulmonary dysplasia, and recurrent wheezing in children less than 6 years of age. Ann Am Thorac Soc. 2013;10(2):S1-S11. doi: 10.1513/AnnalsATS.201301-017ST.
33. Kaminsky DA, Simpson SJ, Berger KI, Calverley P, de Melo PL, Dandurand R, et al. Clinical significance and applications of oscillometry. Eur Respir Rev. 2022;31(163):210208. doi: 10.1183/16000617.0208-2021.
34. Kostorz-Nosal S, Jastrzębski D, Błach A, Skoczyński S. Window of opportunity for respiratory oscillometry: a review of recent research. Respir Physiol Neurobiol. 2023;316:104135. doi: 10.1016/j.resp.2023.104135.
35. Githinji LN, Gray DM, Hlengwa S, Myer L, Zar HJ. Lung function in South African adolescents infected perinatally with HIV and treated long-term with antiretroviral therapy. Ann Am Thorac Soc. 2017;14(5):722-729. doi: 10.1513/AnnalsATS.201612-1018OC.
36. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, et al. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753. doi: 10.1183/13993003.00753-2019.
37. Sarkar S, Jadhav U, Ghewade B, Sarkar S, Wagh P. Oscillometry in lung function assessment: a comprehensive review of current insights and challenges. Cureus. 2023;15(10):e47935. doi: 10.7759/cureus.47935.
38. Stanojevic S, Bowerman C, Robinson P. Multiple breath washout: measuring early manifestations of lung pathology. Breathe (Sheff). 2021;17(3):210016. doi: 10.1183/20734735.0016-2021.
39. Ramsey KA, Stanojevic S, Chavez L, Johnson N, Bowerman C, Hall GL, et al. Global Lung Function Initiative reference values for multiple breath washout indices. Eur Respir J. 2024;64(6):2400524. doi: 10.1183/13993003.00524-2024.
40. Carlsen KH. Lung function during infancy and preschool age. Breathe. 2010;6(3):220-226. doi: 10.1183/18106838.0603.220.

41. Kooi EM, Schokker S, van der Molen T, Duiverman EJ. Airway resistance measurements in pre-school children with asthmatic symptoms: the interrupter technique. Respir Med. 2006;100(6):955-964. doi: 10.1016/j.rmed.2005.09.007.
42. Beydon N. Interrupter resistance: what's feasible? Paediatr Respir Rev. 2006;7(Suppl 1):S5-7. doi: 10.1016/j.prrv.2006.04.022.
43. Hall GL, Wildhaber JH, Cernelc M, Frey U. Evaluation of the interrupter technique in healthy, unsedated infants. Eur Respir J. 2001;18(6):982-988. doi: 10.1183/09031936.01.00221501.
44. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602-615. doi: 10.1164/rccm.9120-11ST.
45. Horváth I, Barnes PJ, Loukides S, Sterk PJ, Högman M, Olin AC, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. Eur Respir J. 2017;49(4):1600965. doi: 10.1183/13993003.00965-2016.
46. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-930. doi: 10.1164/rccm.200406-710ST.
47. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, et al. Reference ranges for spirometry across all ages: a new approach. Am J Respir Crit Care Med. 2008;177(3):253-260. doi: 10.1164/rccm.200708-1248OC.
48. Quanjer PH, Stanojevic S, Stocks J, Hall GL, Prasad KV, Cole TJ, et al. Changes in the FEV_1/FVC ratio during childhood and adolescence: an intercontinental study. Eur Respir J. 2010;36(6):1391-1399. doi: 10.1183/09031936.00164109.
49. Hall GL, Filipow N, Ruppel G, Okitika T, Thompson B, Kirkby J, et al. Official ERS technical standard: Global Lung Function Initiative reference values for static lung volumes in individuals of European ancestry. Eur Respir J. 2021;57(3):2000289. doi: 10.1183/13993003.00289-2020.
50. Stanojevic S, Wade A, Stocks J. Reference values for lung function: past, present and future. Eur Respir J. 2010;36(1):12-19. doi: 10.1183/09031936.00143209.
51. Bhakta NR, Bime C, Kaminsky DA, McCormack MC, Thakur N, Stanojevic S, et al. Race and ethnicity in pulmonary function test interpretation: an official American Thoracic Society statement. Am J Respir Crit Care Med. 2023;207(8):978-995. doi: 10.1164/rccm.202302-0310ST.
52. Bowerman C, Bhakta NR, Brazzale D, Cooper BR, Cooper J, Gochicoa-Rangel L, et al. A race-neutral approach to the interpretation of lung function measurements. Am J Respir Crit Care Med. 2023;207(6):768-774. doi: 10.1164/rccm.202205-0963OC.
53. Bhakta NR, McGowan A, Ramsey KA, Borg B, Kivastik J, Knight SL, et al. European Respiratory Society/American Thoracic Society technical statement: standardisation of the measurement of lung volumes, 2023 update. Eur Respir J. 2023;62(4):2201519. doi: 10.1183/13993003.01519-2022.
54. Stanojevic S, Graham BL, Cooper BG, Thompson BR, Carter KW, Francis RW, et al. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. Eur Respir J. 2017;50(3):1700010. doi: 10.1183/13993003.00010-2017.

55. Deprato A, Ferrara G, Bhutani M, Melenka L, Murgia N, Usmani OS, et al. Reference equations for oscillometry and their differences among populations: a systematic scoping review. Eur Respir Rev. 2022;31(165):220021. doi: 10.1183/16000617.0021-2022.
56. Ruck CE, Smolen KK. Effect of maternal HIV infection on infant development and outcomes. Front Virol. 2022;2:885246. doi: 10.3389/fviro.2022.885246.
57. Slogrove AL, Frigati L, Gray DM. Maternal HIV and paediatric lung health. Paediatr Respir Rev. 2017;21:47-53. doi: 10.1016/j.prrv.2016.08.004.
58. Pike K, Jane Pillow J, Lucas JS. Long term respiratory consequences of intrauterine growth restriction. Semin Fetal Neonatal Med. 2012;17(2):92-98. doi: 10.1016/j.siny.2012.01.003.
59. Arigliani M, Spinelli AM, Liguoro I, Cogo P. Nutrition and lung growth. Nutrients. 2018;10(7):919. doi: 10.3390/nu10070919.
60. Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Vora H, Rappaport EB, et al. Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. Thorax. 2000;55(4):271-276. doi: 10.1136/thorax.55.4.271.
61. Latzin P, Röösli M, Huss A, Kuehni CE, Frey U. Air pollution during pregnancy and lung function in newborns: a birth cohort study. Eur Respir J. 2009;33(3):594-603. doi: 10.1183/09031936.00084008.
62. Aithal SS, Sachdeva I, Kurmi OP. Air quality and respiratory health in children. Breathe (Sheff). 2023;19(2):230040. doi: 10.1183/20734735.0040-2023.
63. Mocelin HT, Fischer GB, Bush A. Adverse early-life environmental exposures and their repercussions on adult respiratory health. J Pediatr (Rio J). 2022;98(Suppl 1):S86-S95. doi: 10.1016/j.jped.2021.11.005.
64. Neophytou AM, Oh SS, White MJ, Mak ACY, Hu D, Huntsman S, et al. Secondhand smoke exposure and asthma outcomes among African-American and Latino children with asthma. Thorax. 2018;73(11):1041-1048. doi: 10.1136/thoraxjnl-2017-211383.
65. Gibbons JTD, Wilson AC, Simpson SJ. Predicting lung health trajectories for survivors of preterm birth. Front Pediatr. 2020;8:318. doi: 10.3389/fped.2020.00318.
66. Colin AA, McEvoy C, Castile RG. Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. Pediatrics. 2010;126(1):115-128. doi: 10.1542/peds.2009-1381.
67. Joss-Moore LA, Albertine KH, Lane RH. Epigenetics and the developmental origins of lung disease. Mol Genet Metab. 2011;104(1-2):61-66. doi: 10.1016/j.ymgme.2011.07.018.
68. Sharma D, Shastri S, Sharma P. Intrauterine growth restriction: antenatal and postnatal aspects. Clin Med Insights Pediatr. 2016;10:67-83. doi: 10.4137/CMPed.S40070.
69. Barker DJ, Godfrey KM, Fall C, Osmond C, Winter PD, Shaheen SO. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. BMJ. 1991;303(6804):671-675. doi: 10.1136/bmj.303.6804.671.
70. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;1(8489):1077-1081. doi: 10.1016/s0140-6736(86)91340-1.

71. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med. 2013;1(9):728-742. doi: 10.1016/S2213-2600(13)70118-8.
72. Fandiño J, Toba L, González-Matías LC, Diz-Chaves Y, Mallo F. Perinatal undernutrition, metabolic hormones, and lung development. Nutrients. 2019;11(12):2870. doi: 10.3390/nu11122870.
73. Misselhorn A, Hendriks SL. A systematic review of sub-national food insecurity research in South Africa: missed opportunities for policy insights. PLoS One. 2017;12(8):e0182399. doi: 10.1371/journal.pone.0182399.
74. Symington E, Norris S, Smuts M. Food and nutrition security of the unborn child: the role of maternal nutrition. In May J, Witten C, Lake L, editors. South African Child Guage. Cape Town: Children's Institute, University of Cape Town; 2020. p. 72-84.
75. Eising JB, Uiterwaal CS, van der Ent CK. Maternal body mass index, neonatal lung function and respiratory symptoms in childhood. Eur Respir J. 2015;46(5):1342-1349. doi: 10.1183/13993003.00784-2014.
76. Palmer AC. Nutritionally mediated programming of the developing immune system. Adv Nutr. 2011;2(5):377-395. doi: 10.3945/an.111.000570.
77. Dogaru CM, Nyffenegger D, Pescatore AM, Spycher BD, Kuehni CE. Breastfeeding and childhood asthma: systematic review and meta-analysis. Am J Epidemiol. 2014;179(10):1153-1167. doi: 10.1093/aje/kwu072.
78. Goyal NK, Fiks AG, Lorch SA. Association of late-preterm birth with asthma in young children: practice-based study. Pediatrics. 2011;128(4):e830-838. doi: 10.1542/peds.2011-0809.
79. Escobar GJ, Ragins A, Li SX, Prager L, Masaquel AS, Kipnis P. Recurrent wheezing in the third year of life among children born at 32 weeks' gestation or later: relationship to laboratory-confirmed, medically attended infection with respiratory syncytial virus during the first year of life. Arch Pediatr Adolesc Med. 2010;164(10):915-922. doi: 10.1001/archpediatrics.2010.177.
80. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics. 2006;117(4):1253-1261. doi: 10.1542/peds.2005-1368.
81. den Dekker HT, Sonnenschein-van der Voort AMM, de Jongste JC, Anessi-Maesano I, Arshad SH, Barros H, et al. Early growth characteristics and the risk of reduced lung function and asthma: A meta-analysis of 25,000 children. J Allergy Clin Immunol. 2016;137(4):1026-1035. doi: 10.1016/j.jaci.2015.08.050.
82. Duijts L, Ramadhani MK, Moll HA. Breastfeeding protects against infectious diseases during infancy in industrialized countries. a systematic review. Matern Child Nutr. 2009;5(3):199-210. doi: 10.1111/j.1740-8709.2008.00176.x.
83. Ogbuanu IU, Karmaus W, Arshad SH, Kurukulaaratchy RJ, Ewart S. Effect of breastfeeding duration on lung function at age 10 years: a prospective birth cohort study. Thorax. 2009;64(1):62-66. doi: 10.1136/thx.2008.101543.
84. Lelijveld N, Kerac M, Seal A, Chimwezi E, Wells JC, Heyderman RS, et al. Long-term effects of severe acute malnutrition on lung function in Malawian children: a cohort study. Eur Respir J. 2017;49(4):1601301. doi: 10.1183/13993003.01301-2016.
85. Korten I, Usemann J, Latzin P. "Lung sparing growth": is the lung not affected by malnutrition? Eur Respir J. 2017;49(4):1700295. doi: 10.1183/13993003.00295-2017.

86. Arigliani M, Canciani MC, Mottini G, Altomare M, Magnolato A, Loa Clemente SV, et al. Evaluation of the Global Lung Initiative 2012 reference values for spirometry in African children. Am J Respir Crit Care Med. 2017;195(2):229-236. doi: 10.1164/rccm.201604-0693OC.
87. Lelijveld N, Kirkby J. Spirometry in undernourished children in sub-Saharan African. J Public Health Emerg. 2018;2:9. doi: 10.21037/jphe.2018.02.02.
88. Ayele BA, Tiruneh SA, Ayele AA, Zemene MA, Chanie ES, Hailemeskel HS. Prevalence and determinants of overweight/obesity among under-five children in sub-Saharan Africa: a multilevel analysis. BMC Pediatr. 2022;22(1):585. doi: 10.1186/s12887-022-03645-z.
89. Parameswaran K, Todd DC, Soth M. Altered respiratory physiology in obesity. Can Respir J. 2006;13(4):203-210. doi: 10.1155/2006/834786.
90. Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence: role of obesity and age at the onset of puberty. Am J Respir Crit Care Med. 2004;170(1):78-85. doi: 10.1164/rccm.200309-1224OC.
91. Forno E, Weiner DJ, Mullen J, Sawicki G, Kurland G, Han YY, et al. Obesity and airway dysanapsis in children with and without asthma. Am J Respir Crit Care Med. 2017;195(3):314-323. doi: 10.1164/rccm.201605-1039OC.
92. Winck AD, Heinzmann-Filho JP, Soares RB, da Silva JS, Woszezenki CT, Zanatta LB. Effects of obesity on lung volume and capacity in children and adolescents: a systematic review. Rev Paul Pediatr. 2016;34(4):510-517. doi: 10.1016/j.rpped.2016.02.008.
93. Forno E, Han YY, Mullen J, Celedón JC. Overweight, obesity, and lung function in children and adults - a meta-analysis. J Allergy Clin Immunol Pract. 2018;6(2):570-581.e10. doi: 10.1016/j.jaip.2017.07.010.
94. Robinson, PD. Obesity and its impact on the respiratory system. Paediatr Respir Rev. 2014;15(3):219-226. doi: 10.1016/j.prrv.2014.06.003.
95. Ofenheimer A, Breyer MK, Wouters EFM, Schiffers C, Hartl S, Burghuber OC, et al. The effect of body compartments on lung function in childhood and adolescence. Clin Nutr. 2024;43(2):476-481. doi: 10.1016/j.clnu.2023.12.010.
96. Ali G, Bui DB, Walters H, Perret J, Erbas B, Thomas P, et al. Lifetime body mass index trajectories and lung function and chronic obstructive pulmonary disease in the Tasmanian longitudinal health study (TAHS). Eur Respir J. 2023;62(Suppl 67): OA890. doi: 10.1183/13993003.congress-2023.OA890.
97. Wang G, Hallberg J, Merid SK, Kumar A, Klevebro S, Habchi B, et al. Body mass index trajectories from birth to early adulthood and lung function development. Eur Respir J. 2025;65(1):2400298. doi: 10.1183/13993003.00298-2024.
98. World Health Organization. WHO report on the global tobacco epidemic, 2023: protect people from tobacco smoke. Geneva: World Health Organization; 2023.
99. Thacher JD, Schultz ES, Hallberg J, Hellberg U, Kull I, Thunqvist P, et al. Tobacco smoke exposure in early life and adolescence in relation to lung function. Eur Respir J. 2018;51(6):1702111. doi: 10.1183/13993003.02111-2017.
100. Constant C, Sampaio I, Negreiro F, Aguiar P, Silva A, Salgueiro M, et al. Environmental tobacco smoke (ETS) exposure and respiratory morbidity in school age children. Rev Port Pneumol. 2011;17(1):20-26.

101. Wongtrakool C, Roser-Page S, Rivera HN, Roman J. Nicotine alters lung branching morphogenesis through the alpha7 nicotinic acetylcholine receptor. Am J Physiol Lung Cell Mol Physiol. 2007;293(3):L611-L618. doi: 10.1152/ajplung.00038.2007.
102. Wongtrakool C, Wang N, Hyde DM, Roman J, Spindel ER. Prenatal nicotine exposure alters lung function and airway geometry through α7 nicotinic receptors. Am J Respir Cell Mol Biol. 2012;46(5):695-702. doi: 10.1165/rcmb.2011-0028OC.
103. Gray D, Czövek D, Smith E, Willemse L, Alberts A, Gingl Z, et al. Respiratory impedance in healthy unsedated South African infants: effects of maternal smoking. Respirology. 2015;20(3):467-473. doi: 10.1111/resp.12463.
104. Vanker A, Gie RP, Zar HJ. Early-life exposures to environmental tobacco smoke and indoor air pollution in the Drakenstein Child Health Study: Impact on child health. S Afr Med J. 2018;108(2):71-72. doi: 10.7196/SAMJ.2017.v108i2.13088.
105. Cook DG, Strachan DP, Carey IM. Parental smoking and spirometric indices in children. Thorax. 1998;53(10):884-893. doi: 10.1136/thx.53.10.884.
106. Hu LW, Yang M, Chen S, Shah K, Hailegiorgis Y, Burgens R, et al. Effects of in utero and postnatal exposure to secondhand smoke on lung function by gender and asthma status: the Seven Northeastern Cities (SNEC) study. Respiration. 2017;93(3):189-197. doi: 10.1159/000455140.
107. Gibbs K, Collaco JM, McGrath-Morrow SA. Impact of tobacco smoke and nicotine exposure on lung development. Chest. 2016;149(2):552-561. doi: 10.1378/chest.15-1858.
108. Torres-Duque C, Maldonado D, Pérez-Padilla R, Ezzati M, Viegi G; Forum of International Respiratory Studies (FIRS) Task Force on Health Effects of Biomass Exposure. Biomass fuels and respiratory diseases: a review of the evidence. Proc Am Thorac Soc. 2008;5(5):577-590. doi: 10.1513/pats.200707-100RP.
109. Masekela R, Vanker A. Lung health in children in sub-Saharan Africa: addressing the need for cleaner air. Int J Environ Res Public Health. 2020;17(17):6178. doi: 10.3390/ijerph17176178.
110. Wichmann J, Voyi KV. Influence of cooking and heating fuel use on 1-59 month old mortality in South Africa. Matern Child Health J. 2006;10(6):553-561. doi: 10.1007/s10995-006-0121-z.
111. Barnes B, Mathee A, Thomas E, Bruce N. Household energy, indoor air pollution and child respiratory health in South Africa. J Energy South Afr. 2009;20(1):4-13. doi: 10.17159/2413-3051/2009/v20i1a3296.
112. Po JY, FitzGerald JM, Carlsten C. Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. Thorax. 2011;66(3):232-239. doi: 10.1136/thx.2010.147884.
113. Laine JE, Bodinier B, Robinson O, Plusquin M, Scalbert A, Keski-Rahkonen P, et al. Prenatal exposure to multiple air pollutants, mediating molecular mechanisms, and shifts in birthweight. Environ Sci Technol. 2020;54(22):14502-14513. doi: 10.1021/acs.est.0c02657.
114. Rice MB, Mein SA. Prenatal air pollution and child lung function: the impossible search for a vulnerable trimester. Am J Respir Crit Care Med. 2020;202(1):15-16. doi: 10.1164/rccm.202003-0764ED.
115. Nandasena S, Wickremasinghe AR, Sathiakumar N. Indoor air pollution and respiratory health of children in the developing world. World J Clin Pediatr. 2013;2(2):6-15. doi: 10.5409/wjcp.v2.i2.6.

116. Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, et al. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351(11):1057-1067. doi: 10.1056/NEJMoa040610.
117. Khreis H, Kelly C, Tate J, Parslow R, Lucas K, Nieuwenhuijsen M. Exposure to traffic-related air pollution and risk of development of childhood asthma: a systematic review and meta-analysis. Environ Int. 2017;100:1-31. doi: 10.1016/j.envint.2016.11.012.
118. Dherani M, Pope D, Mascarenhas M, Smith KR, Weber M, Bruce N. Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. Bull World Health Organ. 2008;86(5):390-398C. doi: 10.2471/blt.07.044529.
119. Kufa-Chakezha T, Shangase N, Lombard C, Manda S, Puren A. The 2022 antenatal HIV sentinel survey: key findings. Public Health Bulletin South Africa; 2023.
120. Global HIV & AIDS statistics - Fact sheet. UNAIDS; 2024.
121. Naidoo K, Hoque M, Buckus S, Hoque M, Jagernath K. Prevention-of-mother-to-child-transmission (PMTCT) program outcomes in South Africa in the pre-COVID and COVID eras. BMC Public Health. 2023;23(1):1395. doi: 10.1186/s12889-023-16214-5.
122. Bailey H, Zash R, Rasi V, Thorne C. HIV treatment in pregnancy. Lancet HIV. 2018;5(8):e457-e467. doi: 10.1016/S2352-3018(18)30059-6.
123. Yeganeh N, Watts DH, Xu J, Kerin T, Joao EC, Pilotto JH, et al. Infectious morbidity, mortality and nutrition in HIV-exposed, uninfected, fomula-fed infants: results from the HPTN 040/PACTG 1043 trial. Pediatr Infect Dis J. 2018;37(12):1271-1278. doi: 10.1097/INF.0000000000002082.
124. Brennan AT, Bonawitz R, Schnippel K, Berhanu R, Maskew M, Long L, et al. Incident tuberculosis in HIV-positive children, adolescents and adults on antiretroviral therapy in South Africa. Int J Tuberc Lung Dis. 2016;20(8):1040-1045. doi: 10.5588/ijtld.15.0488.
125. Chen JY, Ribaudo HJ, Souda S, Parekh N, Ogwu A, Lockman S, et al. Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana. J Infect Dis. 2012;206(11):1695-1705. doi: 10.1093/infdis/jis553.
126. Slogrove AL, Powis KM, Bettinger JA, Cotton MF. Considerations in evaluating infectious morbidity and mortality in HIV-exposed uninfected infants. AIDS. 2018;32(18):2855-2856. doi: 10.1097/QAD.0000000000002049.
127. Martinez DR, Fong Y, Li SH, Yang F, Jennewein MF, Weiner JA, et al. Fc characteristics mediate selective placental transfer of IgG in HIV-infected women. Cell. 2019;178(1):190-201.e11. doi: 10.1016/j.cell.2019.05.046.
128. Cohen C, Moyes J, Tempia S, Groome M, Walaza S, Pretorius M, et al. Epidemiology of acute lower respiratory tract infection in HIV-exposed uninfected infants. Pediatrics. 2016;137(4):e20153272. doi: 10.1542/peds.2015-3272.
129. von Mollendorf C, von Gottberg A, Tempia S, Meiring S, de Gouveia L, Quan V, et al. Increased risk for and mortality from invasive pneumococcal disease in HIV-exposed but uninfected infants aged <1 year in South Africa, 2009-2013. Clin Infect Dis. 2015;60(9):1346-1356. doi: 10.1093/cid/civ059.
130. Schultz ES, Hallberg J, Andersson N, Thacher JD, Pershagen G, Bellander T, et al. Early life determinants of lung function change from childhood to adolescence. Respir Med. 2018;139:48-54. doi: 10.1016/j.rmed.2018.04.009.

131. Diggikar S, Paul A, Razak A, Chandrasekaran M, Swamy RS. Respiratory infections in children born preterm in low and middle-income countries: a systematic review. Pediatr Pulmonol. 2022;57(12):2903-2914. doi: 10.1002/ppul.26128.
132. Simpson SJ, Du Berry C, Evans DJ, Gibbons JTD, Vollsæter M, Halvorsen T, et al. Unravelling the respiratory health path across the lifespan for survivors of preterm birth. Lancet Respir Med. 2024;12(2):167-180. doi: 10.1016/S2213-2600(23)00272-2.
133. Simpson SJ, Logie KM, O'Dea CA, Banton GL, Murray C, Wilson AC, et al. Altered lung structure and function in mid-childhood survivors of very preterm birth. Thorax. 2017;72(8):702-711. doi: 10.1136/thoraxjnl-2016-208985.
134. Caskey S, Gough A, Rowan S, Gillespie S, Clarke J, Riley M, et al. Structural and functional lung impairment in adult survivors of bronchopulmonary dysplasia. Ann Am Thorac Soc. 2016;13(8):1262-1270. doi: 10.1513/AnnalsATS.201509-578OC.
135. Doyle LW, Adams AM, Robertson C, Ranganathan S, Davis NM, Lee KJ, et al. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. Thorax. 2017;72(8):712-719. doi: 10.1136/thoraxjnl-2016-208524.
136. Kotecha SJ, Dunstan FD, Kotecha S. Long term respiratory outcomes of late preterm-born infants. Semin Fetal Neonatal Med. 2012;17(2):77-81. doi: 10.1016/j.siny.2012.01.004.
137. Du Berry C, Nesci C, Cheong JLY, FitzGerald T, Mainzer R, Ranganathan S, et al. Long-term expiratory airflow of infants born moderate-late preterm: a systematic review and meta-analysis. EClinicalMedicine. 2022;52:101597. doi: 10.1016/j.eclinm.2022.101597.
138. World Health Organization. Born too soon: decade of action on preterm birth. Geneva: World Health Organization; 2023
139. Ramokolo V, Malaba T, Rhoda N, Kauchali S, Goga A. A landscape analysis of preterm birth in South Africa: systemic gaps and solutions. S Afr Health Rev. 2019;2019(1):133-144.
140. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP. Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study. Thorax. 2015;70(6):592-594. doi: 10.1136/thoraxjnl-2014-206242.
141. Allinson JP, Chaturvedi N, Wong A, Shah I, Donaldson GC, Wedzicha JA, et al. Early childhood lower respiratory tract infection and premature adult death from respiratory disease in Great Britain: a national birth cohort study. Lancet. 2023;401(10383):1183-1193. doi: 10.1016/S0140-6736(23)00131-9.
142. Agustí A, Noell G, Brugada J, Faner R. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. Lancet Respir Med. 2017;5(12):935-945. doi: 10.1016/S2213-2600(17)30434-4.
143. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of spirometry 2019 update. An official American Thoracic Society and European Respiratory Society technical statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88. doi: 10.1164/rccm.201908-1590ST.

Chapter 2: Lung function in preschool children in low and middle income countries: an under-represented potential tool to strengthen child health

---

Chaya S[1], Zar HJ[1], Gray DM[1]. Lung function in preschool children in low and middle income countries: an under-represented potential tool to strengthen child health. Front Pediatr. 2022;10:908607. doi: 10.3389/fped.2022.908607.

Affiliations:

1. Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town, Cape Town, South Africa

Author contributions:

SC, DG and HZ conceived the idea. SC and DG reviewed the literature. SC and DG drafted the manuscript; HZ provided further input into the manuscript. All authors reviewed, contributed and approved the final manuscript.

Word count: 3324

Abstract

Background: The burden of respiratory disease is high in low-middle income countries (LMIC). Pulmonary function tests are useful as an objective measure of lung health and to track progression. Spirometry is the commonest test, but its use is limited in preschool children. Other lung function methods have been developed but their use in LMIC has not been well described.

Aim: To review the use of preschool lung function testing in children in LMIC, with particular reference to feasibility and clinical applications.

Methods: Electronic databases "PubMed", "Scopus"," Web of Science", and "EBSCO host" were searched for publications in low and middle income countries on preschool lung function testing, including spirometry, fractional exhaled nitric oxide (FeNO), oscillometry, interrupter technique, tidal breathing and multiple breath washout (MBW), from 1 January 2011 to 31 January 2022. Papers in English were included and those including only children ≥6 years were excluded.

Result: A total of 61 papers from LMIC in Asia, South America, Africa, Eurasia or the Middle East were included. Of these, 40 included spirometry, 7 FeNO, 15 oscillometry, 2 interrupter technique, and 2 tidal breathing[1]. The papers covered test feasibility (19/61), clinical application (46/61) or epidemiological studies (13/61). Lung function testing was successful in preschool children from LMIC.[2] Spirometry was the most technically demanding and success gradually increased with age.

Conclusion: Preschool lung function testing is under-represented in LMIC for the burden of respiratory disease. These tests have the potential to strengthen respiratory care in LMIC, however access needs to be improved.

[1] 0 MBW papers were identified

[2] Age feasibility for lung function measurements in preschool children: spirometry: ≥6 years; oscillometry: ≥3 years; tidal breathing: ≥3 years; interrupter technique: ≥6 years; FeNO: ≥ 5 years

2.1 Introduction

Childhood respiratory disease is a common cause of morbidity and mortality globally [1]. The burden of acute and chronic respiratory disease is especially high in low-middle income countries (LMIC) [2], may result in impaired lung function and set a trajectory for chronic illness into adulthood [3, 4]. However, access to respiratory diagnostic and management tools such as lung function are limited in many LMIC [5].

Lung function attained in early life is important for respiratory health, with low lung function associated with subsequent risk of respiratory disease [6]. Pulmonary function tests are an objective measure of lung health which can be used to diagnose and track lung disease and assess response to treatment. In recent years non-invasive tests have been developed and guidelines produced for preschool children, facilitating its use in assessing respiratory health in early life [7, 8].

Lung function tests used in preschool children include spirometry, bronchial response testing, multiple breath washout (MBW), fractional exhaled nitric oxide (FeNO), oscillometry and other tests which measure resistance, including the interrupter technique (R_{int}) and plethysmography. With increased recognition of the importance of maximizing early life respiratory health and the growing availability of tools to do so, their use in LMIC is of particular interest.

Of the preschool lung function tests, spirometry is the most commonly used and most widely available. Even though use is limited in very young or uncooperative children, it is feasible in children as young as 3 years [9]. Spirometry measures lung volumes at maximal expiration and is able to assess airflow obstruction, response to bronchodilator therapy and lung

volumes on forced expiration. The commonly reported measures are the forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}) [10]. Spirometry predominantly reflects airflow in large and medium sized airways, and is a poor measure of peripheral small airways or early lung disease [11]. Current international recommendations for spirometry collection and interpretation in young children are available [10. 12].

Oscillometry (or the forced oscillation technique, FOT) is a simple, non-invasive technique which is performed during tidal breathing. Minimal co-operation is required thereby making this a popular measurement in preschool children. This measures the impedance of the respiratory system, which includes resistance and reactance across a range of frequencies reflecting the entire respiratory system including the small airways [13]. The novel intra-breath measurement may be a more sensitive measure of small airway disease, thus allowing early detection of disease [14, 15]. Recent international recommendations for oscillometry have been published on methodology, technical standards and future developments for use in children [7, 16, 17].

The interrupter technique measures the resistance of the respiratory system requiring minimal cooperation. The technique involves a sudden interruption of flow during tidal breathing, this allows for alveolar and pressure at the mouth to equilibrate therefore alveolar pressure can be estimated [18, 19]. Different methods have been used to perform the test making the comparison of results difficult thus highlighting the need for test standardization. Furthermore, research is still required to determine the best algorithm to calculate pressure at the mouth during occlusion and the cut-off value for R_{int} post bronchodilator needs to be established [7].

Multiple breath washout (MBW) is used to assess ventilation homogeneity. It measures the functional residual capacity (*FRC*) and the lung clearance index (*LCI*) in preschool children [8]. It is more sensitive than spirometry for the detection of peripheral airway disease and has been successfully used in the monitoring of children with cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) [7, 20]. It correlates with high resolution CT scan in CF patients as well as in children with asthma, bronchiolitis obliterans or chronic lung disease of prematurity [8, 21].

Fractional exhaled nitric oxide (FeNO) is a non-invasive marker of T-helper cell type 2 (TH2) eosinophilic airway inflammation. Tests can be performed with high repeatability and accuracy [22-24]. Its main use is an adjunct to the diagnosis of TH2 type asthma and guiding the use of inhaled corticosteroid (ICS) treatment. In addition, FeNO can also assist in the differential diagnosis of other conditions such as cystic fibrosis (CF), PCD, scleroderma, obstructive sleep apnoea syndrome and hepatopulmonary syndrome. Fractional exhaled nitric oxide levels are low in PCD; however measurement of nasal NO improves the diagnostic accuracy and is a useful screening tool for PCD [23, 25]. Normal values for FeNO are published for children from 4 years of age [24], as well as international applications and the use of FeNO [23, 24, 26]. However, there are limited data on the use and feasibility of such measures in LMIC, despite the high burden of lung disease and need for objective tools to diagnose and monitor these.

We aimed to review the use of preschool lung function testing in children in LMIC, with reference to feasibility and clinical applications, to identify opportunities for optimizing diagnosis and management of childhood respiratory disease in these settings.

2.2 Methods

We reviewed published literature of preschool lung function testing in LMIC, which included children between the ages of 3 to 5 years. We included published papers from 1 January 2011 to 31 January 2022 that included lung function testing in the preschool age group from a World Bank defined LMIC.

The search was conducted on the following electronic databases: PubMed, Scopus, EBSCOhost (Cinahl, Africa wide information, Health source- Nursing/Academic Edition) and Web of Science including the search terms: "Respiratory Function Test∗" OR "Lung function test∗" OR "pulmonary function test∗" OR "respiratory function test∗" OR "multiple-breath washout" OR "Forced oscillation technique" OR" tidal breathing" OR "fractional excretion of nitric oxide" OR Spirometry OR Oscillometry OR "impulse oscillometry" OR "interrupter technique" OR "interrupter resistance" AND "preschool child∗". Full search strategy can be seen in Supplementary Table S2.1. Reviews, editorials, case reports and conference proceedings were excluded. Any papers including only children ≥ 6 years were excluded. Abstracts of identified documents were reviewed and screened by SC, with second author DG assisting with inclusion queries. All included papers were reviewed by SC.

2.3 Results

A total of 626 papers were screened of which 61 were eligible for inclusion, Supplementary Figure S2.1. They included papers from 4 regions: 30 (49.2%) from Asia, 17 (27.9%) from South America, 9 (14.7%) from Africa and 5 (8.2%) from Europe and the Middle East. Five lung function tests were most commonly reported: 40 (65.6%) spirometry, 7 (11.5%) FeNO, 15 (24.6%) oscillometry, 2 (3.3%) interrupter technique and 2 (3.3%) tidal breathing measurements. There were no papers including MBW. The papers covered test feasibility

(19, 31.1%), clinical applications (46, 75.4%) and epidemiological studies (13, 21.3%). Clinical studies focused on development of reference tools (19/46, 41.3%) and on specific diseases: asthma (15/46, 32.6%), of which 4 papers in addition to asthma included allergic rhinitis, air pollution, obesity and allergic bronchopulmonary aspergillosis (ABPA); CF (5/46, 10.9%); 1 bronchiolitis obliterans, 1 recurrent wheeze, 1 systemic sclerosis. Epidemiological studies assessed the impact of air pollution (10/13, 77%); electronic waste (e-waste) (2/13, 15.4%) [27, 28] and the effect of antenatal omega 3 fatty acid supplementation (1/13, 7.7%) on lung function. E-waste is accumulated discarded or broken electronic devices which is becoming the largest amount of waste in the world [27, 28]. As spirometry, FeNO, oscillometry or tidal breathing were most commonly used they were the focus of this review.

2.3.1 Spirometry

The majority (40, 68%) of papers included spirometry, (Table 2.1). Spirometry was used to develop reference equations, diagnose and manage respiratory diseases including asthma or CF, assess the impact of air pollution, electronic exposures (e-exposures) or socio-economic status on lung function. Twenty (50%) studies were from Asia, 9 (22.5%) from South America, 7 (17.5%). From Africa and 4 (10%) from Europe and the Middle East.

Table 2.1 Details of included studies using spirometry (*n*=40)

Authors, Year	Country	Age	No. of patients	Study type	Theme	Main finding
Zhu et al. [63]	China	5-12yr; little group-divided into groups 5-7 years	121		asthma, allergic rhinitis	Spirometry was used to assess factors associated with FeNO. A greater peak expiratory flow in addition to a greater age, height/weight and level of total IgE are related to higher FeNO levels
He et al. [49]	China	5-13 years	43		air pollution	No significant associations were noted between personal $PM_{2.5}$ exposure and spirometry.
Kang et al. [56]	China	4-12 years	286-asthma, 301-control		Asthma	A BDR threshold of ≥7.5% may be more valuable compared to ≥12% in childhood asthma
Leung et al. [55]	China	2-7 years	1341		Asthma	The minor allele SNP (rs408223), of CDHR3 was associated with lower $FEV_{0.5}$ ($\beta = -2.411$, $P = 0.004$), and $FEV_{0.5}/FVC$ ($\beta = -1.292$, $P = 0.015$)
Sun et al. [52]	China	3-10 years	112	experimental study	Asthma	Pulmonary function indices (*FVC*, FEV_1 and *PEF*), were significantly higher ($p < 0.001$), in the observational group (treated with montelukast and budesonide), than the control group (budesonide alone), Treating cough variant asthma with montelukast combined with budesonide is more effective than budesonide alone.
Wang et al. [58]	China	2-5 years	120	randomized, double-blind placebo-controlled trial	acute asthma	Treating acute asthma exacerbation with montelukast compared to placebo demonstrated no significant difference in the *PEF* and FEV_1.
Zeng et al. [28]	China	5-7 years	206		e-waste exposure	Taken together, birth weight and chest circumference may be good predictors for lung function levels in preschool children
Zeng et al. [27]	China	5-7 years	206		e-waste exposure	Children living in the exposed area have lower lung function (*FVC* and FEV_1). Levels compared to unexposed

						children. Haemoglobin levels may be a good predictor for lung function- one unit of haemoglobin (1 g/L). Decline was associated with 5 mL decrease in *FVC* and 4 mL decrease in FEV_1
Jian et al. [35]	China	4-80 years	7115	cross-sectional study	reference equation	This study established new reference values for the Chinese population 4 to 80 years. The "South East Asian" and "North East Asian" GLI reference equations under or overestimated the FEV_1, *FVC*, and FEV_1/FVC. Local Chinese equations underestimated *FVC* and FEV_1
Sonnappa et al. [44]	India and UK	5-12 years	1039	prospective cross-sectional study	socio-economic circumstance	Spirometry differences were assessed between children from urban, semiurban, and rural schools. There were significant reductions in FEV_1 and *FVC* in Indian-semiurban and Indian-rural children when compared with Indian-urban children.
Kumari et al. [57]	India	5-15 years	106	cross-sectional	asthma, ABPA	Percentage predicted values of FEV_1 and FEF_{25-75} were lower in asthmatic children with allergic bronchopulmonary aspergillosis (ABPA). Compared to no ABPA, but this did not reach statistical significance. *PEF* that was significantly higher in children with aspergillus sensitization (AS). Compared to those without AS ($P = 0.046$).
Kumar et al. [50]	India	5-18 years	620	cross-sectional study	asthma, obesity	Obese children with asthma (Group 1). Had significantly lower lung function compared to non-obese asthmatic children (Group 2). FEV_1, *FVC*, $FEF_{25-75\%}$, *PEF* for Group-1 were 66.3 ± 9.9, 63.5 ± 4.2, 54.2 ± 5.7, 67.4 ± 8.4. FEV_1, *FVC*, $FEF_{25-75\%}$, *PEF* for Group-2 were 74.07 ± 3.5, 77.4 ± 7.2, 60.1 ± 2.1, 71.6 ± 2.4. *P* values were < 0.001, < 0.001, < 0.001, < 0.05 respectively
Gulla et al. [92]	India	138-120 months	46	retrospective control study	cystic fibrosis	Children with viral infection (Group I). Had adverse outcome in form of greater worsening of Shwachman clinical scores, number of pulmonary exacerbations

						requiring antibiotic usage, need for intravenous antibiotics, hospitalization rates and mortality. Spirometry decreased in both groups decrease in lung function in both groups but was not significant
Bolla et al. [37]	India	5-15 years	790	cross-sectional study	reference equation	Separate equations in males and females were generated with age, weight and height as predictors. No comparison to other reference equations were made.
Lum et al. [42]	India and UK	5-17 years	8124	observational	reference equation	"GLI-Black" equations were most useful for interpreting South-Asian data and "GLI-Other" for North Indian data. When using GLI-predicted values from White Europeans, FEV_1 and *FVC* in South-Asian children were approximately 15% lower. There was an association between socio-economic circumstances (SEC), and lung function. Lung volumes were significantly lower in those living in rural areas or exposed to poorer SEC.
Asrul et al. [45]	Malaysia	5 and 6 years	120	cross-sectional comparative study	air pollution	There was a significant difference in indoor air quality between urban and suburban preschools. *FVC* and FEV_1 among urban children were significantly lower compared to the suburban children. Exposures to indoor air pollutants, especially $PM_{2.5}$ increases the risk of getting lung function abnormalities.
Choo et al. [46]	Malaysia	4-6 years	630	cross-sectional comparative study	air pollution	Urban area preschools have higher CO, PM_{10} and $PM_{2.5}$ concentration compared to from suburban and rural areas. *FVC*, FEV_1, *FVC*% predicted and FEV_1% predicted values were significantly lower among children from urban and suburban area preschools compared to rural preschools.
Kamaruddin et al. [93]	Malaysia	5-6 years	100	cross-sectional comparative study	air pollution	Significant associations between PM_{10} and VOCs with FEV_1% were noted (PR = 5.55, 95% CI = 2.189–14.07), (PR = 6.15, 95% CI = 2.565–14.73), respectively in exposed compared to unexposed children.

Rawi et al. [47]	Malaysia	5-6 years	11	cross sectional study	air pollution	Studied preschools had a significantly higher PM and CO concentration compared to the comparative preschools. *FVC*, FEV_1, *FVC*% and FEV_1% predicted values were significantly lower among studied group.
Asif et al. [39]	Pakistan	5-14 years	3275	cross-sectional study	reference equation	Reference range equations were developed with predictors that included age, height, and weight. Separate equations for males and females were generated. No comparisons made to other studies.
Ventura et al. [59]	Brazil	1-15 years(median age 3.75 years)	38 with CF,31 control	longitudinal study	cystic fibrosis	Pasclerosisrticipants with higher C-reactive protein/albumin ratio at the baseline had higher odds of $FEV_1 \leq 70\%$ after three years of follow-up.
Veras et al. [31]	Brazil	6 years and younger	74	cross-sectional descriptive	feasibility	The spirometry success rate was 82%. Performance improved with age.
França et al. [32]	Brazil	4-6 years	195		reference equation	Reference range generated using height as a predictor. One equation for males and females. No comparison to GLI 2012
Jones et al. [34]	Brazil	3-12 years	1990	cross-sectional observational study	reference equation	Equation generated significantly from those currently in use in Brazil-Underestimate *FVC* and FEV_1 values.
Burity et al. [33]	Brazil	3-6 years	425	prospective study	reference equation	Full expiratory curves are more difficult to obtain in preschool children. In addition to height, gender also influenced the measures of *FVC* and FEV_1
Matos et al. [53]	Brazil	4-12 years	1129	cross-sectional study	Asthma	Overweight children have less respiratory capacity, and was associated with lower FEV_1/FVC ratios (PR =1.37; 95% CI 1.14, 1.64)
França et al. [30]	Brazil	4-6 years	47		Asthma	83% success rate for performing spirometry
Ardura-Garcia et al. [64]	Ecuador	5-15 years	264	cohort study	Asthma	Spirometry did not predict asthma recurrence.

Heinzerling et al. [48]	Guatemala	5-8 years	506	prospective cohort study	air pollution	A significant decrease in *PEF* [173 mL/min/year (95% CI −341 to −7)], and a non-significant decrease in FEV_1 growth were observed with later stove installation at 18 months compared with stove installation at birth
Bougrida et al. [38]	Algeria	5-16 years	208		reference equation	Several predictors in the reference range and these include height, weight, age, gender BSA, BMI. Separate equations for males and females. There were significant differences in FEV_1 between the measured and predicted values from published reference equations except for a USA reference equation.
Jiffri et al. [54]	Egypt	1-15 years	120 asthma,120 controls		Asthma	There is an association between the TNFA −308G>A polymorphism and susceptibility to asthma. Spirometry used to classify patients into asthma severity namely mild intermittent asthma, mild persistent asthma, moderate persistent asthma, or severe persistent asthma
Akodui et al. [40]	Nigeria	5-12 years	100	cross-sectional study	sickle cell anaemia, reference equation	Preferred proxy for spirometry indices in children with sickle cell anaemia may be arm span
Thacher et al. [94]	Nigeria	5-11 years	299	cross-sectional study	asthma, air pollution	The relationship between smoke exposure and airway obstruction in households that did and did not use firewood daily was not significant (mean FEV_1/FEV_6 of 0.95 and 0.97, respectively; $P = 0.41$). There was a significant decline in predicted FEV_1 with age ($p < 0.001$)
Corten et al. [95]	South Africa	5-8 years	12	cross-sectional study	cystic fibrosis	There were significant correlations between *PEF* and manual dexterity and between *FVC* % predicted and balance scores Poorer lung function may affect motor development.

Smith et al. [43]	South Africa	5-95 years	4223	cross-sectional population-based study	reference equation	GLI2012 “Other” had the best fit for Black African individuals and Mixed Ethnicity group when using z-scores. The Caucasian individuals demonstrated a good fit with the GLI2012 “Caucasian” equation and participants of Asian ancestry demonstrated a good fit to the “Southeast Asian” and “Black” equation.
Sibanda et al. [96]	Zimbabwe	1-94 years	240 (49 between 1-16 years of age)	observational study	systemic sclerosis	The mean FEV_1/FVC ratio for all the patients combined was Significantly higher than predicted for age, gender, ethnicity, and BMI suggesting a restrictive pattern. The severity of the restrictive changes varied with the types of autoantibodies detected.
Ghasempour, M. et al. [51]	Iran	5-15 years	73	description-observation	Asthma	The average FEV_1/FVC parameter in the cough variant asthma group was 89.44 ± 13.07, and 72.35 ± 8.47 in the classic asthma group, with a significant difference between the two groups ($p < 0.05$). Patients with cough variant asthma $FEF_{25-75\%}$ were lower than expected. Spirometry can be used in the diagnosis of cough variant asthma.
Tabatabaie et al. [36]	Iran	4-10 years	495		reference equation	Reference range equations were generated for both males and females using height and age as predictors. When compared to previous published international equations significant differences were noted.
Al-Qerem et al. [29]	Jordan	3-5 years	765	random sampling	reference equation	The GLI 2012 for Caucasians is a reasonable fit for Jordanian preschool aged children.
Sasihuseyinoglu et al. [60]	Turkey	mean age7.83 years	80	retrospective study	cystic fibrosis	There were significant correlations between the Bhalla score and FEV_1, FVC, and $FEF_{25-75\%}$

PR, Prevalence Ratio; CI: Confidence Interval; PM, particulate matter; $PM_{2.5}$: particulate matter 2.5; PM_{10}, particulate matter 10; *PEF*, peak expiratory flow; SNP, single-nucleotide polymorphism; $FEV_{0.5}$, forced expiratory volume in 0.5-second; *FVC*, forced vital capacity

Success rates for spirometry in preschool children increased with age [29]. Children between 4-6 years of age achieved a success rate between 82–85% [30-32], while children between 3-5 years and 3-6 years of age, success rates were 68.4% and 42% respectively [29, 33].

