THE ASSOCIATION OF AMBIENT NITROGEN DIOXIDE AND PARTICULATE MATTER EXPOSURE ON INFANT LUNG FUNCTION

By

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Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy (Occupational and Environmental Health) in the Discipline of Occupational and Environmental Health, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

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PREFACE

The study described in this thesis was carried out in the Discipline of Occupational and Environmental Health, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, between January 2016 – December 2019 under the supervision of Professor Rajen N Naidoo and Professor Prakash M Jeena.

This study is original work done and reported by the author. The study has not been used in any form, by any persons or submitted to any tertiary institution for the award of a degree. In cases of publication, due acknowledgement has been accorded to all contributing authors.

Signed:  Date: **17 January 2022**

Sheena Muttoo

DECLARATION 1: PLAGIARISM

I, Sheena Muttoo, declare that:

i. The research reported in this thesis, except for where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

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Supervisor: Professor Rajen N Naidoo

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Co-supervisor: Professor Prakash M. Jeena

Signed: _____  _____ Date: **17 January 2022**

DECLARATION 2: PUBLICATIONS

The publication (published, in print and/or submitted) that constitute this thesis and the contribution I made to each of the manuscripts are presented here:

I (Ms S Muttoo) with my supervisors (Professor Rajen N Naidoo and Professor Prakash M Jeena) conceptualised the papers presented, with additional support from Professor Martin Röösli. Dr Hasheel Tularam and Dr Lisa Ramsay developed the hybrid land use regression-dispersion model, with support from Professor Bert Brunekreef, Dr Kees de Hoogh and Mr Kees Meliefste. Professor Anna-Carin Olin, Dr Hanne Krage-Carlsen and Professor Graciéla Mentz assisted with statistical analyses and presentation of results.

I also performed the lung function testing using the Ecomedics Software and analysis using the Wbreath software together with the clinical technologist Ms Giselle Naidoo. Mrs Kareshma Asharam provided all of the participant data and follow-up questionnaire information from the MACE cohort datasets. I completed all the statistical analyses with guidance and support from Dr Aweke Mitku and Professor Graciéla Mentz. The supervisors and all co-authors critically reviewed the manuscripts.

Published Abstract

1. **Muttoo S,** Naidoo RN, Jeena PM, Asharam K. Air Pollution Exposure and Infant Lung Function in the MACE Cohort, South Africa. Environmental Epidemiology. 2019; 3:280.
2. Naidoo R, Jeena P, **Muttoo S,** Ramcharan K, Naidoo V. Infant Lung Function and Exposure to Oxides of Nitrogen in a South African birth cohort. European Respiratory Journal 2017;50:PA1229.

Manuscripts

1. Muttoo S, Jeena PM, Röösli M, Olin A.C, Carlsen H.K, Asharam K, Naidoo G, Mitku AA, Naidoo RN. **Low Birth Weight and Maternal Smoking as predictors of Infant Lung Function from a South African Birth Cohort within Low-Socioeconomic Communities.** Submitted to the *South African Journal of Child Health* (SAJCH01957). 2021. Status: Accepted.
2. Muttoo S, Jeena PM, Röösli M, de Hoogh K, Naidoo RN. **A review of ambient air pollution exposure assessment methods in determining childhood respiratory health effects in children under five.** Submitted to the journal *Environments* (Environments-1676721). 2021. Status: Accepted.

3. Muttoo S, Jeena PM, Röösli M, de Hoogh K, Meliefste K, Tularam H, Olin A.C, Carlsen H.K, Mentz G, Asharam K, Naidoo RN. **Effect of Short-term Exposure to Ambient Nitrogen Dioxide and Particulate Matter on Repeated Lung Function Measures in Infancy: A South African Birth Cohort.** Submitted to the journal *Environmental Research* (ER-21-6681). 2021. Status: Accepted.

Publications

1. Tularam H, Ramsay LF, ***Muttoo S***, Brunekreef B, Meliefste K, de Hoogh K, et al. A hybrid air pollution / land use regression model for predicting air pollution concentrations in Durban, South Africa. Environmental Pollution. 2021:116513.
2. Mitku AA, Zewotir T, North D, Jeena P, Asharam K, ***Muttoo S***, Naidoo RN. The spatial modification of the non-linear effects of ambient oxides of nitrogen during pregnancy on birthweight in a South African birth cohort. Environmental Research. 2020; 183:109239.

Statement of Contribution

- I performed the lung function testing and analysis.
- I collated all participant information from the MACE cohort for the selected participants that were involved in this work.
- I compiled the database with all relevant information for data analysis
- I performed all the statistical analysis, only seeking support and advice on complex aspects.
- I worked with Dr. Tularam in reviewing and validating the exposure assessment models developed, and I further ran the models for the required time periods for the purposes of this study in determining two-week averages.
- I conceptualised and compiled all manuscripts included in this thesis.

Signed:  Date: **17 January 2022**

Sheena Muttoo

PRESENTATIONS AT CONFERENCES AND SYMPOSIUMS

International:

1. **Muttoo S**, Jeena PM, Mitku A, Asharam K, Naidoo RN. Infant Lung Function and Exposure to Oxides of Nitrogen in a South African Birth Cohort, 31st Annual Conference, International Society for Environmental Epidemiology (ISEE), Utrecht, the Netherlands, 25-28 August 2019.
2. Naidoo R, Jeena P, **Muttoo S**, Ramcharan K, Naidoo V. Infant Lung Function and Exposure to Oxides of Nitrogen in a South African Birth Cohort. European Respiratory Society International Congress, Milan, Italy, 9 – 13 September 2017.

National:

1. **Muttoo S**, Jeena PM, Mitku A, Asharam K, Naidoo RN. Prenatal air pollution exposure & impact on lung function in early childhood. Joint Congress: Pan African Thoracic Society / South African Thoracic Society (PATS/SATS) 2018, Durban, South Africa, 12-15 April 2018.
2. **Muttoo S**, Jeena PM, Asharam K, Mitku A, Naidoo RN. Infant Lung Function and Oxides of Nitrogen Exposure: The Mother and Child in the Environment Study. 13th Annual Conference, Public Health Association of South Africa (PHASA), Johannesburg, 1-4 September 2017.

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I gratefully acknowledge the efforts of the MACE Project Manager Mrs Kareshma Asharam and her team of field workers, as well as the lung testing technician Ms Giselle Naidoo for their extensive support in field data collection towards this project. The support of the Drakenstein Child Lung Health Study (DCHLS) team, namely Professor Heather Zar, Dr Diane Gray, Ms Lauren Willemse and Mr Carven Jacobs are further acknowledged for their assistance in the initial training of our team and providing the necessary standard operating procedures and ongoing support when required, for us to smoothly conduct our testing in a standardised manner.

Finally, I thank my supervisors Professor Rajen N. Naidoo and Professor Prakash M. Jeena for their consistent support, guidance, mentorship and advice throughout this project and the extensive administrative support of the Discipline of Occupational and Environmental Health and the University of KwaZulu-Natal for allowing me a workspace and necessary resources for project completion that is gratefully acknowledged.

ABBREVIATIONS

AP	Air Pollution
BILD	Bern Infant Lung Study
DCLHS	Drakenstein Child Lung Health Study
FRC	Functional Residual Capacity
LCI	Lung Clearance Index
LF	Lung function
MACE	Mother and Child in the Environment
MBW	Multiple Breath Washout
MTEF	Mean Tidal Expiratory Flow
MTIF	Mean Tidal Inspiratory Flow
MV	Minute Ventilation
ND	North Durban
NO	Nitric Oxide
NO_2	Nitrogen Dioxide
NOx	Nitrogen Oxides
PM	Particulate Matter
PM_1	Particulate Matter with particle size <0.1µm
PM_{10}	Particulate Matter with particle size <10µm
$PM_{2\,5}$	Particulate Matter with particle size <2.5µm
RR	Respiratory Rate
SD	South Durban
TB	Tidal Breathing
tPTEF/tE	Time to Peak Tidal Expiratory Flow Over Total Expiratory Time
TV	Tidal Volume

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ABSTRACT

Background: Vulnerable groups such as infants are particularly susceptible to exposure to air pollutants, including nitrogen dioxide (NO_2) and particulate matter with aerodynamic diameter less than 10 microns (PM_{10}), as structural and developmental changes in respiratory physiology are still occurring in-utero and progressively during the first few years of life after birth. Important and sensitive markers of lung growth and development include tidal volume (TV), functional residual capacity (FRC), and lung clearance index (LCI).

Objective: This study aimed to determine if short-term exposure windows to air pollutants nitrogen dioxide and particulate matter, induces an acute response in infant lung function (ILF) and if this response is age-sensitive, given the critical period of lung growth and development in the first two years of life.

Methods: ILF measures were determined by tidal breathing and multiple breath washout assessments without sedation in infants aged six weeks (n= 70), six (n= 72), twelve (n= 61), and twenty-four months (n=69). In preliminary descriptive analysis (Manuscript I), several risk factors were considered in multivariate models including low birth weight (LBW) and maternal smoking. To assess short-term air pollutant impacts on infant lung function (Manuscript III), individual exposure to NO_2 and PM_{10} (two-week average preceding the test date) was determined by hybrid modelling combining land use regression and dispersion modelling. These were included in linear mixed models adjusting for the repeated measures design for the outcome measures and an age*exposure interaction was introduced to obtain effect estimates for each age group.

Results: In multivariate analyses LBW was associated with a lower TV at 6 weeks (β: -5.99mL (95%CI: -9.59; -2.39)), 6 months (β: -15.02mL (95%CI: -22.48; -7.57)) and 12 months (β: -23,7mL (95%CI: -35.55; -11.85)), compared to children with normal birthweight. This was similarly observed for LBW and minute ventilation at 6 weeks (β: -157.78mL/min (95%CI: -338.95; 23.38)), 6 months (-β: 325.57mL/min (95%CI: -619.06; -32.08)) and 12 months (β: -527,58mL/min (95%CI: -947,85; -107,32)), though these observations were less evident at the 24-month age group. Air pollutant exposure-outcome associations assessed by linear mixed models showed reduced tidal volume per unit increase in PM_{10}, as observed at 6 weeks (β: -0.4mL (95%CI: -0.9; 0.0), p=0.065), 6 months (β: -0.5mL (95%CI: -1.0; 0.0), p=0.046) and 12 months (β: -0.3mL (95%CI: -0.7; 0.0), p=0.045). PM_{10} was related to an increase in respiratory rate and minute ventilation, while a reduction was observed for FRC for the same age groups, though not statistically significant for these outcomes. Such associations were however less evident for exposure to NO_2, with inconsistent changes observed across measurement parameters and age groups.

Conclusion: From descriptive analyses, low birth weight was the main predictor for low tidal volumes and minute ventilation at 6 weeks, while observed differences were smaller at 12 and 24 months. Exploration of exposure-outcome associations showed that short-term (two-week average) exposure to PM_{10} results in acute lung function impairments among infants from a low-socioeconomic setting, after adjustment for relevant covariates, while the association with NO_2 is less convincing.

CHAPTER 1

1.1.Introduction

Epidemiological studies have shown that exposure to ambient air pollutants is associated with adverse effects on lung growth and development[1-3]. Both cross-sectional and longitudinal assessments have demonstrated air pollution-related lung function decline in children, suggesting a causative association[4,5]. Airborne pollutants present a significant health risk as they are easily inhalable and reach the respiratory system, thus lung function is an important objective marker for respiratory health and predictor of respiratory morbidity[6]. Ambient air pollutants primarily associated with urban development include nitrogen dioxide (NO_2) and particulate matter (PM_{10}), which are known markers of industrial and traffic emissions.

Children are notably more vulnerable to air pollution insults as their lungs are still developing at birth[7], with physiological and structural changes still occurring in the first few years of life. Thus, lung function assessment in early life is critical to understanding the determinants of long-term respiratory health. Studies have shown that lung function deficits experienced early in life may persist into adulthood[8] and predispose individuals to the onset of respiratory symptoms and illness such as airway inflammation, wheezing, chronic obstructive pulmonary disease (COPD)[2,9,10] and cardio-vascular effects[11]. The identified predictors associated with reduced lung function in infants include maternal smoking during pregnancy[12-14], maternal HIV exposure[15], nutrition, genetics[16], preterm birth[17,18], low birth weight[19] and air pollution[3].

Measures of tidal breathing and multiple breath washout are relevant when assessing lung function in this age group. Tidal breathing measures have been utilized in clinical and research settings to determine tidal volume, breathing frequency and minute ventilation, to assess the control of breathing and airway mechanics[20,21]. Measures of functional residual capacity (FRC) and lung clearance index (LCI) are important markers of lung growth and development. The determination of FRC is useful when assessing for abnormalities present, and for the interpretation of volume-dependent lung function parameters such as indices of gas mixing efficiency[22], while the LCI is considered the most robust and sensitive parameter of ventilation homogeneity. To quantify the degree of functional abnormalities of the lung, an accurate user-friendly technique to measure lung volume and ventilation distribution is necessary. This is achieved by gas dilution multiple breath washout (MBW) techniques, in which the FRC is determined by measuring areas of the lung that readily communicate with the central airways during tidal breathing[23].

Lung function assessment in infants without sedation is limited to research settings, potentially due to challenges around ethical consent, infant cooperation[24], sleep state[21], the use of bulky equipment and the moderately invasive nature of testing[25]. However recent applications of MBW testing without sedation have been reported in the Bern Infant Lung Development cohort study[26] (BILD) in Switzerland, a multicentre European & Australian study[18] and the Drakenstein Child Lung Health Study[25] (DCLHS) conducted in Cape Town, South Africa. These studies demonstrate the feasibility of lung function testing in the early years of life, allowing for the assessment of the determinants of lung development during this vulnerable phase of lung growth.

To assess the dose-response effect of air pollution exposure on lung function outcomes, quantification of the air pollutant of interest is required. In epidemiological studies, this has been achieved by employing methods of exposure assessment which vary in their complexity and capability in achieving robust measures of exposure[27]. Air pollution exposure assessment is used to determine the concentration of pollutants an individual comes into contact with and the duration of exposure. This is achieved by accounting for known meteorological, topographical and geographical attributes that influence the dispersion of air pollutants, which is captured by air pollution modelling approaches[28]. Exposure assessment has played a significant role in our understanding of air pollution-related health effects, particularly with respect to early/subclinical and low-dose effects[8].

As part of the Mother and Child in the Environment (MACE) cohort study, the current study aimed to determine the effect of short-term (2-week average) exposure to nitrogen dioxide (NO_2) and particulate matter (PM_{10}), as determined by hybrid modelling, on lung growth and maturation. These were determined by measures of tidal breathing and multiple breath washout, among infants aged six weeks, six, twelve and twenty-four months.

1.2. Background

1.2.1. Lung Growth and Development

Lung growth and development are controlled by various factors that modulate the timing and pattern of cellular proliferation and differentiation, morphogenesis[29,30] and maturation. This results in changes that affect both structure and functional capabilities of the respiratory system[31]. The earliest stage of lung development may have a principle role in determining postnatal mortality and morbidity of the foetus[30], thus the timing of insults during development is considered critical in the subsequent effects observed. As lung development occurs over the perinatal period, factors affecting lung growth can have

significant consequences whether they occur during the pre-or postnatal periods of life[29]. Lung function, is thus, an important objective marker for respiratory health and predictor of respiratory morbidity[6].

Lung morphogenesis and development of the airway commences at 4-7 weeks of gestation and progresses to the alveolar phase by 36 weeks of gestation, with alveolarization continuing into early adulthood. In early pregnancy, cellular differentiation and branching morphogenesis may be disturbed, while in late pregnancy impairment of structural and functional growth of the lung may occur. Repair mechanisms of the developing tissue may be less efficient in comparison to the mature lung and thus has increased vulnerability to respiratory insults[32].

Morphological changes to the lung have functional consequences on lung volume, ventilation homogeneity and mechanics of the respiratory system. For example, end-expiratory volume (functional residual capacity) has been shown to be diminished in pre-term infants, with further evidence of these infants having increased ventilation homogeneity (higher LCI)[33]. However, in wheezing infants, the LCI has been shown to be lower compared to non-wheezing infants, while an increase in FRC is observed[34]. Physiologically, dynamic regulation in maintaining end-respiratory lung volume above the airway closure has implications for chest wall compliance, as the elasticity of the lungs improves with age. Thus early impediment may have detrimental effects on the mechanical and structural properties of the lung and subsequent airflow[35]. The structure of the lungs is considered an important determinant of ventilatory function, and thus related to the mechanical properties of the lung. This is reflected in the relationship of volume, flow and time during expiratory manoeuvres. The relationship of expiratory flow rates at a given residual volume (a measure sensitive to airway size) to lung volume (a measure sensitive to lung size) is a further measure of this structural/functional relationship[36].

The breathing pattern of newborns, specifically the first breaths taken, are deeper and longer than subsequent breaths and characterized by a short deep inspiration followed by a prolonged expiratory phase. This is referred to as expiratory braking and assists in developing and maintaining the FRC during the immediate postnatal period when the lung is partially fluid-filled and the chest wall highly compliant [37]. At the neonatal stage of life, several events including maturation of the neurologic control of breathing, as well as changes in the mechanical properties of the respiratory system (e.g., changes in lung and chest wall compliance and lung volume), influence respiration. The degree and speed of these occurrences do however vary between infants and this results in greater overall variability in the measurement of respiratory rate. Tidal volume is reported to remain invariant (~6 mL/kg) from birth to adulthood, while respiratory rate progressively declines with growth. The newborn has higher ventilatory needs, which are met by increasing breathing frequency (respiratory rate of up to 40 breaths/min)[35]. In healthy infants, respiratory rate decreases with postnatal age. This decrease is further

accompanied by an increase in the duration of inspiratory and expiratory time, a function in airway size[38].

Some of the identified risk factors associated with changes in these lung function parameters include birth outcomes (low birth weight and preterm birth) and respiratory outcomes (bronchopulmonary dysplasia (BPD), cystic fibrosis, wheezing, and asthma). Schmalish et al. reported that FRC was significantly lower (p=0.036) in former BPD infants compared to non-BPD infants, among a sample of 55 preterm infants (gestational age < 30 weeks) with birth weight <1,5kg, though was not shown to impact tidal breathing parameters after age correction[39]. Hulskamp et al.[18] found that pre-term controls (n=59) had lower mean (SD) FRC (64.3mL (15.6)), in comparison to full-term controls (n=64), at (79.6mL (14.5)). Among this sample, tidal volume was also noted to be significantly smaller among infants with chronic lung disease 18.7mL (6.1), and preterm controls (21.8mL (7.0)) compared to term controls (29.7mL (5.9)).

The rapid development and growth of the lung early in life, suggest increased vulnerability shortly after birth. Studies have further cited specific periods or "windows" of exposure as having a critical impact on developmental changes, as lung immaturity and physiology of the growing foetus[40,41], and that of very young children predisposes them to increased susceptibility to insults by toxicants[11]. Normal lung development is essential for long-term respiratory health, as significant developmental changes in respiratory physiology are known to occur progressively during the first years of life after birth[42,43]. Thus, early clinical assessment of lung function is critical in determining the early life risk factors that predispose lung impairment.

1.2.2. Air Pollution

Ambient air pollution is generated from various anthropogenic, industrial, and transportation processes, that emit harmful gases and particulates into the atmosphere. Airborne pollutants present a significant health risk as they are inhalable and reach the respiratory system, and have thus been linked to various acute and chronic lung health effects in both children and adults.

Particulate air pollution is a mixture of solid, liquid, or solid and liquid particles in suspended air. Particulate matter (PM) is distinguished by the varying particle sizes, "thoracic" particles smaller than 10µm in diameter (PM_{10}) can penetrate the lower respiratory system and "respirable" particles smaller than 2.5µm enter the gas exchange region of the lung, as well as PM_1 (with particle size <0.1 µm) with an increasing degree of lung penetration[44]. Major contributing sources include combustion processes, industrial activity, factories, power plants, motor vehicles, construction, fires, and natural windblown

dust[45]. The composition of PM varies, as they can absorb and transfer a multitude of pollutants, with major components including metals, organic compounds, material of biologic origin, ions, reactive gases, and the particle carbon core[46]. PM causes lung inflammation and mucous secretion by acting on airway epithelial cells and alveolar macrophages with the potential of leading to airway remodelling[47]. PM exposure has been associated with acute respiratory effects[48] and morbidity[49].

Nitrogen dioxide (NO_2) is a gaseous pollutant. Traffic is the major source of ambient outdoor exposure of NO_2 in urban areas, while additionally, emissions emerge from the combustion of fossil fuels from stationary sources (heating, power generation, industrial activities)[50], and indoor sources from anthropogenic gas/kerosene fuelled heating practices[51]. NO_2 that is inhaled, mainly affects the respiratory system, thus increasing susceptibility to respiratory infections[46]. When inhaled, NO_2 penetrates deep into the lungs, thus the small airways are the primary site of NO_2 induced lung damage. The major health endpoints associated with NO_2 exposure include increased incidence in lower respiratory tract infections in children and increased airway responsiveness in asthmatics[49,51].

The association between exposure (acute and chronic) to air pollutants and adverse respiratory outcomes, specifically lung function deficits is well documented in older age groups[52,53], but less so in very young children. Given the interaction between the environment and the individual, the respiratory tract has increased vulnerability to the adverse effects of air pollutants, this is particularly the case for very young children, as their immune system and lung structure is not fully developed at the onset of exposure[40,54]. Current evidence of even low dose effects of air pollution below the prescribed air quality guidelines[55] have been linked to adverse health outcomes[56]. The adverse impacts of NO_2 and PM exposure have been repeatedly linked to respiratory health effects[57,58].

In the BILD study[59], pregnancy exposure to air pollution and changes in tidal breathing, lung volume, and airway inflammation in 241 healthy infants aged 5 weeks was assessed. Exposure to NO_2 and PM_{10} was averaged throughout pregnancy (conception to infant birth date) as well as during each trimester of pregnancy. Minute ventilation was observed to be higher in infants with increased pre-natal PM_{10} exposure (24.9 mL/min per $\mu g/m^3$ PM_{10}). Exhaled nitric oxide (eNO) was increased in infants with higher prenatal NO_2 exposure (0.98 ppb per $\mu g/m^3$ NO_2). Post-natal exposure to air pollution did not modify these findings. There was no association was observed for prenatal exposure to O_3 and lung function measures. This study demonstrates that the pregnancy window and subsequent in-utero exposures predispose diminished lung function in the first few weeks of life.

Mortimer et al.[60] report on prenatal and lifetime exposure in 232 asthmatic children, aged 6-11 years, assessing pollutant-induced lung remodelling. This study found that second-trimester exposure to NO_2 negatively affected forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) with effect estimates (β) of -0.01L and -1.82L respectively and standard error (SE) of <0.01. First

trimester exposure to PM_{10} was negatively associated with peak expiratory flow (PEF) rate (β = -0.01L/min; SE = 0.003). The study showed trimester-specific exposure to air pollutants PM_{10} and NO_2, resulted in adverse impacts on lung function at age six to eleven years, indicative of the long-term exposure effects beyond the pregnancy window.

Usemann et al.[61] found that exposure to "higher" levels of NO_2 during pregnancy, which was still at least four times lower than prescribed standards of the World Health Organisation[55], was associated with a significant decrease in FEV_1 per interquartile increase in NO_2. FEV_1 decreased by (z-score change (95% CI)), −1.07 (−1.67 to −0.47); −1.02 (−1.66 to −0.39); −0.51 (−0.86 to −0.17) and −0.80 (−1.33 to −0.27), respectively as assessed in the first and second years of life and from birth until follow up.

Although the absolute effects of air pollutant exposures on respiratory outcomes seem relatively small, a decline in lung function may be most significant for those with early-life lung function impairments (e.g., preterm infants) or those with increased susceptibility (e.g., infants with cystic fibrosis). Studies demonstrating even low-dose effects highlight the risk that air pollution exposure poses to lung health with outcomes evidently dependant on timing and "windows" of exposure[8].

1.2.3. Environmental Tobacco Smoke

Environmental tobacco smoke (ETS) (also known as second hand smoke or passive smoke), is the by-product of cigarette smoke mixed with exhaled mainstream smoke. ETS contains over 4000 chemical components including polycyclic aromatic hydrocarbons, carbon monoxide, nicotine, and particulate matter, with known carcinogenic properties[11].

Smoke exposure in infants and young children occurs both antenatally in-utero or postnatally from maternal smoking or by passive smoke exposure. Maternal smoke exposure or ETS exposure of pregnant mothers has been linked to premature delivery, childhood malformations, and low birth weight[62]. It has further been associated with significant risks in the development and/or exacerbation of asthma, airway hyper-responsiveness, and other respiratory symptoms such as cough, wheeze, and mucus secretion[29]. The harmful effects of ETS exposure begin even before birth, as the developing lung may be exposed to ETS in utero through transplacental transfer[11], with circulating nicotine from the mother's blood reaching the foetus by permeating the placenta[62]. Such effects may also occur during early childhood through breastfeeding and exposure in the environment[11].

Several mechanisms may alter the lung and airway structure, from maternal or ETS exposure. This includes diminished placental blood flow with subsequent reduced delivery of oxygen and nutrients to the foetus, stimulation of nicotine receptors that alter airway and alveolar architecture, and alteration to foetal breathing pattern which directly influences foetal lung development[63]. Research has further indicated that effects of in-utero smoke exposure show evidence of altered pulmonary function soon after birth prior to any significant post-natal exposure, with changes such as altered tidal breathing patterns and decreased forced expiratory flows observed[64]. Hoo et al. reported on respiratory function in preterm infants whose mothers smoke during pregnancy and found that time to peak tidal expiratory flow as a proportion of total expiratory time (tPTEF:tE) was significantly lower in infants exposed to tobacco smoke in utero, with a mean (SD) of (0.369% (0.109)) when compared with those who were not exposed (0.426% (0.135), $p < 0.02$). Additionally, maximal forced expiratory flow was also reduced in exposed infants with a mean (SD) of 85.2mL/s (41.7) versus 103.8mL/s (49.7) ($p=0.07$) in unexposed infants. After adjusting for sex, ethnic group, body size, postnatal age, and socioeconomic status, tPTEF:tE remained significantly diminished in infants exposed prenatally to tobacco smoke ($p< 0.05$) [50]. As in-utero exposure to maternal smoking and ETS exposure during childhood are highly correlated, both exposures should be considered when assessing adverse effects of tobacco smoke on pulmonary function[65]. The effects of pre-and postnatal tobacco smoke exposure may be difficult to disentangle as mothers who smoke during pregnancy may likely continue to do so thereafter. Thus, the relative contribution of in-utero exposure to maternal smoking and postnatal ETS exposure, to persistent deficits in lung function remains unclear.

Evidence-based epidemiological research has however demonstrated a strong association between respiratory illness in young children and ETS exposure, with some studies further suggesting that exposure during the perinatal period may result in adverse effects on lung function that can persist into adulthood[29,63,65,66]. Maternal and ETS exposure are thus important risk factors for lung function and development.

1.2.4. Lung Function Measurement

From a clinical perspective measurement of respiratory parameters in infants has historically been attempted using spirometry to assess tidal volume and respiratory rate. Previous conventional methods of application included whole-body plethysmography, known for its extensive resource and technical demands[21]. This was used to measure FRC, with later modification allowing measurement of airway resistance. Measurement of ILF using gas dilution/washout techniques was also historically reported [21,67], however further development and clinical was restricted primarily due to technical limitations[68].

The development of innovative gas-analysers and computerised advancements has since emerged as a more suitable tool for routine clinical assessment and research studies[68].

Challenges in undertaking lung function testing in very young children aged below two years, relate to sleep state, sedation, ethical concerns, posture, adaptive equipment, and patient cooperation[20]. Developmental changes that may impact the assessment of lung function in infants include chest wall compliance, dynamic elevation in FRC and the influence of the upper airways. During the first few months of life, infants modulate both expiratory time and flow to maintain adequate FRC. Alterations in respiratory rate, expiratory time and the emptying time of the lung with growth may all have an impact on the interpretation of changes in these various measurement parameters[20].

A combination of improvements in analyser technology and data analysis software, as well as the growing need for robust infant lung function techniques, have been a major driving force for renewed interest in infant lung function assessment. Current non-invasive approaches of early-life lung function assessment include gas dilution techniques that determine FRC by measuring areas of the lungs that regularly communicate with the central airways during tidal breathing. One such method is the multiple breath washout technique that is able to measure lung volume and ventilation distribution in infants[21,23].

1.2.5. Multiple Breath Washout

Multiple breath washout (MBW) in clinical and research settings is used to quantify FRC and assess ventilation distribution (or gas mixing efficiency) in an individual and has recently been popularised for its application in the infant age group[69]. Testing is performed in non-sedated conditions, where the participant is required to breathe tidally while washing tracer gas from the lungs. Most studies have reported on the use of Sulphur Hexafluoride (SF_6) as the inert gas used during testing[23]. This circumvents the tendency for infants to hyperventilate, leading to increased variability in tidal volume (TV), which is known to occur when 100% oxygen is used, as is required for MBW testing by nitrogen washout[69]. SF_6 is a synthetic, colourless, odourless, tasteless, biochemically inert gas and has low solubility in water and body tissues. At concentrations required in clinical testing, it is non-toxic and, thus acceptably used as tracer gas for MBW, provided there is a sufficient supply of oxygen available, 4% SF_6 presents with no side effects on humans[70].

MBW testing is preceded by the measurement of tidal breathing to quantify indices such as respiratory rate, tidal volume, and breath timing parameters. Furthermore, associated measures of tidal breathing have been reported to be early predictors of poor respiratory outcomes in longitudinal studies[69]. Important measurement indices of MBW testing include the LCI and FRC. The LCI is defined as the

number of lung volume turnovers necessary to clear the lungs from the tracer gas to 2.5% (1/40) of the starting concentration[71]. LCI is calculated as the cumulative expired volume (CEV) divided by the FRC[71]. The two parameters, FRC and LCI are reported as the mean (SD) of 2-3 subsequent washout manoeuvres[70].

The determination of FRC is relevant in the assessment of lung growth and development, in health and disease and for interpretation of volume-dependent lung function parameters[18], such as respiratory compliance forced expiratory flow and indices of gas mixing efficiency. As per the American Thoracic Society (ATS)/ European Respiratory Society (ERS), the term FRC refers to the volume of gas in the lungs at end expiration[22].

Though several parameters may be calculated as indices of ventilation homogeneity the LCI is the most robust and sensitive parameter. The LCI is a measure of ventilation homogeneity in the lungs, changes in the small airways lead to inhomogeneous ventilation of different parallel airways, which results in inhomogeneous emptying of peripheral lung units, with consistent changes in ventilation distribution. This ventilation inhomogeneity (VI), is reflected by a delay in the clearance of tracer gas during MBW, and maybe an indication of inflammation or airway obstruction[71]. Studies have demonstrated that the LCI is constant during childhood and adolescence, with variability over time being low and thus being suitable for monitoring lung disease[72]. In the presence of disease the LCI increases, with increasing severity[71]. Longitudinal studies are relevant in establishing how LCI tracks disease progression, given the sensitivity of small airways dysfunction, which makes the LCI a valuable measure of airway physiology[71]. The particular sensitivity of LCI in cystic fibrosis, which is widely reported in the literature, reflects the underlying lung pathology of airway inflammation and obstruction. The range of LCI in healthy subjects appears to be relatively narrow across a wide age range and is seemingly unaffected by height or gender. Additionally, the LCI has good intra-assessment reproducibility with a coefficient of variation of 3-8% for repeated measures[71].

1.2.6. Tidal Breathing

Tidal breathing refers to inhalation and exhalation during restful breathing. The range of tidal breathing is determined both by the depth of inspiration and by the endpoint of passive exhalation at FRC[73]. Tidal breathing measures have been used in clinical and research settings to determine tidal volume, breathing frequency and minute ventilation and the assessment of the control of breathing and airway mechanics[20,21]. Various measurements are assessed during tidal breathing, however quantitative separation of these individual components, together with a meaningful interpretation of their relative role within the tidal breathing process, remains a significant challenge. The most robust and meaningful

parameters are respiratory rate (RR), tidal volume (TV), and minute ventilation (MV). RR and TV are reportedly easy to measure but difficult to interpret as the process of measurement may influence the infants breathing pattern. MV is measured to assess the control of breathing[21] and as it is the product of RR and TV, MV is affected by alterations occurring in either of these parameters[74]. To maintain the required minute ventilation, the human body may increase either the volume of each breath or frequency of breathing or both[74]. Decreases in MV can result from abnormalities of respiratory drive and airway obstruction[17]. Airflow V'(t) and volume V(t) determined by numerical integration of V'(t) are the basic signals of a tidal breath analysis. Both signals plotted together in an x-y diagram represent the tidal breathing flow-volume loop (TBFVL)[75].

Additionally, the measurement of time to reach peak expiratory flow (tPTEF) as a proportion of total expiratory time (tPEF/tE) has also been identified as an important indicator of airway obstruction. In obstructive airway disease, tPTEF is shorter, and the duration of the expiration is longer as compared to healthy controls, leading to a decreased tPEF/tE ratio. For healthy infants within the age group of 3-12 months the tPEF/tE ranges between 0.26-0.29. The index tPEF/tE has been demonstrated to be useful in predicting wheezing in newborns, in differentiating between asthmatic and non-asthmatic children and distinguishing healthy controls from children with chronic lung disease[67]. Mean tPTEF:tE is considered to be a useful index of lower airway obstruction[76].

The table below demonstrates differences in measured values between European Caucasian infants and African infants as observed in the BILD[49,50] and DCLHS[25] cohort respectively.

Table 1: Comparison of ILF measurement outcomes between BILD and the DCLHS study

	BILD (N=269)		**DCLHS (N=363)**	
	Mean (SD)	**Median (IQR) Intra-subject CV***	**Mean (SD)**	**Median (IQR) Intra-subject CV***
Tidal breathing N successful test (%)	**285(96)**		**356 (98)**	
Tidal volume (ml)	32.4 (5.5)	8.6 (7.1: 10.8)	34.9 (6.3)	7.7 (6.3-9.9)
Respiratory rate (breaths per min)	45.2(10.5)	9.1 (7.4; 11.3)	48.1 (11.9)	8.0 (6.7-10.2)
Minute ventilation (ml/min)	1,420 (277)	7.5 (6.1;10.1)	1627.0 (307.6)	7.1 (5.9-9.0)
tPTEF/tE	34.8 (10.7)	23.8 (20.2; 28.4)	39.8 (12.1)	20.1 (16.8-25.2)
Multiple breath washout N successful tests (%)	**201(68)**		**345 (95)**	
Functional residual capacity (ml),	102 (16)	6.3 (4.4:8.3)	77.9 (17)	5.4 (3.3-7.8)
Lung clearance index	6.75 (0.6)	5.8 (3.6;8.0)	7.2 (0.4)	4.0 (2.4-5.8)
*Coefficient of variation				

1.2.7. Determinants of Lung Function

Several risk factors for adverse lung function or respiratory outcomes in childhood have been identified including birth weight[78], prematurity[18], early life respiratory illness such as wheezing[79], maternal factors such as HIV infection[15,80] and socio-economic status (SES)[81], environmental factors such as tobacco smoke exposure[82] and air pollution[3]. It has been reported that factors that occur in-utero may not only restrict foetal weight gain and thus birthweight but would consequently constrain the growth of the lungs and airways[78] increasing the risk of early-life respiratory morbidity. Hence, the association between suboptimal lung function in childhood and later life has been increasingly linked with events occurring during foetal development and early infancy.

When assessed together, parameters of lung size such as FRC and TV have been reported to be independently related to body size (weight and/or length)[83]. Perinatal factors including birth weight, gestational age and nutrition have been associated with reduced lung function over time[31]. A European and Australian multicentre study found that on average full-term infants had a lower standard deviation of FRC (1.3mL/kg) in comparison to preterm infants (1.88mL/kg, p=0.004), suggesting poor lung mechanics, while no significant differences were observed for indices of ventilation inhomogeneity[18]. Premature birth and low birth weight are important determinants of intrauterine growth and development, and predictors of morbidity[32].

It has further been reported that diminished lung function in the first few months of life precedes and is predictive of wheezing in early childhood. Martinez et al.[84] reported that infants who wheezed during the first year of life, and had at least one additional lower respiratory tract illness, had 10% lower FRC ($p<0.05$) compared to never wheezers. Thus, suggesting that initial airway function predisposes recurrent wheezing in the first year of life. Dezeteux et al.[85] observed that maternal smoking and poor airway function observed in early life are independently associated with lower levels of airway function at the end of the first year, and related to early life reduced small airway calibre[86]. Diminished airway function is thus mediated by impaired development during early life, hence lower respiratory tract illness such as wheezing is an important risk factor in lung function assessment.

Maternal smoking during pregnancy has consistently been reported as a risk factor for poor lung function[64]. The DCHLS study showed that infants (aged 6-10 weeks) with maternal smoke exposure, had lower TV (-1.6mL (95%CI: -3.0 to -0.1) p=0.04), and higher LCI (0.1 turnovers (95%CI: 0.01 to 0.3) p =0.03)[87]. The Western Australian pregnancy cohort[14] study found lower values of tPTEF/tE, in 461 healthy infants with a median age of 58 hours. The outcome tPTEF/tE was reported to be independently associated with respiratory rate (0.018 per 10 breaths/min, $p<0.01$), age (–0.008 per 10

hours, p<0.01) and maternal smoking during pregnancy (–0.049 per >10 cigarettes daily, p<0.05). Tager et al.[88] reported on lung function in the first 18 months of life in 159 infants in a longitudinal study, and found that maternal smoking during pregnancy was associated with a reduction in FRC of 9.4 +/- 4.3 mL (p = 0.029) and after controlling for the effects of growth (length). In this study postnatal ETS exposure was not significantly associated with reduced FRC[88]. The data presented suggest that antenatal maternal smoking may play a greater role than postnatal and childhood exposure on the observed effects on lung function in early life. Studies have found that the effects of maternal and/or ETS exposure tracks into later life[9,89], however a limitation of most studies is their inability to separate the effects of direct in-utero and passive postnatal exposure to constituents of cigarette smoke as independent predictors of lung function deficits.