A number of reference ranges were generated for individual population groups in LMIC, which were compared to either local reference ranges and/or The Global Lung Function Initiative (GLI) 2012 equation [32-39]. Many resulted in an over estimation/underestimation of lung function for the population assessed, highlighting the complexity of population differences in lung function. A Nigerian study determined reference equations for children with sickle cell anemia [40]. Published international equations also used arm span to determine height for spirometry [41]. The GLI 2012 "Caucasian" provided a reasonable fit for Jordanian children [29]. When assessing spirometry in two groups of healthy children with Indian ancestry, one living in UK and the other in India, the GLI equations for best fit differed: the "GLI-Black" equation was most useful for interpreting the South-Asian data and "GLI-Other" for North Indian data [42]. Similarly in a South Africa population the GLI 2012 "GLI-Caucasian" provided a good fit for the Caucasian population, "GLI-Black" and "GLI-Southeast Asian" was a good fit for the Indian population and "GLI-Other" fit the Black African and Mixed ancestry populations well [43]. These findings highlight the importance of multiple factors, including environment and socioeconomic exposures that impact population differences [42, 43].

Numerous environmental factors were reported to impact on lung function. Children living in rural areas or exposed to poorer socio-economic circumstances had lower lung function compared to those in urban areas [42, 44]. Exposures to volatile organic compounds,

particulate matter 10 (PM_{10}) or carbon monoxide (CO) were associated with a decrease in FEV_1 and FVC in exposed preschool children [45-47].

In a study in Guatemala, where CO was used as a proxy for $PM_{2.5}$, timing of chimney stove installation was compared to cooking over open fires, and showed a decrease in PEF of 173 ml/min/year (95% CI -341 to -7) with chimney stove installation at 18 months compared to installation at birth [48]. A Chinese study failed to demonstrate any significant association between $PM_{2.5}$ exposure and any of the spirometry lung function measures, however an increase in oscillometry resistance was noted suggesting that oscillometry may be a more sensitive measure [49]. In addition to air pollution, e-waste is a growing concern. Chinese children living in e-waste exposed areas had significantly lower birth weight, chest circumference and spirometry lung function compared to those in unexposed areas [27, 28].

The majority of included papers used spirometry in the clinical diagnosis and management of pediatric obstructive lung disease including asthma and CF. The studies investigated genetic predisposition to asthma, and management of acute, poorly controlled and cough-variant asthma (CVA) and the impact of obesity [50-53]. Genes associated with increased susceptibility to asthma and lower spirometry indices were identified in Chinese and Egyptian children [54, 55]. One study assessed current definitions of BDR, suggesting that a BDR of >7.5% may be more valuable in young children rather than the adult defined 12% [56]. Another study included assessing impact of ABPA on spirometry of asthmatic children [57]. In a Chinese study acute asthma did not respond to adding montelukast to the regular regimen [58], however it was noted that in children with CVA, cough associated with chronic airway allergic inflammation without wheeze, had a significant improvement in FEV_1, FVC, and PEF ($p < 0.001$) with montelukast and budesonide compared to budesonide alone, while

another study noted that FEV_1/FVC was normal in CVA compared to patients with asthma ($p < 0.001$) [51, 52].

Spirometry was also used in South American, Turkish and South African children to monitor lung function in cystic fibrosis [59], and showed good correlation between CT scan Bhalla score and FEV_1, FVC, FEF_{25-75} [60].

2.3.2 Fractional exhaled nitric oxide

Of the 7 papers using FeNO, 4 (57%) clinical studies measured FeNO to assess risk and treatment response of asthma or recurrent wheeze; and 3 epidemiological studies assessed the impact of IAP on airway inflammation in children. These included studies mainly from Asia (5 from China, 1 from Thailand) and 1 from Ecuador, South America. There were no published preschool studies from Africa. Included studies are summarized in Table 2.2.

Table 2.2 Detail of included studies that assessed fractional exhaled nitric oxide (n = 7), oscillometry (n = 15), interrupter technique (n = 2) and tidal breathing (n = 2)

Authors, Year	Country	Age	No. of patients	Study type	Theme	Main finding
Fractional exhaled nitric oxide						
Zhang et al. [62]	China	5 years	507	cross-sectional study	air pollution	Indoor and outdoor $PM_{2.5}$ levels in day care centres were associated with higher levels of FeNO. FeNO levels were also associated with current wheeze and physician diagnosed pneumonia.
Han et al. [65]	China	4-11 years (4-6 and 7-11 years)	142	cross-sectional descriptive study	Asthma	Family management (FM), describes how family members cooperate and integrate the management of childhood chronic disease into their daily family life. FM was closely related to asthma control and could significantly predict FeNO value and C- ACT score.
Li et al. [22]	China	32-48.7 months	88		recurrent wheeze	sRAGE may be a novel biomarker of inflammation of the respiratory tract. There was a significant negative correlation between serum sRAGE and FeNO ($p < 0.001$). In the high-risk asthma group, sRAGE levels increased significantly while FeNO decreased significantly after Pulmicort therapy
Zhu et al. [63]	China	5-12yr; little group classified as 5-7 years.	121		asthma, allergic rhinitis	Height and total IgE are well correlated with FeNO in asthmatic children greater age, height/weight, peak expiratory flow (*PEF*), and higher level of total IgE ($p < 0.001$) are associated with higher FeNO levels
He et al. [49]	China	5-13 years	43		air pollution	An increase in 24-h personal $PM_{2.5}$ exposure one day prior to the clinic visit was associated with a significant increase in of FeNO (airway inflammation), of 9.6%
Siwarom et al. [61]	Thailand	29-72 months	436	randomised control study	air pollution	The mean FeNO levels were statistically different in each season ($p < 0.001$). FENO levels had a strong association with high benzene levels (OR 5.9; 95%CI 1.5–22.9; p-value = 0.01).
Ardura-Garcia, C.et. al. [64]	Ecuador	5-15 years	264	cohort study	Asthma	FeNO level did not predict asthma recurrence.

Oscillometry						
Zhang et al. [78]	China	3-14 years	120	retrospective study	upper airway obstruction	R_5 in the OSAHS group was significantly higher than that in the non-OSAHS group ($P = 0.0025$)
He et al. [49]	China	5-13 years	43		air pollution	An increase in 24-h personal $PM_{2.5}$ exposure one day prior to the clinic visits was associated with a significant increase in total airway resistance (R_5) of 6.3%, small airway resistance ($R_5{-}R_{20}$) of 15.8%
Li et al. [82]	China	<14 years	42	case review	bronchiolitis obliterans	In children with bronchiolitis obliterans impulse oscillometry showed an increase in Z_5 (147.5 ± 19.3% of the predicted value, normal: less than 120% of the predicted value), R_5 (140.4 ±12.8% of the predicted value, normal: less than 120% of the predicted value), and X_5 (226.5 ± 13.4% of the predicted value, normal: less than 120% of the predicted value). This suggesting increased peripheral airway resistance.
Udomittipong et al. [70]	Thailand	3-7 years	291	cross-sectional study	reference equation	Reference values for respiratory impedance using FOT were generated using height and arm span were generated.
Udomittipong et al. [73]	Thailand	3-6 years	150		Asthma	Cut-off values for evaluating bronchodilator response in FOT were determined: R_{rs6}: −23%, R_{rs8}: −20%, R_{rs10}: −20%, X_{rs6}: 36%, X_{rs8}: 60%, and X_{rs10}: 43%.
Gupta et al. [69]	North India	2-18 years	345	prospective interventional study	Asthma	Oscillometry is a useful tool to assess lung function and airway reversibility in asthmatic children. It can provide an objective measurement in children unable to perform spirometry.
Medeiros et al. [74]	Brazil	3-6 years	76	cross -sectional study	respiratory symptoms	IOS in children with respiratory symptoms were higher pre-bronchodilator for R_5 Hz and $R_{5–20}$ Hz compared to those children without respiratory symptoms.
Duenas-Meza et al. [77]	Colombia	3-5 years	96	cross-sectional study	reference equation	Normal IOS reference range equations were determined, and height was the only predictor. A fall in R_5 Hz of 28% or an increase in X_5 Hz of 36% postbronchodilator can be considered as an upper limit of normal.

Gutiérrez-Delgado et al. [68]	Mexico	birth cohort until 5 years	772	double blind, randomized, placebo-controlled study	Intervention with DHA	Prenatal DHA supplementation did not influence IOS values with respect to resistance and reactance at 6, 8, and 10 Hz
Gochicoa-Rangel et al. [71]	Mexico	2.7–15.4 years	283		reference equation	Reference range equations were derived for impulse oscillometry. Predictors include age and height. Marked differences were noted between the derived reference equation when compared to other studies. Separate reference ranges for male and females were generated.
Gochicoa-Rangel et al. [75]	Mexico	4-15 years(mean age 8.6)	224	cross-sectional study	reference equation	Due to the robust adjustment of the equation derived from Gochicoa-Rangel et al. [71] this equation has been recommended for clinical and research purposes in a Mexican population.
Shackleton et al. [72]	Mexico	3-5.2 years	584	double-blind, randomised, placebo-controlled clinical trial	reference equation	Reference ranges for FOT were generated for Mexican children. Height was the only predictive factor and the same equation for males and females was used. An Australian reference range equation overestimate lung function in Mexican children.
Dubowski et al. [67]	Ghana	4 years	112	Prospective study	infection exposure	Infants exposed to a less diverse NPM (nasopharyngeal microbiota) had a higher small airway resistance (R_5–R_{20} = 17.9%, 95% CI 35.6, 0.23; p = 0.047). Compared to a more diverse phenotype
Dutta et al. [66]	Nigeria	2 years (mean age 2.9 years)	223	randomised control trial	air pollution	Increase in postnatal household air pollution ($PM_{2.5}$). were significantly associated with higher airway reactance at 5 Hz (X_5 Hz; P= 0.04)
Er et al. [76]	Turkey	3-7 years	151		reference equation	Reference values for IOS in healthy Turkish children were determined. Resistance was significantly correlated with height and reactance was significantly correlated with age ($p < 0.05$). Separate equations were derived for males and females.

Interrupter technique						
Rocha et al. [79]	Brazil	5 to 18 years (mean 10,79 years)	38	cross-sectional study	cystic fibrosis	Interrupter resistance (R_{int}) correlates well with spirometry. There was a strong correlation between inverse R_{int} and FEV_1 (r = 0.8; $p < 0.001$), and moderate correlation between inverse R_{int} and $FEF_{25–75\%}$ (r = 0.74; $p < 0.001$). R_{int} was not accurate in evaluating bronchodilator response.
Gochicoa et al. [80]	Mexico	24 days to 6.6 years	264	prospective, cross-sectional descriptive study	reference equation	Reference values for interrupter technique (R_{int}), was determined in Mexican children. There was an inverse relationship between R_{int} and height. Females had a higher R_{int} than males ($P = 0.054$).
Tidal breathing						
Kumar et al. [81]	India	3 years	310	prospective birth cohort study	acute respiratory infections	The ratio of tidal expiratory flow (*TEF*) at 25 or 50% of tidal expiratory volume to peak TEF (TEF_{50} or TEF_{25}/peak *TEF*) at 3 years was significantly increased in children who had an acute respiratory infection in infancy.
Li et al. [82]	China	<14 years	42	case review	bronchiolitis obliterans	The tidal breathing analysis revealed a decreased $t_{PTEF\%}$ t_E (18.2 ± 0.26%, normal: more than 40%), or $V_{PEF\%}V_E$ (21.7 ± 0.32%, normal: more than 40%).

FeNO, fractional exhaled nitric oxide; OR, odds ratio; CI, 95% confidence interval; $PM_{2.5}$, particulate matter 2.5; C-ACT, Childhood Asthma Control Test; sRAGE, Receptor for Advanced Glycation End products; Z_5, magnitude of respiratory impedance; R_5 :total respiratory resistance; X_5: distal capacitive reactance; obstructive sleep apnea hypopnea syndrome (OSAHS); R_5Hz, total resistance at 5 Hz; R_{20} Hz :central resistance at 20 Hz; $R_5–R_{20}$ Hz, difference between resistance at 5Hz and 20 Hz; IOS, Impulse oscillometry; $PM_{2.5}$: particulate matter 2.5; CI:95% confidence interval; R_{rs6}, R_{rs8}, R_{rs10}, Resistance at 6 Hz, 8Hz and 10Hz; X_{rs6}, X_{rs8}, and X_{rs10}, Reactance at 6 Hz, 8 Hz and 10Hz; DHA, docosahexaenoic acid; $t_{PTEF\%}$ t_E, ratio of time to reach peak tidal expiratory flow to total expiratory time $V_{PEF\%}V_E$, ratio of volume to reach peak expiratory flow to total expiratory volume.

The success rate for performing FeNO in preschool children ranged between 86–99%. Thai children living in a metropolitan area attending day care, average age of 50.1 months (range 29–72 months), achieved a success rate between 86–93% with data collected over 3 time points [61]. A cohort of 507 Chinese children aged 5 years achieved a success rate of 99% [62]. Over half of studies (57%) used a Niox Mino analyser (Aerocrine, Solna, Sweden) to measure FeNO using the single breath technique. Most studies used the recommended normal standards [26]. No studies have explored population differences in FeNO.

All 3 studies assessing impact of air quality on respiratory health found that environmental pollution, including benzene and $PM_{2.5}$, were associated with high FeNO [49, 61, 62].

Studies from China and one from South America, used FeNO measurement in the management of preschool asthma [63–65], noting the strong association with IgE mediated inflammation and ICS efficacy [63]. However, it was also suggested that FeNO may be less useful in the preschool age group for detecting ICS response, as the mean FeNO level was significantly higher only in the "older" age group than the cut off values reported in other studies for the diagnosis of asthma [63]. FeNO was not able to predict recurrence [64].

2.3.3 Oscillometry and interrupter technique

Oscillometry has been successfully used in LMIC to assess the impact of early life exposures and for clinical management of children with respiratory disease. These include: 6 studies from Asia, 6 from South America, 2 from West Africa and 1 from the Middle East summarized in Table 2.2. The studies used a range of commercially available equipment including both impulse oscillometry (IOS) and airwave oscillometry (AOS).

Oscillometry has proven to be effective in assessing the impact of early life exposures on preschool lung function [49, 66]. An increase in household $PM_{2.5}$ exposure increased airway reactance in Nigerian children, similarly high personal exposure to $PM_{2.5}$ was associated with an increase in small airway resistance ($R_{5–20}$), total airway resistance (R_5) and resistance frequency dependence ($R_{5–20}$), representing small airway disease, in asthmatic Chinese children living outside Shanghai [49, 66].

In a Ghanaian longitudinal cohort, infants exposed to a less diverse nasopharyngeal microbiome had a higher small airway resistance compared to a more diverse microbiome at 4 years of age [67]. Further, in a Mexican birth cohort study, prenatal omega 3 fatty acid supplementation in pregnancy did not influence preschool lung function at 36, 48 or 60 months [68].

Oscillometry was easily performed by preschool children with the overall success rate ranging between 74–98% in studies making it a particularly attractive option [69–73]. As a clinical tool oscillometry for preschool children is supported by studies that are able to detect differences in prebronchodilator lung function of preschool children with respiratory symptoms compared to those without respiratory symptoms [74].

Reference data for Mexican, Thai, Turkish and Colombian children have been collected [70–72, 75–77], facilitating the use in diagnosis of respiratory disease. It is also a useful tool to assess airway reversibility in asthmatic children and cut off values for bronchodilator response were proposed [69, 73]. A Chinese study in children with obstructive sleep apnea hypopnea syndrome (OSAHS) demonstrated an increase in total airway resistance in children with OSAHS compared to children with snoring but without OSAHS [78].

The interrupter technique was used in 2 South American studies, one of which looked at the development of reference values for newborn, infants and preschool children, while the other measured R_{int} in children with cystic fibrosis and found that R_{int} correlated well to spirometry FEV_1 and $FEF_{25–75}$, but not accurate in determining bronchodilator response [79, 80].

2.3.4 Tidal breathing

Tidal breathing was used in 2 studies, one an Indian birth cohort and the other a Chinese retrospective cohort study [81, 82], (Table 2.2). Tidal breathing measurements were in keeping with an obstructive pattern in patients with bronchiolitis obliterans [82]. Indian children who had an acute respiratory tract infection in infancy had increased ratios of tidal expiratory flow (*TEF*) at 25 or 50% of tidal expiratory volume to peak *TEF* (TEF_{50} or TEF_{25}/peak *TEF*) at 3 years suggesting increased airway resistance [81].

2.4 Conclusion

Preschool lung function tests used in LMIC were feasible with high success rates. Success with spirometry increased with age [29], and oscillometry had higher feasibility compared to spirometry. All tests were useful for clinical application and epidemiological studies. Comprehensive preschool testing including spirometry, FeNO, oscillometry and tidal breathing were only reported from China, and only 19 countries of 137 registered LMIC (14%) were represented in the review, a large discrepancy as the majority (80%) of children live in LMIC, where the burden of early life respiratory disease and exposures known to cause illness are high. These include air pollution, maternal smoking, a high infectious load including tuberculosis, and high rates of preterm birth [2, 83–86]. Lung functions tests assessing these vulnerable groups are lacking. A number of studies explored the effects of air pollution; and the studies suggest that air pollutants increase airway inflammation and may in

part explain the association between household air pollution and recurrent wheeze [83]. Given the burden of early life exposures and the need to identify preventative measure, priority should be given to strengthening access to lung function assessment tools.

The majority of clinical studies focussed on asthma diagnosis and management, including defining BDR in young children. The prevalence of asthma in LMIC is high, with a temporal increase in severe asthma [87]. Of particular concern is that up to 40% of children in LMIC with severe symptoms are undiagnosed [87, 88]. Oscillometry was useful, and more sensitive than spirometry, in measuring airway resistance and cut off values for BDR response was also determined. FeNO has been identified as a useful adjunct in diagnosis and informing treatment in asthmatic patients [89]. Access to these tests to improve diagnosis and management is needed for the many children living in LMIC.

Other clinical uses have included management of chronic lung conditions like CF, bronchiolitis obliterans, OSAHS, obesity and systemic sclerosis all of which are associated with respiratory complications. Lung function tests assessing vulnerable groups such as children born preterm, those living with HIV or exposed in utero and those with a history of pulmonary TB and other early life lower respiratory tract infections are lacking in children from LMIC.

The importance of reference range equations were highlighted in these studies. A healthy standard is needed for lung function tests to distinguish between health and disease in different populations. The GLI reference equations attempt to address this, however unique environmental exposures including in-utero exposures influence lung development and this needs to be considered when developing reference equations [90]. Research studies assessing

impact of exposures require appropriate patient samples and statistical modeling and should include both exposed and unexposed control groups.

There are currently no published data for MBW in preschool children in LMIC settings, however data on infant testing have been published in South Africa [3, 91]. There is limited data on tidal breathing and the interrupter technique. Impulse and airway oscillometry use different input signals and this may impact results especially at high impedance, which is associated with disease. Furthermore, FeNO single breath and tidal breathing measures have different interpretation of normal ranges, and the results are not interchangeable. These factors may affect the interpretation and comparison of data.

Challenges to accessing lung function testing need to be addressed, these include lack of trained personnel as each lung function test requires specialized training skills and lack of financial resources to support development and implementation of these tests. Spirometry and oscillometry are relatively inexpensive, whereas FeNO and MBW are currently more expensive further limiting access. Equipment maintenance and poor access locally to consumables and technical support incur further costs. This reduces research outputs and lack of robust data to better diagnose, prevent and manage respiratory disease in these settings [5].

In conclusion, preschool testing in LMIC is feasible, both epidemiologically and clinically. It has the potential to be useful in strengthening the diagnosis, management and prevention of respiratory diseases in younger children but is underutilized. Spirometry still remains a key clinical and epidemiological tool in LMIC, however has limitations especially in young children. Understanding and addressing the challenges for improving access to these tools is needed in order to strengthen the prevention, early diagnosis and management of childhood

respiratory disease in LMIC.

Funding:

DG is funded by the Wellcome Trust (204755/z/16/z). HJZ is funded by the SA-Medical Research Council.

References

1. Liu L, Villavicencio F, Yeung D, Perin J, Lopez G, Strong KL, et al. National, regional, and global causes of mortality in 5-19-year-olds from 2000 to 2019: a systematic analysis. Lancet Glob Health. 2022;10(3):e337-e347. doi: 10.1016/S2214-109X(21)00566-0.
2. Zar HJ, Ferkol TW. The global burden of respiratory disease-impact on child health. Pediatr Pulmonol. 2014;49(5):430-434. doi: 10.1002/ppul.23030.
3. Gray DM, Turkovic L, Willemse L, Visagie A, Vanker A, Stein DJ, et al. Lung function in African infants in the Drakenstein Child Health Study. Impact of lower respiratory tract illness. Am J Respir Crit Care Med. 2017;195(2):212-220. doi: 10.1164/rccm.201601-0188OC
4. Chan JY, Stern DA, Guerra S, Wright AL, Morgan WJ, Martinez FD. Pneumonia in childhood and impaired lung function in adults: a longitudinal study. Pediatrics. 2015;135(4):607-616. doi: 10.1542/peds.2014-3060.
5. Masekela R, Zurba L, Gray D. Dealing with access to spirometry in Africa: a commentary on challenges and solutions. Int J Environ Res Public Health. 2018;16(1):62. doi: 10.3390/ijerph16010062.
6. Belgrave DCM, Granell R, Turner SW, Curtin JA, Buchan IE, Le Souëf PN, et al. Lung function trajectories from pre-school age to adulthood and their associations with early life factors: a retrospective analysis of three population-based birth cohort studies. Lancet Respir Med. 2018;6(7):526-534. doi: 10.1016/S2213-2600(18)30099-7.
7. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 2007;175(12):1304-1345. doi: 10.1164/rccm.200605-642ST.
8. Robinson PD, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, et al. Preschool multiple-breath washout testing. An official American Thoracic Society Technical Statement. Am J Respir Crit Care Med. 2018;197(5):e1-e19. doi: 10.1164/rccm.201801-0074ST.
9. Nystad W, Samuelsen SO, Nafstad P, Edvardsen E, Stensrud T, Jaakkola JJ. Feasibility of measuring lung function in preschool children. Thorax. 2002;57(12):1021-1027. doi: 10.1136/thorax.57.12.1021.
10. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of spirometry 2019 update. An official American Thoracic Society and European Respiratory Society technical statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88. doi: 10.1164/rccm.201908-1590ST.
11. Knihtilä H, Kotaniemi-Syrjänen A, Mäkelä MJ, Bondestam J, Pelkonen AS, Malmberg LP. Preschool oscillometry and lung function at adolescence in asthmatic children. Pediatr Pulmonol. 2015;50(12):1205-1213. doi: 10.1002/ppul.23188.
12. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-1343. doi: 10.1183/09031936.00080312.
13. Bates JH, Irvin CG, Farré R, Hantos Z. Oscillation mechanics of the respiratory system. Compr Physiol. 2011;1(3):1233-1272. doi: 10.1002/cphy.c100058.
14. Gray DM, Czovek D, McMillan L, Turkovic L, Stadler JAM, Vanker A, et al. Intra-breath measures of respiratory mechanics in healthy African infants detect risk of

respiratory illness in early life. Eur Respir J. 2019;53(2):1800998. doi: 10.1183/13993003.00998-2018.
15. Hantos Z. Intra-breath oscillometry for assessing respiratory outcomes. Curr Opin Physiol. 2021;22:100441. doi: 10.1016/j.cophys.2021.05.004.
16. Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003;22(6):1026-1041. doi: 10.1183/09031936.03.00089403.
17. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, et al. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753. doi: 10.1183/13993003.00753-2019.
18. Fenu G, Calogero C, Lombardi E. Lung Function Tests in Preschool Children. Turk Thorac J. 2015;16(4):185-188. doi: 10.5152/ttd.2015.5038.
19. Beydon N. Interrupter resistance: what's feasible? Paediatr Respir Rev. 2006;7(Suppl 1):S5-7. doi: 10.1016/j.prrv.2006.04.022.
20. Kinghorn B, McNamara S, Genatossio A, Sullivan E, Siegel M, Bauer I, et al. Comparison of multiple breath washout and spirometry in children with primary ciliary dyskinesia and cystic fibrosis and healthy controls. Ann Am Thorac Soc. 2020;17(9):1085-1093. doi: 10.1513/AnnalsATS.201905-375OC.
21. Rosenfeld M, Allen J, Arets BH, Aurora P, Beydon N, Calogero C, et al. An official American Thoracic Society workshop report: optimal lung function tests for monitoring cystic fibrosis, bronchopulmonary dysplasia, and recurrent wheezing in children less than 6 years of age. Ann Am Thorac Soc. 2013;10(2):S1-S11. doi: 10.1513/AnnalsATS.201301-017ST.
22. Li Y, Wu R, Tian Y, Bao T, Tian Z. Fraction of exhaled nitric oxide and soluble receptors for advanced glycation end products are negatively correlated in children with recurrent wheezing. Asian Pac J Allergy Immunol. 2017;35(1):33-37. doi: 10.12932/AP0746.
23. Horváth I, Barnes PJ, Loukides S, Sterk PJ, Högman M, Olin AC, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. Eur Respir J. 2017;49(4):1600965. doi: 10.1183/13993003.00965-2016.
24. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602-615. doi: 10.1164/rccm.9120-11ST.
25. Ferrante G, Malizia V, Antona R, Corsello G, Grutta S. The value of FeNO measurement in childhood asthma: uncertainties and perspectives. Multidiscip Respir Med. 2013;8(1):50. doi: 10.1186/2049-6958-8-50.
26. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-930. doi: 10.1164/rccm.200406-710ST.
27. Zeng X, Xu X, Boezen HM, Vonk JM, Wu W, Huo X. Decreased lung function with mediation of blood parameters linked to e-waste lead and cadmium exposure in preschool children. Environ Pollut. 2017;230:838-848. doi: 10.1016/j.envpol.2017.07.014.
28. Zeng X, Xu X, Zhang Y, Li W, Huo X. Chest circumference and birth weight are good predictors of lung function in preschool children from an e-waste recycling area. Environ Sci Pollut Res Int. 2017;24(28):22613-22621. doi: 10.1007/s11356-017-9885-5.

29. Al-Qerem WA, Jarab AS. Applicability of GLI 2012 spirometry equation among preschool aged Jordanian. Respir Med. 2021;182:106397. doi: 10.1016/j.rmed.2021.106397.
30. França DC, Camargos PA, Martins JA, Abreu MC, Avelar e Araújo GH, Parreira VF. Feasibility and reproducibility of spirometry and inductance plethysmography in healthy Brazilian preschoolers. Pediatr Pulmonol. 2013;48(7):716-724. doi: 10.1002/ppul.22743.
31. Veras TN, Pinto LA. Feasibility of spirometry in preschool children. J Bras Pneumol. 2011;37(1):69-74. doi: 10.1590/s1806-37132011000100011.
32. França DC, Camargos PA, Jones MH, Martins JA, Vieira Bda S, Colosimo EA, et al. Prediction equations for spirometry in four- to six-year-old children. J Pediatr (Rio J). 2016;92(4):400-408. doi: 10.1016/j.jped.2015.10.005.
33. Burity EF, Pereira CA, Rizzo JA, Brito MC, Sarinho ES. Reference values for spirometry in preschool children. J Pediatr (Rio J). 2013;89(4):374-380. doi: 10.1016/j.jped.2013.01.002.
34. Jones MH, Vidal PCV, Lanza FC, Silva DCFMF, Pitrez PM, Olmedo APBF, et al. Reference values for spirometry in Brazilian children. J Bras Pneumol. 2020;46(3):e20190138. doi: 10.36416/1806-3756/e20190138.
35. Jian W, Gao Y, Hao C, Wang N, Ai T, Liu C, et al. Reference values for spirometry in Chinese aged 4-80 years. J Thorac Dis. 2017;9(11):4538-4549. doi: 10.21037/jtd.2017.10.110.
36. Tabatabaie SS, Boskabady MH, Mohammadi SS, Mohammadi O, Saremi P, Amery S, et al. Prediction equations for pulmonary function values in healthy children in Mashhad city, North East Iran. J Res Med Sci. 2014 Feb;19(2):128-33. doi: 10.1111/j.1440-1843.2004.00623.x
37. Bolla KC, Shanmuganathan A, Narasimhan M, Ganga N, Fathima Zehra Razvin H. Prediction equations for spirometry parameters of healthy school-going children in South India. Ann Trop Med Public Health. 2020;23:15. doi: 10.36295/ASRO.2020.231514
38. Bougrida M, Bourahli MK, Aissaoui A, Rouatbi S, Mehdioui H, Ben Saad H. Spirometric reference values for children living in Constantine (Eastern region of Algeria). Tunis Med. 2012;90(1):51-61.
39. Asif M, Mustafa G, Aslam M, Altaf S. Predictors of pulmonary function test values for Pakistani children, aged 5-14 years. J Pak Med Assoc. 2017;67(9):1323-1326.
40. Akodui OS, Faleti AO, Adekanmbi FA, Ogunlesi TA. Predicted reference equations for spirometry indices as a proxy from anthropometry measurements of Nigerian children with sickle cell anaemia aged 5-12 years. J Trop Pediatr. 2021;67(1):fmaa095. doi: 10.1093/tropej/fmaa095.
41. Quanjer PH, Capderou A, Mazicioglu MM, Aggarwal AN, Banik SD, Popovic S, Tayie FA, Golshan M, Ip MS, Zelter M. All-age relationship between arm span and height in different ethnic groups. Eur Respir J. 2014;44(4):905-912. doi: 10.1183/09031936.00054014.
42. Lum S, Bountziouka V, Quanjer P, Sonnappa S, Wade A, Beardsmore C, et al. Challenges in collating spirometry reference data for South-Asian children: an observational study. PLoS One. 2016;11(4):e0154336. doi: 10.1371/journal.pone.0154336.
43. Smith SJ, Gray DM, MacGinty RP, Hall GL, Stanojevic S, Mphahlele R, et al. Choosing the better Global Lung Initiative 2012 equation in South African population groups. Am J Respir Crit Care Med. 2020;202(12):1724-1727. doi: 10.1164/rccm.202005-2085LE.

44. Sonnappa S, Lum S, Kirkby J, Bonner R, Wade A, Subramanya V, et al. Disparities in pulmonary function in healthy children across the Indian urban-rural continuum. Am J Respir Crit Care Med. 2015;191(1):79-86. doi: 10.1164/rccm.201406-1049OC.
45. Asrul S, Juliana J. Indoor air quality and its association with respiratory health among preschool children in urban and suburban area. Malays J Public Health Med. 2017;2017:78-88.
46. Choo CP, Jalaludin J, Hamedon TR, Adam NM. Indoor air quality assessment and lung functions among children in preschool at Selangor, Malaysia. Adv Environ Biol. 2015;9:1-9.
47. Rawi NA, Jalaludin J, Chua PC. Indoor air quality and respiratory health among Malay preschool children in Selangor. Biomed Res Int. 2015;2015:248178. doi: 10.1155/2015/248178.
48. Heinzerling AP, Guarnieri MJ, Mann JK, Diaz JV, Thompson LM, Diaz A, et al. Lung function in woodsmoke-exposed Guatemalan children following a chimney stove intervention. Thorax. 2016;71(5):421-428. doi: 10.1136/thoraxjnl-2015-207783.
49. He L, Norris C, Cui X, Li Z, Barkjohn KK, Brehmer C, et al. Personal exposure to $PM_{2.5}$ oxidative potential in association with pulmonary pathophysiologic outcomes in children with asthma. Environ Sci Technol. 2021;55(5):3101-3111. doi: 10.1021/acs.est.0c06114.
50. Kumar SPS, Jain S. Effect of obesity on asthma severity in urban school children of Kanpur, India: an analytical cross-sectional study. J Community Health. 2020;45(2):342-347. doi: 10.1007/s10900-019-00749-z.
51. Ghasempour M, Bilan N, Vafaei I. The role of spirometry in the diagnosis of cough variant asthma in children aged 5 to 15 years. Int J Pediatr. 2017;5:6329-6336. doi: 10.22038/ijp.2017.26518.2276.
52. Sun W, Liu HY. Montelukast and budesonide for childhood cough variant asthma. J Coll Physicians Surg Pak. 2019;29(4):345-348. doi: 10.29271/jcpsp.2019.04.345.
53. Matos SM, Jesus SR, Saldiva SR, Prado MS, D'Innocenzo S, Assis AM, et al. Overweight, asthma symptoms, atopy and pulmonary function in children of 4-12 years of age: findings from the SCAALA cohort in Salvador, Bahia, Brazil. Public Health Nutr. 2011;14(7):1270-1278. doi: 10.1017/S1368980011000462.
54. Jiffri EH, Elhawary NA. The impact of common tumor necrosis factor haplotypes on the development of asthma in children: an Egyptian model. Genet Test Mol Biomarkers. 2011;15(5):293-299. doi: 10.1089/gtmb.2010.0157.
55. Leung TF, Tang MF, Leung ASY, Kong APS, Liu TC, Chan RWY, et al. Cadherin-related family member 3 gene impacts childhood asthma in Chinese children. Pediatr Allergy Immunol. 2020;31(2):133-142. doi: 10.1111/pai.13138.
56. Kang XH, Wang W, Cao L. A clinical study to determine the threshold of bronchodilator response for diagnosing asthma in Chinese children. World J Pediatr. 2019;15(6):559-564. doi: 10.1007/s12519-019-00293-9.
57. Kumari J, Jat KR, Lodha R, Jana M, Xess I, Kabra SK. Prevalence and risk factors of allergic bronchopulmonary aspergillosis and aspergillus sensitization in children with poorly controlled asthma. J Trop Pediatr. 2020;66(3):275-283. doi: 10.1093/tropej/fmz066.
58. Wang X, Zhou J, Zhao X, Yi X. Montelukast treatment of acute asthma exacerbations in children aged 2 to 5 years: a randomized, double-blind, placebo-controlled trial. Pediatr Emerg Care. 2018;34(3):160-164. doi: 10.1097/PEC.0000000000001184.
59. Ventura JC, Hauschild DB, Moreira EAM, Pereira LCR, Rosa AF, Barbosa E, et al. C-reactive protein/albumin ratio is associated with lung function among

children/adolescents with cystic fibrosis: a three-year longitudinal study. Sao Paulo Med J. 2018;136(1):29-36. doi: 10.1590/1516-3180.2017.0109100917.
60. Sasihuseyinoglu AS, Altıntaş DU, Soyupak S, Dogruel D, Yılmaz M, Serbes M, et al. Evaluation of high resolution computed tomography findings of cystic fibrosis. Korean J Intern Med. 2019;34(2):335-343. doi: 10.3904/kjim.2017.287.
61. Siwarom S, Puranitee P, Plitponkarnpim A, Manuyakorn W, Sinitkul R, Arj-Ong Vallipakorn S. Association of indoor air quality and preschool children's respiratory symptoms. Asian Pac J Allergy Immunol. 2017;35(3):119-126. doi: 10.12932/AP0838.
62. Zhang X, Fan Q, Bai X, Li T, Zhao Z, Fan X, et al. Levels of fractional exhaled nitric oxide in children in relation to air pollution in Chinese day care centres. Int J Tuberc Lung Dis. 2018;22(7):813-819. doi: 10.5588/ijtld.17.0601.
63. Zhu Z, Xia S, Chen X, Guan WJ, Guo ZJ, Sun BQ. Factors associated with exhaled nitric oxide in children with asthma and allergic rhinitis. Clin Respir J. 2020;14(1):9-15. doi: 10.1111/crj.13093.
64. Ardura-Garcia C, Arias E, Hurtado P, Bonnett LJ, Sandoval C, Maldonado A, et al. Predictors of severe asthma attack re-attendance in Ecuadorian children: a cohort study. Eur Respir J. 2019;54(5):1802419. doi: 10.1183/13993003.02419-2018.
65. Han L, Shangguan J, Yu G, Li T, Wu Y, Zhou Y, et al. Association between family management and asthma control in children with asthma. J Spec Pediatr Nurs. 2020;25(2):e12285. doi: 10.1111/jspn.12285.
66. Dutta A, Alaka M, Ibigbami T, Adepoju D, Adekunle S, Olamijulo J, et al. Impact of prenatal and postnatal household air pollution exposure on lung function of 2-year old Nigerian children by oscillometry. Sci Total Environ. 2021;755(Pt 2):143419. doi: 10.1016/j.scitotenv.2020.143419.
67. Dubowski K, Kaali S, Jack D, Prah RKD, Clemente JC, Tawiah T, et al. Infant nasopharyngeal microbiota subphenotypes and early childhood lung function: evidence from a rural Ghanaian pregnancy cohort. Int J Environ Res Public Health. 2021;18(14):7276. doi: 10.3390/ijerph18147276.
68. Gutiérrez-Delgado RI, Barraza-Villarreal A, Escamilla-Núñez C, Hernández-Cadena L, Garcia-Feregrino R, Shackleton C, et al. Effect of omega-3 fatty acids supplementation during pregnancy on lung function in preschoolers: a clinical trial. J Asthma. 2019;56(3):296-302. doi: 10.1080/02770903.2018.1452934.
69. Gupta N, Sachdev A, Gupta D, Gupta S. Assessment of airway reversibility in asthmatic children using forced oscillation technique - a single-center experience from North India. Lung India. 2021;38(3):229-235. doi: 10.4103/lungindia.lungindia_354_20.
70. Udomittipong K, Srisukhon W, Nimmannit A, Komoltri C. Respiratory impedance reference values for forced oscillation technique predicted by arm span and height in Thai preschool children. Pediatr Allergy Immunol Pulmonol. 2017;30:97-102. doi: 10.1089/ped.2017.0747.
71. Gochicoa-Rangel L, Torre-Bouscoulet L, Martínez-Briseño D, Rodríguez-Moreno L, Cantú-González G, Vargas MH. Values of impulse oscillometry in healthy Mexican children and adolescents. Respir Care. 2015;60(1):119-127. doi: 10.4187/respcare.03374.
72. Shackleton C, Barraza-Villarreal A, Chen L, Gangell CL, Romieu I, Sly PD. Reference ranges for Mexican preschool-aged children using the forced oscillation technique. Arch Bronconeumol. 2013;49(8):326-329. English, Spanish. doi: 10.1016/j.arbres.2013.01.014.

73. Udomittipong K, Triwatanawong J, Nimmannit A, Komoltri C. Cut-off values for positive bronchodilator response in healthy Thai preschool children using forced oscillation technique. Asian Pac J Allergy Immunol. 2020;38(4):258-263. doi: 10.12932/AP-280718-0381.
74. Medeiros D, Castro P, Bianca ACD, Sarinho E, Araújo JF, Correia M Jr, et al. Impulse oscillometry: pulmonary function assessment in preschool children. Expert Rev Respir Med. 2020;14(12):1261-1266. doi: 10.1080/17476348.2020.1813573.
75. Gochicoa-Rangel L, Del Río-Hidalgo R, Hernández-Ruiz J, Rodríguez-Moreno L, Martínez-Briseño D, Mora-Romero U, et al. Validating reference equations for impulse oscillometry in healthy Mexican children. Respir Care. 2017 Sep;62(9):1156-1165. doi: 10.4187/respcare.05247.
76. Er İ, Günlemez A, Baydemir C, Kılıçbay F, Ersu R, Uyan ZS. Impulse oscillometry reference values and correlation with predictors in Turkish preschool children. Turk J Pediatr. 2019;61(4):560-567. doi: 10.24953/turkjped.2019.04.013.
77. Duenas-Meza E, Correa E, López E, Morales JC, Aguirre-Franco CE, Morantes-Ariza CF, et al. Impulse oscillometry reference values and bronchodilator response in three- to five-year old children living at high altitude. J Asthma Allergy. 2019;12:263-271. doi: 10.2147/JAA.S214297.
78. Zhang J, Zhao J, Chen M, Liu S, Zhang X, Zhang F, et al. Airway resistance and allergic sensitization in children with obstructive sleep apnea hypopnea syndrome. Pediatr Pulmonol. 2016;51(4):426-430. doi: 10.1002/ppul.23264.
79. Rocha A, Donadio MV, Avila DV, Hommerding PX, Marostica PJ. Using the interrupter technique to evaluate airway resistance in cystic fibrosis patients. J Bras Pneumol. 2012;38(2):188-193. English, Portuguese. doi: 10.1590/s1806-37132012000200007.
80. Gochicoa LG, Thomé-Ortiz LP, Furuya ME, Canto R, Ruiz-García ME, Zúñiga-Vázquez G, et al. Reference values for airway resistance in newborns, infants and preschoolers from a Latin American population. Respirology. 2012;17(4):667-673. doi: 10.1111/j.1440-1843.2012.02156.x.
81. Kumar P, Mukherjee A, Randev S, Medigeshi GR, Jat KR, Kapil A, et al. Effect of acute respiratory infections in infancy on pulmonary function test at 3 years of age: a prospective birth cohort study. BMJ Open Respir Res. 2020;7(1):e000436. doi: 10.1136/bmjresp-2019-000436.
82. Li YN, Liu L, Qiao HM, Cheng H, Cheng HJ. Post-infectious bronchiolitis obliterans in children: a review of 42 cases. BMC Pediatr. 2014;14:238. doi: 10.1186/1471-2431-14-238.
83. Vanker A, Barnett W, Workman L, Nduru PM, Sly PD, Gie RP, et al. Early-life exposure to indoor air pollution or tobacco smoke and lower respiratory tract illness and wheezing in African infants: a longitudinal birth cohort study. Lancet Planet Health. 2017;1(8):e328-e336. doi: 10.1016/S2542-5196(17)30134-1.
84. Masekela R, Vanker A. Lung health in children in sub-Saharan Africa: addressing the need for cleaner air. Int J Environ Res Public Health. 2020;17(17):6178. doi: 10.3390/ijerph17176178.
85. Marangu D, Zar HJ. Childhood pneumonia in low-and-middle-income countries: an update. Paediatr Respir Rev. 2019;32:3-9. doi: 10.1016/j.prrv.2019.06.001.
86. Pusdekar YV, Patel AB, Kurhe KG, Bhargav SR, Thorsten V, Garces A, et al. Rates and risk factors for preterm birth and low birthweight in the global network sites in six low- and low middle-income countries. Reprod Health. 2020;17(Suppl 3):187. doi: 10.1186/s12978-020-01029-z.

87. Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S, et al. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax. 2009;64(6):476-483. doi: 10.1136/thx.2008.106609.
88. Baard CB, Franckling-Smith Z, Munro J, Workman L, Zar HJ. Asthma in South African adolescents: a time trend and risk factor analysis over two decades. ERJ Open Res. 2021;7(2):00576-2020. doi: 10.1183/23120541.00576-2020.
89. Gaillard EA, Kuehni CE, Turner S, Goutaki M, Holden KA, de Jong CCM, et al. European Respiratory Society clinical practice guidelines for the diagnosis of asthma in children aged 5-16 years. Eur Respir J. 2021;58(5):2004173. doi: 10.1183/13993003.04173-2020.
90. Schluger NW. The vanishing rationale for the race adjustment in pulmonary function test interpretation. Am J Respir Crit Care Med. 2022;205(6):612-614. doi: 10.1164/rccm.202112-2772ED.
91. Gray D, Willemse L, Visagie A, Czövek D, Nduru P, Vanker A, et al. Determinants of early-life lung function in African infants. Thorax. 2017;72(5):445-450. doi: 10.1136/thoraxjnl-2015-207401.
92. Gulla KM, Balaji A, Mukherjee A, Jat KR, Sankar J, Lodha R, et al. Course of illness after viral infection in Indian children with cystic fibrosis. J Trop Pediatr. 2019;65(2):176-182. doi: 10.1093/tropej/fmy033.
93. Kamaruddin AS, Jalaludin J, Choo CP. Indoor air quality and its association with respiratory health among malay preschool children in Shah Alam and Hulu Langat, Selangor. Adv Environ Biol. 2015;9:17-26.
94. Thacher JD, Emmelin A, Madaki AJ, Thacher TD. Biomass fuel use and the risk of asthma in Nigerian children. Respir Med. 2013;107(12):1845-1851. doi: 10.1016/j.rmed.2013.09.009.
95. Corten L, Morrow BM. Motor performance in South African children with cystic fibrosis. Phys Occup Ther Pediatr. 2020;40(2):192-200. doi: 10.1080/01942638.2019.1647329.
96. Sibanda EN, Dube Y, Chakawa M, Mduluza T, Mutapi F. Systemic sclerosis in Zimbabwe: autoantibody biomarkers, clinical, and laboratory correlates. Front Immunol. 2021;12:679531. doi: 10.3389/fimmu.2021.679531.

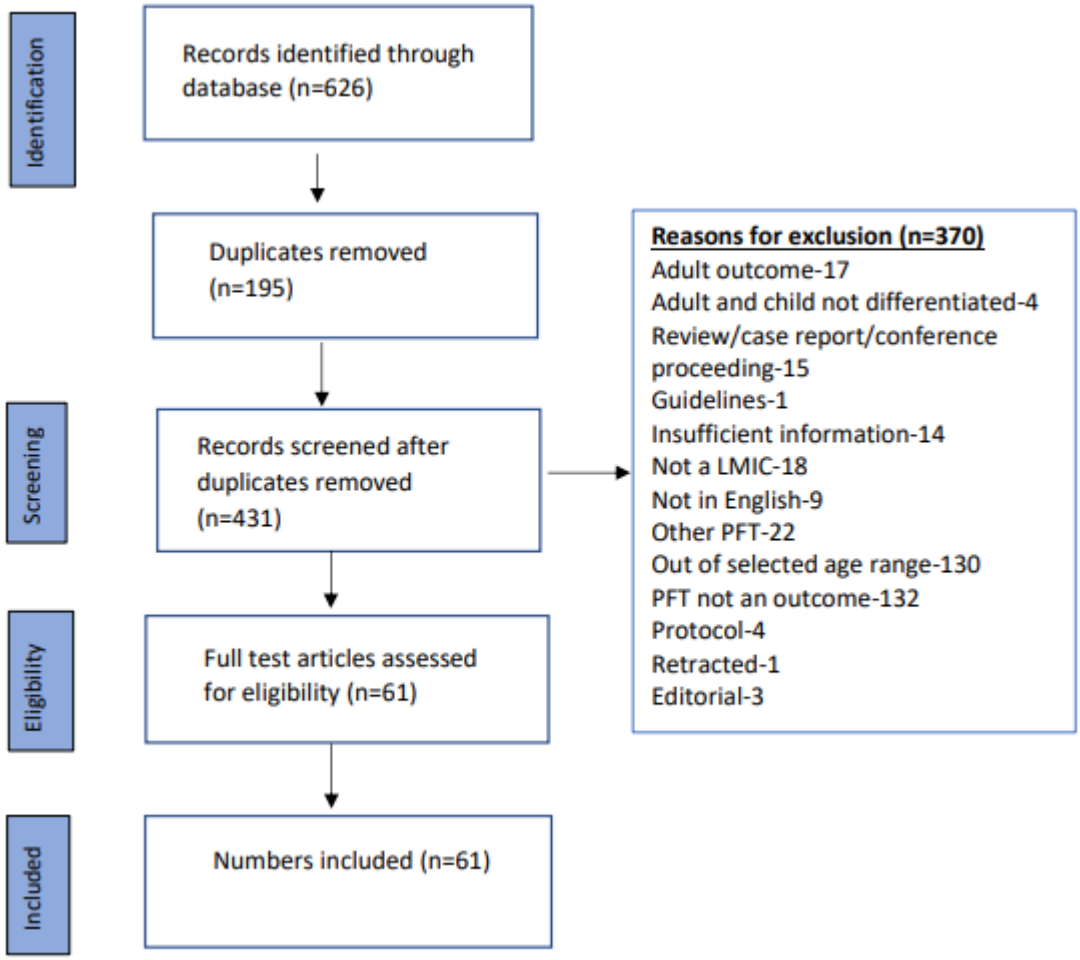
Supplementary information

Table S2.1 Search strategy

Search	Database	
“Respiratory Function Test*” OR “Lung function” OR “Lung function test*” OR “pulmonary function” OR “pulmonary function test*” OR “respiratory function” OR “respiratory function test*” OR “Respiratory outcome*” OR “pulmonary outcome*” OR “lung outcome*” OR “multiple-breath washout” OR “multiple breath washout” OR MBW OR “multiple-breath nitrogen washout” OR “multiple breath nitrogen washout” OR MBNW OR FOT OR “Forced oscillation technique” OR” tidal breathing” OR “fractional excretion of nitric oxide” OR Feno OR Spirometry OR Oscillometry OR “impulse oscillometry” ” OR “interrupter technique” OR “interrupter resistance” AND" preschool child*" OR "pre-school child*"	***Scopus-EMBASE**	**Total papers**: 355
	*** EBSCO host**	**Total papers** :41 - CINAHL:6 - Africa-Wide Information :2 - Health Source: Nursing/Academic Edition:33
	***Web of science**	**Total papers** :44 -core collection:40 -Sielo:4
"Respiratory Function Tests" OR Lung function OR Lung function test OR Lung function tests OR pulmonary function OR pulmonary function test OR pulmonary function tests OR respiratory function OR respiratory function test OR respiratory function tests OR Respiratory outcome OR respiratory outcomes OR pulmonary outcome OR pulmonary outcomes OR lung outcome OR lung outcomes OR multiple-breath washout OR multiple breath washout OR MBW OR multiple-breath nitrogen washout OR multiple breath nitrogen washout OR MBNW OR oscillometry OR FOT OR Forced oscillation technique OR tidal breathing OR “fractional excretion of nitric oxide” OR Feno OR spiro OR spirometry OR "Spirometry" OR "Oscillometry" OR “impulse oscillometry ” OR “interrupter technique” OR “interrupter resistance” AND "preschool child" OR "preschool children" OR "pre-school child" OR "pre-school children" OR "child, preschool"	*** PubMed**	**Total papers** :186

*Limits to 10 years

Figure S2.1 Flow diagram of final papers included



Chapter 3: Normal values of respiratory oscillometry in South African children and adolescents

---

Chaya S[1], MacGinty R[1], Jacobs C[1], Githinji L[1], Hlengwa S[1], Simpson SJ[2,3], Zar HJ[1], Hantos Z[4], Gray DM[1]. Normal values of respiratory oscillometry in South African children and adolescents. ERJ Open Res. 2023;9(2):00371-2022. doi: 10.1183/23120541.00371-2022.

Affiliations:

1. Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town, Cape Town, South Africa
2. Wal-yan Respiratory Centre, Telethon Kids Institute, Perth, Australia
3. School of Allied Health, Curtin University, Perth, Australia
4. Department of Anaesthesiology and Intensive Therapy, Semmelweis University, Budapest, Hungary

Author contributions:

SC contributed to the conceptualisation, methodology, data collection, analyses and writing of original draft. RM contributed to data curation and analysis. CJ contributed to data collection and manuscript. LG and SH contributed to data collection. ZH contributed to methodology, data acquisition, analysis and developed the oscillometry aspects of study. SS

contributed to methodology, analysis and review of the original draft. HJZ contributed to conceptualisation, funding acquisition, methodology, supervision and review of original draft. DMG contributed to lung function conceptualisation, data collection, funding acquisition, methodology, supervision, analyses and writing of original draft. All authors contributed to the final manuscript.

Word count: 3167

Abstract

Introduction: Noninvasive measurement of respiratory impedance by oscillometry can be used in young children aged from 3 years and those unable to perform forced respiratory manoeuvres. It can discriminate between healthy children and those with respiratory disease. However, its clinical application is limited by the lack of reference data for African paediatric populations. The aim of the present study was to develop reference equations for oscillometry outcomes in South African children and adolescents.

Methods: Healthy subjects, enrolled in the Drakenstein Child Health Study, HIV-uninfected adolescents in the Cape Town Adolescent Antiretroviral Cohort and healthy children attending surgical outpatient clinics at Red Cross War Memorial Children's Hospital were measured with conventional spectral (6–32 Hz) and intra-breath (10 Hz) oscillometry. Stepwise linear regression was used to assess the relationship between respiratory variables and anthropometric predictors (height, sex, ancestry) to generate reference equations.

Results: A total of 692 subjects, 48.4% female, median age of 5.2 years (range: 3–17 years) were included. The median (interquartile range (IQR)) for weight for age z-score and height for age z-score was −0.42 (−1.11–0.35) and −0.65 (−1.43–0.35), respectively. Stepwise regression demonstrated that all the variables were significantly dependent on height only. Comparison to previous reference data indicated slightly higher resistance and lower compliance values in the smallest children.

Conclusion: We established the first respiratory oscillometry reference equations for African children and adolescents, which will facilitate use in early identification and management of respiratory disease. Our results suggest differences in oscillometry measures by ancestry but also highlight the lack of standardisation in methodology.

3.1 Introduction

Measurement of lung function in early childhood is important for the diagnosis and management of lung disease, to promote optimal lung growth and development. Early life lung function predicts later morbidity and mortality [1, 2]. Spirometry is currently the most commonly performed lung function test, but its use is limited in young children as it requires a forced expiratory manoeuvre, mostly only feasible in children ≥5 years of age. In addition, it is relatively insensitive to detect early lung disease and is a poor measure of peripheral airway function [3].

Oscillometry is an attractive, feasible option in preschool children as it is a simple noninvasive test, requires minimal cooperation and can be used to follow lung function across the life course. Oscillometry measures the response of the respiratory system to an external small-amplitude oscillatory signal of medium (e.g. 4–40 Hz) frequencies which is superimposed on tidal breathing. The oscillatory pressure–flow relationship reflects the mechanical impedance of the respiratory system (Z_{rs}), which consists of two components, namely resistance (R_{rs}) and reactance (X_{rs}) [4, 5].

The conventional multifrequency or spectral values of Z_{rs} are generally obtained for a number of consecutive whole breaths (or, more recently, as mean values for the inspiratory and expiratory phases). In contrast, the novel intra-breath measurements, collected with a single-frequency tracking signal, follow the changes in R_{rs} and X_{rs} *within* the breathing cycle [6]. In particular, intra-breath oscillometry focuses on the zero-flow points (end inspiration and expiration); these Z_{rs} values are less influenced by the breathing pattern, which is often variable in young children and reflects less the contribution of the flow-dependent extrapulmonary airways. Owing to the ability to measure R_{rs} and X_{rs} at specific points of the

respiratory cycle and thus estimate the tidal changes in respiratory mechanics, intra-breath oscillometry has proved more sensitive than standard measures to assess airway obstruction, ventilation inhomogeneity, asthma control and respiratory disease risk [7–10].

Accurate interpretation of lung function measurements depends on the availability of a robust reference standard specific to the population assessed. Population differences in lung function such as anthropometric, sociocultural and environmental characteristics are well recognised [11–13]. Most oscillometry reference standards are specific for Caucasian populations from Europe, North America and Australia between the ages of 2 and 16 years [14–27]. Studies of non-Caucasian participants include Mexican, Thai, Emirati, Korean, Taiwanese, Turkish and Indian population groups with an age range between 3 and 17 years [28–34]. While reference equations derived from Caucasian data may be adequate for Caucasian South Africans, the most recent census describes the South African population as multi-ancestry: 80.7% Black African, 8.8% mixed ancestry (which would include African ancestries, Asian, Caucasian, amongst others) and 2.6% Indian/Asian [35]. Currently, no oscillometry reference equations exist for African populations, despite the high burden of respiratory disease in the region. Additionally, normative data on the novel intra-breath oscillometry measures are scant [8] and are not available for paediatric populations beyond infancy [9, 36]. Recent technical standards for oscillometry equipment and testing, developed by a European Respiratory Society (ERS) task force have highlighted the lack of appropriate paediatric reference standards, especially for underrepresented populations [37].

The aim of this study was to develop reference values for spectral and intra-breath oscillometry measures in healthy South African children and adolescents.

3.2 Methods

3.2.1 Participants

Healthy children and adolescents were enrolled from three South African groups: the Drakenstein Child Health Study (DCHS), a birth cohort study [38]; the Cape Town Adolescent Antiretroviral Cohort (CTAAC), including a healthy HIV-uninfected control group [39]; healthy children with no history of respiratory illnesses attending surgical outpatient clinics at Red Cross War Memorial Children's Hospital, Cape Town (HCSOC). Participants from the DCHS birth cohort were tested annually (collected 2015–2020) from 3 to 7 years, with one randomly selected time point per individual included in this study to remove any bias in the sample. Participants from CTAAC (11–15 years) and HCSOC (8–17 years) were tested between 2018 and 2020. All participants were of African ancestry, self-identifying as either Black African or mixed ancestry and from predominantly low socioeconomic communities [38, 39]. Socioeconomic status was determined from questionnaires completed at study visits and was based on household income, including accessed social grants. Household smoking was self-reported.

All children were healthy at the time of testing. Prior to testing they were screened for respiratory symptoms (cough, wheeze, difficult breathing) using a clinical and symptom study questionnaire based on the validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Those with acute lower respiratory tract illness (LRTI) or any respiratory illnesses in the previous month were excluded from testing. LRTI was defined as per the World Health Organization (WHO) case definition [40]. Children with any chronic respiratory conditions (self-reported or doctor diagnosed) including recurrent or persistent wheeze as well as chronic illnesses such as HIV infection, cardiac or neurological disorders

were also excluded.

3.2.2 Ethics

The study was approved by the University of Cape Town Faculty of Health Sciences (048/2020; 082/2018; 423/2012). Parents or legal guardians gave written informed consent in their first language and assent was given by all youth 7 years and older.

3.2.3 Lung function measurements

Oscillometry data were obtained using the same custom-made equipment (INCIRCLE wavetube system, University of Szeged, Hungary) [41, 42] by a trained team of three technologists[3]. Measurements were made with the individual sitting comfortably, breathing through a mouthpiece and filter, nose clip in place and the cheeks firmly supported, in accordance with published consensus guidelines [37]. The oscillometry system operated with either a pseudo-random signal in the 6–32 Hz range (conventional oscillometry) or a 10 Hz intra-breath tracking frequency; the latter corresponds to a 0.1-s temporal resolution allowing identification of the zero-flow Z_{rs} values (see below). Measurements consisted of a maximum of five 16-s epochs of multifrequency oscillations to yield a minimum of three acceptable measurements and one 16-s intra-breath recording, repeated if necessary to obtain a minimum of five regular breaths, i.e. without any vocal cord noise, apnoea, irregular breathing pattern, glottic closure, leak or sighs.

Conventional oscillometry measures included R_{rs} at 6 Hz (R_6), 8 Hz (R_8) and 10 Hz (R_{10}), X_{rs} at 6 Hz (X_6), 8 Hz (X_8) and 10 Hz (X_{10}), frequency dependence of R_{rs} (R_6–R_{20}), resonance frequency (F_{res}) and the absolute area of the X_{rs} *versus* frequency plot between 6 Hz and F_{res}

[3] The team of technologists testing patients were the same as highlighted in Chapter 1

(A_x). Additionally, mean respiratory system resistance (R), inertance (I) and compliance (C) were determined from model fitting to the measured Z_{rs} data in the frequency range 10–20 Hz for R and 6–32 Hz for I and C [41–43]. This procedure is illustrated in supplementary figure S3.1.

The intra-breath measurements were characterised by R_{rs} at end inspiration (R_{eI}) and at end expiration (R_{eE}), X_{rs} at end expiration (X_{eE}) and end inspiration (X_{eI}), and their tidal changes R_{eE}–R_{eI} (ΔR) and X_{eE}–X_{eI} (ΔX).

3.2.4 Statistical Analysis

Data were analysed using STATA 14.1 (STATA Corporation, College Station, TX, USA) and presented as frequencies, proportions, median and interquartile range (IQR) as appropriate. A natural logarithmic transformation was used for R, R_6, R_8, R_{10}, C, F_{res}, A_x, R_{eE} and R_{eI}. The effect of sex on oscillometry outcomes was investigated using Wilcoxon rank-sum test (Mann–Whitney U-test), and the relationship between the oscillometry outcomes and anthropometric covariates (sex, height (Ht) and ancestry) were explored using a backward stepwise linear regression. A reference equation for each outcome was generated and presented with the adjusted R^2 and standard error of the estimate (SEE) to allow z-score calculation: z-score=(measured value − predicted value)/SEE.

In order to assess the effect of puberty (particularly as numbers in this age group were relatively low) on the reference equation, backward stepwise regressions with anthropometric data for sex, Ht and ancestry were used to generate a reference equation in children between 3 and 7 years of age from the DCHS cohort.

Bayesian Information Criteria (BIC) models were used to select the best model fit for each of the oscillometry outcomes. In addition, diagnostic checks were done to ensure that the assumptions of linear regression were not violated. This included testing for the presence of multicollinearity using variance inflation factor, normality of residuals using histograms, kernel density and quantile–quantile plots, and homoscedasticity using residual versus fitted plots.

3.3 Results

A total of 692 children between the ages of 3 and 17 years were included in the study; 573 (82.8%) were from the DCHS cohort, 38 (5.5%) and 81 (11.7%) from the CTAAC and HCSOC sites, respectively, all representative of the same population group. All were of African ancestry, 361 (52%) were self-identified Black Africans and 331 (48%) of mixed ancestry. Demographic details and anthropometry of cohort including the weight for age z-score and Ht for age z-score data are summarised in table 3.1 and detailed in supplementary table S3.1. A total of 13 children (2%) were severely stunted (≤3 standard deviations below the mean) and four children (0.6%) were severely underweight (≤3 standard deviations below the mean). Six children (0.9%) were obese (≥3 standard deviations above the mean). Notably, 29% of mothers self-reported smoking.

Table 3.1 Demographics of all children and adolescents (n=692)

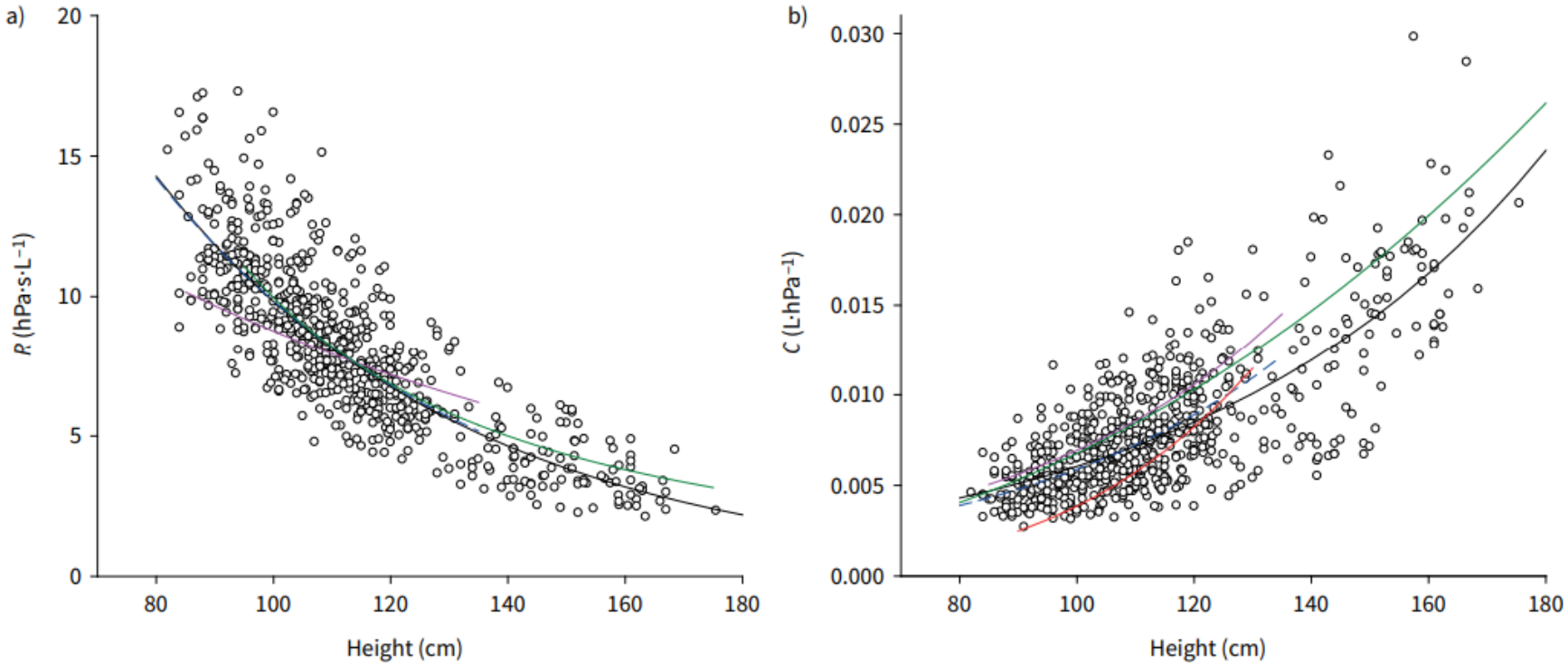
Individual characteristics	
Sex, female	335 (48.4)
Age years	5.2 (4.2–7.2)
Site	
CTAAC	38 (5.5)
Healthy surgical (HCSOC)	81 (12.7)
DCHS	573 (82.8)
Ancestry	
Black African	361 (52.2)
Mixed ancestry	331 (47.8)
Weight kg	18.20 (15.25–23.10)
WAZ	−0.42 (−1.11–0.35)
Height cm	110 (101–120)
HAZ	−0.65 (−1.43–0.05)
BMI $kg \cdot m^{-2}$	15.50 (14.53–17.04)
BMI-Z	−0.04 (−0.70–0.76)
Housing, informal settlement[#,¶]	255/611 (58.3)
Maternal smoking[#]	179/611 (29.3)

Data are presented as median (IQR) or n (%). CTAAC: Cape Town Adolescent Antiretroviral Cohort; HCSOC: Healthy Children at Surgical Outpatient Clinics; DCHS: Drakenstein Child Health Study; BMI: body mass index; WAZ: weight for age z-score; HAZ: height for age z-score; BMI-Z: body mass index z-score. [#]: information not available for HCSOC. [¶]: shelter constructed outside of the formal housing delivery system [46]; remainder classified as urban.

The conventional and intra-breath impedance measures are shown for all age groups in supplementary table S3.2. Values of F_{res} were available (i.e. fell in the 6–32 Hz range) in 514 (74.3%), less in the youngest and in most of the older children. The *R* and *C* data exhibited marked Ht dependences (figure 3.1); the compensatory parameter *I* has less physiological importance, and its values are not reported here. In figure 3.1, regressions on *R* and *C versus* Ht established in earlier work using model fitting are also plotted for comparison. The changes in various Z_{rs} measures with Ht are represented in supplementary figures S3.2a and b, exhibiting a decrease in R_6 and increase in X_6 with Ht. As shown in supplementary figure S3.2c–e, F_{res}, A_x and R_6–R_{20} decreased with increasing Ht. The intra-breath measures are plotted against Ht in supplementary figure S3.3. ΔR and ΔX exhibited large scatters but were predominantly positive (supplementary figure S3.3c and f, respectively).

Stepwise regression analysis demonstrated the significant association with Ht for all variables; R_6, X_8, X_{eE} and X_{eI} were also found to be associated with sex; X_8 and X_{10} with ancestry. However, as demonstrated by the BIC model (supplementary table S3.3), these additional associations offered negligible contribution to predictive models. Thus, only Ht was included in all regression models. The reference equations are compiled in table 3.2. An online tool using these equations for z-score calculation is available from supplementary material. The limits of normal are +1.64 z-score for *R* values, F_{res}, A_x, $R_{6–20}$, and −1.64 z-score for *X* values.

Figure 3.1 a) Respiratory system resistance (*R*) and b) compliance (*C*) versus height in healthy children and adolescents. Black solid and blue dashed lines indicate the regressions on the total population (n=692) and the 3- to 7-year age range (n=573), respectively. *R* and *C versus* height regressions from previous work are also plotted for comparison: HANTOS et al. [22]* (green), MAZUREK et al. [25]* (red) and SHACKLETON et al. [41]* (pink).



*Comparisons to previous studies using the same device

Table 3.2 Reference equations for children and adolescents 3 to 17 years of age

Outcome	Equation	Adj R^2	SEE
R_6 hPa·s·L^{-1}	exp(4.34–0.0189·Ht)	0.723	0.214
R_8 hPa·s·L^{-1}	exp(4.29–0.0190·Ht)	0.735	0.210
R_{10} hPa·s·L^{-1}	exp(4.27–0.0191·Ht)	0.747	0.204
X_6 hPa·s·L^{-1}	3.46–727·Ht^{-1}	0.405	1.168
X_8 hPa·s·L^{-1}	3.31–647·Ht^{-1}	0.425	0.995
X_{10} hPa·s·L^{-1}	2.73–531·Ht^{-1}	0.359	0.938
F_{res} Hz	exp(3.74–0.0062·Ht)	0.230	0.211
R_6–R_{20} hPa·s·L^{-1}	5.67–0.0311·Ht	0.177	1.227
A_x hPa·L^{-1}	exp(6.35–0.0287·Ht)	0.416	0.624
R hPa·s·L^{-1}	exp(4.16–0.0187·Ht)	0.739	0.205
C L·hPa^{-1}	exp(0.099 +0.0168·Ht)	0.523	0.295
R_{eE} hPa·s·L^{-1}	exp(4.36–0.0204·Ht)	0.734	0.225
R_{eI} hPa·s·L^{-1}	exp(4.32–0.0208·Ht)	0.729	0.233
X_{eE} hPa·s·L^{-1}	2.17–409·Ht^{-1}	0.174	1.178
X_{eI} hPa·s·L^{-1}	3.22–577·Ht^{-1}	0.397	0.943
ΔR hPa·s·L^{-1}	2.26–0.0144·Ht	0.040	1.272
ΔX hPa·s·L^{-1}	1.80–0.0117·Ht	0.050	0.928

Adj R^2: adjusted R^2; SEE: standard error of the estimate; R_6, R_8 and R_{10}: resistance at 6, 8 and 10 Hz; X_6, X_8 and X_{10}: reactance at 6, 8 and 10 Hz; F_{res}: resonance frequency; R_6–R_{20}: difference between resistance at 6 Hz and resistance at 20 Hz; A_x: area under the reactance curve; R: resistance from model fitting; C: compliance from model fitting; R_{eI}: resistance at end inspiration; R_{eE}: resistance at end expiration; ΔR: R_{eE}–R_{eI}; X_{eI}: reactance at end inspiration; R_{eE}: reactance at end expiration; ΔX: X_{eE}–X_{eI}.

To assess the effect of puberty on the reference range equations, stepwise regression in children from the DCHS cohort was done; the coefficients obtained (supplementary table S3.4) remained very close to that of the reference equations for the entire cohort, with a moderate decrease in adjusted R^2 attributable to the narrower Ht range. The consistency of reference equations between the full and reduced ranges in Ht is also illustrated in figure 3.1 and supplementary figures S3.2 and S3.3. Overall, the deviations between the full and reduced Ht range predictions are significant only in ΔR (Figure S3.2), and mild in C (figure 3.1), X_6 and A_x (supplementary figure S3.2), R_{eI}, X_{eE} and X_{eI} (supplementary figure S3.3). Excellent agreements were found for R and R_6 between the full and reduced Ht range predictions.

The comparison between R_6 predicted with the current equation and other published reference equations for different populations is illustrated in figure 3.2 [14–26, 30, 34]. Initially, we considered reference data from previous studies only if 1) R_{rs} values at around 5–6 Hz were analysed, 2) Ht was the single independent variable and 3) higher-order than linear relationship to Ht was assumed. The main features of these studies are summarised in table 3.3. Our R_6 values are similar to the R_{rs} plots of the other studies at the medium Ht range. Data from nine additional studies that assumed the linear R_{rs} versus Ht relationship (supplementary table S3.5) are shown in supplementary figure S3.4; these reference lines are rather scattered and fall outside the nonlinear regressions and illustrate the inadequacy of the linear Ht dependence, especially in the wide Ht range.

Figure 3.2 Comparison of respiratory resistance (R_{rs}) *versus* height relationships established in the present and previous studies (see table 3.3 for details).

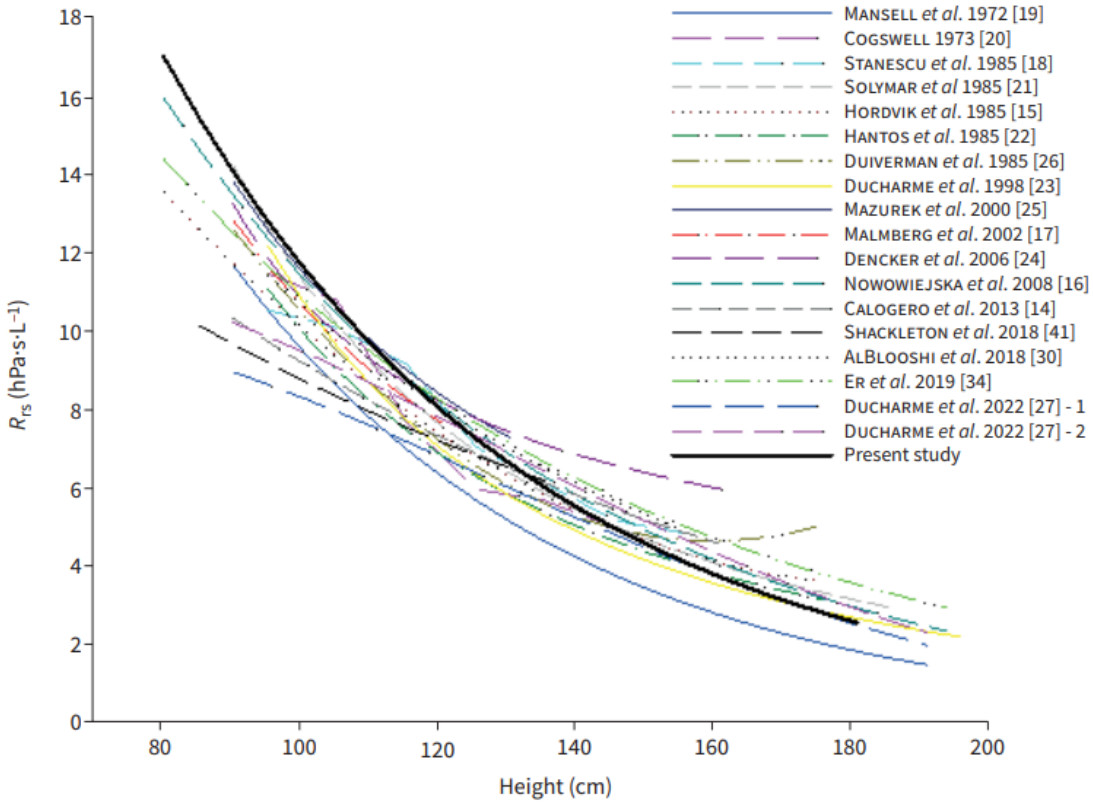


Table 3.3 Summary of reference studies on resistance (*R*) *versus* height (Ht) relationships[#]

Author(s)	Year	Frequency Hz	Device	Country/race	No. of subjects	Age range years	Reference equation
MANSELL et al. [19]	1972	5	Custom made	Canada	79	3–17	R_5=exp(1.877–0.0089·Ht)
COGSWELL [20]	1973	5–7	Custom made	UK	204	3–12	$R_{5–7}$ *versus* Ht range data
STANESCU et al. [18]	1979	4–9	Custom made	Belgium	130	3–14	R_4 *versus* Ht range data
SOLYMAR et al. [21]	1985	2–12	Custom made	Sweden	218	2–18	R_4=antilog(1.053–2.18·log(Ht))
HORDVIK et al. [15]	1985	2–26	Jones Oscillaire	USA/C	138	2–16	R_6=9.2·Ht2–34.1·Ht+35.2
HANTOS et al. [22]	1985	3–10	Custom made	Hungary	121	4–16	R(3–10)=1.28·105·Ht−2.05
DUIVERMAN et al. [26]	1985	2–26	Custom made	The Netherlands/C	255	2.3–12.5	R_6=0.0017·Ht2–0.541·Ht+47.73
DUCHARME et al. [23]	1998	8–16	Custo Vit R	Canada/mixed	199	3–17	R_8=exp(10.99–2.37·ln(Ht))
MAZUREK et al. [25]	2000	4–32	Custom made	Poland	127	2.5–7.5	R_6=exp(2.4422–1.7447·ln(Ht))
MALMBERG et al. [17]	2002	5–35	Jaeger IOS	Finland	109	2–7	R_5=exp(2.115–1.786·ln(Ht))
DENCKER et al. [24]	2006	5–35	Jaeger IOS	Finland-Sweden/C	360	2–11	R_5 *versus* Ht curve
NOWOWIEJSKA et al. [16]	2008	5–35	Jaeger IOS	Poland	626	3–18	R_5=exp(−0.0169·Ht+1.818)
CALOGERO et al. [14]	2013	4–48	Chess i2M	Australia–Italy/C	760	2–13	R_6=exp(3.3738–0.01155·Ht)
SHACKLETON et al. [41]	2018	6–26	Custom made[¶]	Australia/Hungary/C	319	3–6	R_6= exp(3.3501–0.01033·Ht)
ALBLOOSHI et al. [30]	2018	5–37	Tremoflo C-100	UAE/Emirati	291	4–12	R_5=exp(3.786–0.014·Ht)
ER et al. [34]	2019	5–35	Jaeger IOS	Turkey/Turkish	151	3–7	R_5=antilog(0.527–0.005·Ht)
DUCHARME et al. [27] – 1	2022	5–37	Resmon Pro	Canadian/mixed	271	3–17	R_5=exp(−0.1509+0.00809·Ht-0.0000824·Ht2)
DUCHARME et al. [27] – 2	2022	5–37	Tremoflo C-100	Canadian/mixed	292	3–17	R_5=exp(−0.0252+0.00809·Ht-0.0000817·Ht2)

Units in the reference equations are as originally reported: R in $cmH_2O \cdot s \cdot L^{-1}$, $hPa \cdot s \cdot L^{-1}$ or $kPa \cdot s \cdot L^{-1}$; Ht in cm or m. C: Caucasian (when stated). #: only studies that used nonlinear formulae are included; those assuming linear relationship are added in supplementary table S3.5. ¶: identical device to that used in the present study.

3.4 Discussion

This is the first study to report oscillometry data in healthy African children and adolescents that includes both conventional and intra-breath measures. Our findings compare favourably with previously published normative data from other populations, suggesting that standardisation of methodology is a key factor accounting for cohort differences, while indicating the role of population differences.

The vast majority of normative data derived since 1972 includes predominantly Caucasian populations, covering various age ranges, and utilises a variety of oscillometry equipment. Additionally, the predictions employed different statistical models and anthropometric variables, further hindering direct comparison.

We therefore limited the comparison of the present data to studies that reported Ht as the only independent variable and used a nonlinear Ht dependence of Z_{rs} measures, as appropriate. The inappropriateness of the linear R_{rs} *versus* Ht relationship is highlighted in supplementary figure S3.4.

To our knowledge, figure 3.2 represents the most comprehensive survey on the Ht dependence of R_{rs} values in children and adolescents, although the permissive inclusion of the different lowest frequencies (4, 5 or 6 Hz) or frequency ranges for model fitting increases the variability. The roughly inverse relationships between R_{rs} and Ht exhibit some variability between the normative studies, and our data, which covers one of the widest Ht ranges, is consistent with this (see figure 3.2). We note that some nonlinear models, such as polynomial regressions, may predict unrealistic inflections in the R_{rs} *versus* Ht relationships towards lower Ht [27] or higher Ht [26]. Apart from this, in the lowest Ht range (<120 cm), our 6-Hz

R_{rs} values are among the highest, together with lower-frequency (4 and 5 Hz) measurements expected to result in higher R_{rs} [16, 21], and that obtained with a special (head generator) device [25] leads to higher values than the uncorrected R_{rs}. A more rigorous comparison covering only Z_{rs} data at 10 Hz is presented in supplementary figure S3.5; the relative position of our R_{10} values remains similar to that shown in figure 3.2, whereas our X_{10} data are rather in the middle of the smaller set of available X_{10} predictions. There appears to be a systematic difference between our predictions and those based on the same oscillometry setup employed in a population of Caucasian children [41]. Comparison of F_{res} *versus* Ht regressions reveal a wide scatter between studies, in which our data take a midposition.

Ethnic differences in oscillometry measurements obtained with the same device have been noted [28]; ancestry, environmental and body habitus differences, which influence Ht, were the most likely suggested reasons accounting for this discrepancy. Moreover, differences in Ht between populations appear to be greatest in preschool years [44]. The fact that our cohort had a higher R_{rs} at Ht <120 cm possibly indicates that the younger children in our study may have smaller lungs for a given Ht compared to other healthy reference populations. We noted a higher R_{rs} with a predominantly lower X_{rs} in oscillometry variables in females compared to males, similar to findings by others [9, 14]. However, we found that sex was not independently predictive after adjusting for Ht. Difference in Z_{rs} between females and males in childhood and adolescence may be primarily driven by smaller lung volumes and narrower airways in females compared to males [45, 46].

Many early life factors influence lung growth and development, including environmental smoke exposure and socioeconomic status (SES) [11, 38]. Our study population was from a predominantly low SES community with high smoke exposure; 29% of mothers in our cohort

smoked [38, 39]. However, this subtle difference in R_{rs} at small Ht should be interpreted with care as measurement accuracy has been shown to be rather variable between commercial oscillometry devices at high load impedances, such as Z_{rs} in small children [47]. It is worth noting that the reference equipment in this device comparison study [47] was the wavetube setup [41, 42] employed in the present investigation. Efforts are underway to align and standardise equipment signalling and processing, including the development of consensus guidelines [37, 41, 47, 48].

In addition to the conventionally reported R_{rs} and X_{rs} values at the oscillation frequencies, F_{res} and A_X are increasingly used to determine the elasticity and ventilation inhomogeneity of the respiratory system, whereas $R_{6–20}$ reflects peripheral inhomogeneity and airway obstruction [47]. With the exception of F_{res}, these measures are very sensitive to the value of the lowest oscillation frequency, which is rather variable between devices and hence different studies; this is another argument calling for urgent standardisation effort. We have added the mean R_{rs} (R) and C parameters from model fitting [42, 43] and propose these measures as more robust descriptors of the resistive and reactive behaviour of the respiratory system than the *R*rs and *X*rs readings at individual frequencies. Reports on R and C in paediatric populations are scant in the literature [22, 25]; the most important comparison with a previous study [41] that employed the same oscillometry device and evaluation procedure (figure 3.1) reinforces the single-frequency findings on the relatively high resistance and low compliance values in our preschooler population.

This study is one of the first to develop comprehensive reference equations for the novel intra-breath oscillometry measurements in the paediatric population [7–9]. Intra-breath measures have been shown to be a measure of airway obstruction in preschool children with

wheezing and altered in children with asthma [7]. We have also previously shown that these measurements were able to predict healthy infants at risk for lower respiratory tract infections [9]. The clinical utility of the intra-breath measures together with standardised conventional spectral variables in children need to be fully established, and ongoing work is recommended in this area to facilitate diagnosis of respiratory disease with more precision.

Strengths of this study include the large sample of healthy children with data collected using the same equipment and methodology. The age range of children extended from preschool to adolescence provides us with a tool useful through childhood and adolescence. The availability of an online tool for calculation of the lower/upper limits of normal and z-score simplifies this further, facilitating its use for users in the field.

A limitation of this study is the relatively small sample size in the 8- to 17-year-old age interval, a time of variable lung growth particularly between sexes, thus assessing the impact of puberty was limited. Since there is a remarkable consistency in the Ht dependencies of the major oscillometry measures between the full (3–17 years) and the lower (3–7 years) age ranges, these reference equations aim to guide clinical practice until they are updated by using more balanced patient cohorts. In addition, these normative values are based only on data from a single province, the Western Cape, of South Africa; therefore this may not necessarily be generalisable to the rest of the Southern Africa region, although recent multi-province data collection in healthy individuals shows concordance in spirometry measurements [45].

In conclusion, we have established the first respiratory impedance reference equations for South African children and adolescents with an online tool to facilitate its use in early

identification and management of respiratory disease. While our results reveal differences in oscillometry measures by ancestry, they also highlight the lack of standardisation in methodology.

Acknowledgements:

We thank the study staff in Paarl and Cape Town, the study data and laboratory teams, and the clinical and administrative staff of the Western Cape Government Health Department at Paarl and Red Cross War Memorial Children's Hospitals. We thank the families and children who participated in this study. We thank Dorottya Czövek (First Department of Paediatrics, Semmelweis University, Budapest, Hungary) for support with DCHS preschool oscillometry measurement setup and Gergely Makan (Department of Technical Informatics, University of Szeged, Szeged, Hungary) for data management support.

Funding:

The study received funding from the Bill and Melinda Gates Foundation (grant number OPP1017641, OPP1017579); The Wellcome Trust (098479/Z/12/Z; 204755/Z/162); MRC South Africa, The National Research Foundation, South Africa; National Research, Development and Innovation Office (OTKA grant 128701), Hungary; European Respiratory Society (INCIRCLE CRC-2013-02); National Institute of Health R01HD074051; Harry Crossley Clinical Research Fellowship.

References

1. Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet. 2007;370(9589):758-764. doi: 10.1016/S0140-6736(07)61379-8.
2. Duong M, Islam S, Rangarajan S, Leong D, Kurmi O, Teo K, et al. Mortality and cardiovascular and respiratory morbidity in individuals with impaired FEV1 (PURE): an international, community-based cohort study. Lancet Glob Health. 2019;7(5):e613-e623. doi: 10.1016/S2214-109X(19)30070-1.
3. Santus P, Radovanovic D, Pecchiari M, Ferrando M, Tursi F, Patella V, et al. The relevance of targeting treatment to small airways in asthma and COPD. Respir Care. 2020;65(9):1392-1412. doi: 10.4187/respcare.07237.
4. Skylogianni E, Douros K, Anthracopoulos MB, Fouzas S. The forced oscillation technique in paediatric respiratory practice. Paediatr Respir Rev. 2016;18:46-51. doi: 10.1016/j.prrv.2015.11.001.
5. Bates JH, Irvin CG, Farré R, Hantos Z. Oscillation mechanics of the respiratory system. Compr Physiol. 2011;1(3):1233-1272. doi: 10.1002/cphy.c100058.
6. Hantos Z. Intra-breath oscillometry for assessing respiratory outcomes. Curr Opin Physiol. 2021;22:100441. doi: 10.1016/j.cophys.2021.05.004.
7. Czövek D, Shackleton C, Hantos Z, Taylor K, Kumar A, Chacko A, et al. Tidal changes in respiratory resistance are sensitive indicators of airway obstruction in children. Thorax. 2016;71(10):907-915. doi: 10.1136/thoraxjnl-2015-208182.
8. Lorx A, Czövek D, Gingl Z, Makan G, Radics B, Bartusek D, et al. Airway dynamics in COPD patients by within-breath impedance tracking: effects of continuous positive airway pressure. Eur Respir J. 2017;49(2):1601270. doi: 10.1183/13993003.01270-2016.
9. Gray DM, Czovek D, McMillan L, Turkovic L, Stadler JAM, Vanker A, et al. Intra-breath measures of respiratory mechanics in healthy African infants detect risk of respiratory illness in early life. Eur Respir J. 2019;53(2):1800998. doi: 10.1183/13993003.00998-2018.
10. Chiabai J, Friedrich FO, Fernandes MTC, Serpa FS, Antunes MOB, Neto FB, et al. Intrabreath oscillometry is a sensitive test for assessing disease control in adults with severe asthma. Ann Allergy Asthma Immunol. 2021;127(3):372-377. doi: 10.1016/j.anai.2021.06.005.
11. Stanojevic S, Wade A, Lum S, Stocks J. Reference equations for pulmonary function tests in preschool children: a review. Pediatr Pulmonol. 2007;42(10):962-972. doi: 10.1002/ppul.20691.
12. Lum S, Bountziouka V, Sonnappa S, Wade A, Cole TJ, Harding S, et al. Lung function in children in relation to ethnicity, physique and socioeconomic factors. Eur Respir J. 2015;46(6):1662-1671. doi: 10.1183/13993003.00415-2015.
13. Calogero C, Simpson SJ, Lombardi E, Parri N, Cuomo B, Palumbo M, et al. Respiratory impedance and bronchodilator responsiveness in healthy children aged 2-13 years. Pediatr Pulmonol. 2013;48(7):707-715. doi: 10.1002/ppul.22699.

14. Hordvik NL, König P, Morris DA, Kreutz C, Pimmel RL. Normal values for forced oscillatory respiratory resistance in children. Pediatr Pulmonol. 1985;1(3):145-148. doi: 10.1002/ppul.1950010305.
15. Nowowiejska B, Tomalak W, Radliński J, Siergiejko G, Latawiec W, Kaczmarski M. Transient reference values for impulse oscillometry for children aged 3-18 years. Pediatr Pulmonol. 2008;43(12):1193-1197. doi: 10.1002/ppul.20926.
16. Malmberg LP, Pelkonen A, Poussa T, Pohianpalo A, Haahtela T, Turpeinen M. Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children. Clin Physiol Funct Imaging. 2002;22(1):64-71.
17. Stănescu D, Moavero NE, Veriter C, Brasseur L. Frequency dependence of respiratory resistance in healthy children. J Appl Physiol Respir Environ Exerc Physiol. 1979;47(2):268-272. doi: 10.1152/jappl.1979.47.2.268.
18. Mansell A, Levison H, Kruger K, Tripp TL. Measurement of respiratory resistance in children by forced oscillations. Am Rev Respir Dis. 1972;106(5):710-714. doi: 10.1164/arrd.1972.106.5.710.
19. Cogswell JJ. Forced oscillation technique for determination of resistance to breathing in children. Arch Dis Child. 1973;48(4):259-266. doi: 10.1136/adc.48.4.259.
20. Solymar L, Aronsson PH, Sixt R. The forced oscillation technique in children with respiratory disease. Pediatr Pulmonol. 1985;1(5):256-261. doi: 10.1002/ppul.1950010507.
21. Hantos Z, Daróczy B, Gyurkovits K. Total respiratory impedance in healthy children. Pediatr Pulmonol. 1985;1(2):91-98. doi: 10.1002/ppul.1950010207.
22. Ducharme FM, Davis GM, Ducharme GR. Pediatric reference values for respiratory resistance measured by forced oscillation. Chest. 1998;113(5):1322-1328. doi: 10.1378/chest.113.5.1322.
23. Dencker M, Malmberg LP, Valind S, Thorsson O, Karlsson MK, Pelkonen A, et al. Reference values for respiratory system impedance by using impulse oscillometry in children aged 2-11 years. Clin Physiol Funct Imaging. 2006;26(4):247-250. doi: 10.1111/j.1475-097X.2006.00682.x.
24. Mazurek H, Willim G, Marchal F, Haluszka J, Tomalak W. Input respiratory impedance measured by head generator in preschool children. Pediatr Pulmonol. 2000;30(1):47-55. doi: 10.1002/1099-0496(200007)30:1<47::aid-ppul8>3.0.co;2-w.
25. Duiverman EJ, Clément J, van de Woestijne KP, Neijens HJ, van den Bergh AC, Kerrebijn KF. Forced oscillation technique. Reference values for resistance and reactance over a frequency spectrum of 2-26 Hz in healthy children aged 2.3-12.5 years. Bull Eur Physiopathol Respir. 1985;21(2):171-178.
26. Ducharme FM, Smyrnova A, Lawson CC, Miles LM. Reference values for respiratory sinusoidal oscillometry in children aged 3 to 17 years. Pediatr Pulmonol. 2022;57(9):2092-2102. doi: 10.1002/ppul.25984.
27. Shackleton C, Barraza-Villarreal A, Chen L, Gangell CL, Romieu I, Sly PD. Reference ranges for Mexican preschool-aged children using the forced oscillation technique. Arch Bronconeumol. 2013;49(8):326-329. English, Spanish. doi: 10.1016/j.arbres.2013.01.014.