The effects of antenatal in-utero and postnatal exposure to air pollution have been frequently reported in older children[52,90-92] with limited research in infancy. Some studies have cited the shorter windows of exposure in the first few weeks of life as being limiting to the extensive assessment of "postnatal" exposures, while others report in-utero exposures to show significant effects on lung function soon after birth, however, clinical evidence is still lacking in demonstrating an appropriate dose-response effect. The DCHLS cohort reported on household benzene exposure, measured during the antenatal home visit using Markes thermal desorption tube that was left in the home for a two-week period. Infant exposure in-utero had a lower tPTEF/tE ratio, 3% (95% CI −5.2% to −0.7%, p=0.01) compared with unexposed infants[87]. The BILD study reported on higher minute ventilation observed per unit increase in PM_{10} (24.9 mL/min per $\mu g/m^3$ PM_{10}), among healthy infants[3].

Several studies from high-income countries have assessed the effects of known confounders (or risk factors) on lung function, but there is limited data available from low to middle-income settings with novel risk factors that relate to HIV exposure and markers of socioeconomic status that are important in the African context. Exposure to HIV in utero, but not infection, could affect lung growth through direct impacts from the virus itself, consequential immune suppression, or exposure to antiretroviral therapy (ART). Recent work by the DCHLS cohort indicated that in-utero exposure to HIV resulted in lower TV in HIV exposed infants (n=175) in comparison to unexposed infants (n=682) with a mean difference of (1.13mL; CI: 0.02–2.23; p<0.045) between exposure groups at 6 weeks, suggesting an effect on control of breathing[15]. It was further reported that the observed effect may be mediated through maternal HIV or due to ART treatment taken by the mother (to prevent mother to child transmission), resulting in dysregulation of metabolic pathways[87]. Though the effect of SES as a risk factor for poor lung function, was further assessed in this study, it was not found to be associated with the lung function outcomes. Distinguishing SES related effects may be difficult in the absence of a control population with high income, and comparison to other studies is limited as several other factors influence lung function.

The links between in-utero and early postnatal risk factors on lung function outcomes, as evidenced by these studies further highlight the complexity of contributing factors and our understanding of the determinants or predictors of early-life lung function. It is clear that critical windows of exposure and novel risk factors that are population-specific should be considered in the assessment of lung function. Additionally, sophisticated exposure assessment methods with spatio-temporal capabilities may assist in our understanding of the acute and chronic dose-response effects of air pollution exposure on early life function, which is currently lacking in the literature.

1.2.8. Environmental Exposure Assessment

Exposure assessment has played a significant role in our understanding of air pollution-related health effects, particularly with respect to early/subclinical and low-dose effects. Air pollution exposure assessment seeks to determine the concentration of pollutants an individual comes into contact with and the duration of exposure. Continuous measurement of personal exposure to air pollution over the duration of the outcome of interest is considered the gold standard for exposure quantification. In most instances, this may not be practically achievable, particularly in large population studies, attributed to input costs and logistical feasibility[8].

An alternative to personal exposure measurement and a simplistic method of assigning exposure is the use of monitored data from regulatory continuous monitoring networks, with exposure assignation based on the nearest monitor to the participant's address point. This method is merited for its availability of direct measurements, consistency of monitoring method, lack of input costs and availability of data for longer periods[28], allowing exploration of temporal trends. Monitored data is typically used directly as a surrogate of exposure in epidemiological studies, averaged over space and/or time, or as input to source apportionment models to estimate source impacts[27]. Several limitations however constrain its application when assessing intra-urban variations, as exposure is aggregated at a population level and does not take into consideration spatial contrasts in the distribution of ambient air pollutants. Regulatory monitored data has however been used to supplement modelling methods in exposure assessment with good temporal viability.

The BILD study[93] assessed air pollution exposure in relation to frequency, severity and duration of respiratory symptoms during the first year of life. In this prospective cohort of 366 infant's respiratory health was assessed weekly by telephone interviews. Daily mean levels of PM_{10}, NO_2, and Ozone (O_3) were obtained from local monitoring stations, with lag estimates constructed in the 1-10 days preceding the interview. The study found significant associations in the week after respiratory tract infections (risk ratio, 1.13 (95%CI: 1.02–1.24) per 10 μg/m^3 PM_{10} levels) and in infants with diminished lung

function. Additionally, during elevation of PM_{10} (>33.3 μg/m^3), duration of respiratory tract infections increased by 20% (95% CI: 2–42%). Though the study was able to demonstrate an association of effect with prenatal air pollution exposure and higher respiratory need, the authors do acknowledge the limitations of using central site monitoring for air pollution exposure assessment but found it useful in assessing fluctuations in pollutants for short-term assessment in their time-series analysis.

In the Southern Californian Children's Health Study[94] the effect of daily variation in ambient air pollutants on fractional exhaled nitric oxide (FeNO) was assessed in children aged 5-7 years. Daily 24-hour cumulative lagged averages of $PM_{2.5}$ (1–8 days), PM_{10} (1–7 days) and O_3 (at 10:00–18:00 hours over 1–23 days) were significantly associated with 17.42% ($p<0.01$), 9.25% ($p<0.05$) and 14.25% ($p<0.01$) higher FeNO levels over the interquartile range of 7.5 μg/m^{-3}, 12.97μg/m^{-3} and 15.42 ppb, respectively. They were able to establish a link between short-term increases in air pollution levels and airway inflammation. Later work by this cohort further assessed the long-term variations in air pollutants on longitudinal changes in FeNO and reported that long term averages of NO_2 and $PM_{2.5}$ were associated with significant longitudinal changes in FeNO in children after adjustment for short-term effects of air pollution as cited in their previous work[95].

From these studies, it is evident that central site monitoring could effectively be used in time-series analysis and tracks temporal variability in both short-term and long-term exposure windows (when adjusting for short-term effects). However, it is not a robust measure of personal exposure and is constrained by its lack of consideration of spatial contrasts. Furthermore, the general sparse distribution of monitoring networks limits data extrapolation for populations that do not reside in close proximity to a monitoring station. The use of central site monitoring data is also greatly dependant on the availability, quality and validation of data outputs.

As transportation has become one of the largest sources of criteria pollutants with links to health effects, the relative contribution of emissions from traffic has significantly increased in comparison to point sources. Although spatially diffuse throughout urban areas, significant variability occurs at localised spatial scales. Regulatory air pollutant monitoring may significantly under-estimates the range and spatial heterogeneity of traffic emissions and ambient air pollutants in general[96]. Reports on the use of surrogate measures of exposure such as distance to nearest road or industry and known proximity-based methods are also at risk of underestimating exposure.

Efforts to improve on the use of routine monitoring data in air pollution epidemiological studies have included a variety of alternative approaches for exposure assessment. Some studies have reported on the use of interpolation methods (such as inverse distance weighting and kriging), that provide a more spatially resolved pattern than a simple average, however, the assumed smooth spatial change of concentrations may be inadequate for application in urban areas, as local sources such as road networks

may have increased variability than that assumed by interpolation. Interpolation methods have primarily been applied at a regional level and may be beneficial for components with relatively small-scale local sources[28].

Air pollutant concentrations and their spatial distribution are influenced by local geographic characteristics, land use types, as well as time-varying meteorological parameters[97]. Thus models with higher spatial and temporal resolution are critical in exposure assessment[98]. The increasing ability of Geographic Information Systems (GIS) technology in demonstrating spatial variability, has seen various models developed around this concept of exploring the quantitative relationship between ground-based measurements and GIS-based variables (geographic predictors). Additionally, a number of statistical methods have been incorporated in these models, such as mixed effects models[99], artificial neural networks[100] and general linear or non-linear regressions[101].

Statistical approaches are used to combine various methods of exposure estimation, thus overcoming the limitations of singular methods or data restrictions. For example applications incorporating Bayesian and other statistical methods tend to combine measurements with predictive models or satellite observations for extrapolation and enhancement of spatial and/or temporal resolution. Bayesian maximum entropy (BME) is a geostatistical method for analyzing spatial/temporal data with input data on generalizable characteristics and site-specific knowledge[102]. BME modelling uses Bayesian analyses to blend different sources of data with varying temporal and spatial resolutions, with the objective of combining all the data into a blended estimate which tends to be more precise (less uncertainty)[103]. BME has been shown to have complimentary benefits when used with LUR modelling[103-105]. BME applications in exposure assessment may be useful where emissions inventory and meteorological data cannot be attained, as such datasets are not required for modelling[27].

Remote sensing or satellite estimates have been combined with air quality modelled data or geographic predictors for use in epidemiological studies. Remote sensing techniques can simulate air pollutant distribution over an area based on the relation between air pollutant concentrations and aerosol optical depth (AOD), measured by the ozone-monitoring instrument (OMI) onboard the Aura satellite and the moderate resolution imaging spectroradiometer (MODIS) instruments. Monitoring methods are based on the scattering of specific wavelengths of sunlight. A major challenge to inferring near-surface pollutant concentrations from satellite retrieved column AOD data is to accurately describe the nonlinear and spatiotemporally varying relationship between air pollutants and AOD, which depends on aerosol chemical composition, vertical profiles, aerosol optical properties, and the ambient environment[106]. Methods used to link satellite AOD with air pollutant exposures are often classified into two categories: statistical[107] and geophysical[108]. A two-stage process is also used with a geophysical approach followed by a statistical approach. However, a specific challenge with using

satellite estimates is its coarse spatial coverage within the applied domain, with few studies reporting on fine-scale estimates (e.g. within $\leq$1km)[109].

GIS approaches such as land use regression (LUR) modelling rely on the use of monitored data and geographical attributes (e.g., road networks, population/housing density, land use /land cover and industrial proximity) of the surrounding monitoring site, to determine intra-urban variability in air pollution. However, these do not account for short-term meteorological variation which directly influences pollutant gradients and emissions dispersion[110]. The characterization of air pollution exposure by LUR has been widely documented[111-119], with recent studies demonstrating model advancements by incorporation of time-varying emissions and meteorological data[97], such applications include integrating dispersion modelling[120,121], chemical transport modelling[122-124] and satellite data[125-128].

Air quality models, such as dispersion/chemical transport models, vary in spatial and temporal scales, but generally, incorporate data on source emissions and meteorology. Different air quality models exist at varying spatial scales of the modeling domain (regional / urban / local scale), and in the level of detail incorporated into the process equations. Chemical transport modelling (CTM) such as the Community Multiscale Air Quality (CMAQ)[129] model is able simulate air pollutant concentrations over large spatial domains with a fine temporal resolution. This is based on emissions inventories and meteorological conditions and a detailed understanding of chemical properties, but the spatial resolution of the simulated results tend to be relatively coarse[104]. Dispersion models such as the Atmospheric Dispersion Model System (ADMS), calculate the geographic distribution of air pollutants by combining emissions data (from point, line and area sources), geophysical properties and meteorological conditions of the study domain[130]. They are able to simulate near-source pollutant behaviour as a function of source characteristics (such as stack height and size) temporally varying meteorological data (including wind speed, direction and atmospheric stability) and terrain features[110]. Dispersion models calculate concentrations assigned at any time scale, by adjusting for the interacting spatiotemporal effects of sources and meteorology[130].

The recent application of combined approaches (referred to as "hybrid models") have demonstrated significant improvements in spatiotemporal modelling of air pollutants in comparison to stand-alone approaches. Recent reports of hybrid modelling applications combining land use regression (spatial characteristics) with dispersion modelling (temporal characteristics) show an overall improvement in the model explained variance of the combined approach in comparison to the use of singular methods. Korek et al.[121] developed a hybrid LUR-DM using 93 bi-weekly observations of NO_x at 32 sites in the greater Stockholm area, Sweden. The DM was based on spatially resolved topographic, physiographic and emissions data, with hourly meteorological data from a diagnostic wind model. The model was

developed by stepwise linear regression and showed improvement when using a combined approach of both methods (R^2 = 0.89) in comparison to the DM without covariates (R^2 = 0.68, $p<0.0001$). Michanowicz et al.[110] integrated atmospheric dispersion modelling (AERMOD) based predictions for $PM_{2.5}$ into LUR models that were derived from 36 unique sites selected to represent different source and elevation profiles as well as seasonal gradients. The study found that the integrated LUR/AERMOD hybrid model improved the out-of-sample prediction by 2-10% in comparison to LUR alone. An additional study further demonstrated good agreement between model comparisons of LUR and DM as independent approaches with no differential effects on the outcome measures[4]. Overall hybrid modelling approaches improve intra-urban exposure estimates, particularly in areas with large industrial sources, sharp elevation gradients and complex meteorology (e.g., frequent inversion events).

In summary, the selection of exposure assessment method is dependent on epidemiological study design (e.g., time-series vs cohort), health outcome of interest and assessment period (e.g., long-term vs short-term or acute vs chronic exposure effects), the pollutant of interest and their spatial and temporal profiles. The role of the pollutant in indoor infiltration and human activity patterns are further important considerations[27,131]. Robust measures of exposure are critical in exposure-health outcomes epidemiological studies, and methods that incorporate both spatial and temporal gradients would likely yield better results than simplistic approaches (interpolation, central monitoring sites and proximity-based measures), with a further benefit of reducing exposure misclassification. The combination of multiple approaches takes into consideration the limitations of singular methods in comparison to their combined use, while leveraging their individual strengths for a stronger modelling component.

1.3. Study Aims and Objectives

1.3.1. Problem Statement

Epidemiological studies have shown that air pollution has an adverse effect on lung function[3], this has been repeatedly studied in adults and older children[41,58], with limited reports on such effects among infants[3]. Growing evidence of early lung impairment having implications on long term respiratory health, highlights the increased vulnerability of infants at this critical stage of growth and development, with some studies further showing that sensitive windows (e.g., antenatal, perinatal, and postnatal) of exposure may play a role in the nature and severity of the health outcome observed. Cross-sectional assessment of lung function parameters at varying age groups, has the potential to provide improved insight on age-sensitivity to short-term air pollutant exposure. This is particularly relevant for early detection of abnormalities. Lung function assessment as early as six weeks is an important proxy

marker of in-utero exposure effects, that may manifest shortly after birth. Assessing the variability of exposure over a shorter-time frame may further help us better understand acute respiratory dose-response effects.

Exposure to ambient air pollutants is likely to impact the developing lung antenatally, as well as at critical time windows postnatally, throughout infancy and early childhood. The challenge in understanding the role played by ambient pollutants during this cycle of growth and development is appropriate characterization of exposure over this period. In epidemiological studies, investigating the short-term acute response in exposure-outcome relationships requires simultaneous (and sometimes continuous) measures of both exposure and outcome. Thus, we address this challenge by employing hybrid modelling methods to achieve fine-scale spatio-temporally resolved estimates (two-week average), to better understand the short-term effects of air pollution on lung function measures among infants. Early detection of causal factors and association of effect will help us better understand the dose-response effects of ambient air pollutant exposure on lung growth and maturation in infants.

1.3.2. Research Question

Is infant lung growth and maturation adversely affected by short-term exposure to ambient air pollutants nitrogen dioxide and particulate matter, and is this association age-sensitive within the first two years of life?

1.3.3. Overall Aim

The overall aim of this study was to determine if short-term exposure (two-week lagged average) windows to air pollutants nitrogen dioxide and particulate matter, induces an acute response in infant lung function and if this response is age-sensitive.

1.3.4. Specific Objectives

- To assess lung function in infants at the ages six weeks, six, twelve and twenty-four months.
- To estimate short-term exposure to nitrogen dioxide and particulate matter, as determined by a combination of land use regression modelling and dispersion modelling.

- To establish a causal link between short-term exposure to air pollutants and acute response in lung function measures during age-sensitive windows.
- To describe maternal, antenatal and postnatal behavioural, environmental and personal risk factors of adverse lung function.

In Manuscript I as per the stated objectives, lung function was assessed in infants at the ages six weeks, six, twelve and twenty-four months, and a detailed description on maternal, antenatal and postnatal behavioural, environmental and personal risk factors are described in relation to lung function.

In Manuscript II a detailed review is undertaken to evaluate different types of exposure assessment methods when assessing lung function in children under five years of age, this manuscript establishes suitability of relevant approaches to assess a causal link between short-term exposure to air pollutants and acute response in lung function measures during age-sensitive windows.

In Manuscript III as per the stated objectives lung function was assessed in infants at the ages six weeks, six, twelve and twenty-four months, and a detailed description on maternal, antenatal and postnatal behavioural, environmental and personal risk factors are described in relation to lung function. Furthermore, air pollutant estimates were established for short-term exposure to nitrogen dioxide and particulate matter, as determined by a combination of land use regression modelling and dispersion modelling. The findings of this work demonstrate a causal link between short-term exposure to air pollutants and acute response in lung function measures during age-sensitive windows.

1.4. Methodology

This study is nested within the Mother and Child in the Environment (MACE) prospective birth cohort (**Appendix F**). A repeated cross-sectional study design of infant lung function assessment is presented. Infant participants from the larger cohort had a lung function test at ages six weeks, six, twelve and twenty-four months. In addition, two-week average air pollutant estimates (preceding the lung function test visit) were established for nitrogen dioxide and particulate matter, for individual participants to assess an acute-dose response effect.

1.4.1. Sample Population

In this study infant participants, at varying age groups were selected from the MACE cohort. In the MACE cohort, pregnant females were recruited in their first trimester of pregnancy at public sector antenatal clinics in the Merebank, Bluff, Wentworth and Austerville areas in south Durban, and in the Newlands East and Newlands West areas, located in the north Durban area. The study areas were further characterized by the level of urban and industrial pollution in the different regions.

Our sampling strategy for infant participants was systematic, in that all MACE infant participants were pre-booked and scheduled to attend clinical visits at the respective age groups of 6 months, 12 and 24 months. Similarly 6-week old participants were also scheduled for testing, provided they met the age criteria however they did not undergo clinical consultation as this was not part of the MACE protocol, their attendance was only for the purpose of lung function testing at this age. All participants further underwent a pre-screening by field workers prior to test bookings using a questionnaire to determine if they were ill in the prior week, were on medication, recently vaccinated (childhood immunization) or currently teething (these factors were considered to affect either sleep state or success of the test). Though we tried to maintain consistency in our repeat follow ups to achieve the sample size several factors limited our success, these included: (1) the testing facility was based at a paediatric ward in the hospital and thus on days where MACE participants were present for testing, tests had to be cancelled if the ward room was occupied for hospital use; (2) any patient cancellations were due to either the infant being ill or transportation issues; (3) though multiple participants on occasion would attend clinical visits we could only test selected individuals due to our logistical limitations and testing capacity that was a maximum of three participants per day and to try to conclude testing by 3pm in the afternoon to accommodate participant transportation, in addition the participants that were selected for testing on that particular day were those that were booked for appointments during the morning time-slots, as attendance to clinical visits were staggered throughout the day based on the physicians availability; (4) The duration of testing and our ability to achieve a maximum testing rate was also based on equipment disinfection and pre-calibration between test attempts, infant co-operation, sleep state (non-rapid eye movement) and potential equipment or software technical failures were also limiting factors; (5) there were several complexities related to follow up visits and the difference in test visit numbers in comparison to the total sample, as we were conducting tests among all age groups, concurrent bookings and daily testing capacity played a significant role here, firstly (a) there was one study participant that left the study after the 6 week test as she had relocated, at 6 months 23 follow up opportunities, 45 at 12 months, and 42 at 24 months were missed attributed to our logistical constraints and capacity limitations, (b) however we overall had a low rebooking rate (7%) and a low cancellation rate (5%).

1.4.2. Study Area

The residential areas (**Appendix A**) selected within the MACE cohort were primarily based on the industrial profile of the respective regions, with the north Durban area identified to be less industrialized, while the south Durban region was identified to be highly industrialized.

The south Durban region is characterised by various emission sources including approximately six hundred industries, among these two major oil refineries, a sugar manufacturing plant, a paper manufacturing plant, a cluster of chemical industries, major petrochemical and chemical storage facilities, metal smelting industries, breweries, paint and textile industries, and sewage treatment works among others[132]. It also includes the Port of Durban, one of the busiest ports in Africa, and is a focal point of many of the city's major transport routes[132,133]. In addition, the topographical and meteorological conditions enhance air quality impacts, as the basin shape is conducive to pollution accumulation, particularly during surface temperature inversions in winter[134]. Prevailing winds carry gaseous pollution, soot and oil spray from industries in the direction of local residences and schools[135].

Air pollutant dispersion is largely driven by unique geographical, topographical and meteorological influences, with significant urban and regional variability. Thus, accounting for these differences over space and time is critical to understanding the role and influence of exposure to ambient air pollutants on respiratory health. Taking into cognisance the complex industrial profile, emission sources and dispersion characteristics, air quality monitoring networks alone may not be substantive in accounting for individual-level variability in this region, thus exposure modelling is required to achieve this.

1.4.3. Data Collection

Descriptive Data

Descriptive data were obtained from questionnaires administered within the MACE cohort. Detailed interviews were conducted during each trimester of pregnancy, the early post-natal period and at regular intervals during infancy. Information such as gestational age, weight and length were obtained at birth. Additionally, maternal data including socio-economic status, HIV status, maternal weight gain, smoking during pregnancy and delivery type were recorded. Environmental risk factors were determined by data collected on housing type, household primary heating source, environmental tobacco smoke exposure and proximity to emissions sources (determined by calculating the distance from the residential address to the nearest road and industry within a GIS framework). Further details on data collected within the MACE cohort are reported elsewhere[136,137].

Lung Function Measurement

Infant lung function measurements were performed in unsedated infants at six weeks, six, twelve and twenty-four months, during behaviourally defined quiet natural sleep[138]. Tests were performed at the King Edward VIII Hospital, in Durban, KwaZulu-Natal, following standard operating procedures and guidelines (**Appendix E**), aligned with the ERS / ATS standards[139]. Screening questionnaires were completed by the fieldworker prior to the test date, evaluating infant health prior to testing. Participants were also clinically assessed on the day of the test to exclude respiratory infections, which if present resulted in a rescheduling of the test. The principal investigator and a clinical pulmonary technologist were trained in the lung function testing techniques. Multiple breath washout and tidal breathing measures were assessed (**Manuscript I & III**). Participants were further provided with feedback from testing, indicating if the test results were normal or if clinical intervention was required (**Appendix D**).

Exposure Assessment

A comprehensive environmental exposure assessment was conducted within the larger MACE cohort[140,141]. The exposure assessment model derived is based on a hybrid approach combining both land use regression (LUR) and dispersion modelling (DM). Several exposure assessment methods were further evaluated in a review (**Manuscript II**), with the aim of identifying which methods best suit modelling short-term temporal changes in air pollutant effects and hybrid models were identified as an important advancement in the field, towards achieving refined spatio-temporal estimates. It is also noted that validation methods are important when modelling for exposure prediction to ensure accuracy in the modelled outputs. The hybrid LUR-DM model was applied in the current study to achieve time-varying estimates of exposure per pollutant for each two-week period preceding the lung function test date, providing temporally resolved estimates per participant and per testing occasion (**Manuscript III**). This model was validated by the leave one out cross validation technique in which one site is excluded from the model (*n-1*) and the estimate predicted for the excluded site, this is repeated *n* number of times as per the number of measurement sites available.

1.4.4. Statistical Analysis

Descriptive statistics and regression analyses were performed using STATA 15 for Windows (STATA Corporation, College Station, TX, USA). This data was presented as means and standard deviations (**Manuscript I and III**), with range values present for lung function measurements (**Manuscript I**). Cross-sectional multivariable models were derived for each outcome measure (tidal volume, minute

ventilation, respiratory rate, meant tidal inspiratory flow, mean tidal expiratory flow and tPTEF/tE, functional residual capacity and lung clearance index), stratified by each age group (six weeks, six, twelve and twenty-four months) (**Manuscript I**). To assess the effects of short-term exposure (two-week average preceding the test date) to ambient air pollutants NO_2 and PM_{10} on lung function outcomes among the various age groups, linear mixed effects models were developed (**Manuscript III**).

1.4.5. Ethical Consent

This study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (**Appendix B** research ethics approval number: BE431/17). Study participation was voluntary and written informed consent (**Appendix C**) was obtained from the parent or guardian of all infant participants at enrolment. Furthermore, lung function testing in infant participants was performed without sedation and in compliance with the principles of applicable ethical guidelines on research involving human participants.

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CHAPTER 2

MANUSCRIPT I

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ABSTRACT

Background: Early assessment of infant lung function (ILF) is necessary to improve our understanding of factors that determine long term respiratory health. The aim of this study was to identify predictors of lung function among infants aged 6 weeks, 6, 12 and 24 months, from low socio-economic settings, enrolled within the Mother and Child in the Environment (MACE) study.

Methods: ILF tests were performed assessing multiple breath washout and tidal breathing during spontaneous sleep. Several risk factors, relating to infant growth, maternal and environmental exposures were assessed cross-sectionally against the lung function parameters in multivariable models for each age group.

Results: Maternal smoking during pregnancy affected the ratio of time to peak expiratory flow; showing a decline across the age groups, in comparison to non-smokers, while being statistically significant at 6 weeks (β: -24.6% (95%CI: -43.92; -4.59)) and 12 months (β: -12,68% (95%CI: -25.25; -0.11)]. Low birth weight was associated with a lower tidal volume at 6 weeks (β: -5.99 mL/kg (95%CI: -9.59; -2.39)), 6 months (β: -15.02 mL/kg (95%CI: -22.48; -7.57)) and 12 months (β: -23,7 mL/kg (95%CI: -35.55; -11.85)), compared to those with normal birthweight. This was further observed for minute ventilation at 6 weeks (β: -157.78 mL/min (95%CI: -338.95; 23.38)), 6 months (β: -325.57 mL/min (95%CI: -619.06; -32.08)) and 12 months (β: -527,58 mL/min (95%CI: 947,85; -107,32)), though less evident at 24 months.

Conclusion: Low birth weight was the main predictor for low tidal volumes and minute ventilation at 6 weeks, with smaller differences observed at 12 and 24 months. Lung function development early in life is primarily driven by infant size and post-natal growth factors consistent with other studies.

INTRODUCTION

The physiological development of infant's lungs undergoes dynamic changes in the first few months of life and early childhood. Many parameters, such as tidal volume, respiratory rate and minute ventilation, which define the functional characteristics of the lungs, change at different scales in relation to lung size. Measurements of functional residual capacity performed in infants, have in previous studies been shown to be significantly correlated with lung volume and infant anthropometry (weight and length)[1,2]. After birth, lung size increases proportionally with body size and is affected by age and gender[3].

The association between early lung development and airway function is complex and is influenced by exposure to prenatal and postnatal risk factors. Small for gestational age babies,[4] tend to have reduced lung function[5,6] and an increased risk of respiratory morbidity and mortality[7,8]. Birth length and weight have been associated with asthma in later childhood[8]. However, inconsistencies across studies suggest that normal physiologic growth and development patterns of infant airways are not fully understood[9]. Epidemiological studies measuring premorbid lung function in infancy indicates the importance of exposure to risk factors during foetal and early postnatal life[10]. In low to middle-income settings, with the high burden of lower respiratory tract infections among infants[11], several environmental factors including air pollutant exposure[12] and environmental tobacco smoke exposure, as well as maternal factors, such as maternal smoking during pregnancy[13-15], maternal HIV status[16] and socio-economic factors (housing type, level of income etc.) require investigation for their effect on infant lung function.

Routine infant lung function testing remains a challenge as it requires specialised equipment and software, as well as thorough patient preparation. Recent advancements in inert-gas-washout techniques have allowed for lung function assessment in uncooperative infants and very young children without sedation[17,18]. Multiple breath washout (MBW) testing is useful in the early detection of structural alterations and is a more sensitive marker of small airway functioning than conventional lung function tests[19,20]. Application of this test has been successful among infants and children[21]. Some studies have further demonstrated that school-aged children with asthma had increased ventilatory inhomogeneity even when spirometry results were reflected as normal[22-24]. This suggests that conventional lung function tests may not necessarily be sensitive nor specific in detecting small airway flow obstruction unless major changes are present[25-27]. The lung clearance index (LCI), as determined by MBW testing, has been identified as an important index reflecting ventilation inhomogeneity and small airway dysfunction. It has further been identified as a valuable tool to detect disease progression in cystic fibrosis (CF)[28,29].

Previous studies have shown that the LCI is reproducible and more sensitive than measures of forced expiratory ventilation (FEV_1) at identifying early lung disease in children[2,30,31]. Aurora et al. showed LCI to be an early predictor of deteriorating lung function in children with CF, with a significantly high

LCI difference observed for CF as compared to controls (mean: 2.7 (95%CI: 1.9, 3.6); p=0.001)[32]. Korten and colleagues showed that infants with CF had a consistently higher respiratory rate than controls (mean difference: 4.15 breaths/min; (95% CI 2.86–5.44); p < .001)[33]. It has also been shown that LCI may be influenced by large changes in tidal volume, respiratory rate or functional residual capacity[28]. Since LCI is a ratio of cumulative expired volume and FRC, it is also independent of small changes in FRC over the physiological range. In addition, as this is normalised for FRC, the normal range of LCI is largely unaffected by age, height or gender of the subject. Measures of FRC and LCI reflect lung size and ventilation homogeneity, respectively, and are thus reflective of growth and maturation[34]. Tidal breathing measures are important markers of the control of breathing and airway mechanics which are determined by tidal volume, breathing frequency and minute ventilation[35,36]. Studies have demonstrated that FRC and tidal flows (volume dependent parameters) are important proxies of early lung health[12,34,37,38].

This study aims to identify predictors of lung function among infants aged 6 weeks, 6, 12 and 24 months, from low socio-economic settings, enrolled within the Mother and Child in the Environment (MACE) study.

METHODS

Selection of Study Participants

This study is nested within the MACE cohort in Durban, KwaZulu-Natal, South Africa. Details on the selection of pregnant women into the cohort are described elsewhere[39]. The clinical protocols of the assessments of the infants within this cohort required testing at the ages of 6 weeks, 6 months, 12 and 24 months. Infants and children from these varying age groups were invited to attend the lung function assessment centre. All those that attended were subjected to standardized interviews, clinical evaluation and lung function testing. This study has been approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BE431/17). Maternal or primary caregiver written consent was obtained prior to lung function testing. The data collection process within the larger cohort entailed detailed questionnaire interviews at recruitment, at each trimester of pregnancy, early post-natal, and at regular intervals during infancy.

The selection of participants into the study was dependent on the MACE clinical protocols. All infant participants were pre-booked and scheduled to attend clinical visits at the age of 6 weeks, 6 months, 12 and 24 months. All participants underwent a pre-screening by field workers prior to test bookings using a questionnaire to determine if they were ill in the prior week, were on medication, recently vaccinated (childhood immunization) or currently teething (these factors were considered to affect either sleep state or success of the test). No appointment was scheduled if there were any positive responses to these

enquiries. Logistical issues sometimes determined test selection and participation – only a limited number of tests could be conducted on a day, dependant on factors such as time taken to fall asleep (as tests were conducted in non-sedated infants), or requirement of a repeat test attempt if the first attempt failed to meet test quality standards, furthermore some infants could not be tested on the days of the appointment due to such delays.

Data Collection

Data pertaining to infant, maternal, and environmental risk factors were extracted from detailed questionnaire interviews, that were conducted as part of the MACE cohort study, at recruitment, each trimester of pregnancy, early post-natal and regular intervals during infancy. Extracted data from the MACE dataset included information on maternal, environmental and infant risk factors, which we considered in our analyses. Information on child sex, gestational age, birth weight and length were obtained from post-natal questionnaires. Time-variant data such as age, weight, length (reported as postnatal weight and length gain) and self-reported wheezing were recorded at each test date. Low birth weight was defined as birth weight<2.5 kg [40], while preterm birth was defined as gestational age <37 weeks[41] as per the World Health Organization guidelines. Postnatal child weight and length gain were calculated as the difference between the absolute measures taken at the test occasion and time of birth. Maternal smoking during pregnancy, HIV status and income status (classified as low if annual income <USD2000 and high if above this) were obtained from antenatal and postnatal questionnaires. Environmental exposure data were obtained from recruitment questionnaires and included housing type (formal vs informal), environmental tobacco smoke exposure and proximity to the nearest road and industry (based on calculations of GPS coordinates of the residential location). In addition to questionnaire data, clinical assessments evaluating growth and developmental milestones and detailed respiratory evaluation were undertaken for infant participants at their 12 and 24 months visit over the period January 2017 to September 2019.

Lung Function Measurements

Screening questionnaires were completed by the fieldworker prior to the test date, evaluating infant respiratory health prior to testing. Participants were clinically assessed on the day of the test to exclude respiratory infections, which if present, resulted in a rescheduling of the test within at least one week of the initial scheduled date. Both the principal investigator and a clinical pulmonary technologist were trained in the infant lung function testing techniques. Lung function measurements were performed in unsedated infants, aged 6 weeks, 6, 12 and 24 months. Testing was completed during quiet natural sleep in the supine position[42], using the Exhalyser D with ultrasonic flow meter (Ecomedics AG,

Duernten, Switzerland) in accordance with the ERS/ATS standards[18,43]. Lung function measures were conducted over the period January 2017 to September 2019. All tests were analysed using the software Wbreath v2.0 (Ndd Medizintechnik AG).

Quality control was maintained by calibration of the equipment between participant testing and adjustment for case characteristics mainly temperature, and the consistent use of standardized input parameters such as the ambient temperature and humidity[44]. Acceptability of test results was assessed post-testing and during analysis, based on published guidelines[18,43].

Tidal breathing measurements were assessed for 30 regular breaths of tidal breathing during non-rapid eye movement sleep (behaviorally defined quite natural sleep)[45] from a two-minute recording[46]. An acceptable test was required to have a homogenous breath pattern. Test outputs with sighs, apnoea or interruption to the breathing pattern, as well as leak during testing, were excluded as part of the quality control for test acceptability and as per current guidelines[18,47]. The main outcome parameters measured from tidal breathing included tidal volume, respiratory rate, minute ventilation, mean tidal inspiratory flow (MTIF) and mean tidal expiratory flow (MTEF) and time to peak tidal expiratory flow over total expiratory time (tPTEF/tE %).

Multiple breath washout measurements were performed using 4% Sulphur Hexafluoride (SF_6) tracer gas and ultrasonic flowmeter (Spiroson, Ecomedics, Duernten, Switzerland), of which three consecutive tests were performed. Acceptability was based on functional residual capacity (FRC) means within 25% across three successful tests or 10% for two successful tests and lung clearance index (LCI) within one turnover of each other. Additional quality checks for multiple breath washout tests included five homogenous breaths before wash in, a 10-breath plateau before the commencement of washout and 10 breaths after washout, as the SF_6 returns to baseline[46].

Blind analyses of all tests were performed by two independent researchers who were trained and competent in both testing and analysis and the results were compared for differences. Where differences were observed, side by side analysis was further conducted to achieve a high level of accuracy.

Statistical Analysis

Descriptive statistics and regression analyses were performed using STATA 15 for Windows (STATA Corporation, College Station, TX, USA). Data are presented as means and standard deviations (SD), with range values presented for the lung function measurement data. For the lung function outcomes, the intra-subject coefficient of variation (CV) is provided. The coefficient of variation was calculated to determine the consistency of multiple testing across the different age groups, and this was completed for each participant and each measurement parameter.

Although some participants contributed to more than one time point, no longitudinal analyses have been performed because of the small sample with repeated measures. We treated each lung function test at specific age groups as a sub-sample of the total study sample. Thus, cross-sectional multivariable linear regression models are presented by individual age groups (6 weeks, 6, 12 and 24 months) and by individual outcomes (tidal volume, respiratory rate, minute ventilation, MTIF, MTEF, tPTEF/tE, FRC and LCI) per age group. The normality of data was tested by Q-Q plots per outcome measure for each age group. The candidate predictors included in the bivariate analyses were selected based on their association with lung function outcomes as reported in the literature[38]. These included anthropometric measures, preterm birth[37,48], low birth weight[49], infant wheezing[50] and maternal smoking during pregnancy[13-15,51], maternal HIV[16], socio-economic status and environmental exposures[12]. The inclusion of variables in the final multivariable linear regression models was focused on growth variables (age, sex and length gain), and statistical significance ($p<0.1$). In the bivariate analyses, low birth weight and maternal smoking were consistently significant across measurement parameters and were thus included in the models. Models were further tested for collinearity, and a variance inflation factor of <5 was considered acceptable.

A strong correlation was observed between the growth variables, for example, birth weight and weight at test date were correlated, as was birth length and length at test date. Thus, these variables were not entered simultaneously into regression models. Though birth weight is an important physiological predictor of infant lung function, we wanted to assess the effects of low birth weight as a risk factor for lung function. Among the other risk factors assessed in bivariate analyses, maternal smoking was the most consistent, among all parameters and age groups. While other risk factors (e.g., those related to SES factors such as housing type, maternal income etc.) appeared sporadically to be statistically significant for certain parameters and age groups. As we wished to achieve consistency across all the parameter predictors and model parsimony, these variables were not considered further in model development. The variables included in our models thus included age at test date, sex, length gain, low birth weight, and maternal smoking.

RESULTS

A total of 165 infant participants had an acceptable lung function test, of whom 79 had more than one test. Of the 302 multiple breath washout and tidal breath assessments performed across varying age groups of 6 weeks, 6, 12 and 24 months, 272 tests (figure 1) were accepted based on the visual quality of the test output and adherence to acceptability criteria as per the ERS/ATS guidelines[18,43]. These tests are stratified by the age groups at the time of testing (figure 1).