28. Udomittipong K, Srisukhon W, Nimmannit A, Komoltri C. Respiratory impedance reference values for forced oscillation technique predicted by arm span and height in Thai preschool children. Pediatr Allergy Immunol Pulmonol. 2017;30:97-102. doi: 10.1089/ped.2017.0747.
29. AlBlooshi A, AlKalbani A, Narchi H, Al-Hamad S, Al-Houqani M, AlBadi G, et al. Respiratory function in healthy Emirati children using forced oscillations. Pediatr Pulmonol. 2018;53(7):936-941. doi: 10.1002/ppul.23985.
30. Gochicoa-Rangel L, Torre-Bouscoulet L, Martínez-Briseño D, Rodríguez-Moreno L, Cantú-González G, Vargas MH. Values of impulse oscillometry in healthy Mexican children and adolescents. Respir Care. 2015;60(1):119-127. doi: 10.4187/respcare.03374.
31. Lee JY, Seo JH, Kim HY, Jung YH, Kwon JW, Kim BJ, et al. Reference values of impulse oscillometry and its utility in the diagnosis of asthma in young Korean children. J Asthma. 2012;49(8):811-816. doi: 10.3109/02770903.2012.716472.
32. Lai SH, Yao TC, Liao SL, Tsai MH, Hua MC, Yeh KW, et al. Reference value of impulse oscillometry in Taiwanese preschool children. Pediatr Neonatol. 2015;56(3):165-170. doi: 10.1016/j.pedneo.2014.09.002.
33. Er İ, Günlemez A, Baydemir C, Kılıçbay F, Ersu R, Uyan ZS. Impulse oscillometry reference values and correlation with predictors in Turkish preschool children. Turk J Pediatr. 2019;61(4):560-567. doi: 10.24953/turkjped.2019.04.013.
34. Statistics South Africa. Mid-Year Population Estimates—P0302. 2019. www.statssa.gov.za/publications/P0302/P03022019.pdf Date last updated: July 2022.
35. Radics BL, Gyurkovits Z, Makan G, Gingl Z, Czövek D, Hantos Z. Respiratory oscillometry in newborn infants: conventional and intra-breath approaches. Front Pediatr. 2022;10:867883. doi: 10.3389/fped.2022.867883.
36. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, et al. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753. doi: 10.1183/13993003.00753-2019.
37. Gray D, Willemse L, Visagie A, Czövek D, Nduru P, Vanker A, et al. Determinants of early-life lung function in African infants. Thorax. 2017;72(5):445-450. doi: 10.1136/thoraxjnl-2015-207401.
38. Githinji LN, Gray DM, Hlengwa S, Myer L, Zar HJ. Lung function in South African adolescents infected perinatally with HIV and treated long-term with antiretroviral therapy. Ann Am Thorac Soc. 2017;14(5):722-729. doi: 10.1513/AnnalsATS.201612-1018OC.
39. World Health Organization (WHO). Integrated management of childhood illness: distance learning course; 2014. https://apps.who.int/iris/handle/10665/104772.
40. Shackleton C, Czovek D, Grimwood K, Ware RS, Radics B, Hantos Z, et al. Defining 'healthy' in preschool-aged children for forced oscillation technique reference equations. Respirology. 2018;23(4):406-413. doi: 10.1111/resp.13186.
41. Sly PD, Hantos Z; INCIRCLE Clinical Research Collaboration. The International Collaboration to Improve Respiratory Health in Children (INCIRCLE) ERS Clinical Research Collaboration. Eur Respir J. 2018;52(6):1801867. doi: 10.1183/13993003.01867-2018.

42. Miller TK, Pimmel RL. Forced noise mechanical parameters during inspiration and expiration. J Appl Physiol Respir Environ Exerc Physiol. 1982;52(6):1530-1534. doi: 10.1152/jappl.1982.52.6.1530.
43. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-1343. doi: 10.1183/09031936.00080312.
44. Smith SJ, Gray DM, MacGinty RP, Hall GL, Stanojevic S, Mphahlele R, et al. Choosing the better Global Lung Initiative 2012 equation in South African population groups. Am J Respir Crit Care Med. 2020;202(12):1724-1727. doi: 10.1164/rccm.202005-2085LE.
45. Harrison P. Urbanization: the policies and politics of informal settlement in South Africa: a historical perspective. Afr Insight. 1992;22:14-22.
46. Dandurand RJ, Lavoie JP, Lands LC, Hantos Z; Oscillometry Harmonisation Study Group. Comparison of oscillometry devices using active mechanical test loads. ERJ Open Res. 2019;5(4):00160-2019. doi: 10.1183/23120541.00160-2019.
47. Lundblad LKA, Siddiqui S, Bossé Y, Dandurand RJ. Applications of oscillometry in clinical research and practice. Can J Respir Crit Care Sleep Med. 2019;5(1):54-68. doi: 10.1080/24745332.2019.1649607.

Supplementary information

Table S3.1 Demographic data stratified by age

Years	**3 (n=104)**	**4 (n=131)**	**5 (n=114)**	**6 (n=128)**	**7 (n=103)**	**8 (n=9)**	**9 (n=7)**	**10 (n=12)**	**11 (n=23)**	**12 (n=11)**	**13 (n=11)**	**14 (n=10)**	**15 (n=9)**	**16 (n=9)**	**17 (n=11)**	**Total (n=692)**
Site																
CTAAC	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (90.0%)	9 (100%)	9 (100%)	11 (100%)	38 (5.5%)
HCSOC	0 (0.0%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	6 (5.8%)	9 (100%)	7 (100%)	12 (100%)	23 (100%)	11 (100%)	11 (100%)	1 10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	81 (11.7%)
DCHS	104 (100%)	131 (100%)	113 (99.1%)	128 (100%)	97 (94.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	573 (82.8%)
Sex																
Female	52 (50.0%)	69 (52.7%)	53 (46.5%)	63 (49.2%)	48 (46.6%)	6 (66.7%)	3 (42.9%)	4 (33.3%)	9 (39.1%)	3 (27.3%)	5 (45.4%)	3 (30.0%)	5 (55.6%)	5 (55.6%)	47 (63.6%)	335 (48.4%)
Male	52 (50%)	62 (47.33%)	61 (53.51%)	65 (50.78%)	55 (53.40%)	3 (33.33%)	4 (57.14%)	8 (66.67%)	14 (60.87%)	8 (72.73%)	6 (54.55%)	7 (70%)	4 (44.44%)	4 (44.44%)	4 (36.36%)	357 (51.59%)
Weight (kg)*	13.50 (12.6; 14.3)	15.3 (14.4; 17.1)	17.8 (16.1; 19.5)	19. 5 (17.4; 21.9)	21.1 (19.2; 23.9)	29.5 (25.5; 30.0)	28.5 (26.4; 37.0)	36.2 (31.0; 40.8)	35.7 (30.7; 42.9)	41.0 (31.1; 49.8)	45.2 (34.2; 49.8)	49.0 (44.0; 62.0)	52.0 (41.0; 69.0)	55.0 (49.0; 61.0)	55.0 (49.0; 61.0)	18.2 (15.3; 23.1)
WAZ*	-0.54 (-1.11; -0.02)	-0.58 (-1.05; 0.25)	-0.37 (-1.09; 0.31)	-0.50 (-1.38; 0.34)	-0.63 (-1.37; 0.34)	0.66 (-0.07; 1.19)	-0.17 (-0.61; 1.33)	0.88 (-0.10; 1.56)	0.28 (-0.74; 0.80)	0.21 (-1.34; 1.10)	0.31 (-1.40; 0.63)	-0.11 (-0.51; 0.91)	-0.77 (-1.58; 1.42)	0.08 (-1.28; 0.62)	-0.04 (-1.42; 0.95)	-0.42 (-1.11; 0.35)
Height (cm)*	92.3 (89.0; 95.0)	101.0 (97.0; 104.5)	107.0 (104.0; 111.4)	114.0 (110.0; 117.5)	120.0 (115.5; 124.0)	127.0 (126.0; 131.0)	132.0 (120.0; 145.0)	140.3 (138.3; 145.0)	141.0 (136.7; 146.0)	151.0 (143.0; 152.0)	144.0 (142.0; 158.5)	156.8 (153.0; 161.0)	158.0 (151.0; 159.0)	160.5 (156.6; 163.0)	161.0 (157.5; 167.0)	110.0 (101.0; 120.0)
HAZ*	-1.18 (-2.00; -0.49)	-0.69 (-1.51; 0.04)	-0.69 (-1.40; 0.16)	-0.64 (-1.26; 0.13)	-0.41 (-1.25; 0.29)	0.01 (-0.38; 0.47)	-0.42 (-1.79; 1.62)	0.27 (-0.22; 0.82)	-0.46 (-1.29; 0.09)	0.03 (-1.13; 0.53)	-1.33 (-1.99; 0.58)	-0.79 (-1.37; -0.20)	-1.46 (-1.49; -0.49)	-0.82 (-1.82; -0.22)	-0.31 (-1.61;	-0.65 (-1.43;

															-0.14)	0.05)
BMI (kg/m^2)*	15.7 (15.0; 16.9)	15.3 (14.5; 16.3)	15.2 (14.4; 16.0)	15.0 (13.9; 16.2)	14.7 (14.1; 15.8)	16.9 (15.6; 18.5)	16.4 (15.4; 19.4)	17.9 (16.6; 20.3)	18.9 (15.6; 20.1)	19.4 (15.9; 21.8)	18.7 (17.0; 24.0)	19.4 (18.0; 24.7)	19.6 (18.4; 27.8)	21.8 (17.9; 23.7)	21.8 (17.9; 23.7)	15.5 (14.5; 17.0)
BMI-Z*	0.22 (-0.38; 1.09)	0.00 (-0.62; 0.73)	-0.04 (-0.61; 0.56)	-0.27 (-1.01; 0.53)	-0.45 (-1.15; 0.19)	0.55 (-0.14; 1.21)	0.10 (-0.44; 1.33)	0.71 (0.10; 1.47)	0.82 (-0.88; 1.27)	0.39 (-0.85; 1.59)	0.15 (-0.87; 1.58)	0.23 (-0.62; 1.67)	-0.09 (-0.63; 1.88)	0.37 (-1.15; 0.86)	0.47 (-0.46; 1.10)	-0.04 (-0.70; 0.76)

CTAAC: Cape Town Adolescent Antiretroviral Cohort; HCSOC: Healthy children at surgical outpatient clinics; DCHS: Drakenstein Child Health Study; BMI: Body mass index; WAZ: weight for age z-score; HAZ: height for age z-score; BMI-Z: Body mass index z-score.

*Median (interquartile range)

Table S3.2 Summary of respiratory impedance variables stratified by age

	Standard											Intrabreath					
Years	R_6	R_8	R_{10}	X_6	X_8	X_{10}	R_6-R_{20}	F_{res}	A_x	R	C	R_{eE}	R_{eI}	X_{eE}	X_{eI}	ΔR	ΔX
3	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=51**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**	**n=104**
	13.23 (11.81; 15.35)	12.50 (11.31; 14.29)	12.05 (10.83; 13.65)	-4.56 (-5.63; -3.57)	-3.79 (-4.69; -3.08)	-3.07 (-3.76; -2.41)	2.76 (1.95; 3.63)	25.21 (22.71; 27.84)	45.22 (34.66; 61.22)	11.39 (10.09; 12.95)	5.05 (4.21; 5.78)	11.69 (10.47; 13.63)	10.29 (9.26; 12.43)	-2.30 (-3.13; -1.48)	-3.10 (-3.75; -2.35)	1.22 (0.66; 2.43)	0.80 (-0.01; 1.37)
4	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=89**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**	**n=131**
	11.71 (9.96; 13.23)	10.72 (9.48; 12.34)	10.27 (9.16; 12.00)	-3.45 (-4.67; -2.67)	-2.91 (-3.88; -2.02)	-2.28 (-3.29; -1.60)	2.47 (1.57; 3.48)	22.01 (18.25; 24.69)	28.90 (19.64; 48.27)	9.70 (8.73; 10.93)	6.03 (4.66; 7.61)	10.02 (8.77; 11.73)	9.23 (8.11; 10.62)	-1.51 (-2.57; -0.78)	-2.33 (-3.12; -1.68)	0.76 (-0.18; 1.62)	0.69 (0.01; 1.41)
5	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=83**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**	**n=114**
	10.14 (8.60; 12.20)	9.58 (8.26; 10.98)	9.20 (7.93; 10.63)	-2.91 (-3.76; -2.38)	-2.48 (-3.37; -1.95)	-2.04 (-2.73; -1.44)	2.17 (1.51; 3.11)	21.02 (18.15; 25.08)	28.26 (16.37; 40.02)	8.40 (7.36; 9.85)	6.79 (5.67; 8.39)	8.66 (7.37; 10.26)	7.94 (6.86; 9.62)	-1.28 (-2.32; -0.73)	-1.95 (-2.60; -1.45)	0.42 (-0.22; 1.20)	0.52 (0.07; 0.97)
6	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=109**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**	**n=128**
	9.05 (8.13; 10.47)	8.58 (7.57; 9.94)	8.30 (7.31; 9.59)	-2.74 (-3.52; -2.15)	-2.18 (-2.74; -1.50)	-1.68 (-2.16; -1.17)	1.86 (1.27; 2.72)	20.71 (17.93; 23.73)	20.08 (14.48; 29.75)	7.68 (6.76; 8.80)	7.44 (6.38; 9.34)	7.71 (6.69; 9.08)	7.54 (6.35; 8.90)	-1.17 (-1.60; -0.56)	-1.70 (-2.18; -1.15)	0.41 (-0.34; 1.03)	0.57 (0.10; 0.87)
7	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=88**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**	**n=103**
	7.62 (6.52; 9.38)	7.33 (6.15; 8.56)	6.90 (6.14; 8.47)	-2.03 (-2.98; -1.69)	-1.61 (-2.31; -1.31)	-1.39 (-1.92; -0.90)	1.71 (1.10; 2.46)	19.28 (16.29; 22.93)	16.48 (9.94; 27.34)	6.43 (5.63; 7.67)	9.66 (7.60; 11.16)	6.57 (5.57; 7.97)	6.30 (5.44; 7.38)	-0.80 (-1.78; -0.36)	-1.39 (-1.91; -0.96)	0.29 (-0.19; 0.63)	0.54 (0.07; 0.89)
8	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=4**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**
	7.14 (6.46; 9.05)	6.30 (5.93; 8.56)	5.78 (5.65; 7.60)	-2.02 (-3.32; -1.95)	-2.44 (-3.50; -2.00)	-2.34 (-3.42; -1.60)	2.29 (1.01; 4.00)	21.07 (17.74; 25.69)	35.40 (17.57; 60.76)	5.76 (5.36; 6.18)	7.41 (5.53; 10.94)	6.10 (5.27; 7.22)	6.44 (4.43; 6.73)	-2.17 (-3.23; -1.20)	-1.57 (-2.51; -1.20)	0.55 (0.06; 1.67)	0.004 (-0.66; 0.45)
9	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=5**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**	**n=7**

	7.02 (5.48; 10.79)	6.46 (4.87; 9.72)	5.89 (4.43; 9.21)	-2.36 (-4.19; -1.36)	-2.54 (-2.96; -1.44)	-1.93 (-3.42; -0.72)	2.40 (1.13; 3.34)	20.17 (17.48; 25.79)	32.12 (8.70; 54.33)	5.04 (3.98; 8.38)	6.99 (5.29; 13.70)	6.11 (3.89; 8.07)	5.12 (4.12; 8.14)	-1.35 (-2.64; -0.50)	-1.71 (-2.91; -0.42)	0.22 (-0.07; 1.00)	-0.08 (-0.28; 0.27)
10	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=8**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**	**n=12**
	5.86 (4.67; 6.63)	5.74 (4.36; 6.58)	5.56 (4.51; 6.16)	-2.32 (-3.26; -1.32)	-1.55 (-2.59; -1.18)	-1.23 (-2.24; -0.85)	1.04 (0.65; 2.21)	19.97 (18.11; 23.99)	20.41 (10.63; 39.11)	5.31 (3.84; 5.80)	9.51 (7.14; 14.92)	5.12 (3.99; 6.90)	4.67 (3.72; 5.33)	-1.16 (-2.31; -0.35)	-1.17 (-1.84; -0.76)	0.75 (0.06; 1.38)	-0.13 (-0.37; 0.26)
11	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=21**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**	**n=23**
	5.15 (4.34; 5.85)	4.62 (3.83; 5.64)	4.42 (3.79; 5.22)	-1.92 (-2.43; -1.46)	-1.61 (-2.04; -1.19)	-1.29 (-1.89; -0.87)	1.64 (0.88; 2.43)	18.57 (16.46; 20.98)	15.77 (10.44; 24.20)	3.89 (3.41; 4.51)	9.84 (7.22; 13.01)	3.98 (3.72; 4.58)	3.55 (3.16; 4.18)	-0.93 (-1.41; -0.51)	-0.89 (-1.58; -0.52)	0.40 (0.20; 0.77)	-0.09 (-0.34; 0.10)
12	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=10**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**
	4.50 (3.73; 6.61)	4.35 (3.75; 6.70)	4.18 (3.33; 5.66)	-1.96 (-2.16; -1.05)	-1.15 (-2.12; -0.81)	-0.91 (-1.70; -0.17)	1.09 (0.36; 2.30)	19.71 (16.04; 24.87)	18.22 (6.17; 19.35)	4.16 (2.44; 5.42)	13.42 (8.91; 17.85)	4.24 (3.14; 5.22)	3.42 (2.51; 3.83)	-0.55 (-0.91; -0.40)	-0.48 (-1.29; -0.36)	0.54 (0.36; 1.79)	-0.10 (-0.20; 0.02)
13	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=9**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**
	4.19 (3.51; 5.57)	3.91 (3.25; 4.53)	3.99 (3.24; 4.20)	-1.56 (-1.89; -1.32)	-1.22 (-1.95; -0.97)	-0.86 (-1.40; -0.60)	0.86 (0.38; 1.61)	17.59 (14.45; 19.18)	11.80 (5.98; 20.48)	3.33 (3.11; 3.85)	12.52 (10.51; 15.45)	3.58 (2.89; 3.96)	2.56 (2.33; 3.79)	-0.69 (-1.05; 0.03)	-0.74 (-1.29; -0.36)	0.58 (0.34; 1.08)	0.07 (-0.31; 0.43)
14	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**	**n=10**
	4.50 (3.51; 5.30)	4.30 (3.23; 5.04)	4.29 (3.30; 4.84)	-1.22 (-1.62; -0.90)	-0.94 (-1.01; -0.35)	-0.63 (-0.83; -0.56)	0.41 (0.01; 0.77)	15.74 (13.00; 20.58)	6.11 (1.40; 9.42)	4.08 (3.42; 4.82)	16.98 (15.29; 18.07)	3.43 (2.98; 4.00	3.27 (2.49; 3.66)	-0.28 (-0.73; 0.31)	-0.50 (-0.67; -0.20)	0.27 (0.11; 0.57)	0.23 (0.15; 0.43)
15	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=8**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**
	3.77 (3.16; 4.74)	4.00 (3.31; 4.66)	3.86 (3.35; 4.54)	-1.19 (-1.47; -0.92)	-0.94 (-1.13; -0.50)	-0.67 (-0.81; -0.43)	0.05 (-0.04; 0.53)	14.00 (11.95; 15.27)	4.77 (3.07; 6.80)	3.82 (3.43; 4.55)	17.27 (14.48; 18.07)	3.63 (3.06; 4.19)	2.78 (2.55; 3.53)	-0.17 (-0.46; 0.16)	-0.45 (-0.54; -0.14)	0.57 (0.34; 0.76)	0.08 (-0.001; 0.27)
16	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=8**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**	**n=9**

	3.39 (3.01; 4.11)	3.02 (2.93; 3.78)	3.18 (2.74; 3.79)	-1.23 (-1.40; -1.19)	-0.66 (-0.70; -0.59)	-0.37 (-0.66; -0.28)	0.23 (0.01; 0.58)	14.83 (13.26; 15.94)	4.18 (4.36; 4.33)	3.19 (2.67; 3.83)	19.24 (17.78; 19.75)	2.97 (2.46; 3.84)	2.50 (2.20; 3.32)	-0.02 (-0.25; -0.02)	-0.42 (-0.75; -0.13)	0.29 (0.15; 0.67)	0.30 (0.03; 0.46)
17	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**	**n=11**
	3.41 (2.88; 4.59)	3.29 (2.32; 4.34)	3.13 (2.39; 4.08)	-1.23 (-1.50; -1.11)	-0.79 (-1.07; -0.38)	-0.39 (-0.77; -0.15)	0.47 (0.06; 0.58)	14.56 (12.23; 16.45)	4.16 (2.23; 7.62)	3.31 (2.37; 4.18)	15.89 (13.85; 17.98)	2.70 (2.00; 3.33)	2.22 (2.01; 2.93)	-0.28 (-0.39; 0.12)	-0.44 (-0.64; -0.07)	0.23 (-0.04; 0.42)	0.17 (0.05; 0.32)
Total	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=514**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**	**n=692**
	9.62 (7.19; 12.26)	9.15 (6.90; 11.33)	8.88 (6.65; 10.79)	-2.83 (-3.95; -1.97)	-2.34 (-3.31; -1.51)	-1.84 (-2.77; -1.18)	2.01 (1.15; 2.99)	20.67 (17.16; 24.57)	24.30 (14.26; 39.53)	8.11 (6.08; 10.11)	7.20 (5.48; 9.85)	8.22 (6.22; 10.60)	7.85 (5.97; 9.62)	-1.23 (-2.23; -0.55)	-1.87 (-2.68; -1.13)	0.53 (-0.04; 1.22)	0.50 (-0.05; 0.97)

R_6, R_8 and R_{10}: resistance at 6, 8 and 10 Hz; X_6, X_8 and X_{10}: reactance at 6, 8 and 10 Hz; F_{res}: resonance frequency; R_6-R_{20}: difference between resistance at 6 Hz and resistance at 20 Hz; A_x: area under the reactance curve; R: resistance from model fitting; C: compliance from model fitting; R_{eI}: resistance at end inspiration; R_{eE}: resistance at end expiration; ΔR: R_{eE}-R_{eI} ; X_{eI}: reactance at end inspiration, R_{eE}: reactance at end expiration; ΔX: X_{eE}-X_{eI}.

Median (IQR) values.

Units: R and X variables: hPa.L.s-1; C: L.hPa-1; F_{res}: Hz; A_x: hPa.L-1.

Table S3.3 Comparison between models adjusted for height only and height, sex and ethnicity

	Model with height only (n=692)			Model with height, sex, and ethnicity (n=692)				
	Height Coefficient (95% CI)	BIC value	Adj. R^2	Height Coefficient (95% CI)	Sex Coefficient (95% CI)	Ethnicity Coefficient (95% CI)	BIC value	Adj. R^2
R_6	**-0.019 (-0.020; -0.018)**	-156.50	0.723	**-0.019 (-0.020; -0.018)**	**-0.032 (-0.064; -0.000)**	0.008 (-0.024; 0.041)	-147.55	0.724
R_8	**-0.019 (-0.020; -0.018)**	-187.05	0.735	**-0.019 (-0.020; -0.018)**	-0.028 (-0.059; 0.003)	0.006 (-0.026; 0.038)	-177.12	0.735
R_{10}	**-0.019 (-0.020; -0.018)**	-227.68	0.747	**-0.019 (-0.020; -0.018)**	-0.023 (-0.054; 0.007)	0.019 (-0.012; 0.049)	-218.14	0.745
X_6	**-727 (-793; -661)**	2189.17	0.405	**-731 (-797; -665)**	0.089 (-0.085; 0.264)	0.166 (-0.009; 0.341)	2197.61	0.407
X_8	**-646 (-702; -590)**	1967.53	0.425	**-649 (-795; -593)**	**0.182 (0.034; 0.330)**	**0.163 (0.014; 0.311)**	1969.75	0.433
X_{10}	**-530 (-583; -478)**	1886.30	0.359	**-533 (-586; -480)**	0.118 (-0.022; 0.258)	**0.141 (0.001; 0.281)**	1892.48	0.364
F_{res}	**-0.006 (-0.007; -0.005)**	-131.29	0.230	**-0.006 (-0.007; -0.005)**	-0.010 (-0.047; 0.026)	-0.004 (-0.042; 0.033)	-119.20	0.227
R_6-R_{20}	**-0.031 (-0.036; -0.026)**	2258.30	0.177	**-0.031 (-0.037; -0.026)**	-0.092 (-0.275; 0.092)	-0.152 (-0.336; 0.033)	2267.66	0.179
Ax	**-0.029 (-0.031; -0.026)**	1322.14	0.416	**-0.029 (-0.031; -0.026)**	-0.052 (-0.146; 0.041)	-0.014 (-0.108; 0.080)	1333.89	0.416
R	**-0.019 (-0.020; -0.018)**	-220.75	0.739	**-0.019 (-0.019; -0.018)**	-0.025 (-0.055; 0.006)	0.023 (-0.008; 0.054)	-212.14	0.740
C	**0.017 (0.016; 0.018)**	284.34	0.523	**0.017 (0.016; 0.018)**	0.038 (-0.006; 0.082)	0.045 (0.000; 0.089)	290.30	0.526
X_{eE}	**-409 (-476; -343)**	2201.57	0.174	**-408 (-474; -341)**	**0.197 (0.021; 0.373)**	0.055 (-0.121; 0.232)	2209.31	0.178
X_{eI}	**-577 (-630; -524)**	1894.15	0.397	**-579 (-632; -526)**	**0.184 (0.044; 0.325)**	0.137 (-0.004; 0.277)	1896.54	0.404
ΔR	**-0.014 (-0.020; -0.009)**	2307.58	0.040	**-0.014 (-0.020; -0.009)**	0.058 (-0.132; 0.249)	-0.101 (-0.292; 0.091)	2319.28	0.039
ΔX	**-0.012 (-0.016; -0.008)**	1870.89	0.050	**-0.012 (-0.016; -0.008)**	0.012 (-0.127; 0.151)	-0.090 (-0.230; 0.050)	1882.34	0.049

R_6, R_8 and R_{10}: resistance at 6, 8 and 10 Hz; X_6, X_8 and X_{10}: reactance at 6, 8 and 10 Hz; F_{res}: resonance frequency; R_6-R_{20}: difference between resistance at 6 Hz and resistance at 20 Hz; A_x: area under the reactance curve; R: resistance from model fitting; C: compliance from model fitting; R_{eI}: resistance at end inspiration; R_{eE}: resistance at end expiration; ΔR: R_{eE}-R_{eI} ; X_{eI}: reactance at end inspiration, R_{eE}: reactance at end expiration; ΔX: X_{eE}-X_{eI}. BIC: Bayesian Information Criterion; Adj R2: adjusted R2. Statistically significant results are printed in bold.
Units: R and X variables: hPa.L.s-1; C: L.hPa-1; F_{res}: Hz; A_x: hPa.L-1.

Table S3.4 Reference equations for children 3 to 7 years of age from the DCHS cohort

Outcome	Equation	Adj R^2	SEE
$\boldsymbol{R_6}$ (hPa.s.L^{-1})	exp(4.23 - 0.0178·Ht)	0.466	0.203
$\boldsymbol{R_8}$ (hPa.s.L^{-1})	exp(4.17 - 0.0178·Ht)	0.479	0.198
$\boldsymbol{R_{10}}$ (hPa.s.L^{-1})	exp(4.14 - 0.0179·Ht)	0.490	0.194
$\boldsymbol{X_6}$ (hPa.s.L^{-1})	5.06-888·Ht^{-1}	0.333	1.211
$\boldsymbol{X_8}$ (hPa.s.L^{-1})	4.81 -798·Ht^{-1}	0.366	1.011
$\boldsymbol{X_{10}}$ (hPa.s.L^{-1})	3.72 -630/Ht	0.291	0 .946
$\boldsymbol{F_{res}}$ (Hz)	exp(3.85 - 0.0074·Ht)	0.116	0.204
$\boldsymbol{R_6}$-$\boldsymbol{R_{20}}$ (hPa.s.L^{-1})	5.69 - 0.0316·Ht	0.066	1.248
$\boldsymbol{A_X}$ (hPa.L^{-1})	exp(7 - 0.0353·Ht)	0.309	0.561
$\boldsymbol{R}$ (hPa.s.L^{-1})	exp(4.12 - 0.0184·Ht)	0.510	0.192
$\boldsymbol{C}$ (L.hPa^{-1})	exp(-0.30 + 0.0207·Ht)	0.385	0.279
$\boldsymbol{R_{eE}}$ (hPa.s.L^{-1})	exp(4.30 - 0.0198·Ht)	0.486	0.216
$\boldsymbol{R_{eI}}$ (hPa.s.L^{-1})	exp(3.93 - 0.0170·Ht)	0.408	0.218
$\boldsymbol{X_{eE}}$ (hPa.s.L^{-1})	3.11 - 502·Ht^{-1}	0.139	1.199
$\boldsymbol{X_{eI}}$ (hPa.s.L^{-1})	3.96 - 651·Ht^{-1}	0.301	0.955
$\boldsymbol{\Delta R}$ (hPa.s.L^{-1})	4.96 - 0.0403·Ht	0.095	1.316
$\boldsymbol{\Delta X}$ (hPa.s.L^{-1})	1.97 - 0.0132·Ht	0.018	0.989

R_6, R_8 and R_{10}: resistance at 6, 8 and 10 Hz; X_6, X_8 and X_{10}: reactance at 6, 8 and 10 Hz; F_{res}: resonance frequency; R_6-R_{20}: difference between resistance at 6 Hz and resistance at 20 Hz; A_X: area under the reactance curve; R: resistance from model fitting; C: compliance from model fitting; R_{eI}: resistance at end inspiration; R_{eE}: resistance at end expiration; ΔR: R_{eE}-R_{eI} ; X_{eI}: reactance at end inspiration, R_{eE}: reactance at end expiration; ΔX: X_{eE}-X_{eI}. Adj R2: adjusted R2; SEE: standard error of the estimate.

Figure S3.1 Illustration of the resistance (*R*) - inertance (*I*) – compliance (*C*) model fitting to measured impedance data. Mean values of resistance (•) and reactance (○) from repeated measurements, whiskers indicate standard deviation. Model fitting curves are plotted in red. *R* was obtained as the mean value in the 10-20-Hz frequency (f) range; *I* and *C* were obtained from fitting the reactance (*X*) data by the model $X = 2\pi fI - 1/(2\pi fC)$. Green and blue lines, respectively, illustrate the inertial (*X*i) and elastic (*X*e) components of the total *X*.

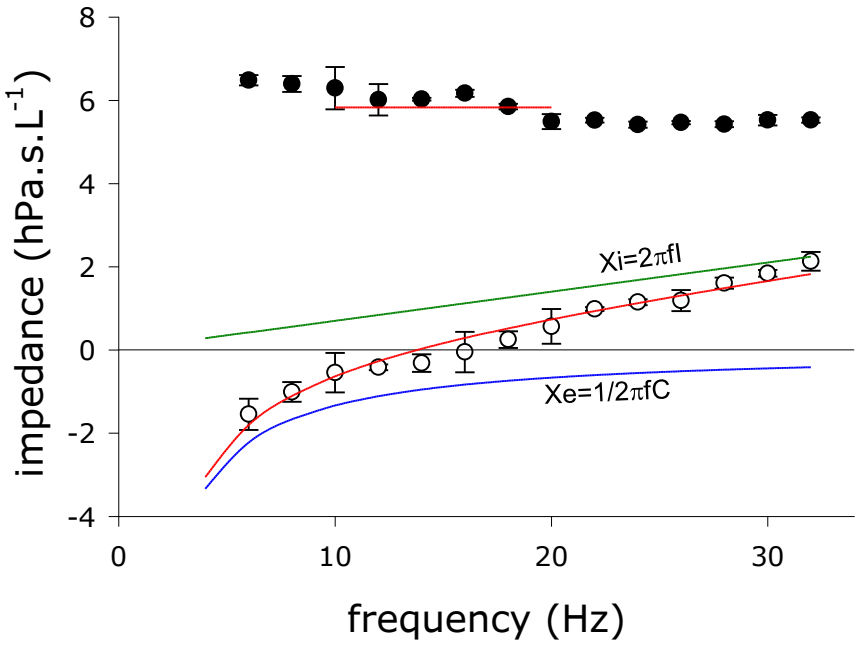


Figure S3.2 Conventional oscillometry measures *vs* height: a) resistance at 6 Hz (R_6) , b) reactance at 6 Hz (X), c) resonance frequency (F_{res}), d) area under the reactance curve (A_x) and e) frequency dependence of resistance (R_6-R_{20}). Solid and dashed lines, respectively, indicate prediction equations for the 3-17-yr and 3-7-yr ranges.

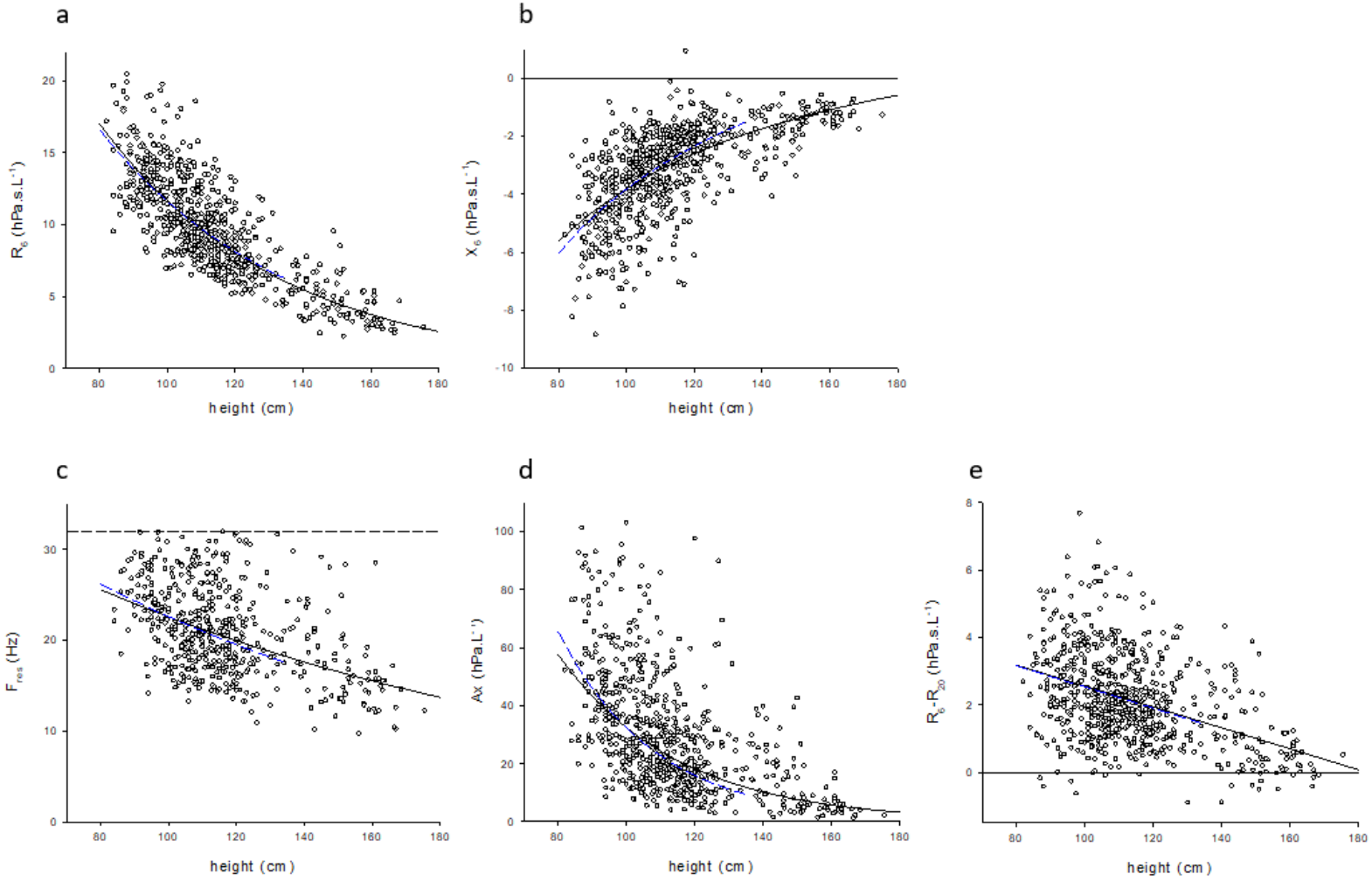


Figure S3.3 Intra-breath oscillometry measures vs height: a) resistance at end expiration (R_{eE}) , b) resistance at end inspiration (R_{eI}), c) tidal change in resistance $\Delta R=R_{eE}-R_{eI}$, d) reactance at end expiration (X_{eE}), e) reactance at end inspiration (X_{eI}) and f) tidal change in reactance ($\Delta X=X_{eE}-X_{eI}$). Solid and dashed lines, respectively, indicate prediction equations for the 3-17-yr and 3-7-yr ranges.

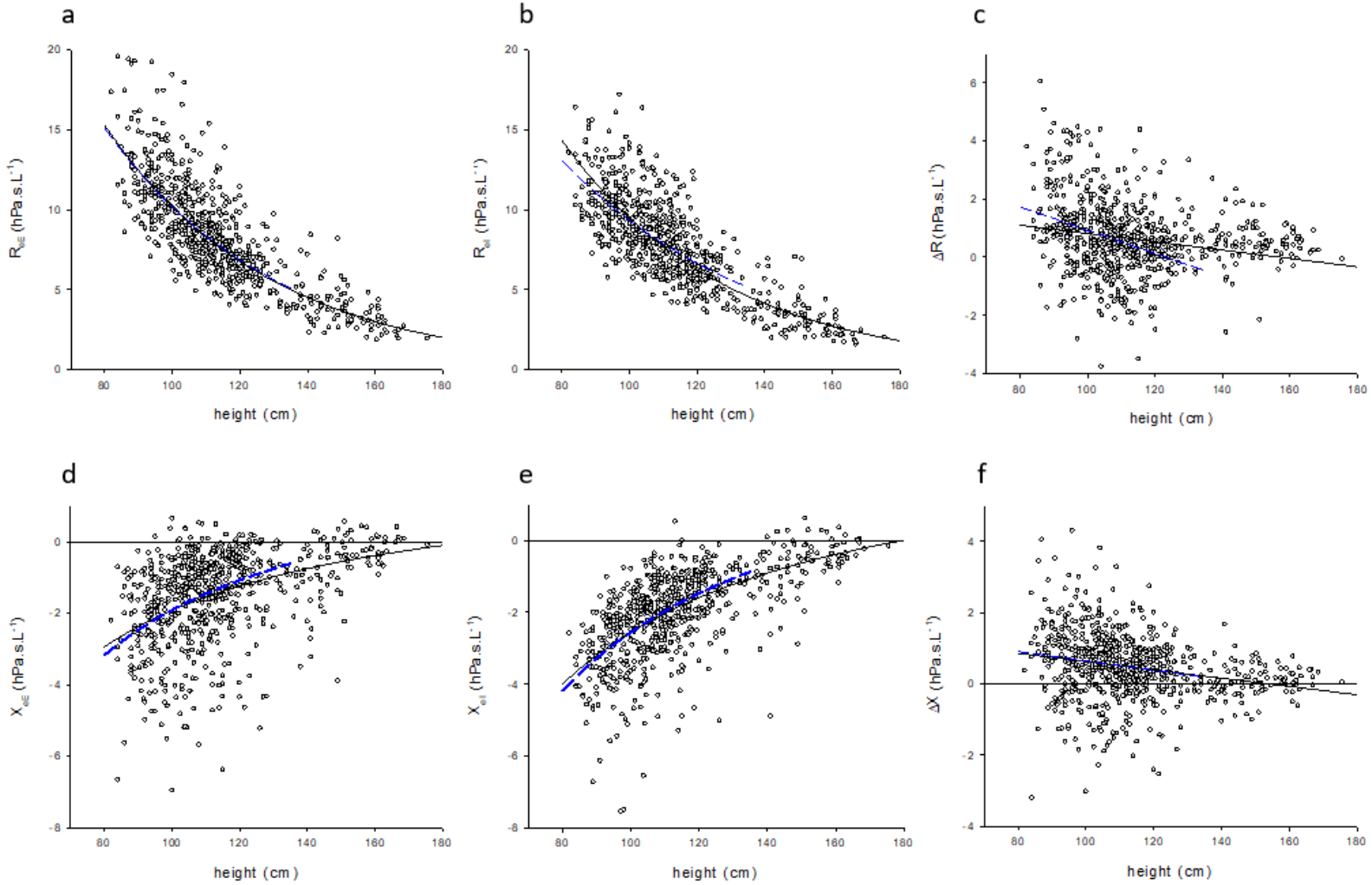


Figure S3.4 Comparison of respiratory resistance (R_{rs}) *vs* height (Ht) relationships with previous studies using linear regression between R_{rs} and Ht (see References). For the individual studies reporting nonlinear R_{rs} vs Ht relationships, see Figure 3.2.

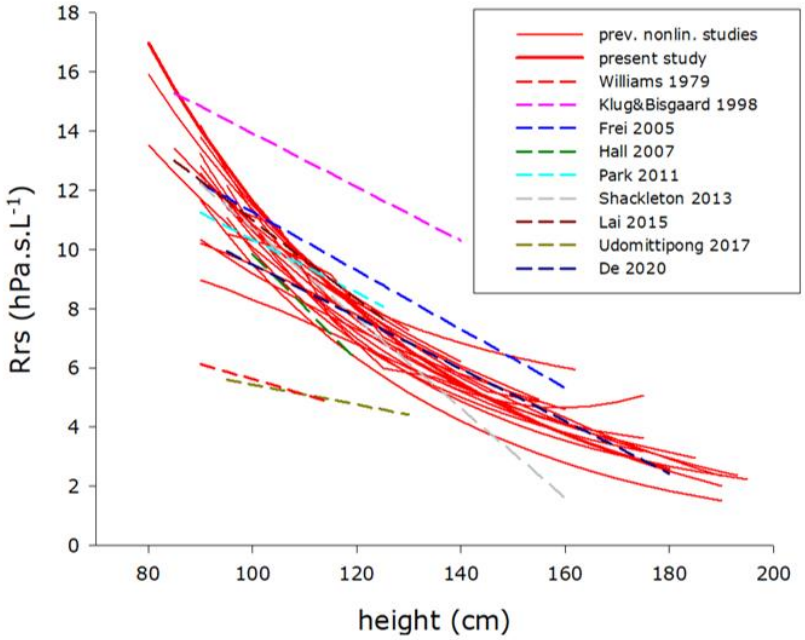


Figure S3.5 Comparison of at 10-Hz resistance (R_{10}, left) and reactance (X_{10}, right) *vs* height (Ht) relationships with previous studies (see References).

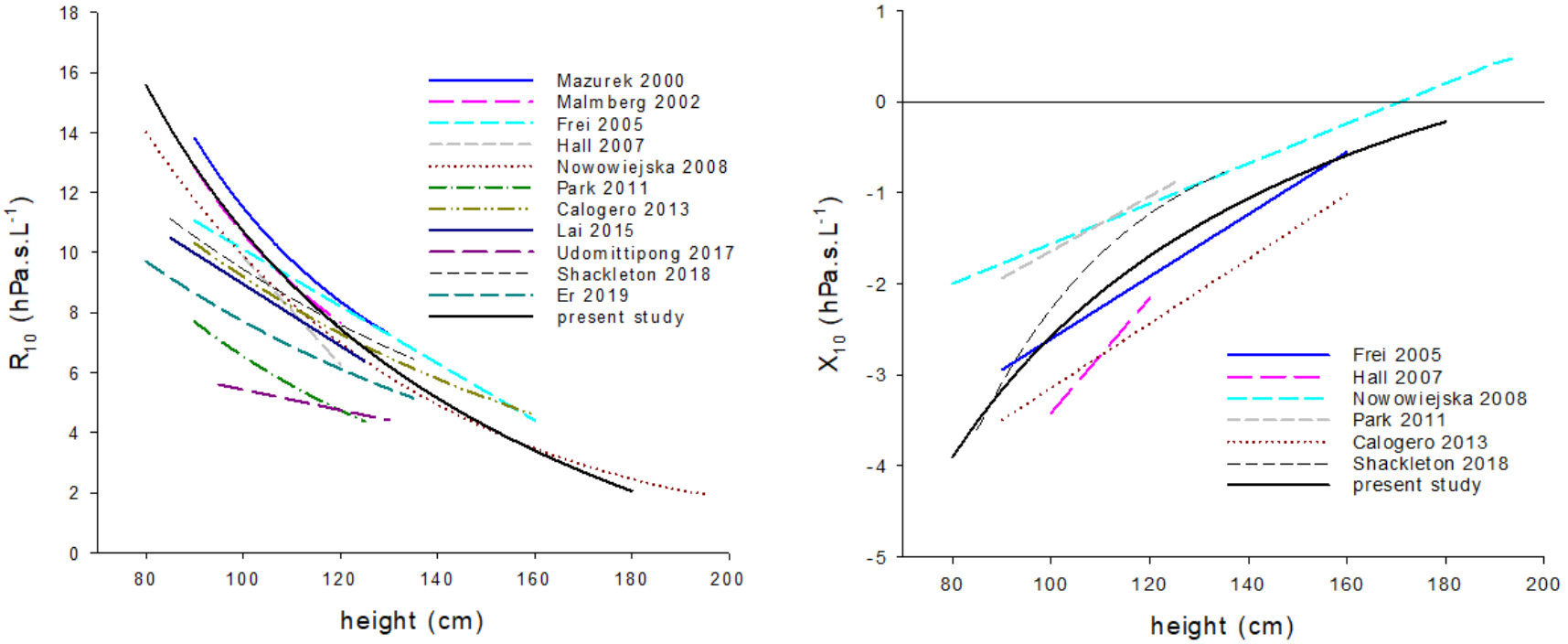


Figure S3.6 Comparison of resonance frequency (F_{res}) *vs* height (Ht) relationships with previous studies (see References).