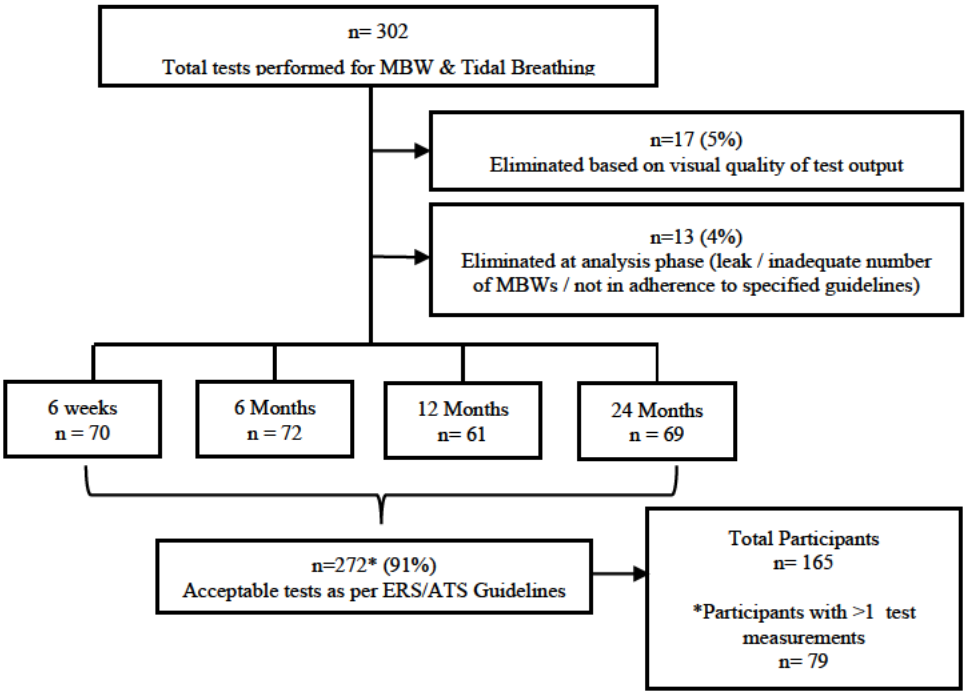

Figure 1: Description and summary of testing

The overall mean (SD) birth weight of participants was 3.12 kg (0.55), while birth length was 50.34 cm (3.65), with equal sex distribution among participants (Table 1). None of the infants were HIV infected even though 33% of mothers were HIV positive (data not shown). There was a low prevalence of maternal smoking during pregnancy (5%). Most participants lived in formal housing (89%) and used electricity as their primary energy source (98%) (data not shown), with the overwhelming majority of mothers (94%) having a low income (USD<2000). A not insubstantial number of participants were of low birth weight (10.9%), and the numbers presenting with this health status at different time points varied from 7.3% at 24 months to 15.7% at six weeks.

Table 1: Descriptive summary of participant data

	Participants (N=165)	**6 weeks (N= 70)**	**6 months (N= 72)**	**12 months (N= 61)**	**24 months (N= 69)**
Female Sex, n (%)	83 (50.3)	35 (50.0)	42 (58.3)	30 (49.2)	35 (50.8)
Gestational age (weeks), mean (SD)	38.9 (1.8)	38.9 (1.5)	39.1 (1.3)	38.9 (1.6)	39.1 (2.1)
Birth weight (kg), mean (SD)	3.1 (0.6)	2.9 (0.5)	3.1 (0.5)	3.2 (0.5)	3.18 (0.6)
Birth length (cm), mean (SD)	50.3 (3.7)	50.3 (4.0)	50.5 (3.8)	50.6 (3.2)	50.12(3.7)
Low-birth weight[1], n (%)	18 (10.9)	11 (15.7)	9 (12.5)	6 (9.8)	5 (7.3)
Maternal smoking during pregnancy, n (%)	8 (4.9)	2 (2.9)	3 (4.2)	4 (6.6)	5 (7.3)
Maternal Income (<USD2000), n (%)	155 (93.9)	66 (95.3)	68 (94.4)	55 (90.2)	65 (94.2)
Age at study date (months) mean (sd)	-	1.1 (0.4)	6.1 (1.4)	12.2 (1.2)	24.4 (1.6)
Postnatal length gain (cm)[2], mean (sd)	-	3.9 (4.5)	16.5 (5.1)	24.7 (5.3)	34.8 (4.9)

[1]low birth weight is classified as birth weight<2.5kg as per the WHO guidelines[40].

[2]postnatal length gain is the difference between length at test date and birth length.

Measured lung function data for each age group are presented in Table 2. The tPTEF/tE parameter showed a large CV, implying a lower level of confidence in this test outcome (Table 2). The mean LCI were below <7.4, consistent with a healthy cohort. While the measured lung parameters are likely to be dependent on independent predictors, the LCI provides a measure of an internally adjusted outcome (akin to the FEV_1/FVC ratio in normal spirometry). Although presented in a single table, the comparisons of measures across the age groups are not appropriate, as these are cross-sectional assessments at the different age groups.

The Q-Q plots showed that the distribution of measured lung function data was approximately normal and appropriate for inclusion into linear models (data not shown). The association between measures of tidal breathing, multiple breath washout and potential predictors for variance in lung function parameters are presented in Table 3 for the 6-week, 6-month, 12-month and 24-month age groups respectively. Among the "a priori" predictors (age, sex and length gain) included in the regression models, age at test date was statistically significant in the 6-week age group for tidal volume, respiratory rate and tPTEF/tE. When assessing the influence of sex differences on lung function measures, females had consistently lower tidal volume, minute ventilation, mean tidal inspiratory flow and mean tidal

expiratory flow, among all age groups though this was statistically significant for only certain age groups. The effect of postnatal length gain was inconsistent across age groups.

Of the predictor variables assessed for inclusion in the regression models, low birth weight and maternal smoking met the inclusion criteria. As observed in Table 3, low birth weight was shown to have a consistent effect on tidal breathing parameters (tidal volume, minute ventilation and inspiratory tidal volume), at age groups, 6 weeks, 6 months and 12 months. These effects however were less evident in the 24 month age group for both low birth weight and maternal smoking. Although low birth weight was not a consistently statistically significant parameter for expiratory tidal flow among all age groups, the effects were significant at 12 months, and showed a consistent increase in effect, in the expected direction. Apart from low birth weight at 12 months, our models poorly predicted the outcomes for the multiple breath washout parameters. Maternal smoking emerged as a consistent predictor in the expected direction for tPTEF/tE. Although it generally contributed to a decline in functional residual capacity, this was not statistically significant across any age group, with an FRC increase at the 6 month age group. Even though we explored several additional socio-demographic covariates in bivariate (data not shown) these did not meet the criteria for inclusion into the multivariable models.

Table 2: Lung function outcome measures in study participants

	6 Weeks (N=70)		6 Months (N=72)		12 Months (N=61)		24 Months (N=69)	
	Mean (SD)	CV% Med (25-75%)	Mean (SD)	CV% Med (25-75%)	Mean (SD)	CV% Med (25-75%)	Mean (SD)	CV% Med (25-75%)
Tidal Breathing								
Tidal volume, mL/kg	33.7 (7.3)	6.74 (5.6 – 8.3)	69.5 (12.0)	6.9 (5.9–8.9)	102.7 (14.8)	6.3 (4.8–7.9)	131.9 (18.9)	5.9 (5.3–7.2)
Respiratory rate, n/min	47.1 (12.6)	7.14 (5.7–8.9)	33.4 (7.9)	6.3 (5.4–7.6)	28.7 (6.0)	6.2 (5.3–7.3)	24.4(3.9)	5.4 (4.4–6.3)
Minute ventilation, mL/min	1517.6 (277.5)	7.11 (5.7-8.9)	2268.1 (435.1)	7.2 (5.8-9.1)	2898.5 (463.7)	7.4 (6.0-9.3)	3162.9 (410.6)	6.80 (5.5-8.3)
Mean inspiratory tidal flow mL/s	55.6 (11.8)	7.26 (5.7–9.1)	86.9 (17.8)	7.8 (6.1–10.3)	112.8 (17.7)	7.4 (6.1–10.2)	125.7 (19.5)	7.0 (4.9–9.1)
Mean expiratory tidal flow mL/s	47.7 (10.2)	9.1 (6.9–10.5)	67.7 (14.7)	8.7 (7.5–10.1)	85.4 (17.5)	8.9 (7.7–10.9)	91.9 (14.4)	8.4 (6.8–10.5)
tPTEF/tE %	43.1 (14.3)	18.2 (14.7–23.5)	30.8 (10.8)	21.9 (17.6–27.4)	30.2 (11.9)	22.3 (18.1–29.4)	28.8 (11.3)	21.5 (18.8–28.7)
Multiple Breath Washout								
Functional residual capacity, mL/kg	84.2 (18.1)	5.8 (3.2–9.0)	146.8 (33.3)	5.2 (3.0–8.4)	204.4 (29.0)	5.1 (3.8–8.1)	250.7 (49.9)	5.85 (2.9 –8.1)
Lung Clearance Index	6.9 (0.4)	3.8 (2.1–5.8)	7.1 (0.5)	4.8 (2.9–6.4)	7.4 (0.6)	4.1 (2.8–5.4)	7.4 (0.6)	3.66 (2.0–5.4)

CV: Coefficient of variation, intrasubject CVs were calculated as the ratio of SD for each outcome parameter over each mean value of the outcome per participant tPTEF/tE %: percentage of time to peak tidal expiratory flow over total expiratory time.

Table 3: Predictors of tidal breathing and multiple breath washout measures from multivariable models stratified by age

	6 Weeks (N=70)	6 Months (N=72)	12 Months (N=61)	24 Months (N=69)
	β(95% CI)	β(95% CI)	β(95% CI)	β(95% CI)
Tidal Breathing Parameters				
Tidal volume, mL/kg				
Age at Test Date, months	**7.36 (4.03; 10.69)**	0.51 (-1.23; 2.24)	1,9 (-1,04; 4,84)	0,9 (-2,17; 3,96)
Female Sex	-1.86 (-4.51; 0.79)	-3.3 (-8.23; 1.63)	**-6,66 (-13,18; -0,15)**	-2,49 (-12,67; 7,68)
Postnatal length gain, cm	**0.41 (0.09; 0.72)**	**1.06 (0.55; 1.58)**	0,59 (-0,1; 1,28)	0,89 (-0,11; 1,88)
Low-birth weight	**-5.99 (-9.59; -2.39)**	**-15.02 (-22.48; -7.57)**	**-23,7 (-35,55; -11,85)**	10,35 (-7,31; 28,01)
Maternal smoking	-1.76 (-9.6; 6.07)	-8.21 (-20.28; 3.87)	3,4 (-9,92; 16,72)	-1,21 (-19,49; 17,07)
Respiratory rate, n/min				
Age at Test Date, months	**-10.62 (-17.46; -3.79)**	-0.17 (-1.49; 1.14)	-0,38 (-1,82; 1,07)	-0,22 (-0,88; 0,44)
Female Sex	-2.64 (-8.08; 2.79)	-2.64 (-6.36; 1.08)	1,17 (-2,04; 4,37)	-1,54 (-3,72; 0,64)
Postnatal length gain, cm	**-0.69 (-1.34; -0.04)**	-0.37 (-0.76; 0.02)	0,01 (-0,33; 0,35)	0,02 (-0,19; 0,24)
Low-birth weight	4.89 (-2.5; 12.29)	4.33 (-1.29; 9.96)	1,72 (-4,11; 7,54)	-1,88 (-5,66; 1,91)
Maternal smoking	0.16 (-15.91; 16.24)	**11.41 (2.29; 20.52)**	0,07 (-6,48; 6,62)	2,44 (-1,48; 6,36)
Minute ventilation, mL/min				
Age at Test Date, months	-33.87 (-201.38; 133.65)	13.62 (-54.8; 82.03)	14,49 (-89,8; 118,78)	-12,71 (-77,19; 51,78)
Female Sex	**-151.39 (-284.56; -18.23)**	**-258.78 (-452.84; -64.73)**	-106,94 (-338,04; 124,16)	**-256,61 (-470,74; -42,48)**
Postnatal length gain, cm	-5.45 (-21.3; 10.4)	13.39 (-6.76; 33.55)	17,69 (-6,68; 42,07)	**23,66 (2,73; 44,59)**
Low-birth weight	-157.78 (-338.95; 23.38)	**-325.57 (-619.06; -32.08)**	**-527,58 (-947,85; -107,32)**	42,24 (-329,39; 413,87)

Maternal smoking	-46.52 (-440.43; 347.39)	454.85 (-20.59; 930.28)	141,57 (-330,96; 614,1)	337,6 (-46,95; 722,16)
Mean inspiratory tidal flow mL/s				
Age at Test Date, months	4.17 (-2.54; 10.87)	0.61 (-2.12; 3.33)	2,13 (-1,67; 5,92)	-0,4 (-3,63; 2,83)
Female Sex	**-8.99 (-14.33; -3.66)**	**-10.04 (-17.78; -2.3)**	-6 (-14,4; 2,41)	-6,24 (-16,96; 4,48)
Postnatal length gain, cm	-0.24 (-0.87; 0.4)	0.73 (-0.07; 1.53)	0,32 (-0,57; 1,2)	0,44 (-0,61; 1,49)
Low-birth weight	-6.5 (-13.76; 0.75)	**-13.62 (-25.33; -1.92)**	**-17,33 (-32,62; -2,04)**	5,93 (-12,67; 24,53)
Maternal smoking	7.6 (-8.18; 23.37)	**21.57 (2.61; 40.53)**	16,78 (-0,41; 33,98)	10,73 (-8,51; 29,98)
Mean expiratory tidal flow mL/s				
Age at Test Date, months	-4.83 (-11.13; 1.47)	0.45 (-1.99; 2.89)	-0,39 (-4,44; 3,65)	-0,48 (-2,69; 1,73)
Female Sex	-0.71(-5.72; 4.3)	**-7.5 (-14.41; -0.59)**	-1,96 (-10,92; 7)	**-9,65 (-16,98; -2,32)**
Postnatal length gain, cm	-0.12(-0.72; 0.48)	0.24 (-0.48; 0.95)	0,81 (-0,14; 1,75)	**1,07 (0,35; 1,79)**
Low-birth weight	-1.7(-8.51; 5.11)	-8.75 (-19.2; 1.7)	**-16,95 (-33,24; -0,66)**	0,29 (-12,43; 13,02)
Maternal smoking	-7.2(-22.01; 7.61)	10.98 (-5.95; 27.91)	-2,11 (-20,43; 16,21)	10,84 (-2,33; 24,01)
tPTEF/tE%				
Age at Test Date, months	**-8.97 (-17.34; -0.61)**	0.68 (-1.17; 2.53)	1,04 (-1,74; 3,81)	-0,07 (-1,95; 1,81)
Female Sex	0.05 (-6.6; 6.7)	-1.15 (-6.39; 4.09)	0,09 (-6,06; 6,24)	-1,16 (-7,4; 5,07)
Postnatal length gain, cm	0.35 (-0.44; 1.14)	**-0.61 (-1.15; -0.06)**	-0,28 (-0,92; 0,37)	0,16 (-0,45; 0,77)
Low-birth weight	4.68 (-4.37; 13.73)	-2.25 (-10.18; 5.67)	3,69 (-7,5; 14,87)	-3,18 (-14,01; 7,64)
Maternal smoking	**-24.26 (-43.92; -4.59)**	-1.41 (-14.25; 11.43)	**-12,68 (-25,25; -0,11)**	-5,81 (-17; 5,39)
Multiple Breath Washout Parameters				
Functional Residual Capacity, mL/kg				

Age at Test Date, months	12.87 (2.49; 23.25)	4.86 (-0.51; 10.23)	6,48 (-0,07; 13,03)	1,55 (-6,12; 9,22)
Female Sex	6.48 (-1.78; 14.73)	-12.48 (-27.71; 2.76)	-13,43 (-27,94; 1,09)	**-31,05 (-56,52; -5,58)**
Postnatal length gain, cm	0.6 (-0.38; 1.58)	0.81 (-0.77; 2.4)	0,09 (-1,44; 1,62)	2,01 (-0,49; 4,49)
Low-birth weight	-6.79 (-18.03; 4.44)	-16.96 (-40; 6.07)	-9,4 (-35,8; 16,99)	32,45 (-11,76; 76,65)
Maternal smoking	-5.66 (-30.08; 18.76)	31.17 (-6.15; 68.49)	-10,3 (-39,98; 19,38)	-31,7 (-77,44; 14,04)
Lung Clearance Index				
Age at Test Date, months	-0.04(-0.3; 0.22)	-0.04 (-0.13; 0.05)	-0,09 (-0,21; 0,04)	-0,01 (-0,12; 0,09)
Female Sex	-0.1(-0.31; 0.11)	0.07 (-0.19; 0.32)	-0,26 (-0,53; 0,01)	0,02 (-0,32; 0,36)
Postnatal length gain, cm	0.02(-0.01; 0.04)	0.02 (-0.01; 0.05)	0,03 (0; 0,06)	0,02 (-0,01; 0,05)
Low-birth weight	-0.2(-0.48; 0.09)	-0.15 (-0.53; 0.24)	**-0,62 (-1,11; -0,13)**	-0,17 (-0,76; 0,42)
Maternal smoking	0.09(-0.53; 0.71)	-0.22 (-0.84; 0.41)	0,17 (-0,38; 0,73)	0,5 (-0,11; 1,12)

Bivariate regression modelling was performed for each of the outcome measures and for each age group, selected covariates with a p-value of <0.1 were included in the subsequent multiple linear regression models.
In bold are effect estimates that are shown in the expected direction and statistically significant (p<0.05)
β- coefficient; CI – Confidence Interval; tPTEF/tE % percentage of time to peak tidal expiratory flow over total expiratory time.

DISCUSSION

In this study of infants from low socioeconomic communities in Durban, South Africa, low birth weight and maternal smoking emerged as important predictors of infant lung function across different lung function parameters in various age groups. These losses were not insubstantial.

Low birth weight was shown to be associated with lower functional residual capacity as well as tidal volume, minute ventilation and tidal flows (MTEF and MTIF) at 6 weeks, 6 and 12 months, however the observed associations were only statistically significant at certain timepoints. Though the impact of low birth weight on lung function is well documented[52] for older children comparison between studies can be difficult due to heterogeneity in lung function, differences in exposures and outcome measures assessed[10]. There are no studies that have focused specifically on low birth weight and the outcome measures on which we report, however, infant size, postnatal length gain and birth weight are known sources of variability in lung function. Lodrup et al.[53] found a decline in tidal breathing (tidal volume and flows) measures with birth weight among 803 infants shortly after birth. Dezeatuax et al.[54] demonstrated that low birth weight for gestational age among 98 participants with a mean (SD) age of 6.6 weeks (2.5) was associated with reduced lung function when measured in early infancy prior to the onset of lower respiratory illness. This study further reported on reductions in forced expiratory flows or volumes as independent of postnatal weight or length. This supports evidence that impaired airway function in infants with low birth weight for gestational age is not only related or attributed to growth factors but may also be due to intrauterine exposure factors[54]. Intrauterine growth restrictions in foetal weight, have been reported to affect the growth of the lungs and airways[52]. Our findings of reduced functional residual capacity (β: -6.79 mL/kg (95%CI: -18.03; 4.44)) in low-birth-weight infants, who are subject to increased risk for structural changes and premorbid lung function, further supports this. This novel finding suggests that infants with low birth weight are likely to have compromised respiratory function during the first 24 months of life; predisposing them to more severe consequences of acute respiratory infections.