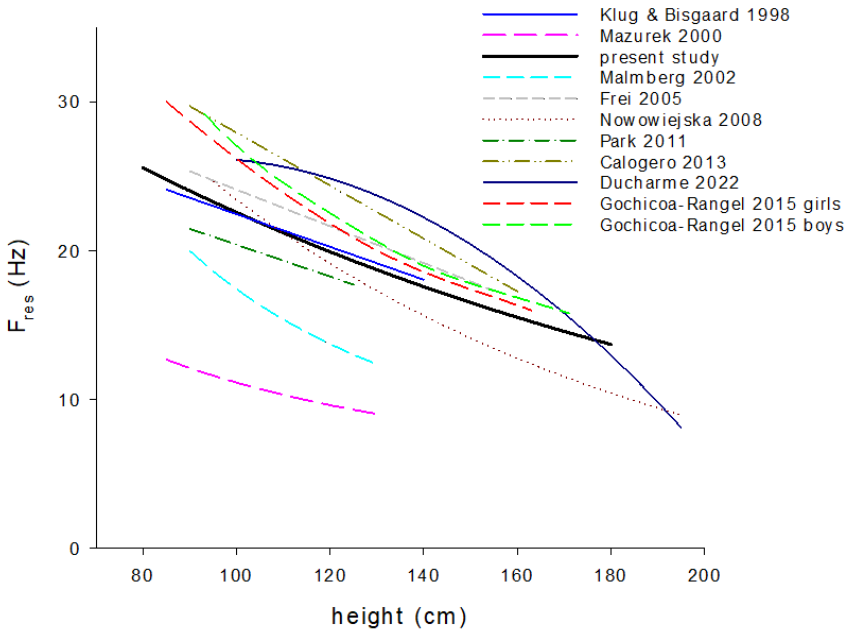


Table S3.5 Summary of reference studies on resistance (R) vs height (Ht) relationships

Author(s) [ref]	year	frequency (Hz)	device	country/race	no. of subjects	age range (yr)	reference equation	units (Zrs; Ht)
Mansell et al. [1]	1972	5	custom made	Canada	79	3-17	R_5=exp(1.877-0.0089·Ht)	$cmH_2O.s.L^{-1}$; cm
Cogswell [2]	1973	5-7	custom made	UK	204	3-12	$R_{5\text{-}7}$ *vs* Ht range data	$cmH_2O.s.L^{-1}$; cm
Stanescu et al. [3]	1979	4-9	custom made	Belgium	130	3-14	R_4 *vs* Ht range data	$cmH_2O.s.L^{-1}$; cm
Solymar et al. [4]	1985	2-12	custom made	Sweden	218	2-18	R_4=antilog(1.053-2.18·log(Ht))	$kPa.s.L^{-1}$; m
Hordvik et al. [5]	1985	2-26	custom made	USA/C	138	2-16	R_6=9.2·Ht^2-34.1·Ht+35.2	$cmH_2O.s.L^{-1}$; cm
Hantos et al. [6]	1985	3-10	custom made	Hungary	121	4-16	$R_{(3\text{-}10)}$=1.28·10^5·$Ht^{-2.05}$	$cmH_2O.s.L^{-1}$; cm
Duiverman et al. [7]	1985	2-26	custom made	The Netherlands/C	255	2.3-12.5	R_6=0.0017·Ht^2-0.541·Ht+47.73	$cmH_2O.s.L^{-1}$; cm
Ducharme et al. [8]	1998	8-16	Custo Vit R	Canada/mixed	199	3-17	R_8=exp(10.99-2.37·ln(Ht))	$kPa.s.L^{-1}$; cm
Mazurek et al. [9]	2000	4-32	custom made	Poland	127	2.5-7.5	R_6=exp(2.4422-1.7447·ln(Ht))	$hPa.s.L^{-1}$; m
Malmberg et al. [10]	2002	5-35	Jaeger IOS	Finland	109	2-7	R_5=exp(2.115-1.786·ln(Ht))	$kPa.s.L^{-1}$; cm
Dencker et al. [11]	2006	5-35	Jaeger IOS	Finland-Sweden/C	360	2-11	R_5 *vs* Ht curve	$kPa.s.L^{-1}$; cm
Nowowiejska et al. [12]	2008	5-35	Jaeger IOS	Poland	626	3-18	R_5=exp(-0.0169·Ht+1.818)	$kPa.s.L^{-1}$; cm
Calogero et al. [13]	2013	4-48	Chess i2M	Australia-Italy/C	760	2-13	R_6=exp(3.3738-0.01155·Ht)	$hPa.s.L^{-1}$; cm
Shackleton et al. [14]	2018	6-26	custom made**	Australia/Hungary	319	3-6	R_6= exp(3.3501-0.01033·Ht)	$hPa.s.L^{-1}$; cm
AlBlooshi et al. [15]	2018	5-37	tremoflo C-100	UAE/Emirati	291	4-12	R_5=exp(3.786-0.014·Ht)	$cmH_2O.s.L^{-1}$; cm
Er et al. [16]	2019	5-35	Jaeger IOS	Turkey/Turkish	151	3-7	R_5=antilog(0.527-0.005·Ht)	$kPa.s.L^{-1}$; cm
Ducharme et al. [17]-1	2022	5-37	Resmon Pro	Canadian/mixed	271	3-17	R_5=exp(-0.1509+0.00809·Ht-0.0000824·Ht^2)	$kPa.s.L^{-1}$; cm
Ducharme et al. [17]-2	2022	5-37	tremoflo C-100	Canadian/mixed	292	3-17	R_5=exp(-0.0252+0.00809·Ht-0.0000817·Ht^2)	$kPa.s.L^{-1}$; cm
Frei et al. [18]	2005	5-35	Jaeger IOS	Canada	222	3-10	R_5=2.117-0.0099·Ht	$kPa.s.L^{-1}$; cm
Hall et al. [19]	2007	4-48	Chess i2M	Australia	149	2-7	R_6=27.86-0.18·Ht	$hPa.s.L^{-1}$; cm
Park et al. [20]	2011	5-35	Jaeger IOS	Korea/Korean	133	3-6	R_5=1.934-0.009·Ht	$kPa.s.L^{-1}$; cm
Shackleton et al. [21]	2013	4-48	Chess i2M	Mexico/Mexican	584	3-5	R_6=25.918-0.152·Ht	$hPa.s.L^{-1}$; cm

Lai et al. [22]	2015	5-35	Jaeger IOS	Taiwan/Taiwanese	150	2-6	R_5=2.4395-0.0134·Ht	kPa.s.L^{-1}; m
Udomittipong et al. [23]	2017	4-48	Quark i2M	Thailand/Thai	233	3-7	R_6=8.834-0.034·Ht	hPa.s.L^{-1}; cm
De et al. [24]	2020	5-19	Resmon Pro	India/Indian	159	5-17	R_5=18.683-0.09·Ht (boys)	cmH_2O.s.L^{-1}; cm

Nonlinear and linear predictions; C: Caucasian

Supplementary references

1. Mansell A, Levison H, Kruger K, Tripp TL. Measurement of respiratory resistance in children by forced oscillations. Am Rev Respir Dis. 1972;106(5):710-714. doi: 10.1164/arrd.1972.106.5.710.
2. Cogswell JJ. Forced oscillation technique for determination of resistance to breathing in children. Arch Dis Child. 1973;48(4):259-266. doi: 10.1136/adc.48.4.259.
3. Stănescu D, Moavero NE, Veriter C, Brasseur L. Frequency dependence of respiratory resistance in healthy children. J Appl Physiol Respir Environ Exerc Physiol. 1979;47(2):268-272. doi: 10.1152/jappl.1979.47.2.268.
4. Solymar L, Aronsson PH, Sixt R. The forced oscillation technique in children with respiratory disease. Pediatr Pulmonol. 1985;1(5):256-261. doi: 10.1002/ppul.1950010507.
5. Hordvik NL, König P, Morris DA, Kreutz C, Pimmel RL. Normal values for forced oscillatory respiratory resistance in children. Pediatr Pulmonol. 1985;1(3):145-148. doi: 10.1002/ppul.1950010305.
6. Hantos Z, Daróczy B, Gyurkovits K. Total respiratory impedance in healthy children. Pediatr Pulmonol. 1985;1(2):91-98. doi: 10.1002/ppul.1950010207.
7. Duiverman EJ, Clément J, van de Woestijne KP, Neijens HJ, van den Bergh AC, Kerrebijn KF. Forced oscillation technique. Reference values for resistance and reactance over a frequency spectrum of 2-26 Hz in healthy children aged 2.3-12.5 years. Bull Eur Physiopathol Respir. 1985;21(2):171-178.
8. Ducharme FM, Davis GM, Ducharme GR. Pediatric reference values for respiratory resistance measured by forced oscillation. Chest. 1998;113(5):1322-1328. doi: 10.1378/chest.113.5.1322.
9. Mazurek H, Willim G, Marchal F, Haluszka J, Tomalak W. Input respiratory impedance measured by head generator in preschool children. Pediatr Pulmonol. 2000;30(1):47-55. doi: 10.1002/1099-0496(200007)30:1<47::aid-ppul8>3.0.co;2-w.
10. Malmberg LP, Pelkonen A, Poussa T, Pohianpalo A, Haahtela T, Turpeinen M. Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children. Clin Physiol Funct Imaging. 2002;22(1):64-71.
11. Dencker M, Malmberg LP, Valind S, Thorsson O, Karlsson MK, Pelkonen A, et al. Reference values for respiratory system impedance by using impulse oscillometry in children aged 2-11 years. Clin Physiol Funct Imaging. 2006;26(4):247-250. doi: 10.1111/j.1475-097X.2006.00682.x.
12. Nowowiejska B, Tomalak W, Radliński J, Siergiejko G, Latawiec W, Kaczmarski M. Transient reference values for impulse oscillometry for children aged 3-18 years. Pediatr Pulmonol. 2008;43(12):1193-1197. doi: 10.1002/ppul.20926.
13. Calogero C, Simpson SJ, Lombardi E, Parri N, Cuomo B, Palumbo M, et al. Respiratory impedance and bronchodilator responsiveness in healthy children aged 2-13 years. Pediatr Pulmonol. 2013;48(7):707-715. doi: 10.1002/ppul.22699.
14. Shackleton C, Czovek D, Grimwood K, Ware RS, Radics B, Hantos Z, et al. Defining 'healthy' in preschool-aged children for forced oscillation technique reference equations. Respirology. 2018;23(4):406-413. doi: 10.1111/resp.13186.

15. AlBlooshi A, AlKalbani A, Narchi H, Al-Hamad S, Al-Houqani M, AlBadi G, et al. Respiratory function in healthy Emirati children using forced oscillations. Pediatr Pulmonol. 2018;53(7):936-941. doi: 10.1002/ppul.23985.
16. Er İ, Günlemez A, Baydemir C, Kılıçbay F, Ersu R, Uyan ZS. Impulse oscillometry reference values and correlation with predictors in Turkish preschool children. Turk J Pediatr. 2019;61(4):560-567. doi: 10.24953/turkjped.2019.04.013.
17. Ducharme FM, Smyrnova A, Lawson CC, Miles LM. Reference values for respiratory sinusoidal oscillometry in children aged 3 to 17 years. Pediatr Pulmonol. 2022;57(9):2092-2102. doi: 10.1002/ppul.25984.
18. Frei J, Jutla J, Kramer G, Hatzakis GE, Ducharme FM, Davis GM. Impulse oscillometry: reference values in children 100 to 150 cm in height and 3 to 10 years of age. Chest. 2005;128(3):1266-1273. doi: 10.1378/chest.128.3.1266.
19. Hall GL, Sly PD, Fukushima T, Kusel MM, Franklin PJ, Horak F Jr, Patterson H, Gangell C, Stick SM. Respiratory function in healthy young children using forced oscillations. Thorax. 2007;62(6):521-526. doi: 10.1136/thx.2006.067835.
20. Park JH, Yoon JW, Shin YH, Jee HM, Wee YS, Chang SJ, Sim JH, Yum HY, Han MY. Reference values for respiratory system impedance using impulse oscillometry in healthy preschool children. Korean J Pediatr. 2011;54(2):64-68. doi: 10.3345/kjp.2011.54.2.64.
21. Shackleton C, Barraza-Villarreal A, Chen L, Gangell CL, Romieu I, Sly PD. Reference ranges for Mexican preschool-aged children using the forced oscillation technique. Arch Bronconeumol. 2013;49(8):326-329. English, Spanish. doi: 10.1016/j.arbres.2013.01.014.
22. Lai SH, Yao TC, Liao SL, Tsai MH, Hua MC, Yeh KW, et al. Reference value of impulse oscillometry in Taiwanese preschool children. Pediatr Neonatol. 2015;56(3):165-170. doi: 10.1016/j.pedneo.2014.09.002.
23. Udomittipong K, Srisukhon W, Nimmannit A, Komoltri C. Respiratory impedance reference values for forced oscillation technique predicted by arm span and height in Thai preschool children. Pediatr Allergy Immunol Pulmonol. 2017;30:97-102. doi: 10.1089/ped.2017.0747
24. De S, Banerjee N, Tiwari RR. Regression equations of respiratory impedance measured by forced oscillation technique for Indian children. Indian J Pediatr. 2020;87(3):192-199. doi: 10.1007/s12098-019-03116-x.
25. Gochicoa-Rangel L, Torre-Bouscoulet L, Martínez-Briseño D, Rodríguez-Moreno L, Cantú-González G, Vargas MH. Values of impulse oscillometry in healthy Mexican children and adolescents. Respir Care. 2015;60(1):119-127. doi: 10.4187/respcare.03374.

Chapter 4: The impact of antenatal and postnatal indoor air pollution or tobacco smoke exposure on lung function at 3 years in an African birth cohort

---

Chaya S[1], Vanker A[1], Brittain K[1], MacGinty R[1], Jacobs C[1], Hantos Z[2], Zar HJ[1], Gray DM[1].

The impact of antenatal and postnatal indoor air pollution or tobacco smoke exposure on lung function at 3 years in an African birth cohort. Respirology. 2023;28(12):1154-1165. doi: 10.1111/resp.14576.

Affiliations:

1. Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town, Cape Town, South Africa
2. Department of Anaesthesiology and Intensive Therapy, Semmelweis University, Budapest, Hungary

Author contributions:

SC contributed to the conceptualisation, methodology, data collection, analyses and writing of original draft. KB and RM contributed to the data curation and analysis. CJ contributed to data collection and manuscript. AV contributed to data collection. ZH contributed to

methodology, analysis and developed oscillometry aspects of study. HJZ contributed to conceptualisation, funding acquisition, methodology, supervision and review of original draft. DMG contributed to lung function conceptualisation, data collection, funding acquisition, methodology, supervision, analyses and writing of original draft. All authors contributed to the final manuscript.

Word count: 3115

Abstract

Background and Objective: Indoor air pollution (IAP) and tobacco smoke exposure (ETS) are global health concerns contributing to the burden of childhood respiratory disease. Studies assessing the effects of IAP and ETS in preschool children are limited. We assessed the impact of antenatal and postnatal IAP and ETS exposure on lung function in a South African birth cohort, the Drakenstein Child Health Study.

Methods: Antenatally enrolled mother–child pairs were followed from birth. Lung function measurements (oscillometry, multiple breath washout and tidal breathing) were performed at 6 weeks and 3 years. Quantitative antenatal and postnatal IAP (particulate matter [PM_{10}], volatile organic compounds [VOC]) and ETS exposures were measured. Linear regression models explored the effects of antenatal and postnatal exposures on lung function at 3 years.

Results: Five hundred eighty-four children had successful lung function testing, mean (SD) age of 37.3 (0.7) months. Exposure to antenatal PM_{10} was associated with a decreased lung clearance index ($p < 0.01$) and postnatally an increase in the difference between resistance at end expiration (R_{eE}) and inspiration ($p = 0.05$) and decrease in tidal volume ($p = 0.06$). Exposure to antenatal VOC was associated with an increase in functional residual capacity ($p = 0.04$) and a decrease in time of expiration over total breath time (t_E/t_{TOT}) ($p = 0.03$) and postnatally an increase in respiratory rate ($p = 0.05$). High ETS exposure postnatally was associated with an increase in R_{eE} ($p = 0.03$).

Conclusion: Antenatal and postnatal IAP and ETS exposures were associated with impairment in lung function at 3 years. Strengthened efforts to reduce IAP and ETS exposure are needed.

4.1 Introduction

Air pollution is a global health concern contributing to the high burden of respiratory disease. Low and middle-income countries (LMICs) are disproportionately affected due to increased reliance on unclean fuel sources such as fossil fuels for household energy [1]. Women and young children are particularly vulnerable to the harmful effects of indoor air pollution (IAP) as they spend more time indoors cooking, often with poor ventilation [2]. IAP and environmental tobacco smoke (ETS) are well described risk factors for childhood respiratory disease and impaired lung function. Solid and alternate fuels emit a mixture of pollutants including particulate matter (PM) and volatile organic compounds (VOCs), all of which threaten respiratory health [3, 4].

In utero exposure to each of ETS and IAP is associated with poor birth outcomes, reduced lung function in infancy through to adulthood and increased childhood respiratory disease [3, 5–7]. We have previously described that in utero exposure to ETS and household benzene reduces lung function 4–6 weeks after birth in the Drakenstein Child Health Study (DCHS), a South African birth cohort study [6, 8, 9].

In addition, postnatal exposures have been associated with decreased lung function in adults and older children [10]. In childhood postnatal exposures have been associated with a decrease in spirometry lung volumes and an increase in resistance and decrease in reactance with oscillometry measurements [10–13].

However, there are limited data from longitudinal cohorts that measure both ante- and postnatal exposure. Many studies to date are retrospective and not conducted with preschool children. A better understanding of the impact of IAP and ETS on early lung development,

particularly in communities with several exposures, is needed to develop targeted preventive strategies.

This study aimed to assess the impact of antenatal and early-life IAP and ETS exposures on lung function at 3-years of age in the DCHS cohort.

4.2 Methods

The DCHS is a prospective birth cohort study of mother–child pairs enrolled from March 2012 to March 2015. This study site is in Paarl, a peri-urban, low socioeconomic community approximately 60 km outside Cape Town, South Africa. Participants were enrolled at two primary health care clinics [14]. Consecutive consenting mothers were enrolled during the second trimester of pregnancy, and mother–child pairs were followed. Study visits synchronized with the national immunization program, and included visits at 6, 10 and 14 weeks, at 6 and 9 months, and then every 6 months from 12 months onward detailed in the Supporting Information [14, 15]. This analysis uses prospectively collected data from 6 weeks through to 3 years of age between July 2012 and October 2015. All children with successful lung function measurements at 3 years were included, although we excluded children born before 32 weeks of gestation and those living with HIV as they generally have poorer lung function for reasons not related to exposure to IAP or tobacco smoke. The study was approved by the University of Cape Town Faculty of Health Sciences human research ethics committee (048/2020; 082/2018; 423/2012).

4.2.1 Clinical data collection

Socioeconomic status (SES) was assessed antenatally using a validated score derived from employment status, maternal educational attainment, household income, assets and market

access [16]. Gestational age at birth was calculated based primarily on antenatal ultrasound performed in the second trimester [17].

Anthropometry was measured at all study visits, and weight and length were converted to z-scores using Anthro software (WHO, Geneva, Switzerland) [18]. Weight-for-age z-scores (WFA), height-for-age z-scores (HFA) and body mass index (BMI) for age z-scores were calculated. WFA < -2 and HFA < -2 were classified as underweight and stunted respectively.

Active surveillance for lower respiratory tract infection (LRTI) was done from birth through 3 years of age; LRTI was defined according to World Health Organization (WHO) criteria with findings confirmed by a trained study doctor or nurse [19].

4.2.2 IAP and ETS exposures

Home visits were conducted antenatally (28–32 weeks gestation) and postnatally (4–6months of age) and PM_{10} and VOCs, benzene and toluene, measured. For PM_{10}, a personal air sampling pump (AirChek 52®; SKC, Eighty Four, PA, USA) was left in the home for 24 h and a 24-h average was obtained. Passive diffusion tubes (Markes® thermal desorption tubes; Llantrisant, UK) which measured VOC were placed in the homes for 2 weeks and a 2-week average was obtained, as previously described [20]. Antenatal ETS exposure was measured using maternal urine cotinine collected at an antenatal visit (28–32 weeks gestation) and at birth using the Immulite^{R} 1000 Nicotine Metabolite Kit (Siemens Medical Solutions DiagnosticsR, Glyn Rhonwy, UK) quantitative urine cotinine test [21]. The highest measured value was used to assign antenatal exposure. Postnatal ETS exposure was determined using the highest infant urine cotinine measurement, done at 6 weeks, and yearly until 3 years of age [20].

Exposure levels for each pollutant were defined using the South African National Ambient Air Quality Standards [22]. Levels for benzene, toluene or PM_{10} were categorized as above threshold if the level was more than 5, 240 or 40 $\mu g/m^3$, respectively [20]. ETS exposure was quantified as no exposure (urine cotinine level <10 ng/mL), moderate (10–499 ng/mL) or high (≥500 ng/mL). Details of IAP measurement and ETS exposure methodology have been previously published and are summarized in the Supporting Information Table S4.1 [20].

4.2.3 Lung function testing

All participants had lung function measurements at 6 weeks and 3 years of age but not within 4 weeks of a respiratory illness. Lung function measurements included intra-breath oscillometry measuring respiratory impedance (Z_{rs}) (resistance and reactance); tidal breathing flow volume loops (TBFVLs) with measures including tidal volume (*TV*), respiratory rate (*RR*) and expiratory flow ratios (ratio of time of expiration to total time [t_E/t_{TOT}] and ratio of time of peak total expiratory flow to time of expiration [t_{PTEF}/t_E]); and multiple breath washout (MBW) measuring the functional residual capacity (*FRC*) and the lung clearance index (*LCI*). All tests were done by the same team which included a respiratory technologist, a nurse and a paediatric pulmonologist. All testing followed international consensus guidelines [9, 23].

Measurements at 6 weeks were performed in unsedated infants during quiet sleep as previously described [9]. Intrabreath oscillometry was performed with custom-built wavetube equipment (University of Szeged, Hungary) using a 16-Hz signal [24]. The measurements of impedance were made in the supine posture, with the head supported in a neutral position, via a facemask and filter. Technically acceptable 30s recordings were collected. Recordings were excluded if they contained breath holds, cries, irregular breathing or leaks around the face

mask [8]. The intra-breath oscillometry measures included were the resistance at end-expiration (R_{eE}) and at end inspiration (R_{eI}), reactance at end-expiration (X_{eE}) and end inspiration (X_{eI}), and the tidal changes R_{eE}-R_{eI} (ΔR) and X_{eE}-X_{eI} (ΔX). Mean resistance (R_{mean}) and reactance (X_{mean}) for the whole breathing periods were also calculated. TBFVL and MBW measurements were collected using the Exhalyzer D with ultrasonic flow meter (Ecomedics, Duernten, Switzerland) and analysed with specialized analysis software (WBreath V.3.28.0; NDD Medizintechnik, Zurich, Switzerland). MBW was done using 4% sulfur-hexafluoride (SF6) as a tracer gas.[4]

At 3 years, lung function tests were performed in awake children. Oscillometry testing was completed using a custom-made oscillometry system (INCIRCLE wavetube system, University of Szeged, Hungary) [25]. Measurement of one 16-s epoch with 10 Hz oscillation frequency was recorded and repeated if necessary to obtain a minimum of five regular breaths, without any artefacts (vocal cord noise, apnoea, irregular breathing pattern, glottic closure, leak or sighs). Tests were conducted with the child sitting comfortably, nose occluded, with the cheeks firmly supported and breathing through a mouthpiece and filter. TBFVL and MBW measurements were collected using Ecomedics Exhalyzer D, Duernten, Switzerland and analysed with specialized analysis software (Spiroware 3.2.1, Zurich, Switzerland). MBW was measured during tidal breathing using inert nitrogen with 100% oxygen washout.[5] Tests were performed with the child sitting and breathing comfortably through a size 2 silicone facemask (Laerdal) and filter (Gibeck Humid-Vent Filter; Perak, Malaysia). The deadspace[6] of the mask was determined by water displacement.

[4] [5] Measuring and quality control were performed as per the latest ATS technical statement: Robinson PD, et al. Preschool multiple-breath washout testing. An official American Thoracic Society technical statement. Am J Respir Crit Care Med. 2018;197(5):e1-e19.

[6] Deadspace indicates deadspace volume corrected for facemask

4.2.4 Statistical analysis

Data were analysed using Stata 14 (StataCorp Inc., College Station, TX). Child characteristics and exposures to IAP and ETS were summarized and compared between children included versus those excluded from analysis using Wilcoxon rank sum (Mann–Whitney) tests for continuous variables and chi-square and Fisher's exact tests for categorical variables. A Pearson's correlation matrix was used to assess the association between each of antenatal and postnatal PM_{10}, benzene, toluene and ETS. The independent effect of both antenatal and postnatal exposure to IAP and ETS on lung function measures at 3 years was examined using linear regression models adjusted for potential confounders. Confounders were selected a priori based on a directed acyclic graph (DAG) and included enrolment site, sex, SES, BMI zscore at the time of testing, and ≥1 episode of LRTI prior to testing (Supporting Information Figure S4.1). Supplementary models explored the impact exposure to IAP and ETS at 3 years adjusted for these a priori selected confounders as well as lung function measured at 6 weeks, to assess the impact of these postnatal exposures adjusted for poor early-life lung function.

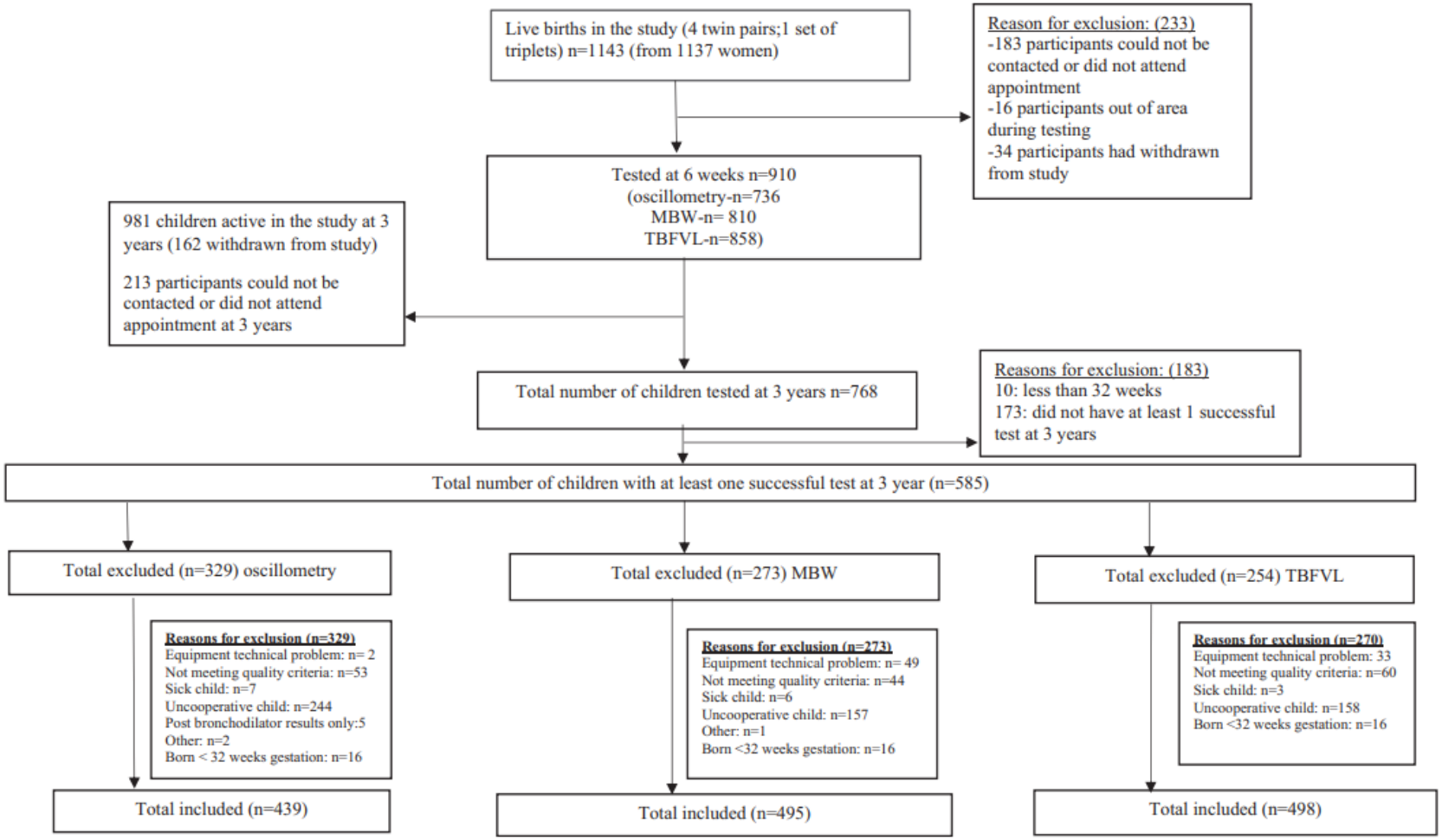
For VOC, we combined benzene and toluene as these exposures were significantly correlated and are by-products of the same alternate fuel combustion.

4.3 Results

Of 768 children eligible for testing at 3 years, 584 (76%) with ≥1 successful lung function measurement (TBFVL, oscillometry and/or MBW) were included. Details of reasons for unsuccessful testing are shown in Figure 4.1. Compared to children excluded from analysis, those included were significantly less likely to be HIV-exposed and to live in informal

housing, and more likely to have antenatal ETS exposure (Supporting Information Table S4.2).

Figure 4.1 Description of all children in the cohort



MBW, multiple breath wash-out; TBFVL, tidal breathing flow volume loop.

The number of good quality trials required for a test to be technically acceptable: more than 30 breaths for TBFVL, at least 5 for oscillometry, and at least 3 for MBW.

The mean age (SD) of the 584 children included was 37.3 (0.7) months (Table 4.1). Of these children, 49% were male, 13% were born late preterm (32–37 weeks), 19% were HIV-exposed but uninfected, 7% were underweight, and 20% were stunted. Episodes of LRTI were common, with 49% experiencing ≥1 episode during the first 3 years of life (mean age at first episode: 8.6 [8.4 months]) of which 52% had recurrent LRTI.

Average lung function values at 6 weeks and 3 years of age are shown in the Supporting Information Table S4.3.

4.3.1 Environmental exposures

Antenatal exposure to PM_{10} and VOC above ambient standards occurred in 44% of children, and postnatal exposure in over one-third (Table 4.1). Antenatal ETS exposure occurred in 82% of children (moderate exposure in 46%; high exposure in 36%), and postnatal exposure in 76% (moderate exposure: 67%; high exposure: 9%). There was a strong correlation between exposures to antenatal benzene and antenatal toluene, as well as between postnatal benzene and postnatal toluene, but there was no correlation between antenatal and postnatal exposures. Strong correlations were however noted between antenatal and postnatal ETS exposure (Supporting Information Table S4.4).

Table 4.1 Characteristics of children with at least one successful test at 3 years of age

	***n* (%)**
Number of children	584
Mean (SD) age in months	37.3 (0.7)
Male sex	289 (49%)
Enrolment site	
Mbekweni	288 (49%)
TC Newman	296 (51%)
Born pre-term (≥32 and <37 weeks gestation)	74 (13%)
HIV-exposed	110 (19%)
Ever breastfed	477 (82%)
Median [IQR] months of breastfeeding	8.0 [2.0, 23.9]
Ever exclusively breastfed	342 (59%)
Median [IQR] months of exclusive breastfeeding	1.6 [0.7, 3.2]
Mean (SD) weight z-score	-0.4 (1.1)
Underweight	42 (7%)
Mean (SD) height z-score	-1.1 (1.1)
Stunted	117 (20%)
Mean (SD) BMI z-score	0.4 (1.2)
Previous LRTI	284 (49%)
Recurrent LRTI	147 (25%)
Mean (SD) age of first LRTI in months (*n* = 284)	8.6 (8.4)
Antenatal exposure to indoor air pollutants above ambient standards	
PM_{10} (*n* = 406)	179 (44%)
Benzene (*n* = 393)	170 (43%)
Toluene (*n* = 393)	33 (8%)
Benzene and/or Toluene (*n* = 393)	171 (44%)
Exposure to Antenatal tobacco smoke	
Antenatal exposure (*n* = 566):	
Moderate exposure	262 (46%)
High exposure	202 (36%)
Postnatal exposure to indoor air pollutants above ambient standards	
PM_{10} (*n* = 285)	103 (36%)
Benzene (*n* = 257)	83 (32%)
Toluene (*n* = 257)	24 (9%)
Benzene and/or Toluene (*n* = 257)	83 (32%)

Exposure to postnatal tobacco smoke	
Postnatal exposure (n = 507):	
Moderate exposure	339 (67%)
High exposure	45 (9%)

Note: Underweight: weight-for-age z-scores < -2. Stunted: height-for-age z-scores < -2.

Abbreviations: LRTI, lower respiratory tract infection; PM_{10}, particulate matter size 10 $\mu g/m^3$.

4.3.2 Impact of IAP and ETS on lung function at 3 years

4.3.2.1. PM10

The impact of PM_{10} on lung function at 3 years is presented in Table 4.2. In unadjusted analyses, antenatal PM_{10} exposure above threshold was associated with decreased *LCI* ($p = 0.009$), and postnatal exposure was associated with decreased *RR* ($p = 0.062$) and increased X_{eI} ($p = 0.067$). In adjusted analyses, the association between antenatal exposure and decreased *LCI* persisted ($p = 0.006$), and antenatal exposure was associated with decreased tidal volume ($p = 0.067$). In addition, postnatal PM_{10} exposure was associated with increased ΔR ($\underline{p} = 0.052$). After additional adjustment for 6-week lung function, no significant associations with postnatal PM_{10} exposure were observed (Supporting Information Table S4.5).

Table 4.2 Impact of ante- and postnatal exposure to PM_{10} above ambient standards on lung function at 3 years of age

	Unadjusted models			Adjusted models		
	n	***β* [95% CI]**	***p*-value**	***n***	***β* [95% CI]**	***p*-value**
R_{eE} (hPa s L^{-1})						
Antenatal exposure	299	0.22 [-0.42, 0.87]	0.499	152	0.49 [-0.42, 1.40]	0.287
Postnatal exposure	203	0.31 [-0.51, 1.12]	0.457		0.60 [-0.33, 1.53]	0.205
X_{eE} (hPa s L^{-1})						
Antenatal exposure	299	-0.13 [-0.53, 0.27]	0.518	152	-0.02 [-0.62, 0.57]	0.943
Postnatal exposure	203	0.20 [-0.31, 0.71]	0.435		0.00 [-0.61, 0.61]	0.998
R_{eI} (hPa s L^{-1})						
Antenatal exposure	299	0.03 [-0.50, 0.57]	0.903	152	0.32 [-0.45, 1.08]	0.416
Postnatal exposure	203	0.01 [-0.70, 0.73]	0.970		0.07 [-0.71, 0.86]	0.855
X_{eI} (hPa s L^{-1})						
Antenatal exposure	299	0.02 [-0.29, 0.32]	0.906	152	0.15 [-0.27, 0.57]	0.472
Postnatal exposure	203	0.37 [-0.03, 0.76]	0.067		0.36 [-0.06, 0.79]	0.096
R_{mean} (hPa s L^{-1})						
Antenatal exposure	299	0.01 [-0.62, 0.64]	0.979	152	0.38 [-0.52, 1.28]	0.409
Postnatal exposure	203	0.30 [-0.52, 1.13]	0.470		0.60 [-0.33, 1.52]	0.202
X_{mean} (hPa s L^{-1})						
Antenatal exposure	299	-0.08 [-0.42, 0.26]	0.633	152	0.02 [-0.49, 0.52]	0.951
Postnatal exposure	203	0.25 [-0.20, 0.70]	0.276		0.05 [-0.46, 0.57]	0.838
ΔR (hPa s L^{-1})						
Antenatal exposure	299	0.19 [-0.20, 0.58]	0.346	152	0.18 [-0.34, 0.70]	0.506
Postnatal exposure	203	0.29 [-0.17, 0.76]	0.217		**0.53 [-0.01, 1.06]**	**0.052***
ΔX (hPa s L^{-1})						
Antenatal exposure	299	-0.15 [-0.49, 0.19]	0.390	152	-0.17 [-0.60, 0.25]	0.423
Postnatal exposure	203	-0.16 [-0.55, 0.23]	0.408		-0.36 [-0.80, 0.07]	0.103
FRC (L)						
Antenatal exposure	342	0.01 [-0.01, 0.03]	0.349	191	0.01 [-0.02, 0.03]	0.604
Postnatal exposure	243	-0.02 [-0.04, 0.00]	0.100		-0.02 [-0.04, 0.01]	0.199
LCI (number of turnovers)						
Antenatal exposure	342	**-0.35 [-0.61, -0.09]**	**0.009**	191	**-0.49 [-0.84, -0.14]**	**0.006**
Postnatal exposure	243	0.24 [-0.08, 0.55]	0.138		0.15 [-0.21, 0.50]	0.421
Respiratory rate (min^{-1})						
Antenatal exposure	340	0.07 [-1.10, 1.24]	0.910	184	0.02 [-1.59, 1.62]	0.981
Postnatal exposure	236	1.42 [-0.07, 2.91]	0.062		0.70 [-0.96, 2.35]	0.408
Tidal volume (mL)						
Antenatal exposure	346	-3.76 [-9.77, 2.24]	0.218	187	-7.87 [-16.29, 0.55]	0.067
Postnatal exposure	239	1.62 [-6.51, 9.75]	0.696		1.35 [-7.31, 10.01]	0.759

t_E/t_{TOT}						
Antenatal exposure	346	-0.27 [-0.99, 0.45]	0.461	187	0.12 [-0.90, 1.15]	0.812
Postnatal exposure	239	0.01 [-0.90, 0.92]	0.980		0.07 [-0.99, 1.13]	0.899
t_{PTEF}/t_E						
Antenatal exposure	339	-0.50 [-3.12, 2.12]	0.708	182	-0.46 [-4.06, 3.14]	0.802
Postnatal exposure	234	2.19 [-1.18, 5.56]	0.201		2.44 [-1.28, 6.16]	0.197

Note: Adjusted model: model of the effect of ante- and postnatal exposure to PM_{10}, adjusted for enrolment site, sex, socioeconomic status, BMI z-score, and previous lower respiratory tract illness (LRTI). PM_{10}, particulate matter size 10 µg/m^3; R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration. Significant results: $p<0.05$; * indicates near significance.

4.3.2.2. VOCs (benzene and/or toluene)

Associations between exposure to VOCs and lung function measures are presented in Table 4.3. In unadjusted analyses, antenatal exposure to VOCs above ambient standards were associated with increased *FRC* ($p = 0.035$) and decreased t_E/t_{TOT} ($p = 0.033$). Although not statistically significant, antenatal exposure was also associated with lower tidal volume ($p = 0.063$) and higher R_{eI} ($p = 0.067$). In addition, postnatal exposure to VOCs above ambient standards was associated with a decrease in resistance (R_{eE}, R_{eI} and R_{mean}), as well as an increased reactance (X_{eI}) and *RR* in unadjusted analyses.

In adjusted analyses, none of the associations between antenatal exposure to VOCs and lung function measures persisted. However, postnatal exposure remained significantly associated with decreased R_{eE} ($p = 0.014$), R_{eI} ($p = 0.050$) and R_{mean} ($p = 0.033$), and an increased *RR* ($p = 0.041$) after adjustment for antenatal exposure and confounding.

When adjusting for 6-week lung function, however, no significant associations were observed between postnatal exposure to VOCs and lung function at 3 years of age (Supporting Information Table S4.6).

Table 4.3 Impact of ante- and postnatal exposure to benzene and/or toluene above ambient standards (vs. below ambient standards for both) on lung function at 3 years of age

	Unadjusted models			Adjusted models		
	n	***β* [95% CI]**	***p*-value**	***n***	***β* [95% CI]**	***p*-value**
R_{eE} (hPa s L^{-1})						
Antenatal exposure	293	0.29 [-0.36, 0.94]	0.378	135	0.35 [-0.63, 1.32]	0.487
Postnatal exposure	181	**-1.12 [-2.01, -0.22]**	**0.015**		**-1.31 [-2.36, -0.26]**	**0.014**
X_{eE} (hPa s L^{-1})						
Antenatal exposure	293	-0.01 [-0.41, 0.40]	0.973	135	0.23 [-0.41, 0.86]	0.482
Postnatal exposure	181	0.45 [-0.11, 1.01]	0.112		0.62 [-0.06, 1.30]	0.073
R_{eI} (hPa s L^{-1})						
Antenatal exposure	293	0.49 [-0.04, 1.02]	0.067	135	0.45 [-0.37, 1.28]	0.276
Postnatal exposure	181	**-0.79 [-1.58, 0.00]**	**0.049**		**-0.88 [-1.75, 0.00]**	**0.050**
X_{eI} (hPa s L^{-1})						
Antenatal exposure	293	0.00 [-0.30, 0.30]	0.991	135	0.28 [-0.16, 0.72]	0.209
Postnatal exposure	181	**0.45 [0.03, 0.88]**	**0.037**		0.31 [-0.16, 0.77]	0.197
R_{mean} (hPa s L^{-1})						
Antenatal exposure	293	0.47 [-0.16, 1.09]	0.141	135	0.58 [-0.36, 1.53]	0.226
Postnatal exposure	181	**-0.92 [-1.83, -0.02]**	**0.046**		**-1.10 [-2.11, -0.09]**	**0.033**
X_{mean} (hPa s L^{-1})						
Antenatal exposure	293	-0.04 [-0.38, 0.30]	0.818	135	0.12 [-0.41, 0.64]	0.658
Postnatal exposure	181	0.41 [-0.08, 0.90]	0.099		0.44 [-0.12, 1.00]	0.120
ΔR (hPa s L^{-1})						
Antenatal exposure	293	-0.20 [-0.59, 0.18]	0.303	135	-0.11 [-0.69, 0.47]	0.711
Postnatal exposure	181	-0.33 [-0.86, 0.21]	0.230		-0.43 [-1.05, 0.19]	0.170
ΔX (hPa s L^{-1})						
Antenatal exposure	293	-0.01 [-0.35, 0.33]	0.960	135	-0.05 [-0.53, 0.42]	0.824
Postnatal exposure	181	0.00 [-0.46, 0.46]	0.997		0.31 [-0.19, 0.82]	0.223
FRC (L)						
Antenatal exposure	331	**0.02 [0.00, 0.04]**	**0.035**	173	0.01 [-0.01, 0.04]	0.362
Postnatal exposure	220	0.01 [-0.02, 0.03]	0.503		0.00 [-0.03, 0.02]	0.751
LCI (number of turnovers)						
Antenatal exposure	331	-0.11 [-0.36, 0.15]	0.416	173	0.05 [-0.32, 0.43]	0.834
Postnatal exposure	220	0.04 [-0.31, 0.38]	0.839		0.08 [-0.31, 0.47]	0.697
Respiratory rate (min^{-1})						
Antenatal exposure	328	0.97 [-0.24, 2.17]	0.114	165	1.24 [-0.47, 2.96]	0.103
Postnatal exposure	210	**1.65 [0.02, 3.27]**	**0.047**		**1.85 [0.08, 3.62]**	**0.041**
Tidal volume (mL)						
Antenatal exposure	334	-5.81 [-11.94, 0.32]	0.063	169	-6.34 [-15.39, 2.71]	0.168
Postnatal exposure	214	-0.56 [-9.20, 8.08]	0.898		-1.89 [-11.25, 7.47]	0.690

t_E/t_{TOT}						
Antenatal exposure	334	**-0.81 [-1.56, -0.06]**	**0.033**	169	-0.58 [-1.64, 0.48]	0.280
Postnatal exposure	214	-0.26 [-1.19, 0.68]	0.591		-0.62 [-1.71, 0.48]	0.267
t_{PTEF}/t_E						
Antenatal exposure	329	-0.56 [-3.27, 2.15]	0.683	164	-0.91 [-4.69, 2.87]	0.635
Postnatal exposure	208	-1.18 [-4.92, 2.56]	0.535		-1.06 [-4.99, 2.87]	0.596

Note: Adjusted model, model of the effect of ante- and postnatal exposure to benzene and/or toluene, adjusted for enrolment site, sex, socioeconomic status, BMI z-score, and previous LRTI. R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

4.3.2.3. ETS

Table 4.4 presents the impact of ETS exposure on lung function. In unadjusted analyses, antenatal exposure to high levels of ETS was associated with decreased tidal volume (p = 0.074), and postnatal exposure with higher R_{eE} (p = 0.026) and R_{mean} (p = 0.087). In addition, both moderate and high exposure to postnatal ETS were associated with decreased t_{PTEF}/t_E in unadjusted analyses (p = 0.066 and p = 0.067, respectively). None of these associations persisted in adjusted analyses, or after adjustment for 6-week lung function (Supporting Information Table S4.7).

Table 4.4 Impact of ante- and postnatal exposure to moderate and high levels of tobacco smoke, versus low levels, on lung function at 3 years of age

	Unadjusted models			Adjusted models		
	n	**β [95% CI]**	***p*-value**	***n***	**β [95% CI]**	***p*-value**
R_{eE} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.11 [-0.87, 0.65]	0.775	370	-0.37 [-1.20, 0.47]	0.387
High		0.34 [-0.44, 1.13]	0.389		-0.37 [-1.35, 0.60]	0.453
Postnatal exposure						
Moderate	379	0.17 [-0.50, 0.84]	0.628		0.02 [-0.75, 0.78]	0.964
High		**1.27 [0.15, 2.39]**	**0.026**		0.70 [-0.62, 2.03]	0.298
X_{eE} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.16 [-0.62, 0.30]	0.493	370	-0.16 [-0.67, 0.34]	0.529
High		-0.09 [-0.56, 0.39]	0.724		-0.03 [-0.63, 0.56]	0.918
Postnatal exposure						
Moderate	379	0.12 [-0.28, 0.53]	0.555		0.16 [-0.30, 0.63]	0.494
High		0.01 [-0.67, 0.68]	0.988		0.01 [-0.80, 0.82]	0.981
R_{eI} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	0.17 [-0.48, 0.81]	0.614	370	-0.03 [-0.74, 0.67]	0.925
High		0.36 [-0.31, 1.03]	0.293		-0.14 [-0.97, 0.69]	0.739
Postnatal exposure						
Moderate	379	0.46 [-0.11, 1.02]	0.115		0.34 [-0.31, 0.99]	0.299
High		0.73 [-0.22, 1.68]	0.130		0.28 [-0.85, 1.40]	0.631
X_{eI} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.12 [-0.49, 0.25]	0.527	370	-0.04 [-0.45, 0.37]	0.855
High		-0.03 [-0.41, 0.36]	0.894		0.03 [-0.45, 0.51]	0.909
Postnatal exposure						
Moderate	379	-0.08 [-0.41, 0.25]	0.628		-0.09 [-0.46, 0.29]	0.647
High		-0.04 [-0.59, 0.50]	0.876		0.02 [-0.64, 0.67]	0.963
R_{mean} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.13 [-0.87, 0.61]	0.728	370	-0.34 [-1.15, 0.47]	0.409
High		0.27 [-0.50, 1.04]	0.486		-0.45 [-1.40, 0.50]	0.355
Postnatal exposure						
Moderate	379	0.16 [-0.49, 0.82]	0.626		0.01 [-0.74, 0.75]	0.986
High		0.95 [-0.14, 2.04]	0.087		0.38 [-0.91, 1.67]	0.561

X_{mean} (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.12 [-0.52, 0.29]	0.572		-0.10 [-0.55, 0.35]	0.671
High		-0.03 [-0.46, 0.39]	0.872	370	0.08 [-0.45, 0.61]	0.765
Postnatal exposure						
Moderate	379	0.09 [-0.27, 0.45]	0.617		0.09 [-0.32, 0.51]	0.657
High		-0.13 [-0.73, 0.48]	0.682		-0.14 [-0.86, 0.58]	0.701
ΔR (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.28 [-0.72, 0.17]	0.226		-0.33 [-0.83, 0.17]	0.192
High		-0.02 [-0.48, 0.45]	0.946	370	-0.23 [-0.82, 0.35]	0.437
Postnatal exposure						
Moderate	379	-0.29 [-0.69, 0.11]	0.154		-0.33 [-0.79, 0.13]	0.164
High		0.54 [-0.13, 1.21]	0.113		0.43 [-0.37, 1.22]	0.292
ΔX (hPa s L^{-1})						
Antenatal exposure						
Moderate	429	-0.04 [-0.43, 0.34]	0.833		-0.12 [-0.54, 0.29]	0.552
High		-0.06 [-0.46, 0.34]	0.768	370	-0.06 [-0.54, 0.42]	0.810
Postnatal exposure						
Moderate	379	0.20 [-0.13, 0.53]	0.226		0.25 [-0.13, 0.63]	0.193
High		0.05 [-0.50, 0.60]	0.861		-0.01 [-0.66, 0.65]	0.986
FRC (L)						
Antenatal exposure						
Moderate	481	0.00 [-0.03, 0.02]	0.700		0.00 [-0.03, 0.02]	0.841
High		-0.02 [-0.04, 0.01]	0.183	418	-0.01 [-0.04, 0.02]	0.399
Postnatal exposure						
Moderate	428	-0.01 [-0.03, 0.02]	0.586		-0.01 [-0.04, 0.01]	0.368
High		-0.02 [-0.06, 0.02]	0.258		-0.03 [-0.08, 0.01]	0.110
LCI (number of turnovers)						
Antenatal exposure						
Moderate	481	-0.03 [-0.34, 0.28]	0.846		0.05 [-0.31, 0.40]	0.796
High		-0.01 [-0.33, 0.31]	0.950	418	0.08 [-0.34, 0.50]	0.711
Postnatal exposure						
Moderate	428	0.01 [-0.27, 0.29]	0.948		0.11 [-0.22, 0.43]	0.527
High		-0.20 [-0.68, 0.27]	0.401		0.03 [-0.54, 0.59]	0.927

Respiratory rate (min^{-1})						
Antenatal exposure						
Moderate	475	0.59 [-0.81, 1.99]	0.407		1.25 [-0.29, 2.78]	0.111
High		0.21 [-1.24, 1.65]	0.777	416	1.60 [-0.22, 3.42]	0.085
Postnatal exposure						
Moderate	427	0.69 [-0.61, 1.99]	0.297		0.89 [-0.56, 2.33]	0.231
High		-1.10 [-3.25, 1.06]	0.317		-0.61 [-3.13, 1.91]	0.637
Tidal volume (mL)						
Antenatal exposure						
Moderate	482	-1.63 [-8.65, 5.38]	0.648		-0.33 [-8.02, 7.37]	0.933
High		-6.61 [-13.86, 0.64]	0.074	423	-1.81 [-10.95, 7.34]	0.698
Postnatal exposure						
Moderate	434	-4.60 [-11.08, 1.88]	0.163		-2.41 [-9.69, 4.87]	0.516
High		-2.74 [-13.50, 8.01]	0.616		5.61 [-7.00, 18.22]	0.382
t_E/t_{TOT}						
Antenatal exposure						
Moderate	482	-0.17 [-0.98, 0.64]	0.686		-0.59 [-1.48, 0.29]	0.188
High		0.03 [-0.80, 0.87]	0.935	423	-0.43 [-1.49, 0.62]	0.418
Postnatal exposure						
Moderate	434	-0.46 [-1.19, 0.27]	0.213		-0.57 [-1.41, 0.27]	0.180
High		0.36 [-0.85, 1.57]	0.559		-0.09 [-1.54, 1.36]	0.903
t_{PTEF}/t_E						
Antenatal exposure						
Moderate	475	0.00 [-3.05, 3.05]	1.000		1.34 [-1.93, 4.62]	0.421
High		-1.09 [-4.23, 2.05]	0.496	417	0.33 [-3.55, 4.22]	0.866
Postnatal exposure						
Moderate	428	-2.52 [-5.20, 0.16]	0.066		-1.89 [-4.99, 1.21]	0.231
High		-4.14 [-8.57, 0.28]	0.067		-3.10 [-8.42, 2.22]	0.253

Note: Adjusted model: model of the effect of ante- and postnatal exposure to tobacco smoke, adjusted for enrolment site, sex, socioeconomic status, BMI z-score, and previous LRTI. R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

4.4 Discussion

In this study, we used comprehensive longitudinal lung function measurements including novel intra-breath oscillometry, TBFVL and MBW to assess the impact of antenatal and postnatal IAP and ETS exposure on lung function at 3 years of age in an African birth cohort. Adjusting for 6 week lung function allowed us to determine the independent effect of these exposures on postnatal lung function. Both antenatal and postnatal IAP and ETS were associated with decreased lung function at 3 years. Antenatal and postnatal PM_{10} exposure were associated with decreased *LCI* and increased ΔR, respectively[7]. Antenatal VOC exposure was associated with increased *FRC*, and postnatal exposure with increased *RR*.[8] Postnatal ETS exposure was associated with increased R_{eE}.[9]

Antenatal PM_{10} was associated with a decreased *LCI*. No previous comparative studies have assessed the impact of antenatal PM_{10} exposure using MBW. An Australian study assessed the effect of ambient ultrafine particles (UFP) in 8–11 year old children, and showed no changes in MBW measurements [26]. This suggests that MBW may not be a sensitive method to assess the effect of PM_{10} on lung function or that PM_{10} exposure antenatally is not associated with long-term impairment measured by MBW and oscillometry[10]. Also of note, PM_{10} and $PM_{2.5}$ deposit variably within in the airways; PM_{10} in the larger and $PM_{2.5}$ in the smaller airways. Particulate size may hence have different effects on lung growth and function. However, the observed association between postnatal PM_{10} exposure and increased resistance, measured using intra-breath oscillometry, suggests increased airway obstruction.

[7] Erratum: Antenatal and postnatal PM_{10} exposure were associated with decreased *LCI* and increased ΔR, respectively.
[8] and postnatal exposure with increased *RR*.
[9] In the adjusted analysis, PM_{10} exposure was associated with increased resistance and decreased *LCI*, and VOC exposure with an increased respiratory rate.
[10] A physiological explanation for a low *LCI* could be a disruption in lung development caused by these exposures, which may lead to non-communicating regions within the lung or "simplification of the lung architecture".

Similar to our findings, $PM_{2.5}$ exposure was not associated with respiratory impedance measured using oscillometry in 2 year-old Nigerian children, however, postnatal exposure was associated with lower reactance, suggesting stiffer lung [27]. Oscillometry measurements in 8–11 year-old children similarly showed an association between exposure to UFPs and lower reactance [26]. Taken together, the results of these studies suggest that postnatal PM exposure impacts negatively on child lung development [26, 27].

The deleterious effects of antenatal ETS exposure on infant lung function and growth are well described [5]. Here, postnatal exposure to ETS was associated with a decrease in t_{PTEF}/t_E and an increase in resistance at 3 years after adjusting for antenatal exposure[11], which may reflect narrowed airways and predispose a child to recurrent LRTI and possibly chronic lung disease [7]. These findings are consistent with previous studies which found that postnatal ETS exposure increased oscillometry resistance in children aged 3–14 years [11, 12]. It is difficult to separate the impact of antenatal and postnatal ETS exposure as they are strongly correlated. However, by adjusting for 6-week lung function we were able to show postnatal effects on lung function in children with continued exposure. This may reflect either an additional effect of postnatal ETS exposure or a persistence of antenatal programming that impairs normal lung development in early life, or likely both.

Antenatal VOC exposure was associated with increased *FRC* and decreased t_E/t_{TOT} at 3 years, suggestive of an obstructive pattern. This extends our prior findings of an association between antenatal benzene exposure and lower expiratory flow ratio, t_{PTEF}/t_E, at 6 weeks [9]. Our findings are also in keeping with other studies reporting increased respiratory symptoms

[11] Here, postnatal exposure to ETS was associated with a decrease in t_{PTEF}/t_E and an increase in resistance at 3 years in the unadjusted analysis.

and reduced lung function associated with VOC exposure [3, 28, 29]. Here, postnatal VOC exposure was further associated with increased *RR* at 3 years. We observed a paradoxical finding of decreased respiratory system impedance, lower resistance (increased airway calibre) and higher reactance (less stiff lungs). A similar pattern of lower resistance was noted in the study by Robinson et al. assessing the effect of UFP exposure on lung function using oscillometry[12] [26]. Prior studies of associations between VOC and respiratory outcomes ranged from some effect to no effect and, while increased benzene exposure could impair respiratory health, comparisons between studies are difficult in view of different methodologies [30, 31]. Our study supports altered lung function after VOC exposure, highlighting the importance of avoiding high exposure, but further studies are needed to better understand the impact of VOC exposure on lung development.

This large longitudinal cohort included the measurement of antenatal and postnatal exposures, comprehensive lung function from birth up to 3 years and robust risk factor assessments. There are only a few longitudinal studies which assess antenatal and postnatal exposures and many use spirometry, thus limited to older children. In addition, this is one of the first studies to assess these impacts in a LMIC setting with high prevalence of risk factors for respiratory disease. However, our findings must be considered in light of several limitations. Separating antenatal and postnatal exposures is challenging, but adjusting for 6-week lung function allowed for the assessment of the independent effects of antenatal and postnatal exposures. However, the sample size precluded a meaningful exploration of potential interactions between antenatal and postnatal exposures. In addition, the small number of children in some of the exposure groups, especially postnatally, limited our ability to detect significant associations, and dichotomising exposure variables reduced statistical

[12] However, in contrast to our findings, a more negative reactance was noted in this setting.

power. We measured exposures at two time points, which may underestimate ongoing exposure to IAP and seasonal variations. We conducted multiple comparisons and cannot rule out the possibility of Type 1 errors, however, our findings are in keeping with the existing literature. We included just over half the cohort in analysis and, although the subsample is mostly representative of the entire cohort, differences in HIV exposure, ETS exposure and housing type were observed. In addition, a complete case analysis was used and that this could lead to bias in the results. Further studies and more data are needed to confirm our findings.

Despite some limitations, this study provides novel data suggesting that antenatal IAP and ETS exposures were associated with decreased lung function at 3 years, with postnatal exposures having deleterious effects independent of antenatal exposures. This highlights the need for public health interventions including educational initiatives, especially for women of child-bearing age to prevent exposure, as well as measures to provide safer, less polluting alternative fuel sources. Our study highlights the vulnerability of children to both antenatal and postnatal IAP and ETS exposure, but further studies are warranted to explore the longer-term effects of these exposures on lung function and to assess the long-term clinical implications of these findings.

Acknowledgements:

We thank the study staff in Paarl, the data and laboratory teams, the clinical and administrative staff of the Western Cape Government Health Department at Paarl Hospital. We thank the families and children who participated in this study. We thank Dr Dorottya Czovek (Department of Paediatrics, Semmelweis University, Budapest, Hungary), Gergely

Makan and Prof Zoltan Gingl (Department of Technical Informatics, University of Szeged, Hungary) for support with DCHS infant and preschool oscillometry measurement setup.

Funding:

The study received funding from the Bill and Melinda Gates Foundation (grant number OPP1017641, OPP1017579); NIH, USA(H3 Africa grants U54HG009824 and U01AI110466), The Wellcome Trust (098479/Z/12/Z; 204755/Z/162); MRC South Africa, The National Research Foundation, South Africa; Hungarian Scientific Research Fund grant(K 128701); European Respiratory Society (INCIRCLE CRC-2013-02); Harry Crossley Clinical Research Fellowship; AV is supported by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development from funding received from the South African National Treasury.

References

1. World Health Organization. WHO global air quality guidelines. Particulate matter ($PM_{2.5}$ and PM_{10}), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide. Geneva: World Health Organization; 2021.
2. Aithal SS, Gill S, Satia I, Tyagi SK, Bolton CE, Kurmi OP. The effects of household air pollution (HAP) on lung function in children: a systematic review. Int J Environ Res Public Health. 2021;18(22):11973. doi: 10.3390/ijerph182211973.
3. Mocelin HT, Fischer GB, Bush A. Adverse early-life environmental exposures and their repercussions on adult respiratory health. J Pediatr (Rio J). 2022;98(Suppl 1):S86-S95. doi: 10.1016/j.jped.2021.11.005.
4. Tran TN, Tran NB, Tran HMT, Tang HK, Ngo XM, Godin I, et al. Influence of type of dwelling on the prevalence of chronic respiratory diseases in Ho Chi Minh City, Viet Nam. Int J Tuberc Lung Dis. 2020;24(3):316-320. doi: 10.5588/ijtld.19.0043.
5. McEvoy CT, Spindel ER. Pulmonary effects of maternal smoking on the fetus and child: effects on lung development, respiratory morbidities, and life long lung health. Paediatr Respir Rev. 2017;21:27-33. doi: 10.1016/j.prrv.2016.08.005.
6. Lee AG, Kaali S, Quinn A, Delimini R, Burkart K, Opoku-Mensah J, et al. Prenatal household air pollution is associated with impaired infant lung function with sex-specific effects. Evidence from GRAPHS, a cluster randomized cookstove intervention trial. Am J Respir Crit Care Med. 2019;199(6):738-746. doi: 10.1164/rccm.201804-0694OC.
7. Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. Lancet. 2015;385(9971):899-909. doi: 10.1016/S0140-6736(14)60446-3.
8. Gray D, Czövek D, Smith E, Willemse L, Alberts A, Gingl Z, et al. Respiratory impedance in healthy unsedated South African infants: effects of maternal smoking. Respirology. 2015;20(3):467-473. doi: 10.1111/resp.12463.
9. Gray D, Willemse L, Visagie A, Czövek D, Nduru P, Vanker A, et al. Determinants of early-life lung function in African infants. Thorax. 2017;72(5):445-450. doi: 10.1136/thoraxjnl-2015-207401.
10. Heinzerling AP, Guarnieri MJ, Mann JK, Diaz JV, Thompson LM, Diaz A, et al. Lung function in woodsmoke-exposed Guatemalan children following a chimney stove intervention. Thorax. 2016;71(5):421-428. doi: 10.1136/thoraxjnl-2015-207783.
11. Lajunen K, Kalliola S, Kotaniemi-Syrjänen A, Malmberg LP, Pelkonen AS, Mäkelä MJ. Environmental tobacco smoke affects lung function of preschoolers with asthma even after a decade. Am J Respir Crit Care Med. 2019;199(4):534-536. doi: 10.1164/rccm.201809-1729LE.
12. Gunlemez A, Er İ, Baydemir C, Arisoy A. Effects of passive smoking on lung function tests in preschool children born late-preterm: a preventable health priority. J Matern Fetal Neonatal Med. 2019;32(14):2412-2417. doi: 10.1080/14767058.2018.1430759.
13. Garcia E, Rice MB, Gold DR. Air pollution and lung function in children. J Allergy Clin Immunol. 2021;148(1):1-14. doi: 10.1016/j.jaci.2021.05.006.
14. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP. Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study. Thorax. 2015;70(6):592-594. doi: 10.1136/thoraxjnl-2014-206242.

15. le Roux DM, Myer L, Nicol MP, Zar HJ. Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study. BMJ Open. 2015;5(12):e009111. doi: 10.1136/bmjopen-2015-009111.
16. Myer L, Stein DJ, Grimsrud A, Seedat S, Williams DR. Social determinants of psychological distress in a nationally-representative sample of South African adults. Soc Sci Med. 2008;66(8):1828-1840. doi: 10.1016/j.socscimed.2008.01.025.
17. Zar HJ, Pellowski JA, Cohen S, Barnett W, Vanker A, Koen N, et al. Maternal health and birth outcomes in a South African birth cohort study. PLoS One. 2019;14(11):e0222399. doi: 10.1371/journal.pone.0222399.
18. World Health Organization. WHO child growth standards: length/-height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. World Health Organization; 2006.
19. World Health Organization (WHO). Integrated management of childhood illness: distance learning course; 2014. https://apps.who.int/iris/handle/10665/104772.
20. Vanker A, Barnett W, Workman L, Nduru PM, Sly PD, Gie RP, et al. Early-life exposure to indoor air pollution or tobacco smoke and lower respiratory tract illness and wheezing in African infants: a longitudinal birth cohort study. Lancet Planet Health. 2017;1(8):e328-e336. doi: 10.1016/S2542-5196(17)30134-1.
21. Siemens. Immulite 1000 Nicotine Metabolite2009. Available from: https://www.siemens-healthineers.com/en-za/immunoassay/systems/immulite-1000-immunoassay-system
22. Government Gazette Republic of South Africa. National Ambient Air Quality Standards. 2009 [cited 2014 Aug]. Available from: https://www.environment.gov.za/sites/default/files/legislations/nemaqa_airquality_g32816gon1210.pdf
23. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, et al. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753. doi: 10.1183/13993003.00753-2019.
24. Gray DM, Czovek D, McMillan L, Turkovic L, Stadler JAM, Vanker A, et al. Intra-breath measures of respiratory mechanics in healthy African infants detect risk of respiratory illness in early life. Eur Respir J. 2019;53(2):1800998. doi: 10.1183/13993003.00998-2018.
25. Sly PD, Hantos Z; INCIRCLE Clinical Research Collaboration. The International Collaboration to Improve Respiratory Health in Children (INCIRCLE) ERS Clinical Research Collaboration. Eur Respir J. 2018;52(6):1801867. doi: 10.1183/13993003.01867-2018.
26. Robinson PD, Salimi F, Cowie CT, Clifford S, King GG, Thamrin C, et al. Ultrafine particle exposure and biomarkers of effect on small airways in children. Environ Res. 2022;214(Pt 1):113860. doi: 10.1016/j.envres.2022.113860.
27. Dutta A, Alaka M, Ibigbami T, Adepoju D, Adekunle S, Olamijulo J, et al. Impact of prenatal and postnatal household air pollution exposure on lung function of 2-year old Nigerian children by oscillometry. Sci Total Environ. 2021;755(Pt 2):143419. doi: 10.1016/j.scitotenv.2020.143419.
28. Rive S, Hulin M, Baiz N, Hassani Y, Kigninlman H, Toloba Y, et al. Urinary S-PMA related to indoor benzene and asthma in children. Inhal Toxicol. 2013;25(7):373-382. doi: 10.3109/08958378.2013.790522.

29. Rumchev K, Spickett J, Bulsara M, Phillips M, Stick S. Association of domestic exposure to volatile organic compounds with asthma in young children. Thorax. 2004;59(9):746-751. doi: 10.1136/thx.2003.013680.
30. Junaidi ES, Jalaludin J, Tualeka AR. The evaluation of exposure to benzene among children in indoor environments: a review. Malays J Med Health Sci. 2019;15(3):151-155.
31. Nurmatov UB, Tagiyeva N, Semple S, Devereux G, Sheikh A. Volatile organic compounds and risk of asthma and allergy: a systematic review. Eur Respir Rev. 2015;24(135):92-101. doi: 10.1183/09059180.00000714.

Supplementary information: Further Methodological Details

The Drakenstein Child Health Study is a longitudinal cohort study assessing the impact of risk factors on child health, including environmental, infectious, nutritional, genetic, psychosocial, maternal, and immunological factors. The study area, a peri-urban, low socioeconomic community, is in Paarl, approximately 60 km outside Cape Town, South Africa. Pregnant women were enrolled in their second trimester and followed through childbirth. All births took place at the regional (Paarl) hospital. Birth information was obtained at the time of delivery by study staff members. Visits were synchronised with the national immunisation program, and included visits at 6, 10 and 14 weeks, at 6 and 9 months, and then every 6 months from 12 months onward. Two home visits (one antenatal and one postnatal) were conducted to investigate environmental risk factors, including measurement of indoor air pollution. Anthropometry measurements were recorded at each visit. Robust surveillance for lower respiratory tract infections (LRTI) was undertaken and all events were comprehensively investigated. Study nurses were trained to diagnose LRTI according to the WHO clinical case definitions. Each case of suspected LRTI was examined by study staff and those with confirmed LRTI had a nasopharyngeal swab for respiratory organisms [1, 2].

Table S4.1 Methods and definitions

Measurement	Definition	Collected
Pollutant measurement equipment		
Particulate matter (PM_{10})	personal air sampling pump – SKC Aircheck 52^{R}	-Between 4 and 6 months of life -All measurements were done in the communal/main living room, away from windows and doors, approximately 1.5 meters from the ground
Volatile organic compounds (VOC) benzene and toluene	Markes^{R} thermal desorption tubes using passive diffusion tubes	
National Ambient Air Quality Standards	Expected exposure for each pollutant based on an averaging period of 1 year for each measure: -PM_{10}: 40 μg/m^3 -benzene: 5 μg/m^3 -toluene: 240 μg/m^3 [3]	-Between 4 to 6 months of life -VOC: An average concentration based on the 2-week duration in the home was obtained for volatile organic compounds; -PM_{10}: 24-hour averages were obtained
Urine cotinine measurement	Urine cotinine tests were performed using the IMMULITER 1000 Nicotine Metabolite Kit (Siemens Medical Solutions Diagnostics^{R}, Glyn Rhonwy, United Kingdom) [4].	Antenatal: Maternal urine was collected at the second antenatal study visit and maternal and infant urine at birth. The higher result was used to classify smoking levels. Postnatal: Infant urine was collected at 6 weeks and yearly
Urine cotinine levels	The highest value from two visits: active smoker (cotinine≥ 500 ng/ml), passive smoker (10-499 ng/ml) or non-smoker (<10 ng/ml) [5]. In children these were referred to as high, moderate or no exposure.	
WHO LRTI case definitions	WHO LRTI case definition: cough or difficulty breathing and age-specific tachypnoea or lower chest wall in-drawing [6]. WHO severe LRTI case definition: any child under 2 months of age with signs of LRTI or in a child of any age with danger signs (cyanosed, unable to drink, seizures, or decreased level of consciousness) [6, 7].	
Surveillance for LRTI	Active surveillance for LRTI in the cohort was undertaken using community field workers, a short message system (SMS) phone system, ongoing monitoring of cases at health facilities and study staff who could always be contacted [2].	

Recurrent LRTI	Recurrent LRTI: 2 or more episodes of LRTI in the past 12 months	
Gestational age	Assessed by antenatal ultrasound in the second trimester, if this was unavailable then symphysis-fundal height, recorded by trained clinical staff at enrolment, or maternal recall of last menstrual period was used [8].	Antenatal 2nd trimester ultrasound/examination

VOC: volatile organic compounds, PM_{10} : particulate matter size10 g/m^3, LRTI: lower respiratory tract infection

Table S4.2 Comparison of baseline characteristics between children included in and excluded from analysis

	Total cohort	Included in analysis	Not included in analysis [1]	P-value
Number of children	1,143	584	559	
Enrolment site				
Mbekweni	634 (55%)	288 (49%)	346 (62%)	
TC Newman	509 (45%)	296 (51%)	213 (38%)	<0.001
Mean (SD) maternal age at enrolment	26.6 (5.7)	26.8 (5.7)	26.4 (5.6)	0.200
Maternal education				
Less than secondary	695 (61%)	358 (61%)	337 (60%)	
Secondary/any tertiary	448 (39%)	226 (39%)	222 (40%)	0.725
Housing type: Informal shack	419 (37%)	189 (33%)	230 (41%)	0.002
Parent employed	580 (51%)	300 (51%)	280 (50%)	0.665
Household income per month (South African Rand)				
<R1000/m	386 (34%)	199 (34%)	187 (34%)	
R1000-5000/m	596 (52%)	305 (52%)	291 (52%)	
>R5000/m	160 (14%)	80 (14%)	80 (14%)	0.946
Male sex	586 (51%)	289 (49%)	297 (53%)	0.218
Delivery mode: Caesarean section	230 (20%)	118 (20%)	112 (20%)	0.996
Gestation at delivery				
<32 weeks	33 (3%)	0 (0%)	33 (6%)	
≥32 and <37 weeks	159 (14%)	74 (13%)	85 (15%)	
≥37 weeks	951 (83%)	510 (87%)	441 (79%)	<0.001
Mean (SD) birth weight z-score	-0.3 (1.1)	-0.3 (1.1)	-0.3 (1.1)	0.446
Mean (SD) birth length z-score	0.4 (1.6)	0.4 (1.6)	0.4 (1.6)	0.635
HIV-exposed	248 (22%)	110 (19%)	138 (25%)	0.016
Antenatal exposure to indoor air pollutants above ambient standards				
PM_{10} (n=767)	345 (45%)	179 (44%)	166 (46%)	0.599
Benzene (n=738)	334 (45%)	170 (43%)	164 (48%)	0.244
Toluene (n=738)	67 (9%)	33 (8%)	34 (10%)	0.429
Benzene and/or Toluene (n=738)	336 (46%)	171 (44%)	165 (48%)	0.240
Exposure to Antenatal tobacco smoke				
Antenatal exposure (n=1,093):				
No exposure	249 (23%)	102 (18%)	147 (28%)	
Moderate exposure	491 (45%)	262 (46%)	229 (43%)	
High exposure	353 (32%)	202 (36%)	151 (29%)	<0.001

[1] Includes children who did not attend 3-year visit, and children who attended visit but had no successful lung function tests; PM_{10}: particulate matter size10 □g/m^3

Table S4.3 Oscillometry and multiple-breath washout measures at 6 weeks and 3 years of age

	6 weeks		3 years	
	n with data	**Mean (SD)**	**n with data**	**Mean (SD)**
R_{eE} (hPa.s.L^{-1})	434	45.56 (12.67)	439	12.36 (2.85)
X_{eE} (hPa.s.L^{-1})	434	-8.23 (8.22)	439	-2.63 (1.72)
R_{eI} (hPa.s.L^{-1})	434	40.60 (13.67)	439	10.86 (2.43)
X_{eI} (hPa.s.L^{-1})	434	-5.30 (5.13)	439	-3.22 (1.38)
R_{mean} (hPa.s.L^{-1})	434	53.91 (18.50)	439	12.77 (2.79)
X_{mean} (hPa.s.L^{-1})	434	-8.96 (7.92)	439	-3.45 (1.52)
ΔR (hPa.s.L^{-1})	434	4.96 (7.65)	439	1.49 (1.68)
ΔX (hPa.s.L^{-1})	434	-2.93 (7.37)	439	0.60 (1.43)
FRC (L)	572	0.08 (0.02)	495	0.46 (0.09)
LCI (number of turnovers)	572	7.15 (0.44)	495	8.23 (1.24)
Respiratory rate (min^{-1})	608	48.89 (11.30)	491	27.92 (5.63)
Tidal volume (mL)	608	34.75 (5.96)	498	179.23 (28.65)
t_E/t_{TOT}	608	54.98 (4.51)	498	57.78 (3.30)
t_{PTEF}/t_E	608	38.10 (12.22)	490	42.96 (12.20)

R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

Table S4.4 Correlation matrix for the different exposures, among children with successful lung function tests

	Antenatal PM_{10}	**Postnatal PM_{10}**	**Antenatal Benzene**	**Postnatal Benzene**	**Antenatal Toluene**	**Postnatal Toluene**	**Antenatal cotinine**	**Postnatal cotinine**
Antenatal PM_{10}	-							
Postnatal PM_{10}	0.013	-						
Antenatal Benzene	-0.010	-0.024	-					
Postnatal Benzene	0.008	0.117	-0.020	-				
Antenatal Toluene	0.027	-0.028	0.500 *	-0.068	-			
Postnatal Toluene	-0.012	0.218 *	0.012	0.720 *	-0.043	-		
Antenatal cotinine	-0.084	0.090	-0.005	-0.017	-0.011	0.060	-	
Postnatal cotinine	-0.030	0.121	-0.009	-0.013	0.018	0.114	0.618 *	-

PM_{10}: particulate matter size $10ug/m^3$; * $p<0.05$

Table S4.5 Impact of postnatal exposure to PM_{10} above ambient standards on lung function at 3 years of age

	Unadjusted models			Adjusted model		
	n	β [95% CI]	P-value	n	β [95% CI]	P-value
R_{eE} (hPa.s.L^{-1})	203	0.31 [-0.51, 1.12]	0.457	119	0.64 [-0.35, 1.63]	0.205
X_{eE} (hPa.s.L^{-1})	203	0.20 [-0.31, 0.71]	0.435	119	0.14 [-0.50, 0.77]	0.672
R_{eI} (hPa.s.L^{-1})	203	0.01 [-0.70, 0.73]	0.970	119	0.62 [-0.27, 1.51]	0.168
X_{eI} (hPa.s.L^{-1})	203	0.37 [-0.03, 0.76]	0.067	119	0.00 [-0.52, 0.52]	0.999
R_{mean} (hPa.s.L^{-1})	203	0.30 [-0.52, 1.13]	0.470	119	0.78 [-0.22, 1.79]	0.125
X_{mean} (hPa.s.L^{-1})	203	0.25 [-0.20, 0.70]	0.276	119	0.05 [-0.48, 0.58]	0.854
ΔR (hPa.s.L^{-1})	203	0.29 [-0.17, 0.76]	0.217	119	-0.03 [-0.61, 0.55]	0.916
ΔX (hPa.s.L^{-1})	203	-0.16 [-0.55, 0.23]	0.408	119	0.20 [-0.32, 0.72]	0.438
FRC (L)	243	-0.02 [-0.04, 0.00]	0.100	189	-0.02 [-0.05, 0.00]	0.092
LCI (number of turnovers)	243	0.24 [-0.08, 0.55]	0.138	189	0.25 [-0.11, 0.62]	0.170
Respiratory rate (min^{-1})	236	1.42 [-0.07, 2.91]	0.062	199	0.77 [-0.70, 2.25]	0.300
Tidal volume (mL)	239	1.62 [-6.51, 9.75]	0.696	202	1.05 [-7.48, 9.59]	0.808
t_E/t_{TOT}	239	0.01 [-0.90, 0.92]	0.980	202	-0.14 [-1.15, 0.88]	0.792
t_{PTEF}/t_E	234	2.19 [-1.18, 5.56]	0.201	198	1.24 [-2.38, 4.86]	0.500

Adjusted model: adjusted for enrolment site, sex, socioeconomic status, BMI z-score, previous LRTI, and lung function parameter at 6 weeks of age.

R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

Table S4.6 Impact of postnatal exposure to Benzene and/or Toluene above ambient standards (versus below ambient standards for both) on lung function at 3 years of age

	Unadjusted models			Adjusted model 2		
	n	β [95% CI]	P-value	n	β [95% CI]	P-value
R_{eE} (hPa.s.L^{-1})	181	**-1.12 [-2.01, -0.22]**	**0.015**	107	-1.01 [-2.16, 0.14]	0.085
X_{eE} (hPa.s.L^{-1})	181	0.45 [-0.11, 1.01]	0.112	107	0.48 [-0.25, 1.22]	0.196
R_{eI} (hPa.s.L^{-1})	181	**-0.79 [-1.58, 0.00]**	**0.049**	107	-0.58 [-1.62, 0.47]	0.279
X_{eI} (hPa.s.L^{-1})	181	**0.45 [0.03, 0.88]**	**0.037**	107	0.54 [-0.03, 1.10]	0.062
R_{mean} (hPa.s.L^{-1})	181	**-0.92 [-1.83, -0.02]**	**0.046**	107	-0.75 [-1.91, 0.42]	0.205
X_{mean} (hPa.s.L^{-1})	181	0.41 [-0.08, 0.90]	0.099	107	0.46 [-0.12, 1.03]	0.117
ΔR (hPa.s.L^{-1})	181	-0.33 [-0.86, 0.21]	0.230	107	-0.44 [-1.15, 0.28]	0.228
ΔX (hPa.s.L^{-1})	181	0.00 [-0.46, 0.46]	0.997	107	-0.04 [-0.68, 0.60]	0.908
FRC (L)	220	0.01 [-0.02, 0.03]	0.503	175	0.00 [-0.03, 0.03]	0.994
LCI (number of turnovers)	220	0.04 [-0.31, 0.38]	0.839	175	0.13 [-0.27, 0.53]	0.527
Respiratory rate (min^{-1})	210	**1.65 [0.02, 3.27]**	**0.047**	182	1.39 [-0.17, 2.96]	0.081
Tidal volume (mL)	214	-0.56 [-9.20, 8.08]	0.898	186	0.87 [-8.07, 9.81]	0.848
t_E/t_{TOT}	214	-0.26 [-1.19, 0.68]	0.591	186	-0.29 [-1.30, 0.73]	0.578
t_{PTEF}/t_E	208	-1.18 [-4.92, 2.56]	0.535	181	-0.86 [-4.73, 3.01]	0.662

Adjusted model: adjusted for enrolment site, sex, socioeconomic status, BMI z-score, previous LRTI, and lung function parameter at 6 weeks of age.

R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

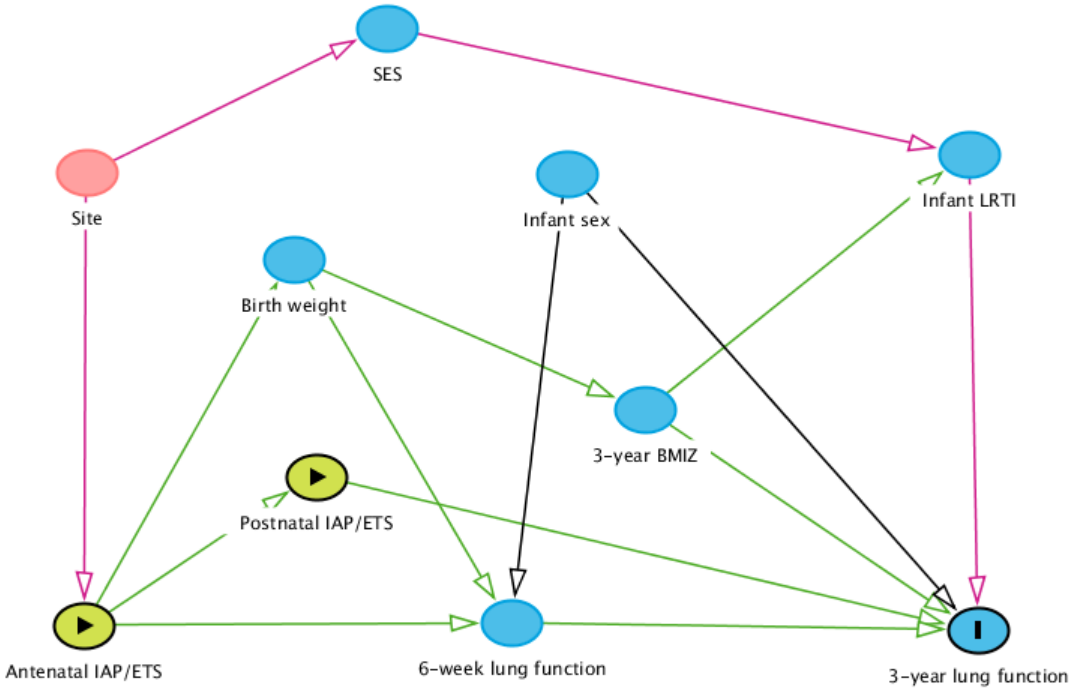
Table S4.7 Impact of postnatal exposure to moderate and high levels of tobacco smoke, versus low levels on lung function at 3 years of age

		Unadjusted models			Adjusted model 2		
		n	β [95% CI]	P-value	n	β [95% CI]	P-value
R_{eE} (hPa.s.L^{-1})	Moderate	379	0.17 [-0.50, 0.84]	0.628	237	-0.32 [-1.16, 0.52]	0.456
	High		**1.27 [0.15, 2.39]**	**0.026**		-0.30 [-1.88, 1.28]	0.707
X_{eE} (hPa.s.L^{-1})	Moderate	379	0.12 [-0.28, 0.53]	0.555	237	0.15 [-0.34, 0.64]	0.552
	High		0.01 [-0.67, 0.68]	0.988		0.54 [-0.39, 1.47]	0.256
R_{eI} (hPa.s.L^{-1})	Moderate	379	0.46 [-0.11, 1.02]	0.115	237	0.07 [-0.68, 0.82]	0.848
	High		0.73 [-0.22, 1.68]	0.130		-0.14 [-1.55, 1.26]	0.840
X_{eI} (hPa.s.L^{-1})	Moderate	379	-0.08 [-0.41, 0.25]	0.628	237	0.08 [-0.35, 0.51]	0.702
	High		-0.04 [-0.59, 0.50]	0.876		0.05 [-0.76, 0.86]	0.906
R_{mean} (hPa.s.L^{-1})	Moderate	379	0.16 [-0.49, 0.82]	0.626	237	-0.37 [-1.21, 0.48]	0.394
	High		0.95 [-0.14, 2.04]	0.087		-0.17 [-1.76, 1.42]	0.835
X_{mean} (hPa.s.L^{-1})	Moderate	379	0.09 [-0.27, 0.45]	0.617	237	0.19 [-0.25, 0.62]	0.401
	High		-0.13 [-0.73, 0.48]	0.682		0.10 [-0.72, 0.92]	0.814
ΔR (hPa.s.L^{-1})	Moderate	379	-0.29 [-0.69, 0.11]	0.154	237	-0.36 [-0.86, 0.13]	0.147
	High		0.54 [-0.13, 1.21]	0.113		-0.07 [-1.01, 0.86]	0.874
ΔX (hPa.s.L^{-1})	Moderate	379	0.20 [-0.13, 0.53]	0.226	237	0.05 [-0.36, 0.47]	0.798
	High		0.05 [-0.50, 0.60]	0.861		0.38 [-0.41, 1.17]	0.345
FRC (L)	Moderate	428	-0.01 [-0.03, 0.02]	0.586	326	-0.01 [-0.04, 0.02]	0.495
	High		-0.02 [-0.06, 0.02]	0.258		-0.03 [-0.08, 0.01]	0.163
LCI (number of turnovers)	Moderate	428	0.01 [-0.27, 0.29]	0.948	326	0.14 [-0.21, 0.49]	0.428
	High		-0.20 [-0.68, 0.27]	0.401		0.12 [-0.47, 0.72]	0.690
Respiratory rate(min^{-1})	Moderate	427	0.69 [-0.61, 1.99]	0.297	350	0.88 [-0.54, 2.31]	0.224
	High		-1.10 [-3.25, 1.06]	0.317		-0.64 [-3.10, 1.82]	0.607
Tidal volume (mL)	Moderate	434	-4.60 [-11.08, 1.88]	0.163	357	0.89 [-6.47, 8.25]	0.813
	High		-2.74 [-13.50, 8.01]	0.616		8.10 [-4.57, 20.77]	0.209
t_E/t_{TOT}	Moderate	434	-0.46 [-1.19, 0.27]	0.213	357	-0.55 [-1.42, 0.32]	0.217
	High		0.36 [-0.85, 1.57]	0.559		-0.06 [-1.55, 1.44]	0.942
t_{PTEF}/t_E	Moderate	428	-2.52 [-5.20, 0.16]	0.066	352	-1.71 [-4.83, 1.42]	0.283
	High		-4.14 [-8.57, 0.28]	0.067		-2.36 [-7.71, 2.99]	0.386

Adjusted model: adjusted for enrolment site, sex, socioeconomic status, BMI z-score, previous LRTI, and lung function parameter at 6 weeks of age.

R_{eE}, resistance at end-expiration; X_{eE}, reactance at end-expiration; R_{eI}, resistance at end inspiration; X_{eI}, reactance at end inspiration, R_{mean}, mean resistance; X_{mean}, mean reactance; ΔR, R_{eE}-R_{eI}; ΔX, X_{eE}-X_{eI}, *FRC*, functional residual capacity; *LCI*, lung clearance index; t_E/t_{TOT}, ratio time of expiration to total time; t_{PTEF}/t_E, ratio time of peak total expiratory flow to time of expiration.

Figure S4.1 Directed acyclic graph constructed to select minimum set of adjustment variables



Supplementary references

1. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP. Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study. Thorax. 2015;70(6):592-594. doi: 10.1136/thoraxjnl-2014-206242.
2. le Roux DM, Myer L, Nicol MP, Zar HJ. Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study. BMJ Open. 2015;5(12):e009111. doi: 10.1136/bmjopen-2015-009111.
3. Government Gazette Republic of South Africa. National Ambient Air Quality Standards. 2009 [cited 2014 Aug]. Available from: https://www.environment.gov.za/sites/default/files/legislations/nemaqa_airquality_g32816gon1210.pdf
4. Siemens. Immulite 1000 Nicotine Metabolite. UK, 2009. Available from: https://www.siemens-healthineers.com/en-za/immunoassay/systems/immulite-1000-immunoassay-system.
5. Vanker A, Barnett W, Brittain K, Gie RP, Koen N, Myers B, et al Antenatal and early life tobacco smoke exposure in an African birth cohort study. Int J Tuberc Lung Dis. 2016;20(6):729-737. doi: 10.5588/ijtld.15.0697.
6. World Health Organization (WHO). Integrated management of childhood illness: distance learning course; 2014. https://apps.who.int/iris/handle/10665/104772.
7. Zar HJ, Barnett W, Stadler A, Gardner-Lubbe S, Myer L, Nicol MP. Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study. Lancet Respir Med. 2016;4(6):463-472. doi: 10.1016/S2213-2600(16)00096-5.
8. Zar HJ, Pellowski JA, Cohen S, Barnett W, Vanker A, Koen N, et al. Maternal health and birth outcomes in a South African birth cohort study. PLoS One. 2019;14(11):e0222399. doi: 10.1371/journal.pone.0222399.

Chapter 5: The effect of moderate to late preterm birth on lung function over the first 5 years of life in a South African birth cohort

---

Chaya S[1], Simpson SJ[2,3], Marozva N[1], Jacobs C[1], Botha M[1], Workman L[1], Hantos Z[4], Zar HJ[1], Gray D[1]. The effect of moderate-to-late preterm birth on lung function over the first 5 years of life in a South African birth cohort. ERJ Open Res. 2025;11(3):00733-2024. doi: 10.1183/23120541.00733-2024.

Affiliations:

1. Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town, Cape Town, South Africa
2. Wal-yan Respiratory Centre, Telethon Kids Institute, Perth, Australia
3. School of Allied Health, Curtin University, Perth, Australia;
4. Department of Anaesthesiology and Intensive Therapy, Semmelweis University, Budapest, Hungary

Author contributions:

SC contributed to the conceptualisation, methodology, data collection, analyses and writing of original draft. LM contributed to the data curation and analysis. NM completed the data

curation and analysis. CJ contributed to data collection and manuscript. MB contributed to data collection and the manuscript. SS contributed to conceptualisation, methodology, supervision and writing of the original draft. ZH contributed to methodology, data acquisition and analysis, developed Oscillometry aspects of study. HJZ contributed to conceptualisation, funding acquisition, methodology, supervision and review of original draft. DMG contributed to lung function conceptualisation, data collection, funding acquisition, methodology, supervision, analyses and writing of original draft. All authors contributed to the final manuscript.

Word count: 3527

Abstract

Background: Preterm birth is associated with increased mortality and morbidity, particularly due to lung disease. Low-middle income countries (LMIC) have the highest rates of preterm birth. Infants born extremely preterm rarely survive, so the largest burden is amongst moderate to late preterm (MLP) infants. The long-term health impact on MLP children in LMIC is poorly understood.

Aim: To assess the effect of MLP birth on lung function trajectories from birth to 5 years in the birth cohort Drakenstein Child Health Study. Methods: Children were followed from birth with lung function measurements at 6 weeks and annually until 5 years. Exposures were longitudinally collected from the antenatal period through 5 years of age. Lung function tests included spectral and intra-breath oscillometry, tidal breathing and multiple breath washout. Mixed-effect models adjusted using a minimum set of variables were used.

Results: Of 923 children, 119 (13%) were born MLP [32-<37 weeks gestation; median gestational age (GA) 35 weeks] and 19 (2%) <32 weeks gestation. Children born MLP had altered lung function trajectories. Spectral oscillometry demonstrated a 13% reduction in compliance (0.87, 95% CI: 0.82; 0.92), 5% increase in resistance at end expiration (1.05, 95% CI: 1.01; 1.09) and 1.74 Hz (95% CI: 1.10; 2.39) increase in resonance frequency over 5 years compared to term children. The proportion of children hospitalised for lower respiratory tract infections (LRTI) was higher in preterm born children (29 (23.6%) MLP; 18 (94.7%) extreme to very preterm; 109 (14%) term born children; p<0.01). LRTI and tobacco smoke exposure were associated with impaired lung function, but the impact of MLP on lung function was independent of these.

Conclusion: MLP born children have impaired lung function trajectories over the first 5 years of life, highlighting the importance of strengthening maternal health, perinatal care and strategies to reduce tobacco smoke exposure and early life LRTI.

5.1 Introduction

Approximately 13.4 million infants are born preterm annually [1], with a particularly high burden in low- and middle-income countries (LMIC), especially Africa and South East Asia [1]. Preterm born infants are grouped as extremely preterm (less than 28 weeks), very preterm (28-32 weeks) or moderate to late preterm (MLP) (32 to 37 weeks), with MLP births making up 85% of global prematurity [2, 3]. Survival rates of preterm infants, especially very preterm are significantly lower in LMIC compared to high income countries (HIC), reflecting limited access to neonatal intensive resources [1]. Resultingly, infants born < 32 weeks gestation rarely survive in LMIC. Poor long-term outcomes in survivors of preterm birth are well reported in all preterm groups in HIC, with earlier gestation correlated with the poorest outcomes [4]. However, data on long-term outcomes of preterm birth are lacking from LMIC settings, where poor outcome may be more widespread in the MLP groups due to social disadvantage, high burden of infectious diseases and limited access to care.

The respiratory system is particularly susceptible to the long term sequalae of prematurity [5]. Studies from survivors of very and extremely preterm birth in HIC report increased respiratory symptoms, increased frequency and severity of lower respiratory tract infection (LRTI) and lower lung function through childhood compared to term born children [6, 7]. However, limited attention has been given to the MLP group. A recent meta-analysis of 15 spirometric studies (n=847 individuals between 5-25 years; all from HIC) showed that MLP-born individuals have higher rates of airway obstruction, with lower forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC and forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}) compared to those born at term [8]. These deficits were further exacerbated in children exposed to passive smoking [9]. However, reliable spirometry is difficult to obtain in young children. As such, other lung function tests

(such as oscillometry and multiple breath washout) which can be performed in young children, may provide viable options to the early assessment of lung disease [10]. Further, these tools assess the peripheral lung, including the smaller airways which are increasingly recognised as an important indicator of early lung disease.