Maternal smoking did not emerge as a consistent predictor of low lung function in our sample unlike other studies[15,38,55,56], but this is likely due to the low prevalence of maternal smoking during pregnancy (4.9%) in our study. However, maternal smoking was associated with a decline in tPTEF/tE, a measure of airway conductance[57], across age groups[58-60]. In a study in Oslo, Norway, adjusted linear regression analysis of tidal breathing parameters showed that infants with a mean age of 2.7 days, exposed to maternal smoking in-utero were shown to have an estimated -0.0021% change in tPTEF/tE, (p=0.03) per unit increase in daily smoking, compared to non-exposed children[53]. Stick et al.[15] assessed lung function in 461 infants shortly after birth with a mean age of 61.9 hours, lower values of tPTEF/tE were observed among infants exposed to maternal smoking during pregnancy (>10 cigarettes daily), (–0·049% (0·022; – 0·005), p<0·05). In comparison with our results, the effects on tPTEF/tE in our

youngest age group of 6-week old infants were (-24.26% (-43.92; -4.59)). While the physiological mechanisms of tPTEF/tE are unclear, these are likely to involve complex interactions between mechanical properties of the lung and chest wall which differ between healthy infants and those with respiratory disease[15]. Studies have demonstrated tPTEF/tE as an important predictor of respiratory morbidity in early childhood[58]. Patterns of tPTEF/tE have also been shown to be an important predictor of subsequent wheeze in infants as well as airway function in adults[13,61,62].

Several studies have shown that exposure to tobacco smoke has been linked to lung function in airway responsiveness (an indicator for asthma development)[63] and risk of wheezing in early life[51,60,64]. It has been reported that tPTEF/tE is diminished in infants who subsequently wheeze[59,65]. Reduced tPTEF/tE precede and predict important childhood pulmonary outcomes, including wheeze and lung function[59,66,67]. Thus, it may be speculated that antenatal factors that reduce tPTEF/tE influence airway development or result in small airways in relation to lung volume.

We explored several socio-economic and demographic factors associated with low-income communities, but we were unable to relate these to the observed measures in tidal breathing and multiple breath washout measures. This could be attributed to the fact that most households had a low income (94%), and our sample lacked sufficient variability in socio-economic status to investigate this variable. Even though 33% of infants were HIV exposed, all HIV-positive mothers received antenatal antiretroviral treatment, and none of the infants were HIV positive. We were unable to replicate the HIV exposure-related adverse lung function outcomes seen in a previous South African study[16] and this may be attributed to our sample size, however, the authors from that study did acknowledge the observed changes were marginal in their study with a mean difference in tidal volume of 1.13 mL ((CI: 0.02–2.23), $p<0.045$) between exposure groups at 6 weeks and with the effects not sustained by 24 months.

There are few studies that report on infant lung function under non-sedation using similar testing methods and the same analytic software, limiting our ability for comparison[37,46,68,69]. The Bern Infant Lung Development (BILD) study tested infants at 5 weeks providing normative and reference values for these tests for a white Middle European population of this age group[68]. Similarly, a European and Australian multicentre study conducted lung function assessment in non-sedated infants aged 4-8 weeks, stratified by level of prematurity[37], including "full-term controls". The Drakenstein Child Lung Health Study (DCLHS) assessed lung function in healthy South African infants, aged 5-11 weeks[46], from a low socio-economic community, with the aim of providing reference data for tidal breathing and multiple breath washout measures. Compared with our findings, the mean (SD) tidal volume at 6 weeks was lower in BILD and the European/Australian multicentre study, reporting 29.7 mL (5.9)[37] and 32.4 mL (5.5)[68] respectively, compared to our values and that reported by the DCLHS of 33.7 mL/ (7.3) and 34.9 mL (6.9)[46]. Respiratory rate was on average within $\pm$1SD across studies, whereas minute

ventilation was higher in South African infants[68] from both studies in comparison to the European and multicentre studies[37,46].

For the multiple breath washout measures in the 6 week age group, mean (SD) FRC was on average higher in the BILD study at 102mL (16)[68]. Our findings (84.2 mL (18.1)) was closer to the range reported by the multicentre study (79.6 mL (14.5)) [37] and the DCLHS study (77.9 mL (17.0))[46]. Mean (SD) LCI across all studies were within one standard deviation of each other: BILD: 6.75 (0.6)[68], MACE: 6.91 (0.4), DCHS: 7.2 (0.4)[46] and the European/Australian study: 7.2 (0.5)[37]. Lung function is known to be dependent on specific population characteristics, thus we observe similarities with the DCHLS study, but note some differences with BILD, which may be attributed to unmeasured socio-demographic differences. Given that the methods, techniques and equipment across these studies were similar, these are unlikely to account for differences seen within these studies. When comparing the data at 12 months of age with the DCHLS study, on average our values were higher (but within $\pm$1SD) for tidal volume, FRC and LCI[69]. Such differences may be attributed to anthropometric differences between studies, for example, the infants from our study were on average taller by 2cm and heavier by 1 kg, with a similar mean age (SD) of 2.2 months (1.2) in comparison to the DCHLS study with a mean age (SD) of 12.6 months (1.0) respectively.

A major strength of our study is that we used state-of-the art measures in infant lung function assessment in a low-income setting. We were further able to undertake such assessments at varying age groups (6 weeks, 6, 12 and 24 months), overcoming challenges in sleep and behavioural patterns between age groups. Lung function assessment without sedation among these age groups is a costly and time-intensive process, and we were able to achieve a high success rate on testing, further ensuring quality control in the test outputs through blind analyses by two independent researchers.

We acknowledge the limitations of the study. The small sample size and distribution of the sample across age ranges requires a cautious interpretation of our results. The uniform socio-economic status could also affect the generalizability of our results. Nonetheless, the characteristics of our study population and the lung function parameter results were comparable to prior studies for selected age groups[38,46,68]. The assessment of lung function in infancy and our understanding and interpretation of these outcome measures still requires further technical optimisation as even in studies with larger sample numbers and with longer observation periods, the observed inter-individual variability of identified outcome measures were not adequately explained.

In conclusion, our study adds to the growing body of knowledge on infant lung function generally, and our key finding of low birth weight and its effect on several lung function parameters is particularly relevant in low socio-economic communities.

Abbreviations

ATS/ERS: American Thoracic Society/ European Respiratory Society; CV: coefficient of variance; FRC: Functional Residual Capacity; LCI: Lung Clearance Index; MACE: Mother and Child in the Environment; MBW: Multiple Breath Washout; TV: Tidal Volume

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Author's contribution

SM, RN and PMJ conceptualized the study aims, objectives and methods. SM, RN, PMJ and KA were responsible for study implementation. MR and ACO provided intellectual contributions on the methods and interpretation of results. HKC and AAM contributed to the statistical methods and analysis. SM, KA and GN were responsible for lung function testing and data acquisition. All authors contributed towards the interpretation of the data and have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

Research ethics approval number BE431/17. Approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee. We confirm that written informed consent was obtained from the parent or guardian of all infant participants at enrolment. Lung function testing in infant participants was performed without sedation and in compliance with the principles of the Declaration of Helsinki and applicable ethical guidelines on research involving human participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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CHAPTER 3

MANUSCRIPT II

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A review of ambient air pollution exposure assessment methods in determining childhood respiratory health effects in children under five

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ABSTRACT

Various epidemiological studies have reported on air pollution exposure-related lung function decline and respiratory health effects in children. Children have increased susceptibility to ambient air pollutants as physiological and structural changes of the lung are still occurring within the first five years of life after birth. Prenatal, perinatal and postnatal growth stages have been identified as critical exposure windows, with the timing of insults during development considered to be critical in subsequent later life lung impairment. Quantification of exposure using robust exposure assessment methods is an important component to understanding the dose-response effects of air pollutant exposure on lung function and respiratory health outcomes. This review examines applications in air pollution exposure assessment methods when evaluating lung function and respiratory health dose-response effects in young children while considering the time-varying effects of critical windows of exposure.

INTRODUCTION

The World Health Organization (WHO) reported 543 000 deaths of children under the age of five years, in 2016, attributed to respiratory tract infections resulting from exposure to air pollution. The burden of impact is particularly high, in low to middle-income settings[1]. Children are notably more vulnerable to air pollution insults as their lungs are still developing at birth[2], with physiological and structural changes still occurring within the first five years of life[3]. Their susceptibility to adverse respiratory health outcomes in early childhood[4] is influenced by factors that occur-in utero[5], perinatally[6] and in the early post-natal[7] developmental stages.

The association between exposure to air pollutants and adverse respiratory outcomes, specifically lung function deficits (as a marker of lung growth) is well documented in older age groups[8-10], while less so in very young children. Lung function is considered an important objective marker for respiratory health and predictor of respiratory morbidity[11]. Children under the age of five, with a still developing lung structure, are particularly at risk for exposure[12,13]. Several studies have linked air pollution exposure during pregnancy, perinatally and postnatally to lung function decline and respiratory health outcomes[9,14-16]. Recent studies have demonstrated that exposure to even low concentrations of air pollution below the prescribed air quality guidelines, have been found to be associated with adverse respiratory outcomes[17]. Specifically, exposure to nitrogen dioxides (NO_2)[18] and particulate matter (PM_{10} and $PM_{2\,5}$)[19], have frequently been reported in relation to respiratory health outcomes[20,21], given their association with urban development and as known markers of traffic and industrial pollution.

Understanding the dose-response effect of an exposure-outcome association for various critical windows of development requires reliable and detailed quantification of the air pollutant of interest. In epidemiological studies, this has been achieved by employing methods of exposure assessment which vary in their complexity and capability in achieving robust measures of exposure. Air pollution exposure assessment seeks to determine the concentration of pollutants an individual comes into contact with and the duration of exposure, over the period in which such exposure is likely to cause the outcome of interest[4]. Exposures are known to vary in space and time[22] with ambient air pollution dispersion also known to be influenced by seasonal gradients, meteorological and topographical differences. In addition, for the growing lung temporal changes in exposure need to be characterized, from exposure in utero through to birth, neonatal, infancy and early childhood. Thus, the selection of exposure assessment techniques should be informed by these considerations.

Studies have further cited specific periods or "windows" of exposure as having a significant impact on developmental changes, as lung immaturity and physiology of the growing foetus[13,23], and that of very young children predisposes them to increased susceptibility to insults by toxicants[24]. Normal lung development is essential for long-term respiratory health, as significant developmental changes in respiratory physiology are known to occur progressively during the first years of life after birth[13,24-27], thus early clinical assessment of lung function is critical in determining the early life risk factors that predispose lung impairment.

This review examines current applications in air pollution exposure assessment methods when evaluating lung function and respiratory health dose-response effects in children under five while considering the time-varying effects of critical windows of exposure.

Windows of susceptibility

The pregnancy period is marked with significant developmental changes that may be affected by exposure to air pollutants. There is a range of respiratory health outcomes reported to be associated with specific windows of exposure to air pollutants NO_2 and PM. These are classified as prenatal[5], perinatal[6] and post-natal[7] exposure windows. The adverse changes are structural and functional, with chronic clinical outcomes occurring over time (figure 1). Damage to the lungs during periods of susceptibility in childhood may result in airway remodelling that might increase vulnerability to later life insults[2]. Reduced airway calibre and airflow restriction resulting from airway remodelling, may manifest as airway

hyperresponsiveness, as well as structural changes to the alveoli and supporting parenchyma[3]. Thus, exposure during these periods could impact the development of alveoli and lung growth.

In addition, as the breathing pattern of children differs from that of adults, this may alter the deposition of inhaled pollutants. Children also have a larger surface area per unit of body weight than adults, and under normal breathing, breathe considerably more air per unit of body weight than adults, thus having a higher breathing rate[2]. Prenatal factors are more likely to affect airway development, while postnatal factors tend to affect airway growth and alveolarization[3]. Diminished airway function identified soon after birth, before any postnatal insult has occurred, may predispose wheezing and diminished lung function in later life. Though studies may not prove causation, they do suggest a dose-response effect from specific exposure windows of development that will have varying effects on pre-or postnatal development.

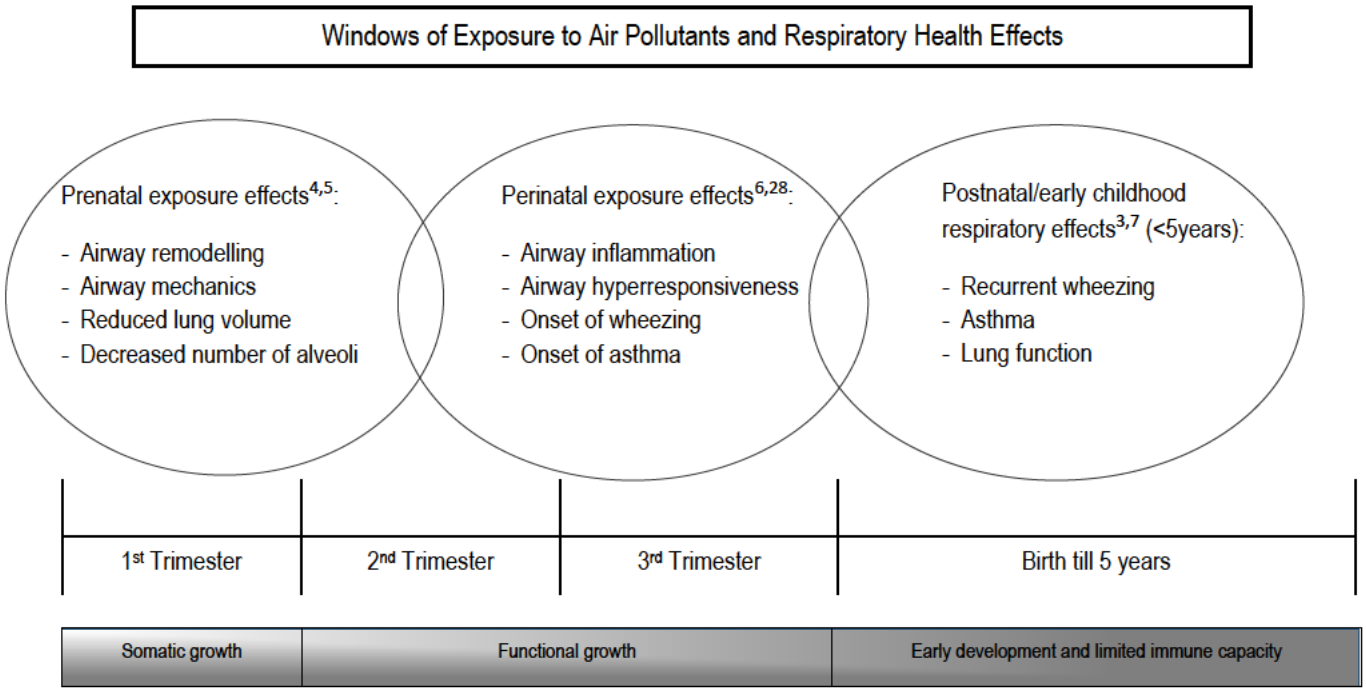

Figure 1: Summary of respiratory health effects of air pollution by exposure windows

In this review, we provide an overview of current methods in environmental exposure assessment and review the approaches used in studies evaluating air pollution-related lung function decline and respiratory health outcomes in children under five years of age.

SELECTION OF STUDIES IN THIS REVIEW

We conducted this review using the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA)[29] approach (Appendix A), in the selection of articles. Relevant articles were identified through searches on electronic databases including PubMed, Science Direct, Scopus and Google Scholar, as well as reviewing those identified in the reference list of individual articles and other reviews. In our search strategy, we used a combination of search terms including "outdoor or ambient air pollution", "traffic pollution", "nitrogen dioxide", "particulate matter", "exposure assessment" and "pregnancy exposure", "childhood exposure", "childhood respiratory health" or "childhood lung function". We further applied filters, limiting this review to those articles published in the "English" language, as "observational studies" or "journal articles", among "human" participants with "age" specified as children under five. The search strategy and identification of articles were done without time limits. The identified articles were further assessed to meet our inclusion criteria of presenting (1) validated exposure assessment methods for air pollutants nitrogen dioxide and/or particulate matter; (2) assessing exposure during specific windows including either trimester of pregnancy, entire pregnancy, infancy or early childhood; (3) with health endpoints including lung function and respiratory health outcomes (presence of lower respiratory tract infections, respiratory symptoms, wheezing and asthma) assessed in children under five years of age. For studies that referenced their exposure assessment from a separate publication, we further assessed these articles and made reference to them where the exposure assessment for the study was evaluated.

RESULTS

The initial search identified 5401 articles through the application of selected search terms, and an additional 24 articles were identified through other sources (e.g., bibliographies of other review studies). Further to this after the application of relevant filters as described above, 3042 articles were excluded. The remaining 2401 were screened based on their title and abstract to meet our inclusion criteria as described above, resulting in an exclusion of a further 2317. The remaining 84 full-text articles were then further assessed, and 67 did not provide adequate details on the exposure assessment. The 17 articles presented in this review met the inclusion criteria for this review (Appendix A).

The selected studies used varying exposure assessment methods. Almost all the studies used routine ambient air quality monitoring networks either as a direct measure of exposure or for model development and validation (n= 10). Only one study used personal monitoring, while others reported the use of proximity-based methods (e.g., distance to nearest emission source) (n=3), inverse distance weighting (n=2), geographic weighted regression (n=1), dispersion modelling (n=1), satellite-based methods (n=2)

and land use regression modelling (n=5). These studies assessed exposure and outcomes at different "windows of susceptibility": antenatally/specific trimesters (n=8), early infancy (n=5) and early childhood (n=6).

Personal Monitoring

Only a single study reported measures of personal monitoring. In a study among 336 Polish pregnant women, personal monitors were used to measure exposure to $PM_{2.5}$ over a single 48-hour period in the second trimester of pregnancy. A subsample of 80 had single repeated measurements in each trimester. The outcome of interest in this study was lung function[30] of the offspring at the age of five years, while a second report on the same cohort considered wheezing[31] among four year old's.

The subset of repeat personal sampling was intended to determine the representativity of the second-trimester sampling for the entire pregnancy. The authors report that within this subset, a consistent trend across the trimesters was present [$PM_{2.5}$ mean (SD) in the 2nd trimester was 42.3 μg/m^3 (30.8 μg/m^3) and 38.5 ug/m3 (29.9 μg/m3) in the 3rd trimester, while the mean difference was not statistically significant (t = 1.015, p = 0.313)][31], strengthening their case of representativity of sampling in the second trimester. However, while the subset sampling provides some confidence in the representativity of exposure, the lack of intra-trimester repeat sampling provides little understanding of variability at this interval. Thus, in measuring the exposure-outcome relationship, the assumption in this study of consistent exposure throughout the pregnancy is responsible for the increased risk, either in adverse lung function or repeated infection. Given that these outcomes are meant to be proxy markers for either abnormal lung growth or development (as opposed to a more acute functional or acute inflammatory response), these once-off exposure measures may be biased.

Routine Air Quality Monitoring Network Data

In the report of a birth cohort in Bern, Switzerland[32], weekly exposure to NO_2 and PM_{10} was assessed from daily mean averages obtained from two local monitoring stations, as well as the development of ten-day lag structures preceding the participant interview date. Because the outcome of interest was acute (respiratory symptoms and infections in children in the first year of life), the use of these short-term exposure measures was used based on the assumption that routine monitoring measurements reflect the temporal exposure fluctuation in the study region. The 366 infants participating in the study were selected from urban and rural settings. An urban and a rural monitoring station from the national network were used, and exposure of infants was assigned based on the geocoded location in proximation to the two

monitoring stations. The use of two monitoring stations to describe exposure among the full cohort of urban and rural resident participants is likely to have resulted in exposure assessment errors. However, the use of the repeated measures design provided a large number of observations (n=19 106), as well as the lagged exposure approach, is expected to produce a reasonable ranking of short-term variability in exposure for evaluating acute exposure-outcome associations.

In describing slightly longer exposure periods (trimesters in pregnancy), a cohort study in Singapore[33], extracted daily 24-hour national averages from eight regulatory monitoring stations (from the national network) for the first trimester of each participant. This was used to explore associations between first-trimester exposure to $PM_{2.5}$ and increased wheezing episodes in the first two years of life. The use of a national average to describe individual exposure is likely to provide very crude estimates for a three-month period, despite Singapore's geographical size. In addition, spatial variability in estimates was not accounted for. Furthermore, the investigation of a relationship between an antenatal measure of exposure and an acute post-natal outcome implies the hypothesis of an anatomical basis for the outcome. In such instances, control of antenatal factors associated with the acute outcome, including exposure is critical.

Proximity and Interpolation Methods

In a study of childhood asthma (at the age of 3-4 years) related to in-utero and first year of life exposure among children in British Columbia[34], multiple approaches to exposure characterisation were undertaken. The proximity estimates included proximity to roadways (defined as the residential address within 50m or 150m of highways and major roads) and proximity to industrial point sources (point sources were assigned an index value based on its pollutant contribution relative to other point sources in the region), with inverse distance weighted (IDW) summation of emissions from other point sources, as a proxy measure of exposure. Effect estimates associated with road proximity failed to reach statistical significance, with confidence intervals including estimates of no effect. However, the IDW models showed statistically significant exposure-outcome associations. In the previously described Swiss BILD cohort[35], among the various methods of exposure characterisation, distance of the residential address to the nearest major road at 6-m width (first class road) and 4-m width (second class road), was used as a proxy for traffic-related exposure. The outcome of interest in this report was lung function in 5-week old newborns. As with the British Columbia cohort, this particular measure of exposure failed to show any association, despite the suggestion of a trend of increasing risk from the second class to the first class road. There are several reasons why this proximity approach may not be adequate for characterisation of exposure: while road distance itself is a crude measure, and road class may improve this, it remains a proxy for vehicle density and vehicle type. These may result in substantial exposure misclassification

bias, although part of the error may be of Berkson type, which does not result in a downward bias but in an increase of the confidence interval[36]. The difference in approaches between studies further shows that there is no standardization on how sources may be defined (e.g., major vs minor roads or heavy vs light industry), or at what distance an effect is likely to occur.

Inverse distance weighted methods were used in another study, investigating in-utero exposure to air pollutants and incidence of asthma, rhinitis and eczema among 3-6-year-old Chinese children. Daily 24-hour average estimates for the duration of the pregnancy, from seven air quality monitoring stations within <5km radius were interpolated based on proximity of the station to the attending kindergarten school[37]. Monthly means were calculated for the entire pregnancy and individual trimesters. There was no association observed for particulate matter generally, while asthma was associated with SO_2 exposure in the single pollutant models and NO_2 was generally associated with all outcomes, but varied across the single and multipollutant models. The absence of effect with particulates and SO_2 could be interpreted as a true absence of effect, or that the IDW approach from the station to the kindergarten school did not sufficiently represent exposure. The primary difference between the British Columbia and China studies is the former determined IDW estimates based on proximity to industrial point sources, while the latter used proximity to air quality monitoring stations to assign exposure, and this may have resulted in misclassification of exposure.

Land Use Regression Models

Land use regression has been used as a predictive modelling tool, that relies on monitored data at selected sites and geographic predictors as input data into a stochastic model that is able to predict measures at unmonitored locations within a study domain. We identified five reports (two of which are from the same cohort), in addition to the British Columbia study described above, that reported on the use of LUR modelling[14,16,38-41]. Among these, three studies[38,40,41] used air quality monitoring networks (AQMN) to develop their models with the number of monitors used ranging from 7 to 78. The outcomes of interest in these studies were asthma. The remaining studies[14,16,39], which focused on lower respiratory tract infections, wheezing and lung function, used passive sampling measures for model development. Apart from the Spanish and Norwegian studies, all studies used a combination of approaches to supplement the LUR approach, thus accounting for spatio-temporal variability. These included the use of satellite-based estimates as observed in the Ontario, Canada[41] and the Boston, USA[40] studies. Inverse distance weighting was additionally used in the latter two studies as well as the British Columbia study[38]. The combination of methods improves upon the spatial and temporal variability in exposure estimates. The studies that did not use supplementary approaches for more temporally refined data, were shown to have exposure

estimates that were highly correlated (between-subjects and between-trimesters). This is likely attributed to limited variability in AQMN data, while studies with fewer sites are subject to increased exposure misclassification due to the sparse distribution of these sites. Furthermore, inadequate representation of the different windows of exposure is unlikely to provide accurate exposure-outcome effect estimates. Studies that used LUR on its own found greater difficulty in disentangling trimester-specific effects given the limited temporal variability in exposure estimates. The success of LUR models is largely dependent on the accuracy of input data.

Dispersion Models

In our review, we identified only one study to use dispersion modelling[42]. In this study of pollutant-related wheezing in Dutch pre-schoolers, ambient pollution exposure in each year of the first three years of life was determined. Measures of traffic intensity and emissions, meteorology patterns, shipping, industry and household data were included as input data in the model, which further adjusted for background concentrations from three regulatory air quality monitors with the incorporation of the Dutch standard methods[43] (inclusion of intra-urban road traffic, traffic on highways, and industrial and other point sources). Thus, accounting for temporal changes in air pollutant exposure. The model was then validated by comparison between predicted annual average PM_{10} and NO_2 concentrations and measured data from the available monitoring stations. This study found no association of effect with the preceding year's average air pollutant exposure, but a statistically increased risk was observed with the preceding monthly averages for NO_2. The lack of a consistent exposure-response effect probably reflects the challenge in characterising the appropriate exposure metric for the outcome of interest: wheezing in early infancy is probably the clinical manifestation of long-term structural damage of the infant airway, and long-term exposure is probably a better exposure metric to understand this relationship. However, wheezing is also an acute outcome as a result of a recent insult (e.g., infection, recent air pollutant exposure etc.), and a shorter (recent) term exposure is more appropriate. In this study, it is likely that the preceding yearly average failed to capture the exposure required for a structural impairment, but better described the acute outcome.

Remote Sensing

In a pregnancy cohort conducted in Boston, USA[40], estimates of $PM_{2.5}$ exposure was determined by spatio-temporal modelling incorporating MODIS (moderate resolution imaging spectroradiometer) satellite-derived AOD (aerosol optical depth) measurements. AOD data account for temporal variability in air

pollutant emissions. The AOD-$PM_{2.5}$ data was calibrated for daily estimates using grid cells and AOD values by mixed modelling with random slopes. The objective was to identify sensitive antenatal exposure windows for the development of asthma by the age of six years in the cohort. The predicted satellite data was further combined with LUR derived data, providing modelled estimates at 10 x 10km^2 spatial resolution, for pregnancy exposure to $PM_{2.5}$. In a retrospective study in Canada[41], satellite estimates were derived at a 1 x 1km^2 spatial resolution, for $PM_{2.5}$ used in combination with a chemical transport model, with further adjustment by geographic weighted regression. The resulting time-varying (during pregnancy, the first year of life and in childhood) exposure estimates were then linked to childhood asthma[41]. The strengths of this exposure characterisation approach were the use of multiple sources and methods of validation achieving fine temporal granularity, with the LUR layered approach providing the required spatial estimates. AOD reflects air pollution in the atmosphere and thus calibration is needed to obtain ground-level air quality data, which may introduce some exposure assessment errors. An increased antenatal pollutant-related risk was found almost across the entire antenatal period, but this was statistically significant in the 16-25 weeks of pregnancy. The relatively narrow confidence intervals (ranging from approximately 0.8- 1.3), although including the null effect, suggests robust effect estimates.

DISCUSSION

Exposure assessment in environmental epidemiology facilitates the investigation of a cause-effect relation between an environmental toxicant and an adverse health outcome. The relevance of its application in pregnancy or childhood studies is based on evidence suggesting early programming effects during the prenatal[4,5], perinatal[6,28] and early postnatal[3,7] windows of development, that may result in structural and physiological changes in the airways and lungs, with implications for long term respiratory health. The sensitivity of specific windows of exposure (trimesters of pregnancy or early childhood) and associated respiratory health outcomes suggest that time integration is critical in exposure assessment in accounting for inter-and intra-subject variability. Individual personal monitoring lacks logistical feasibility for large scale studies, thus modelling methods are necessary to simulate and predict air pollutant exposure based on known characteristics of the surrounding environment. Despite significant advancements in this field a multitude of challenges still exist in addressing individual-level exposure estimation, as opposed to aggregate population-level exposure and accounting for temporal variability in exposure estimates.

The time-point of interest and duration of exposure varies across studies, ranging from specific trimesters or entire pregnancy, shortly after birth or early childhood, depending either on the objectives of the study, or the availability of either exposure or outcome data[44]. Assessing exposure during pregnancy is further

influenced by foetal susceptibility and other maternal and biological risk factors[3]. Exposure assessment has a significant role to play in identifying dose-response effects, thus highly resolved fine-scale spatio-temporal estimates are required. Acute effects such as lower respiratory tract infections, respiratory symptoms or wheezing can be assessed in early infancy and may be linked to in-utero exposure or recent exposure events. Studies assessing long-term health outcomes such as asthma may look at developmental changes over time. Thus, having highly resolved time-relevant exposure data available may help identify when such changes are occurring[4].

Because the lung begins growth and development antenatally and continues to develop postnatally in early infancy, effects of external insults such as air pollution may impact at specific time points, either once-off, or at multiple points, or throughout the developmental period[45]. This raises the complexity for determining exposure-outcome relationships for lung health specifically or other organ health generally, in this perinatal period. Arbitrary choices of cross-sectional exposure metrics, repeated measures of exposure at selected timepoints, or even assumptions of averaging exposure over extended periods are likely to result in exposure misclassification to some extent. As shown in Table 1 at least seven of the studies reviewed reported moderate to high correlation of measured observations, between trimesters.

Air pollutants are likely to covary, given that they are emitted from the same sources or produced by similar atmospheric chemistry or meteorologic processes, however, their chemical and physical properties are likely to yield differing impacts on the severity of health outcome assessed. For example, exposure to particulates is associated with lung inflammation and mucous secretion by acting on airway epithelial cells and alveolar macrophages with the potential of leading to airway remodelling[19]. Nitrogen dioxide exposure is associated with increased incidence in lower respiratory tract infections in children and increased airway responsiveness in asthmatics[46,47]. Correlation between pollutants makes it difficult to assess individual or combined health effects, as estimates may become unstable when adjusting for multiple pollutant effects in regression analysis.

Research has suggested that given that humans are simultaneously exposed to a complex mixture of air pollutants, "multi-pollutant" approaches should be considered[48]. A central aspect of multi-pollutant approaches is to model complex air pollution mixture effects more explicitly to gain better insight into the features that define the toxicity of an air pollution mix[49]. This approach may characterise more fully the complexity of the exposure and the health outcomes, with the potential to identify the most harmful emission sources. While this has yet to be fully explored, new approaches should modify the current methods of specifying air pollutant concentrations (or exposures) in statistical models to estimate health effects.

Conclusion

The usefulness of spatio-temporal modelling methods ultimately depends on the epidemiological study design (e.g. case-crossover vs time-series vs cohort), the health outcome of interest including acute (e.g. respiratory symptoms or infections and lung function) and chronic (wheezing and asthma) outcomes. the pollutant of interest and their specific spatial and temporal patterns[50,51]. Robust measures of exposure are critical in exposure-health outcomes epidemiological studies, and methods that incorporate both spatial and temporal gradients would likely yield better results than simplistic approaches (interpolation, regulatory monitoring data and proximity-based measures), with a further benefit of reducing prediction error or exposure misclassification. Future studies may likely benefit from considering multi-pollutant models, to characterize complex pollutant interactions and account for spatio-temporal variability in the emissions of complex mixtures.

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Table 1: Detailed Summary of Studies Reviewed

Author	Study Area	Study Design / Assessment Age	Health Outcome & Effect Estimates	Pollutant	Pollutant Data / Mean (SD)/ / Median (IQR)	Exposure Estimation Method	Temporal Adjustment	Additional Information
Soh et al., 2018[33]	Singapore	Longitudinal birth cohort – Growing Up in Singapore towards health Outcomes (GUSTO) Assessment age – 2 years	Wheezing episodes	$PM_{2.5}$	$PM_{2.5}$ ($\mu g/m^3$): 17.92 (1.31)* 18.21 (2.97)** 18.24 (2.68)*** 17.17 (2.39)****	a) National AQMN (N=8): daily 24-hour average (2009 – 2013)	Trimesters of pregnancy.	$PM_{2.5}$ between trimesters was moderately correlated and strongly correlated within a trimester, thus multi-trimesters were adjusted for in the models.
Lavigne et al., 2017[41]	Ontario, Canada	Retrospective cohort Assessment age – birth to 6 years	Asthma	NO_2 $PM_{2.5}$	NO_2 (ppb) 13.2 (7.8)* 13.2 (7.8)** 13.2 (7.8)*** 13.1 (7.8)**** 13.1 (7.8)***** 13.0 (7.8)****** $PM_{2.5}$ ($\mu g/m^3$): 7.3 (3.0)* 7.3 (3.0)** 7.3 (3.0)*** 7.3 (3.0)**** 7.3 (3.0)***** 7.3 (3.0)******	a) Satellite AOD estimates at 1x1 km resolution (2006-2012) b) GWR were used to determine $PM_{2.5}$ exposure estimates c) national LUR was developed using AQMN data (N=46), satellite estimates (2005-2011) and spatio-temporal characteristics (road length, industrial land use, mean summer rainfall) to determine NO_2 exposure estimates LUR Adjusted R2 = 0.73 (for 2006)	Trimesters of pregnancy, first year of life; cumulative childhood. A scaling factor used by calculating ratio of monthly mean NO_2 concentrations per monitor used to adjust the LUR estimate by trimesters of pregnancy.	$PM_{2.5}$ was moderately correlated with NO_2 during the entire pregnancy period. Moderate correlations were observed between trimester-specific periods and exposures after birth to $PM_{2.5}$.

						d) IDW – applied to zip codes within 25km of the AQMN to create scaling surface		
Madsen et al., 2016[16]	Norway	Prospective population-based pregnancy cohort – Norwegian Mother and Child Cohort study (MOBA) Assessment age: birth to 18 months	Lower respiratory tract infection and wheezing	NO_2	NO_2 (µg/m^3): 13.6 (6.9)* 13.7 (7.4)** 13.8 (7.5)*** 13.6 (7.3)****	a) LUR – NO_2 measured by passive samplers (Oslo N=14; Arkerhus N=36; Bergen/Hordaland N=46); 3 sampling campaigns of two-weeks each, over the duration of a year (2010 - 2011) during summer, winter and an intermediate season. LUR Adjusted R^2 Oslo = 0.65 LUR Adjusted R^2 Arkerhus = 0.55 LUR Adjusted R^2 Bergen/Hordaland = 0.85	AQMN data (2000-2012) was used for ratio method of back-extrapolation during the pregnancy period - the LUR-modelled yearly estimate multiplied by the ratio between daily NO_2, AQMN data and an annual average for the year of LUR measurement campaign. Daily NO_2 exposure estimates were averaged separately for first, second and third trimester, and entire pregnancy[52].	Exposures by trimester and entire pregnancy exposure were highly correlated. Thus, average NO_2 exposure during entire pregnancy was used as the exposure estimate in analyses.
Deng et al., 2016[37]	Hunan Province, south-central China	Survey study Assessment age: 3-6 years	Asthma	NO_2 PM_{10}	NO_2 (µg/m^3): 46.0 (8.0)* 45.0 (11.0)** 46.0 (11.0)*** 46.0 (10.0)**** PM_{10} (µg/m^3): 110.0 (11.0)* 113.0 (16.0)** 110.0 (15.0)*** 108.0 (18.0)****	a) AMQN (N=7) – daily averages (2005 – 2008). Spatial resolution -1909km^2 b) IDW used to establish individual exposure estimates	Average of the monthly mean concentrations of AP was calculated for trimesters of pregnancy and entire pregnancy.	The pollutants during each trimester were weakly or moderately correlated with each other. Each pollutant was also weakly or moderately correlated between different trimesters. Multi-pollutant models were explored.
Hsu et al., 2015 [40]	Boston, USA	Pregnancy cohort – Asthma	Asthma	$PM_{2.5}$	$PM_{2.5}$ (µg/m^3), median (IQR) 11.2 (10.2–11.8)*	a) MODIS satellite derived AOD measurements at 10 x 10km resolution	Entire pregnancy and distributed lag windows over 6 years	