There is increasing recognition of the role of prematurity in lifelong lung disease, particularly in the development of chronic obstructive pulmonary disease (COPD) [11], with changes in lung development (from prematurity) and adverse early life exposures recently named as COPD etiotypes [12]. As such, the identification of "at-risk" individuals in early life is key. The aim of this study was to assess the impact of prematurity, particularly MLP birth, on lung growth and function, longitudinally, from birth to 5 years in a South African birth cohort. Further, we aimed to identify environmental exposures impacting on lung function trajectories in those born preterm.

5.2 Methods

This was a prospective study of children enrolled in The Drakenstein Child Health Study (DCHS) a longitudinal birth cohort in a peri-urban area outside Cape Town, South Africa. Pregnant mothers were enrolled between 5 March 2012 to 31 March 2015 in their second trimester (20-28 weeks gestation) at public sector clinics and followed through pregnancy as previously described [13].

Maternal health was assessed at enrolment, antenatal follow up visits and at birth using questionnaires and physical examination performed by trained study staff [14]. Antenatal and postnatal maternal smoking as well as postnatal household smoke exposure information was self reported [13]. High blood pressure was defined as a BP > 140/90 mmHg and

preeclampsia as high blood pressure after 20 weeks with proteinuria or other organ dysfunction. Anaemia was defined as haemoglobin < 10 g/dl. Socioeconomic status (SES) was assessed antenatally by questionnaires including information on employment status, maternal educational attainment, household income, assets and market access (bank accounts, shops accessed, retail accounts).

Gestational age was estimated using antenatal ultrasound measurements in the second trimester; if this was unavailable then symphysis-fundal height and maternal recall of the last menstrual period was used. All deliveries were attended by study staff. The WHO definition of preterm birth was used to classify moderate to late (32-<37 weeks gestation), very (28-<32 weeks) and extremely (<28 weeks gestation) preterm birth [2]. Preterm birthweight for gestational age z-score was calculated using the Fenton growth charts [15]. Full-term birth and all anthropometry measured post birth were calculated using WHO growth standards chart (WHO, Geneva, Switzerland) [16]. Those eligible for maternal antenatal steroids were mothers who were in preterm labour with a pregnancy gestation 27 to 34 weeks; infants were eligible for surfactant if they were born at 28 to 34 weeks GA as per the local hospital protocol.

Mothers and infants were followed until discharge. Regular follow up visits were then performed at 6, 10, 14 weeks, 6, 9, 12 months and thereafter 6 monthly at a local clinic [14]. Anthropometry was measured from birth and at all study visits. Lower respiratory tract infection (LRTI) surveillance was established through community health workers, use of cell phones, a dedicated study contact person available 24 hours, and a network of study staff at community-based sites. Each suspected case of LRTI was examined by study staff to confirm the presence of LRTI as previously described [17]. The WHO clinical case definitions of

pneumonia were used to define LRTI [17]. Lung function was done at least 4 weeks after an episode of LRTI. A detailed explanation of each of the variables and outcomes used in this study are presented in table S1.

5.2.1 Lung function measures

Lung function testing was performed at the local hospital study site by the trained study team. Lung function measurements were done at 5-11 weeks and then yearly until 5 years. Tests included oscillometry custom-built wave-tube equipment (University of Szeged, Hungary), tidal breathing flow volume loops (TBFVL) and multiple breath washout (MBW) Exhalyzer®D with an ultrasonic flow metre (Ecomedics, Duernten, Switzerland). Tests were performed in accordance with international guidelines as previously described [18]. Oscillometry measured the respiratory impedance (Z_{rs}), expressed as respiratory system resistance (R_{rs}) and reactance (X_{rs}). An increased R_{rs} and more negative X_{rs} would reflect reduced lung function (impairment in respiratory system mechanics). We measured both standard spectral measures [compliance (C_{rs}), R_{rs}, frequency dependence of R_{rs} (R_6-R_{20}), resonance frequency (F_{res}) and X_{rs} area below Fres (A_X)] and intra-breath measures [R_{rs} at end inspiration (R_{eI}) and at end expiration (R_{eE}), X_{rs} at end expiration (X_{eE}) and end inspiration (X_{eI}), and their tidal changes R_{eE}-R_{eI} (ΔR) and X_{eE}-X_{eI} (ΔX)]. Tidal breathing measures included respiratory rate (*RR*) and tidal volume (*VT*). MBW measures are functional residual capacity (*FRC*) and lung clearance index (*LCI*), an index of ventilation homogeneity. Increased *LCI* indicates ventilation inhomogeneity. Measurements at 5-11 weeks, 1 and 2 years were performed in unsedated infants using a face mask during quiet sleep in supine position. At 3 to 5 years lung function tests were performed in awake children, seated, breathing through a mask for MBW and TBFVL; and a mouthpiece for oscillometry. (See

Supplementary document for detailed methodology)

5.2.2 Ethics

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009; 423/2012; 048/2020) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their first language for their infants to participate, and were re-consented yearly thereafter.

5.2.3 Statistical analysis

Data was analysed using STATA 14.1 (STATA Corporation, College Station, TX USA) and R (R Core team 2021, version 4.1.2). Data were reported as means (and standard deviations), frequencies and proportions as appropriate. Two-tailed ANOVA, Student T-tests, Pearson Chi-squared and Fisher's Exact test, were used to examine differences of participant characteristics between the preterm and full-term group, as well as differences between children included vs excluded from the final analysis. Data that were not normally distributed were transformed (logged).

A univariate analysis was done to investigate the association between exposures and lung function outcomes. The model selection process was done using a directed acyclic graph (DAG) produced on dagitty.net (Figure S1). The DAGs minimal set of variables (sex, height, age, indicator of asleep vs awake and interaction between age and indicator, HIV exposure, birth weight, maternal antenatal smoking, household income, LRTI counts) were used in mixed-effect models to assess the impact of preterm birth on lung function outcomes over a 5-year period. (Table S5.6) Each outcome was included in individual models. Robust standard errors (to account for heteroscedasticity) were used in the estimation.

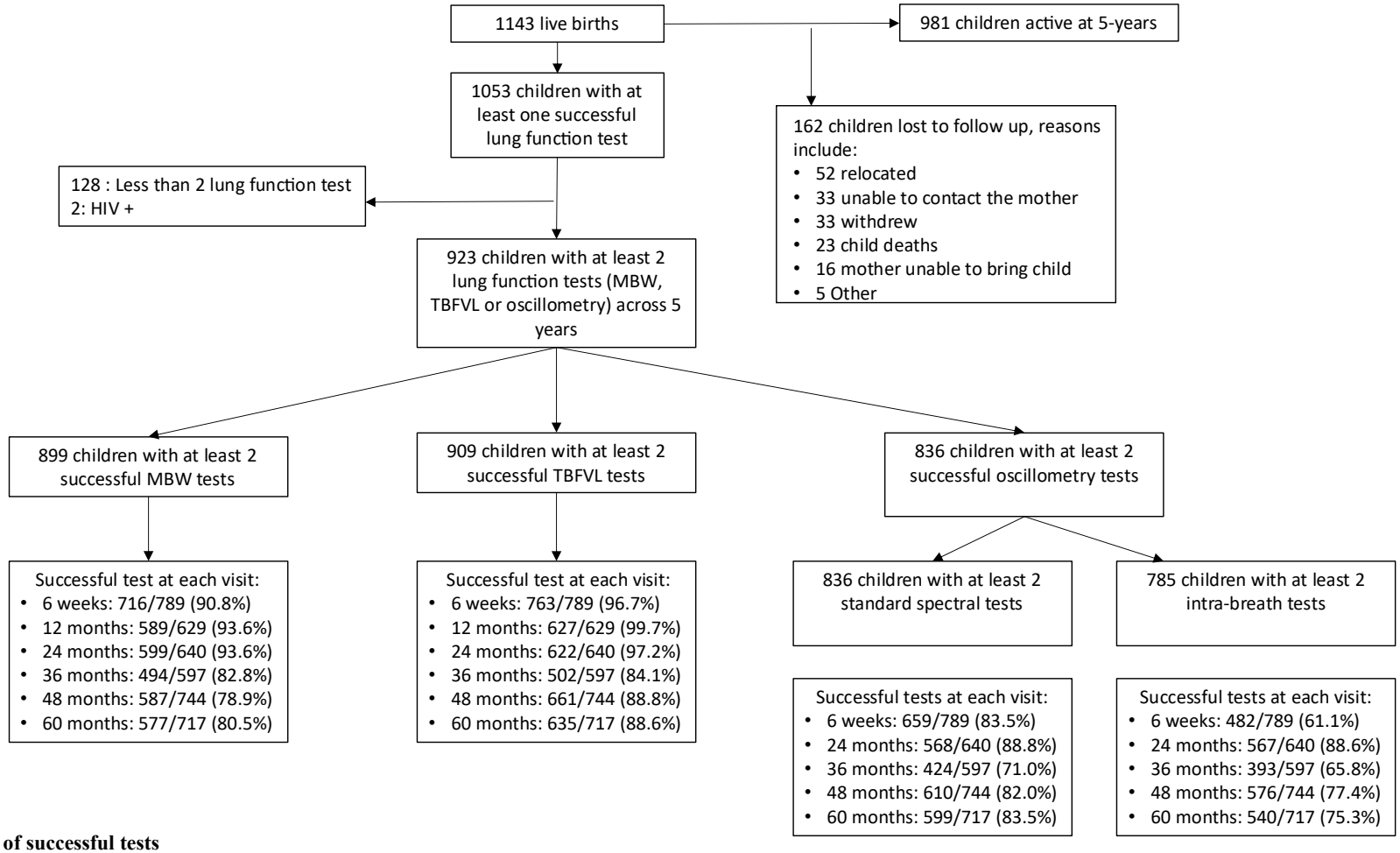
Further, as some of the measures were impacted by whether the child was asleep (during the first 24 months) or awake (from 36 months onwards) at the time of the test, an indicator variable was created to distinguish between whether the child was awake or not. The indicator variable and an interaction between the indicator variable and visit was also considered. (See Supplementary document)

5.3 Results

5.3.1 Characteristics of the cohort

A total of 1137 mothers delivered 1143 live births. Of these 923 (88%) children had at least 2 successful lung function tests over 5 years and were included in analysis: Amongst these, 785 (85%) children were born term (median gestational age (GA): 39 weeks), 119 (13%) MLP (32-<37 weeks gestation; median GA: 35 weeks), and 19 (2%) extremely to very preterm (<32 weeks gestation; median GA: 30 weeks; $p<0.001$). For the purposes of this study, very and extreme preterm birth were combined as the cohort contained only a few extreme preterm births. Cohort retention over the 5 years was 81%. Figure 5.1 shows the study profile.

Figure 5.1 Flow diagram of lung function testing in the Drakenstein Child Health Study



Number of successful tests

Number of LF tests	MBW	TBFVL	Oscillometry	
			Standard spectral	Intra breath
2	126	91	217	249
3	208	200	246	259
4	261	230	252	203
5	206	237	121	74

Median maternal age at the time of delivery, mothers living with HIV and receiving ART were similar among the three gestational age groups. Measures of SES, maternal education and household income, were similar between groups. Mothers with high blood pressure and/or pre-eclampsia had higher rates of MLP preterm births whereas mothers with diabetes had higher rates of very to extreme preterm births. Antenatal steroids in preterm labor were given to 71.4% and 50% of mothers of children born MLP and very preterm respectively. Data on antenatal steroids were not available for all eligible patients. Maternal antenatal and postnatal smoking were present in 28% and 39% and 53% and 63% of MLP and extreme to very preterm born children, respectively (Table 5.1). Characteristics of participants included and excluded are shown in Table S5.2. The two groups were similar except for maternal antenatal, postnatal smoking and postnatal household smoke exposure which were all lower in those excluded ($p<0.001$).

The median birth weight was 2.52 kg for MLP infants, 1.34 kg for extreme preterm infants, and 3.17 kg for term infants, respectively ($p<0.001$). A total of 169 (21.5%) infants were HIV exposed uninfected (HEU), with similar frequency across preterm groups. Two children were HIV-infected and were excluded from the analysis. Height-for-age z-score at birth was similar between preterm and term children, however children born MLP had higher weightfor-age z-score (WAZ) mean (SD) -0.02 (1.24) compared to those born at term -0.33 (1.03).

Surfactant was administered to a total of 6 children, 1 (16.7%) MLP and 5 (33.3%) very to extremely preterm infants. More children in the preterm groups, particularly the <32 week infants, required supplementary oxygen and ventilation after birth compared to those born at term, although numbers were small: the number (%) of children requiring oxygen was 10

(1.3%), 7 (5.9%) and 9 (47.4%) in the term, MLP and extreme to very preterm infants respectively, p=0.391; the number of infants requiring ventilation was 8 (1%), 7 (5.9%) and 11 (57.9%) in the respective groups, p=<0.001.

LRTI during the first 5 years of life was more frequent in children born MLP compared to term, [60 (50.4%) MLP vs 375 (47.8%) term vs 16 (84.2%) extreme to very preterm, p<0.007] and hospitilised [109 (14%) MLP vs 29 (23.6%) term vs 18 (94.7%) extreme to very preterm, p<0.001]. The median age of the first and hospitalised LRTI in the first 5 years of life were lower in MLP children compared to term born, however did not show any significance (Table 5.1).

Table 5.1 Characteristics of included children, n=923

	Full-term (785)	Moderate to late preterm (n=119)	Extremely to very preterm (n=19)	P-value
Maternal and household characteristics				
Number of mothers	783 (85.2%)	117 (12.7%)	19 (2.1%)	
Age at delivery (years)Ω	26.59 (22.53, 31.16)	25.2 (21.47, 30.43)	25.48 (20.36, 33.48)	0.310
BMI at enrollment Ω	26.11 (22.32, 30.82)	24.03 (20.34, 28.24)	23.78 (19.49, 28.04)	<0.001
Antenatal steroids n (%)¥	0	5/7 (71.4%)	6/12 (50%)	0.277
Maternal antenatal smoking	242 (31.0%)	34 (28.57%)	10 (52.6%)	0.106
Maternal postnatal smoking	303 (38.6%)	45 (37.8%)	12 (63.2%)	0.091
Maternal education				
Primary	60 (7.6%)	6 (5.04%)	2 (10.5%)	
Some secondary	422 (53.7%)	70 (58.8%)	14 (73.7%)	
Complete secondary	255 (32.5%)	36 (30.3%)	3 (15.8%)	
Tertiary	48 (6.1%)	7 (5.9%)	0 (0.0%)	0.440
Household income per month				
<1000 ZAR [$54]	273 (34.8%)	41 (34.5%)	10 (52.6%)	
1000-5000 ZAR	410 (52.2%)	64 (53.8%)	8 (42.1%)	
>5000 ZAR [$270]	102 (13.0%)	14 (11.8%)	1 (5.3%)	0.542
Maternal illnesses during pregnancy				
Asthma**	9 (1.2%)	4 (3.4%)	0 (0.0%)	0.169
Diabetes	7 (0.9%)	3 (2.5%)	2 (10.5%)	<0.001
Anemia	189 (24.1%)	18 (15.1%)	1 (5.3%)	0.018
Pre-eclampsia	13 (1.7%)	8 (6.7%)	1 (5.3%)	0.002
High blood pressure	26 (3.3%)	14 (11.8%)	1 (5.3%)	<0.001
Maternal HIV	169 (21.5%)	23 (19.3%)	4 (21.1%)	0.861
Antiretroviral regimen	146 (87.4%)	20 (87%)	3 (75%)	0.496
Child characteristics				
Gestational age Ω	39 (38, 40)	35 (34, 36)	30 (28, 31)	<0.001
Sex – male	400 (51.0%)	62 (52.1%)	8 (42.1%)	0.720
Birth weight (kg)Ω	3.17 (2.84, 3.46)	2.52 (2.13, 2.94)	1.34 (1.04, 1.52)	<0.001
Weight for age z-score birth#	-0.33 (1.03)	-0.02 (1.24)	-0.03 (0.99)	0.008
Height for age z-score birth#	0.40 (1.67)	0.36 (1.38)	0.57 (1.37)	0.890
Birth length	50 (48, 52)	47 (45, 50)	41 (35, 42)	<0.001
Breastfeeding (months)Ω	7 (2, 23.56)	6.03 (2.7, 18.07)	6 (4, 23.72)	0.863

Exclusive breastfeeding (months) Ω	1.61 (0.69, 3.22)	1.84 (0.69, 3.47)	1.84 (1, 4.6)	0.311
Surfactant administered n (%)	0	1/6 (16.7%)	5/15 (33.3%)	0.445
Oxygen supplement n (%)	10 (1.3%)	7 (5.9%)	9 (47.4%)	0.391
(days) ΩΔ	2 (1,7)	1 (1, 2)	7 (7, 13)	0.01
Any ventilation n (%)	8 (1%)	7 (5.9%)	11 (57.9%)	<0.001
(days) Ω	2 (1, 4)	2 (2, 3)	3 (2,9)	0.317
Non-invasive ventilation n (%):	7 (0.9%)	7 (5.9%)	11 (57.9%)	0.132
(days) Ω	1 (1, 3)	2 (2, 3)	3 (2, 9)	0.10
Invasive ventilation n (%):	2 (0.3%)	1 (0.8%)	2 (10.5%)	0.716
(days) Ω	5 (3, 7)	1 (1, 1)	1.5 (1, 2)	0.207
Child exposures				
Household smoke exposure	618 (78.7%)	91 (76.5%)	18 9 (94.7%)	0.195
Any LRTI episode (0-5 years)	375 (47.8%)	60 (50.4%)	16 (84.2%)	0.007
Any Hospitalised LRTI episode (0-5 years)	109 (14.0%)	29 (23.6%)	18 (94.7%)	<0.001
Age (months) of 1st LRTI (0-5 years) Ω	5.52 (2.78, 13.31)	4.77 (2.32, 11.71)	4.61 (2.19, 8.82)	0.325
Age (months) of 1st hospitilised LRTI (0-5 years) Ω	6.91 (2.02, 14.34)	2.43 (1.9, 10.51)	3.07 (2.40, 4.99)	0.270
Number of LRTI episodes#	0.99 (1.53)	1.32 (1.87)	3.37 (2.03)	<0.001

LRTI: LRTI lower respiratory tract infection; CPAP: continuous positive airway pressure; Extremely to very preterm: <32 weeks gestation; moderate to late preterm: 32-37 weeks gestation; full-term: >=37 weeks gestation

**Any maternal self-reported wheeze episode

#Mean and standard deviation reported

Ω Median and IQR

Δ Note: two term-born babies received oxygen for 7 days, and 1 for 10 days resulting in a higher mean

¥ Antenatal steroid information available in 19/53 mothers

5.3.2 Lung function over time

Lung function measurements from 6 weeks to 5 years of age by gestational age group are summarised in Table S3 to S5. These cross-sectional analyses demonstrate deficits in lung function outcomes in preterm compared to term infants in tidal breathing (*VT*, *RR*), MBW (*FRC*), spectral oscillometry (R_{rs}, C_{rs} and F_{res}) and intra-breath oscillometry tests (R_{eE}, R_{eI}, ΔR, X_{eE}, X_{eI}, ΔX) at various timepoints of testing.

Longitudinal analysis of lung function trajectories showed that, independent of baseline lung function and somatic size, children born preterm had lung function trajectories that diverged from the term-born control trajectory through 5 years. With respect to MLP born children we noted a 13% decrease in compliance (0.87, 95% CI 0.82 to 0.92). Reactance decreased by 1.50 and 0.83 hPa.s.L^{-1} in X_{eE} and X_{eI}, respectively (X_{eE}: -1.50 hPa.s.L^{-1}, 95% CI -2.15 to -0.86; X_{eI}: -0.83 hPa.s.L^{-1}, 95% CI -1.23 to -0.44). A 5% increase in R_{eE} (1.05 hPa.s.L^{-1}, 95% CI 1.01 to 1.09) and a 1.74 Hz increase in F_{res} (95% CI: 1.10 to 1.09) were observed over time, compared to children born at term, Table 5.2 and Fig 5.2.

Children born very and extremely preterm had higher resistance trajectories relative to term-born children, a 15% increase in *R* (1.15, 95% CI: 1.03 to 1.30) and 18% increase in R_{eI} (1.18, 95% CI: 1.03 to 1.34) over the first 5 years of life. Trajectories of tidal breathing measures and *LCI* were similar over time between term and preterm children and showed no significance.

Adjusting for additional confounding factors including LRTI, showed similar results, demonstrating an independent effect of prematurity on lung function trajectories throughout the first 5 years of life (Table S5.6).

Table 5.2 Lung function trajectories for moderate to late preterm, extreme to very preterm and full-term born children over the first 5 years of life

	Number	Full-term	Moderate to late preterm	Extremely to very preterm
			*Adjusted coefficient (95% CI)	*Adjusted coefficient (95% CI)
TBFVL				
Tidal volume (mL)	909	Reference	-1.26 (-6.24; 3.72)	-2.96 (-16.44; 10.51)
Respiratory rate (minutes)	909	Reference	0.56 (-0.26; 1.38)	0.41 (-2.35; 3.17)
MBW				
FRC (mL)	889	Reference	-6.01 (-19.07; 7.06)	27.15(-16.95; 71.25)
LCI (n turnovers)#	889	Reference	1.01 (0.94; 1.10)	0.89 (0.65; 1.21)
Oscillometry				
Spectral				
Resistance# (hPa.s.L^{-1})	836	Reference	1.04 (1.00; 1.08)	**1.15 (1.03; 1.30)**
Compliance# (ml.hPa^{-1})	836	Reference	**0.87 (0.82; 0.92)**	0.90 (0.74; 1.09)
F_{res} (Hz)	812	Reference	**1.74 (1.10; 2.39)**	1.65 (-0.92; 4.22)
Intrabreath				
R_{eE}#(hPa.s.L^{-1})	785	Reference	**1.05 (1.01; 1.09)**	1.12 (0.98; 1.29)
R_{eI}# (hPa.s.L^{-1})	785	Reference	1.04 (0.99; 1.09)	**1.18 (1.03; 1.34)**
ΔR (hPa.s.L^{-1})	785	Reference	0.42 (-0.03; 0.88)	-0.36 (-1.31; 0.58)
X_{eE} (hPa.s.L^{-1})	785	Reference	**-1.50 (-2.15; -0.86)**	-1.12 (-2.53; 0.28)
X_{eI} (hPa.s.L^{-1})	785	Reference	**-0.83 (-1.23; -0.44)**	-0.70 (-1.69; 0.30)
ΔX (hPa.s.L^{-1})	785	Reference	**-0.67 (-1.18; -0.18)**	-0.45 (-1.50; 0.60)

LRTI: Lower respiratory tract infection; MBW: Multiple breath washout; TBFVL: Tidal breathing flow volume loop; *FRC*: Functional Residual Capacity; *LCI*: Lung clearance index; 95% CI: 95% confidence interval; F_{res}: Resonant frequency ; R_{eE}: Resistance at end expiration; R_{eI}: Resistance at end inspiration; ΔR: the difference between R_{eE} and R_{eI}; X_{eE}: Reactance at end expiration, X_{eI}: Reactance at end inspiration; ΔX: the difference between X_{eE} and X_{eI};

Full-term: >=37 weeks gestation; Moderate to late preterm: >=32- <37 weeks gestation; Extremely to very preterm: <32 weeks gestation

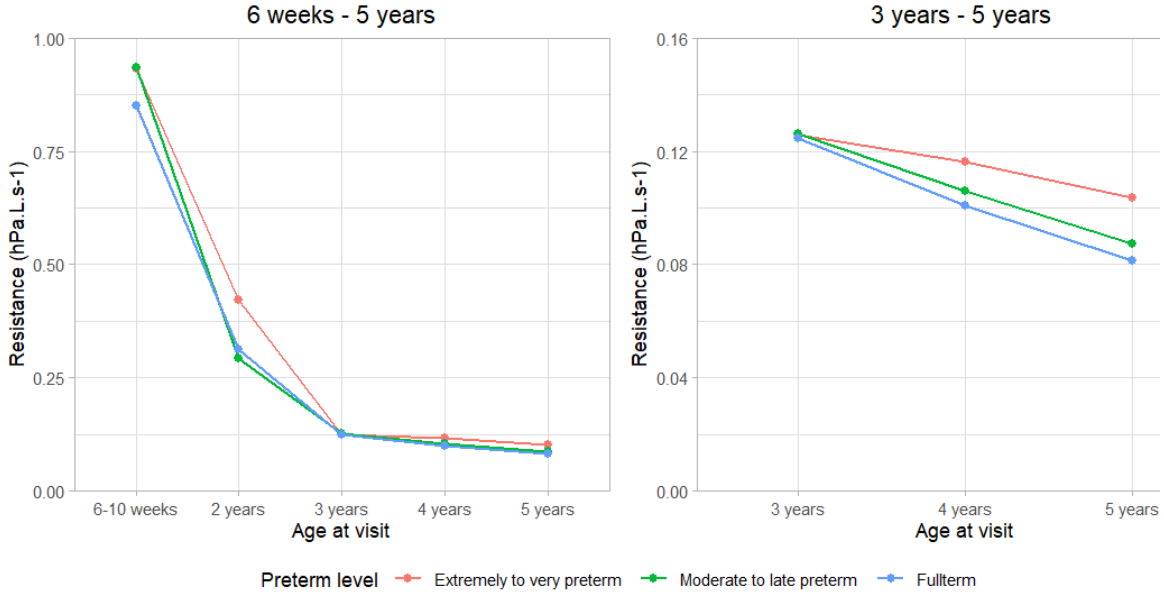
*Adjusted for sex, height, age, indicator of asleep vs awake and interaction between age

#Natural log transformation

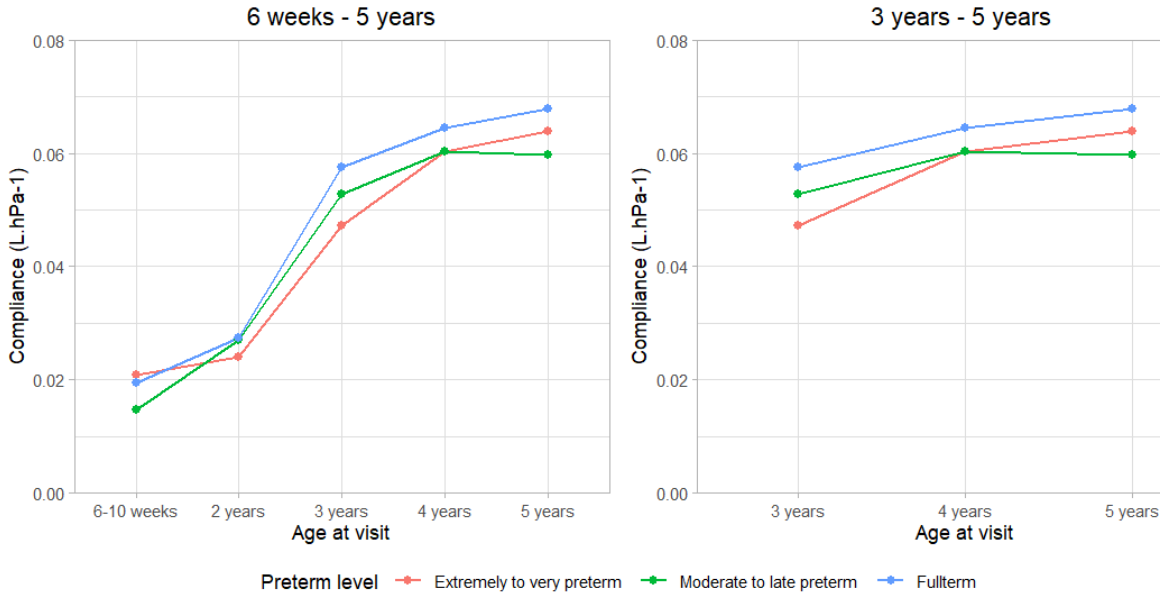
Note: bold indicates a significant association

Figure 5.2 Oscillometry measures through 5 years. Average (a) Resistance and (b) Compliance (adjusted for height) per visit and preterm level trajectories

(a) Resistance



(b) Compliance



5.3.3 Predictors of divergent lung function trajectory after preterm birth

We examined predictors of a divergent lung function trajectory for the moderate to late preterm group, including exposures to adverse early life events such as being born small for gestational age, tobacco smoke exposure, HIV exposure and the number and severity of lower respiratory tract infections (Table 5.3).

Each additional lower respiratory tract infection shifted MLP infants further from an optimal trajectory. Respiratory rate increased by 0.90 breaths per minute (95% CI 0.32 to 1.48) with each LRTI episode and by 2.44 breaths per minute (95% CI 1.09 to 3.78) for each LRTI resulting in hospitalization. *LCI* increased by 1% (95%CI 1.000 to 1.007) with each additional LRTI and respiratory system reactance became more negative over time with each LRTI exposure: X_{eE} reduced by 0.47 hPa.s.L^{-1} (95%CI -0.87 to -0.08) with each additional event.

Antenatal tobacco smoke was also detrimental: moderate to late preterm-born children of smoking mothers had I_{eE} measures that were -1.63 hPa.s.L^{-1} lower over time (95% CI -3.17 to -0.04) than those of non-smoking mothers. Maternal postnatal smoking was associated with an 18% decrease in compliance (95% CI 0.68, 0.99). Postnatal household smoke exposure showed no significance.

Children born moderate to late preterm, whose mothers were living with HIV, had a resonant frequency that was 1.69 Hz higher (95% CI 0.18 to 3.21) than children born moderate to late preterm who were not HIV exposed. In addition, this group had larger decreases in I_{eE} (0.83 hPa.s.L^{-1}, 95% CI: 0.69 to 0.99) and R_{eI} (0.81 hPa.s.L^{-1}, 95% CI: 0.68, 0.97) over time.

The relationship of these exposures to respiratory variables in those <32 weeks are shown in Table S5.6. LRTI episodes, LRTI-hospitalization or maternal antenatal smoking was associated with an increase in *LCI* and LRTI episodes with an increase in F_{res} over 5 years. Household smoke exposure was associated with a decrease in tidal volume and an increase in resistance.

Table 5.3 Exposures associated with lung function trajectories in moderate-late preterm (≥32 & <37 weeks gestation) children over 5 years

	Total LRTI episodes	**Total hospitalized LRTI episodes**	**WAZ at birth**	**Maternal antenatal smoking**	**Maternal postnatal smoking**	**HIV exposure**	**Household smoke exposure**
	Coefficient (95% CI) n	Coefficient (95% CI) n	Coefficient (95% CI) n	Coefficient (95% CI) n	Coefficient (95% CI) n	Coefficient (95% CI) n	Coefficient (95% CI) n
FRC (mL; n=113)	-7.87 (-18.24, 2.5)	-6.70 (-30.87, 17.47)	-4.97 (-21.25, 11.31)	7.23 (-34.15, 48.62)	2.95 (-35.79, 41.68)	8.14 (-40.78, 57.05)	3.32 (-41.03, 47.67)
LCI[#] (n turnovers; n=113)	**1.00 (1.00; 1.01)**	1.00 (0.99; 1.01)	**0.9 (1.00; 1.01)**	0.99 (0.97; 1.01)	0.92 (0.82, 1.03)	1.00 (0.98; 1.02)	0.94 (0.82, 1.07)
Tidal Volume (mL; n=113)	-1.81 (-5.53, 1.9)	-7.17 (-15.77, 1.43)	2.14 (-3.61, 7.9)	-2.94 (-17.84, 11.97)	-6.9 (-21.19, 7.4)	4.56 (-13.02, 22.13)	-2.78 (-19.13, 13.57)
Respiratory rate (1/min; n=117)	**0.90 (0.32, 1.48)**	**2.44 (1.09, 3.78)**	-0.62 (-1.53, 0.29)	-0.51 (-2.86, 1.84)	-0.21 (-2.42, 2)	0.09 (-2.68, 2.87)	0.04 (-2.49, 2.56)
Resistance[#] (hPa.s.L^{-1}; n=110)	1.01 (0.98, 1.05)	1.02 (0.94; 1.12)	0.95 (0.98; 1.01)	1.15 (0.99; 1.35)	1.13 (0.98, 1.31)	0.87 (0.72;1.04)	1.01 (0.85, 1.19)
Compliance[#] (ml.hPa^{-1}; n=110)	0.96 (0.92, 1.01)	0.92 (0.82; 1.03)	1.06 (0.98; 1.15)	0.84 (0.69; 1.03)	**0.82 (0.68, 0.99)**	1.20 (0.95; 1.51)	1 (0.81, 1.24)
F_{res} (Hz; n=105)	0.16 (-0.19, 0.52)	0.91 (-0.09; 1.93)	-0.40 (-0.91, 0.11)	0.25 (-1.17; 1.67)	0.85 (-0.41, 2.1)	**1.69 (0.18; 3.21)**	0.77 (-0.66, 2.19)
R_{eE}[#] (hPa.s.L^{-1}; n=99)	1.02 (0.98; 1.06)	1.04 (0.92; 1.17)	0.97 (0.91; 1.03)	1.12 (0.96; 1.31)	1.13 (0.99, 1.31)	**0.83 (0.69; 0.99)**	1 (0.86, 1.17)
R_{el}[#] (hPa.s.L^{-1}; n=99)	1.02 (0.98; 1.06)	1.03 (0.91 1.16)	0.97 (0.91; 1.02)	1.11 (0.95; 1.29)	1.1 (0.96, 1.27)	**0.81 (0.68; 0.97)**	1.02 (0.87, 1.19)
ΔR (hPa.s.L^{-1}; n=99)	0.12 (-0.13, 0.36)	0.29 (-0.47, 1.05)	-0.15 (-0.52; 0.22)	0.72 (-0.25; 1.68)	**1.01 (0.16, 1.87)**	-0.36 (-1.47, 0.76)	0.23 (-0.76, 1.22)

X_{eE}(hPa.s.L^{-1}; n=99)	**-0.47 (-0.87; -0.08)**	-0.81 (-2.02; 0.43)	0.28 (-0.32, 0.87)	**-1.63 (-3.17; -0.04)**	**-2.48 (-3.9, -1.07)**	1.15 (-0.64, 2.93)	-0.79 (-2.39, 0.81)
X_{eI} (hPa.s.L^{-1}; n=99)	-0.11 (-0.35, 0.13)	-0.48 (-1.22, 0.26)	**0.49 (0.15; 0.84)**	-0.89 (-1.84; 0.06)	**-1.36 (-2.2, -0.51)**	0.55 (-0.55, 1.64)	-0.33 (-1.3, 0.65)
ΔX (hPa.s.L^{-1}; n=99)	**-0.36 (-0.65; -0.07)**	-0.33 (-1.23; 0.57)	-0.23 (-0.67; 0.21)	-0.74 (-1.84; 0.45)	**-1.13 (-2.17, -0.09)**	0.62 (-0.71; 1.95)	-0.47 (-1.65, 0.71)

WAZ: Weight for age z-score birth; *FRC*: Functional Residual Capacity; *LCI*: Lung clearance index; F_{res}: Resonant frequency ; R_{eE}: Resistance at end expiration; R_{eI}: Resistance at end inspiration; ΔR: the difference between R_{eE} and R_{eI}; X_{eE}: Reactance at end expiration, X_{eI}: Reactance at end inspiration; ΔX: the difference between X_{eE} and X_{eI}; LRTI: lower respiratory tract infection; CI: Confidence interval.

Natural log transformation

Note: bold indicates a significant association

5.4 Discussion

This study provides a comprehensive assessment of longitudinal lung function measurements in MLP children from birth through 5 years in a LMIC setting with several adverse early life exposures. MLP infants had lung function trajectories that diverged from term infants over the first 5 years of life, in particular increased resistance and reduced compliance in keeping with narrower airways and stiffer lungs. These impairments in lung function were independent of baseline lung function, early life LRTI and other risk factors including HIV exposure and environmental tobacco smoke exposure, suggesting a sustained impact of MLP birth on healthy lung development.

These findings challenge the previously held paradigm that the lung health of preterm infants "caught up" with term infants in the period after birth [19], especially those born moderate to late preterm. The findings are consistent with recent longitudinal studies of lung function of MLP born children in HIC which describe impaired lung health including poorer lung function and increased airway obstruction compared to term born children [20, 21]. Additionally lung function deficits in this group have been shown to persist into the 6th decade of life and result in increased risk of COPD [22]. Several inflammatory, cellular and genetic mechanisms driving early neonatal lung disease and its persistence into later life have been proposed [5].

A population-based study of children from the United Kingdom measured spirometry at 8-9 years and again at 14-17 years of age and showed that those born MLP had airway obstruction (reduced FEV_1/FVC) at both timepoints, but with improvements in FEV_1 over time [23]. Spirometry requires high levels of co-operation and is not feasible in young children. Consequently, monitoring lung growth and development during the early years of

life is challenging. Importantly, this study offers insights into lung function from the first weeks of life over 5 years in MLP, a time of substantial lung growth and hence a period when the impact of additional insults is likely to be greatest. Several risk factors including environmental tobacco smoke (ETS) exposure and LRTI, particularly an event requiring hospitalisation, contributed to reduced lung function in children born MLP.

In contrast to our observations of worse respiratory impedance, we observed no difference in *FRC* or *LCI* trajectories through 5 years between MLP and term born children. These findings are in keeping with previous reports of cross-sectional studies of older children in HICs [24, 25]. Together, these findings suggest that multiple breath washout may not be the best tool for monitoring prematurity-associated lung disease in those born MLP. Others have reported lower *FRC* and higher *LCI* in very preterm infants (mean GA of 29.5 weeks) without respiratory disease at 40 weeks corrected GA, compared to term born infants (mean GA of 39.8 weeks) [26]. We did not show that in our very preterm group, but are limited by small numbers born <32 weeks gestation and thus underpowered to investigate these impacts. The difference between the findings from <32 weeks and >32 weeks gestation within these studies may be explained by the more mature alveoli in MLP, preserved lung volume and ventilation homogeneity; and lower perinatal and postnatal respiratory morbidity as compared to children born <32 weeks [24].

We described a high burden of LRTI in MLP children, both ambulatory and hospitalised LRTI were higher compared to term infants, as previously described [27]. An immature immune system, insufficient transfer of maternal antibodies [28] and lower lung function [29] may all increase susceptibility to LRTI risk. However, early life LRTI is itself an independent predictor of lower lung function [17, 30] and hence may contribute to lower lung function

described in MLP born children. This is supported by our findings that in MLP LRTIs, particularly those requiring hospitalisation, resulted in a decreased rise in lung function through childhood. These findings emphasize the importance of preventing LRTI in early childhood. Access to preventive strategies including vaccination against respiratory pathogens may be particularly important for children born MLP.

Both antenatal and postnatal smoking were associated with a lower reactance over 5 years in MLP children. This complements the cross-sectional findings of two late preterm studies performed in 3-7 year children which demonstrated higher resistance and lower reactance in late preterm children exposed to passive smoke compared to unexposed children 20 and the observation of accelerated lung function decline in exposed children <32 weeks GA [6]. This highlights ETS as an important target to promote healthy lung development in children especially those born preterm.

Strengths of this study are the large cohort, high cohort retention and comprehensive lung function measurement from 6 weeks to 5 years of age which are novel, particularly in Sub-Saharan Africa which has a high burden of preterm birth. Additionally, the detailed characterisation of maternal health indicators, robust surveillance for LRTI and measures of smoke exposure allowed assessment of the impact of these on lung development in MLP. Limitations include the small number of extreme to very preterm infants. However MLP infants are more likely to survive in LMICs and represent the greatest proportion of preterm births in LMICs, and therefore more appropriately reflect the regions of the world most impacted by preterm birth. Additionally, data on antenatal steroid and surfactant use were incomplete, however the number of eligible patients receiving these interventions were small.

We describe the first longitudinal data on lung function from birth through preschool in South African children born preterm, showing that children born MLP have long-term impairment of healthy lung growth trajectories. This study highlights the importance of strengthening antenatal care to prevent prematurity as well as follow-up in children born MLP prioritising them for preventive strategies such as vaccination. Further longitudinal data are needed to define specific lung function trajectories and the implications of these through adolescents and adulthood, to better understand the long term impact of MLP and identify targets for intervention to mitigate the consequences across the life course.

Acknowledgements:

We thank Dr Samah El- Borei for her assistance in data collection, the children and families participating in the Drakenstein Child Health Study. We acknowledge the study staff, and the clinical and administrative staff of the Western Cape Government Health Department for their support of the study.

Funding:

The study received funding from the Bill and Melinda Gates Foundation (grant numberOPP1017641, OPP1017579); NIH, USA (H3 Africa grants U54HG009824 and U01AI110466), The Wellcome Trust (098479/Z/12/Z; 204755/Z/162);South African Medical Research Council through its Division of Research Capacity Development; The National Research Foundation, South Africa; Hungarian Scientific Research Fund grant (K 128701); European Respiratory Society (INCIRCLE CRC-2013-02); Harry Crossley Clinical Research Fellowship.

References

1. World Health Organization. Born too soon: decade of action on preterm birth. Geneva: World Health Organization; 2023.
2. World Health Organization. Preterm birth fact sheet. Available from: https://www.who.int/news-room/fact-sheets/detail/preterm-birth.
3. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019;7(1):e37-e46. doi: 10.1016/S2214-109X(18)30451-0.
4. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371(9608):261-269. doi: 10.1016/S0140-6736(08)60136-1.
5. Simpson SJ, Du Berry C, Evans DJ, Gibbons JTD, Vollsæter M, Halvorsen T, et al. Unravelling the respiratory health path across the lifespan for survivors of preterm birth. Lancet Respir Med. 2024;12(2):167-180. doi: 10.1016/S2213-2600(23)00272-2.
6. Simpson SJ, Turkovic L, Wilson AC, Verheggen M, Logie KM, Pillow JJ, Hall GL. Lung function trajectories throughout childhood in survivors of very preterm birth: a longitudinal cohort study. Lancet Child Adolesc Health. 2018;2(5):350-359. doi: 10.1016/S2352-4642(18)30064-6.
7. Kotecha SJ, Gibbons JTD, Course CW, Evans EE, Simpson SJ, Watkins WJ, et al. Geographical differences and temporal improvements in forced expiratory volume in 1 second of preterm-born children: a systematic review and meta-analysis. JAMA Pediatr. 2022;176(9):867-877. doi: 10.1001/jamapediatrics.2022.1990.
8. Du Berry C, Nesci C, Cheong JLY, FitzGerald T, Mainzer R, Ranganathan S, et al. Long-term expiratory airflow of infants born moderate-late preterm: a systematic review and meta-analysis. EClinicalMedicine. 2022;52:101597. doi: 10.1016/j.eclinm.2022.101597.
9. Er İ, Günlemez A, Uyan ZS, Aydoğan M, Oruç M, Işık O, et al. Evaluation of pulmonary functions in preschool children born late-preterm. Turk Pediatri Ars. 2017;52(2):72-78. doi: 10.5152/TurkPediatriArs.2017.4187.
10. Chaya S, Zar HJ, Gray DM. Lung function in preschool children in low and middle income countries: an under-represented potential tool to strengthen child health. Front Pediatr. 2022;10:908607. doi: 10.3389/fped.2022.908607.
11. Stolz D, Mkorombindo T, Schumann DM, Agusti A, Ash SY, Bafadhel M, et al. Towards the elimination of chronic obstructive pulmonary disease: a Lancet Commission. Lancet. 2022;400(10356):921-972. doi: 10.1016/S0140-6736(22)01273-9.
12. Agustí A, Celli BR, Criner GJ, Halpin D, Anzueto A, Barnes P, et al. Global Initiative for Chronic Obstructive Lung Disease 2023 report: GOLD executive summary. Am J Respir Crit Care Med. 2023;207(7):819-837. doi: 10.1164/rccm.202301-0106PP.
13. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP. Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study. Thorax. 2015;70(6):592-594. doi: 10.1136/thoraxjnl-2014-206242.

14. Zar HJ, Pellowski JA, Cohen S, Barnett W, Vanker A, Koen N, et al. Maternal health and birth outcomes in a South African birth cohort study. PLoS One. 2019;14(11):e0222399. doi: 10.1371/journal.pone.0222399.
15. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr. 2013;13:59. doi: 10.1186/1471-2431-13-59.
16. World Health Organization. WHO child growth standards: length/-height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. World Health Organization; 2006.
17. Zar HJ, Nduru P, Stadler JAM, Gray D, Barnett W, Lesosky M, Myer L, Nicol MP. Early-life respiratory syncytial virus lower respiratory tract infection in a South African birth cohort: epidemiology and effect on lung health. Lancet Glob Health. 2020;8(10):e1316-e1325. doi: 10.1016/S2214-109X(20)30251-5.
18. Chaya S, Vanker A, Brittain K, MacGinty R, Jacobs C, Hantos Z, et al. The impact of antenatal and postnatal indoor air pollution or tobacco smoke exposure on lung function at 3 years in an African birth cohort. Respirology. 2023;28(12):1154-1165. doi: 10.1111/resp.14576.
19. de-Mir-Messa I, Sardón-Prado O, Sánchez-Solis M, Corcuera-Elosegui P, Korta-Murua J, Pérez-Fernández V, et al. Development of lung function in preterm infants during the first two years of life. Arch Bronconeumol. 2022;58(3):237-245. English, Spanish. doi: 10.1016/j.arbres.2021.07.007.
20. Er I, Gunlemez A, Uyan ZS, Aydogan M, Oruc M, Isik O, et al. Evaluation of lung function on impulse oscillometry in preschool children born late preterm. Pediatr Int. 2016;58(4):274-278. doi: 10.1111/ped.12836.
21. Thunqvist P, Gustafsson PM, Schultz ES, Bellander T, Berggren-Broström E, Norman M, et al. Lung function at 8 and 16 years after moderate-to-late preterm birth: a prospective cohort study. Pediatrics. 2016;137(4):e20152056. doi: 10.1542/peds.2015-2056.
22. Bui DS, Perret JL, Walters EH, Lodge CJ, Bowatte G, Hamilton GS, et al. Association between very to moderate preterm births, lung function deficits, and COPD at age 53 years: analysis of a prospective cohort study. Lancet Respir Med. 2022;10(5):478-484. doi: 10.1016/S2213-2600(21)00508-7.
23. Kotecha SJ, Watkins WJ, Lowe J, Henderson AJ, Kotecha S. Effect of early-term birth on respiratory symptoms and lung function in childhood and adolescence. Pediatr Pulmonol. 2016;51(11):1212-1221. doi: 10.1002/ppul.23448.
24. Yaacoby-Bianu K, Plonsky MT, Gur M, Bar-Yoseph R, Kugelman A, Bentur L. Effect of late preterm birth on lung clearance index and respiratory physiology in school-age children. Pediatr Pulmonol. 2019;54(8):1250-1256. doi: 10.1002/ppul.24357.
25. Vrijlandt EJLE, Reijneveld SA, Aris-Meijer JL, Bos AF. Respiratory health in adolescents born moderately-late preterm in a community-based cohort. J Pediatr. 2018;203:429-436. doi: 10.1016/j.jpeds.2018.07.083.
26. Hjalmarson O, Sandberg K. Abnormal lung function in healthy preterm infants. Am J Respir Crit Care Med. 2002;165(1):83-87. doi: 10.1164/ajrccm.165.1.2107093.

27. Haataja P, Korhonen P, Ojala R, Hirvonen M, Korppi M, Gissler M, et al. Hospital admissions for lower respiratory tract infections in children born moderately/late preterm. Pediatr Pulmonol. 2018;53(2):209-217. doi: 10.1002/ppul.23908.
28. van den Berg JP, Westerbeek EA, van der Klis FR, Berbers GA, van Elburg RM. Transplacental transport of IgG antibodies to preterm infants: a review of the literature. Early Hum Dev. 2011;87(2):67-72. doi: 10.1016/j.earlhumdev.2010.11.003.
29. Gray DM, Czovek D, McMillan L, Turkovic L, Stadler JAM, Vanker A, et al. Intra-breath measures of respiratory mechanics in healthy African infants detect risk of respiratory illness in early life. Eur Respir J. 2019;53(2):1800998. doi: 10.1183/13993003.00998-2018.
30. McCready C, Haider S, Little F, Nicol MP, Workman L, Gray DM, et al. Early childhood wheezing phenotypes and determinants in a South African birth cohort: longitudinal analysis of the Drakenstein Child Health Study. Lancet Child Adolesc Health. 2023;7(2):127-135. doi: 10.1016/S2352-4642(22)00304-2.

Table S5.1 Definitions

	Definition	**Collected/measured**
WHO LRTI/ pneumonia case definitions	LRTI was diagnosed in children with cough or difficulty breathing and age-specific tachypnoea. (≥50 breaths per min for children aged 2–12 months) or if the child had lower chest wall indrawing. Severe pneumonia was diagnosed in children younger than 2 months with tachypnoea (>60 breaths per min) or lower chest wall indrawing, or in children of any age if the child had a general danger sign (ie, cyanosed, unable to drink, seizures, or decreased level of consciousness [1].	A robust surveillance system was implemented at all local clinics and Paarl hospital. Mothers were counselled on key respiratory symptoms and were advised to attend the clinic or contact the study staff whenever a child developed cough or difficulty breathing. Primary health nurses and study staff were trained to recognize World Health Organisation (WHO) defined pneumonia. Training of nursing staff included video-clips demonstrating clinical signs. The study doctor provided regular on-site refresher training and competency assessments [2].
Surveillance for pneumonia/LRTI	Active surveillance for pneumonia/LRTI in the cohort was undertaken using community field workers, a short message system (SMS) phone system, ongoing monitoring of cases at health facilities and study staff who could always be contacted [2].	
Gestational age	Assessed by antenatal ultrasound in the second trimester, if this was unavailable then symphysis-fundal height, recorded by trained clinical staff at enrolment, or maternal recall of last menstrual period was used [3].	Antenatal 2nd trimester ultrasound/examination
Term infants	≥37 weeks gestation	
Moderate to late preterm	32 to 37 weeks gestation	
Very preterm	28 to less than 32 weeks gestation	
Extreme preterm	≤28 weeks gestation [4].	
Maternal smoking	Antenatal and postnatal maternal smoking information was obtained using self-report questionnaires	Questionnaire
Household smoke exposure	Any individual residing in the household who smokes including the mother.	Questionnaire
Socio-economic status	Composite variable, placing participants into relative quartiles. Score is derived from employment status and standardized scores	Based on questionnaire information collected at antenatal study visit (28-32 weeks).

	of: educational attainment, household income, assets and market access [5].	
HIV status	Mothers: point of care rapid HIV antibody test, positive test confirmed with laboratory based combined antibody and antigen test [3].	Mothers: pregnancy booking and labour Exposed infants: 6 weeks PCR, 9 months PCR, ELISA or rapid antibody testing and 18 months by rapid antibody testing as per provincial PMTCT guidelines [6].
Maternal Hypertension	High blood pressure was defined as BP >140/90 mmHg [3].	Single arm measurement using an electronic blood pressure cuff [3]
Eclampsia	Presence of seizures due to pre-eclampsia [3].	
Pre-eclampsia	New onset of hypertension after 20 weeks gestation with proteinuria or other organ dysfunction [3].	
Maternal Diabetes		Assessed using urine dipstick. If urine dipstick was positive then a fasting blood glucose was done. No formal glucose tolerance tests were conducted [3].
Anaemia	Haemoglobin measurement <10 g/dl [3].	Women had haemoglobin levels measured during pregnancy when they attended their antenatal booking visit as standard-of-care. Haemoglobin was measured using rapid tests [7].
Maternal asthma	History of asthma and/or low forced expiratory volume [8].	

Supplementary information

Lung function measurements

Oscillometry was measured with custom-built wave-tube equipment (University of Szeged, Hungary) using a medium frequency signal for conventional spectral measurement (infants: 8-48 Hz, minimum of five technically acceptable 30-s epochs; children: 6-32 Hz, maximum of five 16-s epochs); and a single frequency for intra-breath measurements (infants: 16 Hz, 30-s epoch; children: 10 Hz,16-s epoch). The signal was delivered via a wave-tube as previously described [9, 10] and performed as per the technical guidelines [11].

Spectral oscillometry measures included respiratory system compliance (C_{rs}), resistance (R_{rs}), frequency dependence of R_{rs} (R_6-R_{20}), resonance frequency (F_{res}) and X_{rs} area below F_{res} (A_X). Mean respiratory system resistance (R), inertance (I) and compliance (C) were determined from model fitting to the measured mechanical impedance of the respiratory system in the frequency range 12-32 Hz for R and 8-32 Hz for I and C in infants and 10-20 Hz for R and 6-32 Hz for I and C in children [12, 13]. The intra-breath measurements included R_{rs} at end inspiration (R_{eI}) and at end expiration (R_{eE}), X_{rs} at end expiration (X_{eE}) and end inspiration (X_{eI}), and their tidal changes R_{eE}-R_{eI} (ΔR) and X_{eE}-X_{eI} (ΔX).

Tidal breathing flow volume loop and MBW measurements were collected using the Exhalyzer D with an ultrasonic flow metre (Ecomedics, Duernten, Switzerland) and analysed with specialised analysis software (WBreath V.3.28.0 for 0 to 2 years and Spiroware 3.3.1 for 3-5 years; Zurich, Switzerland). MBW was done using 4% sulfur-hexafluoride (SF6) as a tracer gas during quiet natural sleep for infants (14) and using endogenous nitrogen with 100% oxygen washout for children (3-5 years). The deadspace[13] of the face masks (Laerdal)

[13] Deadspace indicates deadspace volume corrected for facemask

was determined by water displacement. Measurements included: tidal breathing flow volume loops with tidal volume (*VT*) and respiratory rate (*RR*) and for MBW, *FRC* and the *LCI*.

Statistical analysis

The best model was selected using the Akaike information criterion (AIC)/Bayesian information criterion (BIC): these were used to compare models with the variables identified with the DAG (as well as height-for-age z-score and age), with the addition of the indicator variable models where the interaction between the indicator variable and age was also included. In addition, we considered the final model per lung function outcome with the addition of lower respiratory tract infections (LRTI) counts per visit per participant. Further, diagnostic checks were conducted. These included checking for normality in the residuals using histograms, standardized probability (P–P) plot, Quantile–Quantile (Q–Q) plots. Further, the presence of multicollinearity was explored using the variance inflation factor. If any of the outcomes were found to be non-normal, then transformations were explored; these included natural log transformations.

Table S5.2 Characteristics of participants excluded vs those included (having at least 2 successful lung function tests)

	Total (n=1053)	Included (n=923)	Excluded (n=130)	P-value
Maternal and household characteristics				
Number of mothers	1048	919 (87.7%)	129 (12.3%)	
Age (year) at delivery Ω	26.59 (22.53, 31.16)	25.2 (21.47, 30.43)	25.48 (20.36, 33.48)	0.455
BMI at enrollment#	28.29 (6.42)	28.43 (6.43)	27.30 (6.21)	0.067
Maternal antenatal smoking	303 (28.8%)	286 (31.0%)	17 (13.1%)	<0.001
Maternal postnatal smoking	360 (34.5%)	344 (37.4%)	16 (12.9%)	<0.001
Maternal education***				
Primary	79 (7.5%)	68 (7.4%)	11 (8.5%)	0.669
Some secondary	570 (54.2%)	506 (54.8%)	64 (49.6%)	-
Completed secondary	341 (32.4%)	294 (31.9%)	47 (36.4%)	-
Tertiary	62 (5.9%)	55 (6.0%)	7 (5.4%)	-
Household income per month***				
<1000 ZAR [$54]	368 (35.0%)	324 (35.1%)	44 (34.1%)	0.171
1000-5000 ZAR	543 (51.6%)	482 (52.2%)	61 (47.3%)	-
>5000 ZAR [$270]	141 (13.4%)	117 (12.7)	24 (18.6%)	-
Maternal illnesses during pregnancy				
Asthma**	15 (1.4%)	13 (1.4%)	2 (1.5%)	0.907
Diabetes	14 (1.3%)	12 (1.3%)	2 (1.5%)	0.824
Anaemia	233 (22.1%)	208 (22.5%)	25 (19.2%)	0.395
Pre-eclampsia	24 (2.3%)	22 (2.4%)	2 (1.5%)	0.546
High blood pressure	47 (4.5%)	41 (4.4%)	6 (4.6%)	0.929
Maternal HIV	228 (21.7%)	196 (21.2%)	32 (24.6%)	0.381
Antiretroviral regimen	193 (85.8%)	169 (87.1%)	24 (77.4%)	0.313
Child characteristics				
Gestational Age Ω	39 (38, 40)	39 (38, 40)	39 (38,40)	0.462
Sex – male	537 (51.0%)	470 (50.9%)	67 (51.5%)	0.895
Birth weight Ω (kg)	3.10 (2.71, 3.42)	3.1 (2.72, 3.42)	3.01 (2.65, 3.41)	0.553
Weight for age z-score birth#	-0.29 (1.06)	-0.28 (1.06)	-0.33 (1.01)	0.651
Height for age z-score birth#	0.41 (1.61)	0.40 (1.63)	0.50 (1.47)	0.513
Birth length (cm)	50 (48, 52)	50 (48, 52)	50 (48, 52)	0.806
Preterm (<37 weeks gestation)	162 (15.4%)	138 (14.9%)	24 (18.5%)	0.301
Household smoke exposure	780 (74.5%)	724 (78.7%)	56 (44.1%)	<0.001

LRTI: lower respiratory tract infection

*Includes congenital cases

**Any maternal self-reported wheeze episode

***1 participant missing maternal education or household income data

[#]Mean and standard deviation reported

[Ω] Median and IQR

Table S5.3 Summary of multiple breath washout and tidal breathing tests stratified by very, moderate to late preterm and full-term birth at each study visit

	Multiple breath washout (MBW)[#]				**Tidal breathing flow–volume loop (TBFVL)[##]**			
	FRC (mL)		***LCI (n turnovers)***		***Tidal volume (mL)***		***Respiratory rate (minutes)***	
	n	*Mean (SD)*	*n*	*Mean (SD)*	*n*	*Mean (SD)*	*n*	*Mean (SD)*
6 weeks								
Full-term	608	78.43*$^{\Omega}$ (15.96)	608	7.16 (0.44)	647	35.26*$^{\Omega}$ (6.23)	647	48.43* (11.15)
Moderate to late preterm	95	73.47*$^{\Omega}$ (15.47)	95	7.12 (0.42)	102	32.85*$^{\Omega}$ (6.34)	102	50.38* (12.54)
Very preterm	13	65.94* (8.74)	13	6.97 (0.36)	14	27.42* (5.68)	14	54.17* (11.47)
12 months								
Full-term	503	200.86*$^{\Omega}$ (40.72)	503	6.76 (0.57)	536	93.65* (13.80)	536	29.51 (5.13)
Moderate to late preterm	73	183.94*$^{\Omega}$ (46.23)	73	6.79 (0.48)	77	90.60* (15.14)	77	29.49 (4.34)
Very preterm	13	154.64* (47.16)	13	6.86 (0.64)	14	81.13* (18.21)	14	29.16 (5.51)
24 months								
Full-term	508	260.40 (46.02)	508	6.70 (0.47)	526	119.80 (16.34)	526	26.28 (4.34)
Moderate to late preterm	76	251.95 (42.79)	76	6.65 (0.44)	80	118.16 (18.17)	80	26.71 (4.21)
Very preterm	15	252.77 (52.64)	15	6.56 (0.54)	16	114.08 (15.94)	16	25.45 (4.22)
36 months								
Full-term	386	426.46 (79.93)	386	7.01 (0.75)	436	178.87* (28.89)	436	27.83*(5.62)
Moderate to late preterm	52	450.60 (146.59)	52	7.05 (0.87)	54	181.32* (27.26)	53	28.67*(5.70)
Very preterm	4	409.07 (30.96)	4	6.12 (0.84)	9	146.09*(32.12)	9	33.79* (8.11)
48 months								
Full-term	475	484.22 (95.45)	475	6.93 (0.58)	565	202.54 (32.87)	564	26.58 (6.07)
Moderate to late preterm	65	487.13 (100.47)	65	6.87 (0.62)	81	196.83 (35.45)	81	27.43 (6.73)
Very preterm	11	477.10 (70.48)	11	7.12 (0.67)	15	201.52 (43.70)	15	27.21 (8.37)
60 months								
Full-term	456	571.59 (118.03)	456	6.85 (0.55)	535	228.06 (46.08)	532	27.40 (7.99)
Moderate to late preterm	68	558.46 (111.50)	68	6.88 (0.54)	88	222.62 (55.04)	88	28.34 (7.48)

Very preterm	7	600.19 (176.78)	7	6.95 (0.89)	11	209.25 (31.44)	10	25.06 (5.14)

FRC: Functional Residual Capacity; *LCI*: Lung clearance index; SD: standard deviation

[#] Number of Multiple breath washout (MBW) tests with two or more successful tests over 5-year period: 901

[##] Number of Tidal breathing flow volume (TBFVL) tests with two or more successful tests over 5-year period: 910

*Significant difference among the 3 groups ($p<0.005$)

[Ω] Significant difference among 2 groups – Full term vs moderate to late preterm ($p<0.005$)

Table S5.4 Summary of spectral oscillometry tests stratified by very, moderate to late preterm and full-term birth at each study visit (2 successful tests)

	Resistance (hPa.s.L^{-1})		**Compliance (ml.hPa^{-1})**		**F_{res} (Hz)**	
	n	***Mean (SD)***	***n***	***Mean (SD)***	***n***	***Mean (SD)***
6 weeks						
Full-term	572	47.07*$^{\Omega}$ (14.92)	572	1.08*$^{\Omega}$ (0.51)	542	19.62*$^{\Omega}$ (4.42)
Moderate to late preterm	81	50.34$^{*\Omega}$ (13.86)	81	0.80*$^{\Omega}$ (0.32)	70	21.97*$^{\Omega}$ (4.05)
Very preterm	6	46.15* (17.84)	6	1.02* (0.61)	6	22.94* (6.45)
24 months						
Full-term	480	26.34* (10.81)	480	2.31 (0.86)	467	17.75 (4.28)
Moderate to late preterm	75	24.50* (7.14)	75	2.25 (0.80)	71	18.76 (4.82)
Very preterm	13	34.46* (10.46)	13	2.01 (1.19)	12	17.80 (5.03)
36 months						
Full-term	365	11.47 (2.21)	365	5.32 (1.95)	167	24.32 (4.22)
Moderate to late preterm	54	11.58 (2.25)	54	4.86 (1.41)	17	24.33 (3.56)
Very preterm	5	11.35 (1.95)	5	4.23 (1.45)	2	28.99 (1.27)
48 months						
Full-term	519	10.04*$^{\Omega}$ (2.12)	519	6.46 (2.32)	347	22.25 (4.30)
Moderate to late preterm	77	10.54*$^{\Omega}$ (2.12)	77	6.04 (2.16)	42	23.61 (4.63)
Very preterm	14	11.47* (2.31)	14	6.05 (3.19)	6	23.11 (3.98)
60 months						
Full-term	509	8.72*$^{\Omega}$ (1.96)	509	7.33*$^{\Omega}$ (2.46)	390	21.39 (4.62)
Moderate to late preterm	80	9.26*$^{\Omega}$ (1.82)	80	6.43*$^{\Omega}$ (1.88)	51	22.61 (4.54)
Very preterm	10	10.80* (2.77)	10	6.76* (2.75)	4	20.57 (4.18)

F_{res}: Resonant frequency; SD: standard deviation;

Number of standard spectral tests with two or more successful tests over 5-year period: 836

*Significant difference among the 3 groups ($p<0.005$)

$^{\Omega}$ Significant difference among 2 groups – Full term vs moderate to late preterm ($p<0.005$)

Table S5.5 Summary of Intra-breath oscillometry tests stratified by very, moderate to late preterm and full-term birth at each study visit (2 successful tests)

	R_{eE} (hPa.s.L^{-1})		R_{eI} (hPa.s.L^{-1})		ΔR (hPa.s.L^{-1})		X_{eE} (hPa.s.L^{-1})		X_{eI} (hPa.s.L^{-1})		ΔX (hPa.s.L^{-1})	
	n	***Mean (SD)***	***n***	***Mean (SD)***	***n***	***Mean (SD)***	***n***	***Mean (SD)***	***n***	***Mean (SD)***	***n***	***Mean (SD)***
6 weeks												
Full-term	421	44.76*$^{\Omega}$ (12.24)	421	39.78*$^{\Omega}$ (13.32)	421	4.97 (7.41)	421	-7.45*$^{\Omega}$ (7.32)	421	-5.06*$^{\Omega}$ (4.71)	421	-2.40* (6.77)
Moderate to late preterm	55	48.59*$^{\Omega}$ (13.17)	55	43.17*$^{\Omega}$ (13.64)	55	5.42 (6.35)	55	-13.82*$^{\Omega}$ (10.65)	55	-8.52*$^{\Omega}$ (6.39)	55	-5.31* (8.64)
Very preterm	6	60.08* (18.69)	6	53.17* (22.63)	6	6.91 (5.46)	6	-13.79* (6.41)	6	-7.95* (3.56)	6	-5.84* (4.47)
24 months												
Full-term	478	19.84* (7.17)	478	19.89* (7.67)	478	-0.05 (4.07)	478	-0.63$^{*\Omega}$ (3.25)	478	-0.52 (2.97)	478	-0.11*$^{\Omega}$ (2.71)
Moderate to late preterm	76	19.78* (5.56)	76	19.45* (6.28)	76	0.33 (3.37)	76	-1.58*$^{\Omega}$ (3.33)	76	-0.86 (3.22)	76	-0.72*$^{\Omega}$ (2.81)
Very preterm	13	24.74* (9.34)	13	25.21* (10.47)	13	-0.47 (5.23)	13	-0.69* (4.70)	13	-0.10 (3.46)	13	-0.59* (4.65)
36 months												
Full-term	338	12.22 (2.68)	338	10.83 (2.35)	338	1.39$^{\Omega}$ (1.63)	338	-2.55 (1.62)	338	-3.19 (1.31)	338	0.64 (1.36)
Moderate to late preterm	50	12.94 (3.33)	50	10.96 (2.37)	50	1.98$^{\Omega}$ (1.88)	50	-3.05 (2.12)	50	-3.36 (1.54)	50	0.30 (2.01)
Very preterm	5	13.35 (3.06)	5	12.41 (2.86)	5	0.94 (1.45)	5	-3.76* (2.47)	5	-5.28* (2.73)	5	1.53 (1.86)
48 months												
Full-term	491	10.50*$^{\Omega}$ (2.51)	491	9.71 (2.12)	491	0.78 (1.71)	491	-2.01$^{\Omega}$ (1.49)	491	-2.49 (1.19)	491	0.47 (1.25)
Moderate to late preterm	71	11.19*$^{\Omega}$ (2.81)	71	10.13 (2.40)	71	1.07 (1.64)	71	-2.47$^{\Omega}$ (1.74)	71	-2.72 (1.16)	71	0.25 (1.34)
Very preterm	14	11.25* (2.47)	14	10.81 (2.10)	14	0.44 (1.75)	14	-2.10 (1.08)	14	-2.92 (1.15)	14	0.82 (1.25)

60 months												
Full-term	457	8.93$^{\Omega}$ (2.30)	457	8.55* (2.19)	457	0.38 (1.47)	457	-1.45*$^{\Omega}$ (1.19)	457	-2.06*$^{\Omega}$ (1.05)	457	0.62 (1.08)
Moderate to late preterm	74	9.83*$^{\Omega}$ (3.02)	74	9.04* (2.42)	74	0.78 (1.64)	74	-2.01*$^{\Omega}$ (1.79)	74	-2.40 $^{\Omega}$ (1.28)	74	0.39 (1.42)
Very preterm	9	11.01* (4.12)	9	10.47* (3.25)	9	0.55 (1.78)	9	-1.99* (1.88)	9	-2.93* (1.83)	9	0.94 (1.31)

R_{eE}: Resistance at end expiration; R_{eI}: Resistance at end inspiration ; ΔR: the difference between R_{eE} and R_{eI}; X_{eE}: Reactance at end expiration, X_{eI}: Reactance at end inspiration ; ΔX: the difference between X_{eE} and X_{eI}; SD: standard deviation

Number of standard spectral tests with two or more successful tests over 5-year period: 785

*Significant difference among the 3 groups ($p<0.005$)

$^{\Omega}$Significant difference among 2 groups – Full term vs moderate to late preterm ($p<0.005$)

Table S5.6 Adjusted lung function trajectories for moderate to late preterm, extreme to very preterm and full-term born children over the first 5 years of life

	Number	Full-term	Moderate to late preterm	Extremely to very preterm
			*Adjusted coefficient (95% CI)	*Adjusted coefficient (95% CI)
TBFVL				
Tidal volume (mL)	907	Reference	-1.37 (-6.36; 3.62)	-3.54 (-17.43; 10.35)
Respiratory rate (1/min)	907	Reference	0.55 (-0.27; 1.36)	0.33 (-2.46; 3.13)
MBW				
FRC (mL)	889	Reference	-6.90 (-20.26; 6.46)	21.75 (-25.32; 38.83)
LCI# (n turnovers)	889	Reference	0.92 (0.73; 1.15)	1.07 (0.96; 1.02)
Oscillometry				
Spectral				
Resistance# (hPa.s.L^{-1})	834	Reference	1.03 (0.99; 1.07)	**1.14 (1.02; 1.28)**
Compliance# (ml.hPa^{-1})	834	Reference	**0.87 (0.83; 0.92)**	0.90 (0.74; 1.09)
Fres (Hz)	810	Reference	**1.75 (1.10; 2.39)**	1.65 (-0.92; 4.22)
Intrabreath				
R_{eE}#(hPa.s.L^{-1})	577	Reference	**1.06 (1.02; 1.11)**	1.13 (0.96; 1.32)
R_{eI}# (hPa.s.L^{-1})	577	Reference	1.04 (0.99; 1.08)	1.18 (1.02; 1.37)
ΔR (hPa.s.L^{-1})	783	Reference	**0.46 (0.00; 0.92)**	-0.34 (-1.47; 0.78
X_{eE} (hPa.s.L^{-1})	783	Reference	**-0.53 (-0.98; -0.08)**	-0.89 (-2.10; 0.31)
X_{eI} (hPa.s.L^{-1})	783	Reference	-0.16 (-0.49; 0.16)	-0.11 (-0.81; 0.60)
ΔX (hPa.s.L^{-1})	783	Reference	**-0.70 (-1.23; -0.18)**	-0.57 (-1.67; 0.53)

LRTI: Lower respiratory tract infection; MBW: Multiple breath washout; TBFVL: Tidal breathing flow volume loop; *FRC*: Functional Residual Capacity; *LCI*: Lung clearance index; 95% CI: 95% confidence interval; F_{res}: Resonant frequency ; R_{eE}: Resistance at end expiration; R_{eI}: Resistance at end inspiration; ΔR: the difference between R_{eE} and R_{eI}; X_{eE}: Reactance at end expiration, X_{eI}: Reactance at end inspiration; ΔX: the difference between X_{eE} and X_{eI}; Full-term: >=37 weeks gestation; Moderate to late preterm: >=32 - <37 weeks gestation; Extremely to very preterm: <32 weeks gestation

*Adjusted for sex, height, age, indicator of asleep vs awake and interaction between age and indicator, HIV exposure, birth weight, maternal antenatal smoking, household income, LRTI counts per child

#Natual log transformation

Note: bold indicates a significant association

Table S5.7 Exposures associated with lung function trajectories in very preterm (<32 weeks gestation) children over 5 years

	Total LRTI episodes	**Total hospitalized LRTI episodes**	**WAZ at birth**	**Maternal antenatal smoking**	**Maternal postnatal smoking**	**HIV exposure**	**Household smoke exposure**
	Coefficient (95% CI), n	**Coefficient (95% CI), n**	**Coefficient (95% CI), n**	**Coefficient (95% CI), n**	**Coefficient (95% CI), n**	**Coefficient (95% CI), n**	**Coefficient (95% CI) n**
FRC (mL; n=18)	0.26 (-23.79; 24.31)	-3.34 (-45.65; 38.97)	6.84 (-47.91; 61.58)	-31.24 (-129.30; 66.83)	-4.85 (-109.2, 99.5)	-47.61 (-174.73; 79.51)	-68.11 (-195.93, 59.71)
LCI[#] (n turnovers; n=18)	**1.02 (1.01; 1.03)**	**1.01 (1.01; 1.06)**	1.00 (0.96; 1.04)	**1.06 (1.00; 1.12)**	1.29 (0.82, 2.02)	1.06 (0.98; 1.14)	0.96 (0.9, 1.03)
Tidal Volume (mL; n=19)	-0.19 (-8.31; 7.92)	1.25 (-13.00; 15.51)	-3.7 (-21.25; 13.85)	-13.58 (-44.78; 17.61)	-8.25 (-40.89, 24.4)	-7.48 (-44.73; 29.77)	**-44.49 (-80.01, -8.98)**
Respiratory rate (1/min; n=19)	0.36 (-1.11; 1.85)	-0.67 (-3.27; 1.93)	2.09 (-1.20; 5.37)	0.21 (-5.55; 5.96)	-0.93 (-6.96, 5.1)	-1.21 (-8.02; 5.59)	3.71 (-2.95, 10.37)
Resistance[#] (hPa.s.L^{1}; n=14)	1.05 (0.94; 1.17)	1.06 (0.87; 1.3)	1.07 (0.83; 1.40)	1.04 (0.70; 1.54)	0.92 (0.61, 1.39)	0.76 (0.41; 1.40)	**1.68 (1.06, 2.66)**
Compliance[#] (ml.hPa^{1}; n=14)	0.93 (0.81; 1.07)	0.92 (0.71; 1.2)	0.89 (0.63; 1.25)	0.94 (0.57; 1.55)	1.27 (0.75, 2.15)	1.03 (0.47; 2.28)	0.65 (0.35, 1.21)
F_{res} (Hz; n=13)	**1.26 (0.08; 2.44)**	1.18 (-1.36; 3.72)	0.03 (-2.78; 2.83)	3.71 (-0.33; 7.75)	2.6 (-2.49, 7.7)	2.15 (-6.52; 10.83)	-1.32 (-9.73, 7.09)
R_{eE}[#](hPa.s.L^{-1}; n=14)	1.04 (0.91; 1.18)	1.12 (0.89; 1.40)	1.07 (0.79; 1.45)	1.00 (0.62; 1.59)	0.78 (0.48, 1.25)	0.71 (0.36; 1.40)	1.62 (0.96, 2.74)
R_{el}[#] (hPa.s.L^{-1}; n=14)	1.03 (0.91; 1.16)	1.09 (0.89; 1.33)	1.01 (0.76; 1.34)	0.89 (0.58; 1.37)	0.71 (0.47, 1.07)	0.76 (0.40; 1.44)	1.51 (0.91, 2.51)
Δ *R* (hPa.s.L^{-1}; n=14)	0.09 (-0.58; 0.76)	0.29 (-0.95; 1.53)	1.08 (-0.44; 2.59)	2.13 (-0.21; 4.47)	1.35 (-1.21, 3.9)	-1.48 (-5.23; 2.27)	1.43 (-1.7, 4.56)

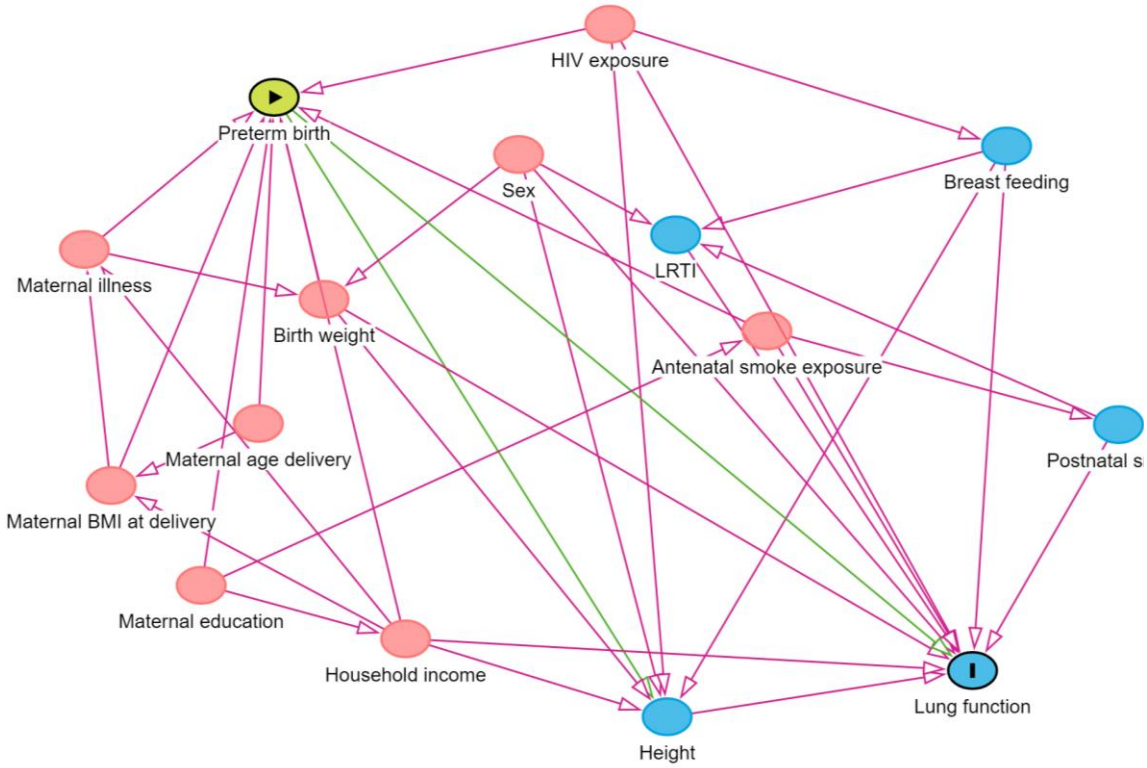
X_{eE} (hPa.s.L^{-1}; n=14)	-0.57 (-1.45; 0.32)	-0.88 (-2.58; 0.81)	0.22 (-2.01; 2.46)	-1.19 (-4.52; 2.15)	1.2 (-2.35, 4.75)	-0.46 (-5.67; 4.76)	-0.55 (-4.91, 3.8)
X_{eI} (hPa.s.L^{-1}; n=14)	-0.07 (-0.74; 0.60)	-0.23 (-1.43; 0.97)	-0.35 (-1.85; 1.14)	-1.01 (-3.32; 1.31)	0.11 (-2.4, 2.61)	-2.72 (-5.98; 0.53)	0.92 (-1.85, 3.69)
ΔX (hPa.s.L^{-1}; n=14)	-0.50 (-1.10; 0.10)	-0.65 (-1.78; 0.49)	0.61 (-0.90; 2.11)	-0.19 (-2.55; 2.17)	1.05 (-1.32, 3.41)	2.27 (-1.15; 5.69)	-1.49 (-4.36, 1.38)

FRC: Functional Residual Capacity; *LCI*: Lung clearance index; F_{res}: Resonant frequency ; R_{eE}: Resistance at end expiration; R_{eI}: Resistance at end inspiration; ΔR: the difference between R_{eE} and R_{eI}; X_{eE}: Reactance at end expiration, X_{eI}: Reactance at end inspiration; ΔX: the difference between X_{eE} and X_{eI}; LRTI: lower respiratory tract infection; CI: confidence interval.

[#] Natural log transformation

** All very preterm children had at least one LRTI episode Note: bold indicates a significant association

Figure S5.1 Direct acyclic graph to determine minimum set of confounding variables to include in adjusted models



Supplementary references

1. World Health Organization (WHO). Integrated management of childhood illness: distance learning course; 2014. https://apps.who.int/iris/handle/10665/104772.
2. le Roux DM, Myer L, Nicol MP, Zar HJ. Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study. BMJ Open. 2015;5(12):e009111. doi: 10.1136/bmjopen-2015-009111.
3. Zar HJ, Pellowski JA, Cohen S, Barnett W, Vanker A, Koen N, et al. Maternal health and birth outcomes in a South African birth cohort study. PLoS One. 2019;14(11):e0222399. doi: 10.1371/journal.pone.0222399.
4. World Health Organization. Preterm birth fact sheet. Available from: https://www.who.int/news-room/fact-sheets/detail/preterm-birth.
5. Myer L, Stein DJ, Grimsrud A, Seedat S, Williams DR. Social determinants of psychological distress in a nationally-representative sample of South African adults. Soc Sci Med. 2008;66(8):1828-1840. doi: 10.1016/j.socscimed.2008.01.025.
6. Gray DM, Wedderburn CJ, MacGinty RP, McMillan L, Jacobs C, Stadler JAM, et al. Impact of HIV and antiretroviral drug exposure on lung growth and function over 2 years in an African Birth Cohort. AIDS. 2020;34(4):549-558. doi: 10.1097/QAD.0000000000002444.
7. Wedderburn CJ, Ringshaw J, Donald K, Joshi S, Subramoney S, Fouche J-P, et al. Effects of maternal and early-life anaemia on child brain development: a South African birth cohort study. Preprint. Available at http://dx.doi.org/10.2139/ssrn.3920258
8. Kariuki SM, Gray DM, Newton CRJC, Vanker A, MacGinty RP, Koen N, et al. Association between maternal psychological adversity and lung function in South African infants: a birth cohort study. Pediatr Pulmonol. 2020;55(1):236-244. doi: 10.1002/ppul.24532.
9. Gray DM, Czovek D, McMillan L, Turkovic L, Stadler JAM, Vanker A, et al. Intra-breath measures of respiratory mechanics in healthy African infants detect risk of respiratory illness in early life. Eur Respir J. 2019;53(2):1800998. doi: 10.1183/13993003.00998-2018.
10. Chaya S, MacGinty R, Jacobs C, Githinji L, Hlengwa S, Simpson SJ, et al. Normal values of respiratory oscillometry in South African children and adolescents. ERJ Open Res. 2023;9(2):00371-2022. doi: 10.1183/23120541.00371-2022.
11. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, et al. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753. doi: 10.1183/13993003.00753-2019.
12. Shackleton C, Czovek D, Grimwood K, Ware RS, Radics B, Hantos Z, et al. Defining 'healthy' in preschool-aged children for forced oscillation technique reference equations. Respirology. 2018;23(4):406-413. doi: 10.1111/resp.13186.
13. Sly PD, Hantos Z; INCIRCLE Clinical Research Collaboration. The International Collaboration to Improve Respiratory Health in Children (INCIRCLE) ERS Clinical Research Collaboration. Eur Respir J. 2018;52(6):1801867. doi: 10.1183/13993003.01867-2018.

14. Gray D, Willemse L, Visagie A, Czövek D, Nduru P, Vanker A, et al. Determinants of early-life lung function in African infants. Thorax. 2017;72(5):445-450. doi: 10.1136/thoraxjnl-2015-207401.

Chapter 6: Conclusion

6.1 Summary and recommendations

This work investigated early life factors that impact lung development in early childhood (birth to 5 years) in a South African birth cohort, the Drakenstein Child Health Study (DCHS). Participants were followed from birth with lung function measured at 6 weeks until 2 years. The current work extended this to measure lung function through the preschool years, from 3 to 5 years of age. Data were collected from almost 1000 child participants with high success rates that improved each year as children aged.

This work demonstrated the utility and feasibility of comprehensive lung function testing in awake preschool children, developed local reference standards and identified factors impacting preschool lung function, providing novel African data during a critical time of life for lung growth and development.

6.2 Summary of chapters

6.2.1 Lung function in preschool children in low- and middle-income countries: an under-represented potential tool to strengthen child health

The burden of respiratory disease is high in low- and middle-income countries (LMICs). Pulmonary function tests are useful as an objective measure of lung health and for tracking progression, though access remains extremely limited in these settings. Spirometry is the most common test available, but it requires a complex manoeuvre, which limits its use in preschool children. Newer lung function tests such as fractional exhaled nitric oxide (FeNO), oscillometry, interrupter technique, tidal breathing and multiple breath washout technique

(MBW) have been developed and successfully used in children, however their use in LMIC has not been well described. Despite being used successfully in both clinical and epidemiological studies, these newer measurements are under-represented in LMIC. These tests have the potential to strengthen respiratory care in LMIC, however access needs to be improved.

6.2.2 Normal values of respiratory oscillometry in South African children and adolescents

A reference range was developed using healthy children from the study cohort and older children and adolescents from Red Cross War Memorial Children's Hospital. The participants were from a LMIC population. This equation facilitates tracking of an individual's lung function with potential to improve early detection of respiratory disease and/or quantify impacts of early life exposures on lung growth and development. It was developed using data from 692 children and adolescents and is applicable for use between 3 and 17 years, using height as the only predictor. This reference range includes one of the largest participant cohorts for oscillometry reference standard, allowing for a robust reference equation. To allow accessibility and promote widespread use it is freely available online, along with a reference range calculator. This enables all users with similar population groups to compare individual results against a reference value and to track lung function, the first for Africa. In addition, the collected data were shared with international collaborators in efforts to harmonise global reference standards. This work provides some of the only African paediatric data for oscillometry to date. Until the Global Lung Initiative (GLI) reference equation is available, this equation provides a reliable reference for oscillometry in LMIC.

6.2.3 Impact of postnatal indoor air pollution on lung function

Findings in this study indicated that both indoor air pollution (IAP) and environmental tobacco smoke (ETS) exposure were associated with decreased lung function at 3 years. Children are vulnerable to these exposures both antenatally and postnatally. Both antenatal and postnatal exposure to particulate matter (PM_{10}) and volatile organic compounds (VOC) were associated with lung function deficits at 3 years. Postnatal exposure to ETS was associated with a decrease in expiratory flows and an increase in resistance at 3 years which may suggest airway narrowing. ETS was also a key modifiable factor. Separating antenatal and postnatal exposures can be challenging, however by adjusting for 6 week lung function, the additional impact of ongoing postnatal exposures was assessed. This study highlighted the need for regular follow-up as well as tracking lung function over time, to identify early decline.

6.2.4 Impact of preterm birth on lung development

This study provided the first data on lung function outcomes in moderate to late preterm (MLP) born African infants, including the first longitudinal findings. This is important as the majority of preterm infants are born in LMIC across Africa and southeast Asia, with MLP infants making up 85% of the global burden of premature births. This group is a growing population. The study findings showed a decrease in compliance, increase in resistance and increase in resonance frequency (F_{res}) over 5 years compared to term born infants. Several early life factors in preterm children were associated with lung function impairment including low birth weight, ETS exposure, HIV exposure, and number and severity of lower respiratory tract infections (LRTI). These children represent a vulnerable group who require close monitoring with regular follow-up. This study highlights the importance of maternal antenatal

care and the need for interventions aimed at decreasing ETS exposure and reduction in early life LRTI.

6.3 Limitations of the study

- This was an observational study with a large overall sample size, but a relatively small exposure group in some instances. For example, there was a relatively small sample group for some of the IAP exposures. These exposures were measured at only two time points, and this may have underestimated ongoing exposures, seasonal variations and the ability to adjust for multiple exposures. These factors may limit the generalisability of the findings to other population groups.
- The oscillometry reference equation was derived from a single group within a South African population. As a result, the data may not be generalisable to other population groups in the Southern Africa. However, spirometry data collected from various regions in South Africa have demonstrated similarity in measurements, suggesting that oscillometry results could show comparable consistency. Further testing in other regions is needed.
- In the current study, the number of extreme to very preterm infants was very small, and data on participants who received interventions such as surfactant and antenatal steroids were incomplete. However, this represented only a small number of eligible participants.

6.4 Implications of the study

- Preschool lung function testing is feasible and useful in tracking respiratory health in a LIMC setting; however, access must be strengthened.

- High-risk groups, such as children with high environmental exposure, LRTI during early life, and those born preterm should be prioritised for follow-up to identify individuals at risk for early lung function decline.
- Educating women and those of child-bearing age on the risk of ETS during and after pregnancy is key. This could be supported by providing resources such as pamphlets and smoking cessation programs.
- Strengthening vaccination programs against LRTI in pregnant women and young children is important for reducing the incidence of respiratory illnesses in vulnerable populations.
- Healthcare professionals should be educated about the impact of preterm delivery, including MLP births, and its effects on lung function. The importance of long-term follow-up for these children should be emphasised.

6.5 Areas of future research

- Lung function testing in other healthy population groups is still needed to contribute to international data and reference equation development.
- Validating the current reference equation in both LMICs and high-income countries.
- Follow-up studies to track lung function from infancy through adolescence in relation to antenatal and early life exposures, aiming to investigate the long-term impact on lung health in this cohort.
- Using these comprehensive lung function measures to better understand the pathophysiology of early childhood lung disease, and how this may strengthen clinical diagnosis and management.
- To identify which lung function measurement/s may be most sensitive for detecting early lung disease.

- To assess the feasibility and clinical benefit of tracking lung function in those with early life respiratory disease with the aim of evaluating disease progression and/or improvement.

6.6 Strategies implemented to ensure high feasibility in preschool lung function testing

- Adequate funding for equipment and staff.
- Research staff from the community:
    - Involved nurses from the community to facilitate comfort and cooperation.
    - Leveraged local staff's cultural understanding to improve communication and trust, which helped build rapport with patients and families, improving retention in the study.
    - Staff were familiar with community members thus increasing both the parent's and child's trust and cooperation.
    - Aligned lung function testing with other study related events to minimise the number of visits.
- Preparation and environment:
    - Brought more than one child at a time.
    - Set up a waiting area where families could connect with each other.
    - Offered snacks to children and parents while waiting, however avoiding those that could interfere with FeNO testing.
    - Created a child-friendly, non-intimidating testing environment, both in the waiting area and in the testing room.
    - Used visual aids or toys to distract and engage the child during testing.

    - Scheduled tests at convenient times considering the child's routine or school schedule, avoiding late morning or afternoon tests around their nap times.
    - Provided incentives such as small toys or stickers after testing to motivate children.
- Technique and equipment:
    - Used age-appropriate communication and play to reduce anxiety.
    - Explained procedures simply and positively to both children and caregivers in their first language.
    - Utilised visual pre-recordings to improve understanding.
    - Used age-appropriate devices, for example for spirometry, with motivations like a candle on a cake to blow out.
- Training and education:
    - Well trained staff in pulmonary function testing, supported by international experts in the field including ongoing collaborations, and on-site visits by experts as well as staff training in established units.
    - Trained staff in child-centred communication and motivational techniques.
- Parental and caregiver involvement:
    - Obtained informed consent and addressed parental concerns beforehand.
    - Encouraged caregivers to support and reassure the child during testing. For example, in 3- and 4-year-old children, had the child sit on the parent's lap during oscillometry.
- Active retention:
    - Visited families at home if they did not attend their scheduled appointment to reschedule.

- Hand delivered lung function booking dates and times if families were not reachable by phone.

6.7 Proposed strategies for integrating lung function testing into routine paediatric care

Integrating preschool lung function testing into routine paediatric care in LMICs requires comprehensive health policy actions and strategic implementation approaches. Policies need to prioritise resource allocation, workforce training and access to equipment at the same time, while leveraging on existing healthcare infrastructure. The aim is to improve early detection of respiratory conditions and optimise management, ultimately decreasing the burden of childhood respiratory diseases in LMIC.

Suggestions include:

- Prioritise lung health: Incorporate lung function screening into national child health programs such as immunisation visits, growth monitoring or well-child clinics, all of which align with broader goals of reducing childhood morbidity and mortality.
- Resource allocation and infrastructure development: Availability of affordable, portable, and open-source equipment—such as low-cost spirometers and oscillometry devices (http://www.open-source-medical-devices.com) that require minimal calibration and are validated for use in young children, allowing widespread use especially in LMIC.
- Capacity building and training: Training community healthcare workers and primary healthcare providers in paediatric lung function assessment and equipment maintenance. Develop training modules on testing techniques and results

interpretation. Incorporate training into existing medical curricula and continuous professional development programs.

- Standardisation of protocols and guidelines: Development of context-specific, evidence-based guidelines specific for LMIC and other high burden settings; and integration of these guidelines into existing paediatric care frameworks.
- Data collection and surveillance: Establish systems for recording and monitoring lung function data to inform policy, track disease burden, and evaluate interventions.
- Community engagement: Educate caregivers about the importance of early lung health assessment. Involve community members in co-creating solutions to raise awareness about lung health and to advocate for improved preventative and clinical services.

6.8 Conclusion

Early life is a critical time for lung growth and a time when lungs are vulnerable to insults with lifelong consequence. Preterm born children, notably MLP born children, have impaired lung function trajectories over the first 5 years of life, highlighting the importance of maternal and perinatal health care. This data demonstrated the impact of various exposures on lung health and highlights the potential of tracking lung function in infancy through childhood.

This study showed that preschool lung function is feasible and can be done with high success in a LMIC setting, highlighting its use as a tool to assess respiratory health in young children. Various factors that impacted lung health were identified, including postnatal environmental exposures, and quantified the effects of various exposures such as pollution and ETS

exposure on lung function. Programs and interventions to mitigate these exposures are urgently needed to prevent lung function decline and to promote healthy lung growth.