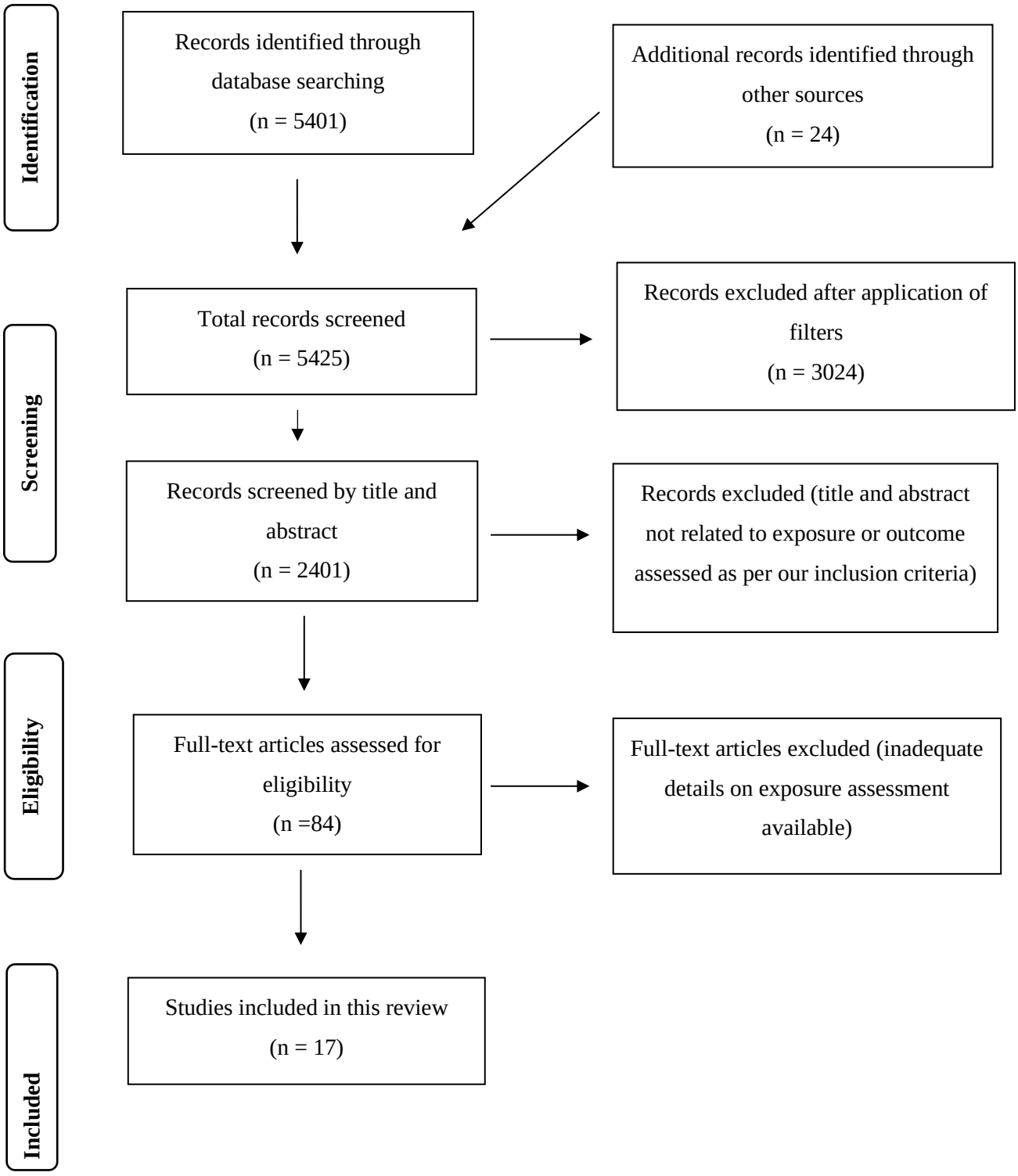
		Coalition on Community, Environment and Social Stress (ACCESS) Assessment age – 6 years				b) LUR derived using AMQN data (N=78) meteorological variables combined with AOD-$PM_{2.5}$ measurement was calibrated daily.		
Morales et al., 2014[14]	Sabadell and Gipuzkoa, Spain	Cohort study – INMA (Environment and Childhood) Study Assessment age: 4-5 years	Lung function	NO_2	NO_2 ($\mu g/m^3$), median (IQR): 25.50 (17.40 – 31.66)* 24.30 (16.76 – 33.48)** 24.23 (16.96 – 25.63)*** 23.87 (16.88 – 33.26)**** 27.87 (19.84 – 33.59) *****	a) LUR – ambient NO_2 measured by passive samplers (N=57) (2005-2006)[53] during 4 sampling campaigns of one week each. LUR Adjusted R^2 Sabadell = 0.75 LUR Adjusted R^2 Gipuzkoa = 0.51	Adjustment using a ratio of daily NO_2 levels from AMQN to establish estimates for entire pregnancy, trimesters of pregnancy and first year of life	NO_2 levels were moderately to highly correlated between trimesters of pregnancy, and highly correlated between the entire prenatal period and the first year of life.
Stern et al., 2013[54]	Bern, Switzerland	Prospective birth cohort – Bern Infant Lung Development Study (BILD) Assessment age: 1 year	Respiratory symptoms	PM_{10} NO_2	PM_{10} ($\mu g/m^3$): Weekly average rural: 19.9 (10) Weekly average urban: 32.6 (13) NO_2 ($\mu g/m^3$): Weekly average rural: 15.2 (7) Weekly average urban: 48.2 (9) PM ($\mu g/m^3$): Urban: 34.2 (4.2)* Rural: 20.8 (2.5)*	a) Swiss National Air Pollution Monitoring Network – daily mean hourly data for PM_{10} and NO_2 (2004 – 2006) b) Proximity measures – distance to nearest major road of 4- to 6-m width	Lag windows of 1-10 days established during the first year of life Lag structures of 1 to 10 days preceding interview, were constructed with shifting windows of weekly mean AP by 1-10 days	

Aguilera et al., 2013[39]	Spain	Birth cohort Assessment age: 12-18 months	Lower respiratory tract infections and wheezing	NO_2	NO_2 (µg/m^3), median Asturias – 21.0* & 22.0***** Gipuzkoa –18.0* & 19.0***** Sabadell – 30.0* & 32.0 ***** Valencia – 38.0* & 38.0*****	a) LUR model developed using ambient NO_2 measured by passive sampling during 4 sampling campaigns of one week each. Asturias (N=67) + 4 AQMN Gipuzkoa (N=86) + 2 AQMN Sabadell (N = 57) + 1 AQMN Valencia (N= 93) + 7 AQMN. LUR model R^2 Asturias = 0.52 LUR model R^2 Gipuzkoa = 0.52 LUR model R^2 Sabadell = 0.75 LUR model R^2 Valencia = 0.73	Exposure estimate derived by multiplying LUR estimate by the ratio between average measured concentration at AQMN over the pregnancy period to establish trimester specific and entire pregnancy estimates.	Levels of each pollutant were moderately to highly correlated between trimesters of pregnancy and highly correlated between the entire prenatal period and the first year of life.
Sonnenschein-van der Voort, 2012[42]	Rotterdam, the Netherlands	Prospective cohort – Generation R study Assessment age: 1-3 years	Wheezing	NO_2 PM_{10}	PM_{10} (µg/m^3): 28.86 (2.11)^ 28.27 (1.57)^^ 27.92 (1.67)^^^ NO_2 (µg/m^3): 38.66 (4.20)^ 37.46 (4.17)^^ 36.22 (4.28)^^^	a) AQMN data (N=3), (taking into account wind conditions and fixed temporal patterns from sources) b) Dispersion modelling[55].	Average annual levels per year over 1-3 years.	
Jedrychowski et al., 2010[31]	Krakow, Poland	Birth cohort Assessment age: 4.5 years	Wheezing	$PM_{2.5}$	$PM_{2.5}$ (µg/m^3), median (IQR) 35.4 (10.3 – 294.9)	a) Personal Environmental Monitoring Samplers over a 48-hour period [2nd trimester (N = 369); 3rd trimester (N=85)]	48-hour measurement extrapolated over specific trimesters (second and third)	

Jedrychowski et al., 2010[30]	Krakow, Poland	Birth cohort Assessment age: 5 years	Lung function	$PM_{2.5}$	$PM_{2.5}$ ($\mu g/m^3$), median (IQR) 32.4 (30.1)	a) Personal Environmental Monitoring Samplers over a 48-hour period [2nd trimester (N = 176)]	48-hour measurement extrapolated over specific trimesters (second and third)	
Clark et al., 2010[38]	British Columbia, Canada	Nested case-control Assessment age: 3-4 years	Asthma	NO_2, PM_{10} $PM_{2.5}$	*Controls* NO_2 ($\mu g/m^3$) LUR – 31.68 (8.64)* & 29.86 (8.85) ***** IDW – 30.74 (8.90)* & 29.86 (8.85) ***** PM_{10} ($\mu g/m^3$) IDW – 11.94 (1.35)* & 12.37 (1.00) ***** $PM_{2.5}$ ($\mu g/m^3$) LUR – 4.67 (2.47)* & 4.50 (2.45) ***** IDW – 4.74 (1.19)* & 5.62 (0.61) ***** *Asthma Cases* NO_2 ($\mu g/m^3$) LUR – 31.73 (8.42)* & 30.68 (9.06) ***** IDW – 31.37 (9.20)* & 30.68 (9.06) ***** PM_{10} ($\mu g/m^3$)	a) LUR models derived using AMQN – 24-hour averages [NO_2 (N=14); PM_{10} (N=19); $PM_{2.5}$ (N=7)] – road density, population density, elevation, and type of land use were used to develop high-resolution (10m) maps b) IDW – summation of emissions from point sources within 10km c) Proximity measures – distance to roadways and industrial point sources within 50 m or 150 m of highways and major roads	Daily average over entire pregnancy and first year of life.	Pregnancy and first-year exposures were moderately to highly correlated. Some of which could be examined together in mutually adjusted models. Multipollutant methods could not be explored due to correlation.

					IDW – 12.03 (1.30)* & 12.42 (1.00) ***** $PM_{2\,5}$ (µg/m^3) LUR – 4.78 (2.46)* & 4.59 (2.40) ***** IDW – 4.71 (1.20)* & 5.62 (0.61) *****			
Latzin et al., 2009[35]	Bern, Switzerland	Prospective birth cohort –BILD Assessment age: 5 weeks	Lung function	NO_2 PM_{10}	PM_{10} (µg/m^3), median (IQR) 221. (20.2 – 23.8)* 20.0 (16.6 – 23.4) ****** NO_2 (µg/m^3), median (IQR) 15.8 (14.7 – 17.0)* 15.1 (10.9 0 19.7)******	a) AQMN in Payern (part of the Swiss National Air Pollution Monitoring Network) – daily mean hourly data for PM_{10} and NO_2 (2004 – 2006). b) Proximity methods – distance to nearest major road of 4- to 6-m width	Entire pregnancy, trimesters of pregnancy and birth till test date (postnatal). Mean daily levels of AP were established over the estimation period.	
*entire pregnancy (prenatal / in-utero); **first trimester; ***second trimester; ****third trimester; *****first year of life, ******cumulative/postnatal ^one year; ^^two year; ^^^three year **Abbreviations**: AP – Air pollutant; AOD – Aerosol Optical Depth; AQMN – Air Quality Monitoring Network; DM – Dispersion Modelling; GWR – Geographic Weighted Regression; IDW – Inverse Distance Weighting; IQR – Interquartile Range; LUR – Land Use Regression; MODIS- Moderate Resolution Imaging Spectroradiometer; NO_2 – Nitrogen Dioxide; PM_{10} - Particulate Matter <10 microns; $PM_{2\,5}$ – Particulate Matter <2.5 microns; SD – Standard Deviation								

APPENDIX A: SELECTION OF STUDIES FOR REVIEW BASED ON THE PRISMA STRATEGY



CHAPTER 4

MANUSCRIPT III

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Effect of short-term exposure to ambient nitrogen dioxide and particulate matter on repeated lung function measures in infancy: A South African birth cohort

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Abstract

Background: The developing lung is highly susceptible to environmental toxicants, with both short- and long-term exposure to ambient air pollutants linked to early childhood effects. This study assessed the short-term exposure effects of nitrogen dioxide (NO_2) and particulate matter (PM_{10}) on lung function in infants aged 6 weeks, 6, 12 and 24 months, the early developmental phase of child growth.

Methods: Lung function was determined by multiple breath washout and tidal breathing measurement in non-sedated infants. Individual exposure to NO_2 and PM_{10}, as two-week average estimates (preceding the test date), was determined by hybrid land use regression and dispersion modelling. Linear mixed models were used to adjust for the repeated measures design for the outcome measures and an age*exposure interaction was introduced to obtain effect estimates for each age group.

Results: There were 165 infants that had lung function testing, with 82 of them having more than one test occasion. Exposure to PM_{10} resulted in a decline in tidal volume at 6 weeks (β: -0.4mL/kg (95%CI: -0.9; 0.0), p=0.065), 6 months (β: -0.5mL/kg (95%CI: -1.0; 0.0), p=0.046) and 12 months (β: -0.3mL/kg (95%CI: -0.7; 0.0), p=0.045). PM_{10} was related to an increase in respiratory rate and minute ventilation, while a decline was observed for functional residual capacity for the same age groups, though not statistically significant for these outcomes. Such associations were however less evident for exposure to NO_2, with inconsistent changes observed across measurement parameters and age groups.

Conclusion: Our study suggests that PM_{10} results in acute lung function impairments among infants from a low-socioeconomic setting, while the association with NO_2 is less convincing.

Abbreviations: ATS/ERS, American Thoracic Society/ European Respiratory Society; CV, coefficient of variance; FRC; Functional Residual Capacity; LCI, Lung Clearance Index; MACE, Mother and Child in the Environment; MBW, Multiple Breath Washout; TV, Tidal Volume.

INTRODUCTION

Epidemiological studies have shown that both long- and short-term exposures to air pollution have been linked to adverse respiratory health outcomes[1,2]. Lung growth and development shortly after birth is predictive of long-term respiratory health. During the neonatal growth stage, several events occur including maturation of the neurologic control of breathing, as well as changes in the mechanical properties of the respiratory system (e.g., lung volume), which influence respiration. Compensatory adjustments of these parameters are seen in the disease states including exposure to air pollutants[3]. The structure of the lungs is an important determinant of ventilatory function, and thus related to mechanical properties, that are reflected in the relationship of volume, flow and time during expiratory manoeuvres[4].

The timing of exposure, to air pollutants, during development, appears to be critical to the subsequent effects observed[5,6]. As lung development occurs over the entire perinatal period, exposure effects may have significant consequences whether they occur during pre-or postnatal life[5,7], as is evidenced by prior studies demonstrating in-utero[8,9] and early life[10-12] effects from air pollutant exposure. Additionally, research suggests that early life exposures may have long term consequences on lung health and compensatory measures in respiratory morbidity[8,11,13,14].

Important markers of early-life lung function include functional residual capacity (FRC), lung clearance index (LCI) and tidal breathing indices, which are used to evaluate lung function abnormalities. For example, end-expiratory volume analogous to FRC has been shown to be diminished in pre-term infants, with increased ventilation homogeneity (higher LCI)[15]. In wheezing infants, the LCI has been shown to be lower compared to non-wheezing infants, with a functional increase in FRC observed[16]. Newborns have higher ventilatory needs than older children, and these are met by increasing breathing frequency (higher respiratory rate)[3]. In healthy infants' respiratory rate decreases with postnatal age, and this is accompanied by an increase in the duration of inspiratory and expiratory time[17]. Tidal volume is reported to remain invariant from birth to adulthood, while minute ventilation declines with postnatal age[5]. These ventilatory characteristics are thus considered "a priori" when assessing early-life lung function outcomes. The sensitivity of these measures, in assessing lung function impairments, has been well established in studies assessing cystic fibrosis[18,19].

There is little known about the effects of short-term exposure to air pollutants on lung function shortly after birth. In a Swiss birth cohort[20], pregnancy exposure to PM_{10} was associated with higher minute ventilation at 5 weeks of age. Maximal forced expiratory flow (a measure of peripheral airway function) shortly after birth was reduced in infants exposed to maternal smoking during pregnancy, studies have frequently reported on antenatal exposure and early childhood lung function relationships, but there are

no studies that have evaluated the responsiveness of lung function in the first two years of life in relation to short-term air pollutant exposure, which is subject to temporal fluctuations.

Though exposure effects on respiratory health for NO_2 and PM_{10} have repeatedly been shown[21,22] in older age groups, short-term exposure effects on lung function shortly after birth and in early childhood has not been reported. This is probably due to the challenge of assessing short term exposure to air pollution and measuring lung function outcomes in infants and children younger than two years of age[7].

In this study, we used a hybrid land use regression and dispersion model[23] to predict short-term (two-week average) exposure estimates for nitrogen dioxide (NO_2) and particulate matter (PM_{10}) with the aim of evaluating short-term changes in lung function at the ages of 6 weeks, 6, 12 and 24 months, among selected infants from the existing Mother and Child in the Environment (MACE) cohort.

METHODS

Study Design

This study used a repeated cross-sectional design. Infants had a lung function test performed by multiple breath washout and tidal breathing at 6 weeks, 6, 12 and 24 months, with clinical assessments at 6, 12 and 24 months. Additionally, exposure to air pollutants NO_2 and PM_{10} was determined by spatio-temporal modelling. Average estimates were calculated for each two-week period preceding the test date, providing individual measures of exposure for each test occasion.

Study Location & Participants

In this study infant participants, at varying age groups were selected from the MACE cohort, in Durban, KwaZulu-Natal, South Africa. We included all infants from the existing cohort that were eligible and available for testing, and who consented to participate. Pregnant females were enrolled at public sector antenatal clinics from low-socio-economic communities in the north and south Durban regions, with varying levels of ambient pollution. The cohort selection is described elsewhere[24]. This study was approved by the University of KwaZulu-Natal Biomedical Ethics Committee (BE431/17) with prior written consent obtained from the mother or primary caregiver.

Lung Function Measurements

Lung function measurement included tidal breath assessment and multiple breath washout using the Exhalyser D with ultrasonic flow meter (Ecomedics AG, Duernten, Switzerland) and sulphur

hexafluoride (SF_6) tracer gas. Testing was conducted in non-sedated infants during behaviourally defined quiet sleep, with non-rapid eye-movement[25], at the ages 6 weeks, 6, 12 and 24 months. The tests were performed by two independent researchers trained and competent in both testing and analysis of the test outputs. Measurements were completed over the period January 2017 to December 2019, and in adherence with the American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines[26,27]. Analysis of all tests performed was conducted using the Wbreath v2.0 (Ndd Medizintechnik AG) software. The main outcome measures assessed included functional residual capacity, lung clearance index, tidal volume, respiratory rate, minute ventilation, mean tidal inspiratory flow, mean tidal expiratory flow and time to peak tidal expiratory flow over total expiratory time (tPTEF/tE).

Covariate Data Extraction

Data was sourced from the cohort questionnaire-based interviews. Candidate predictors of lung function were based on previous literature and the availability of data in the MACE cohort. The data included participant demographic information, maternal data relating to socioeconomic status, human immunodeficiency virus (HIV) status, maternal weight gain, smoking during pregnancy and delivery type as reported elsewhere[28].

Information on child characteristics was obtained from the post-natal questionnaire data, relating to child sex, gestational age, birth weight and length. Time-varying characteristics such as age, weight and length/height were obtained at the lung function test session. Clinical assessments and a detailed respiratory evaluation were conducted by a paediatric pulmonologist and a physician, in which growth and developmental milestones were examined at 6, 12 and 24 months.

Environmental exposure data were obtained from the recruitment questionnaires including housing type (formal vs informal), primary heating source (electrical vs gas/paraffin) and environmental tobacco smoke exposure (ETS). While individual-level air pollutant exposure was derived from modelled estimates.

Air Pollution Exposure Assessment

A comprehensive environmental exposure assessment was conducted within the larger MACE cohort and is described in detail elsewhere[23,29]. In brief, air pollution exposure estimates for NO_2 and PM_{10} were derived from hybrid-modelling, which included both land use regression (LUR) and dispersion modelling (DM). Single pollutant LUR models were developed according to the ESCAPE (European

Study of Cohorts for Air Pollution Effects)[30] method, following the principles of stepwise linear regression in which monitored air pollutant levels were regressed against a predefined set of geographic predictor variables accounting for the influence of vehicle emissions, population and housing density, proximity to industry and the harbour as well land use characteristics. To achieve temporal coverage within the modelling framework a DM component was introduced into the regression model, to estimate time-varying exposure. DM derived data account for near-source pollutant behaviour based on source characteristics (e.g. stack/mixing height), time- and space -varying effects of meteorological conditions (e.g. wind speed, wind direction and atmospheric stability) as well as complex terrain and coastal interactions[31]. The models were further validated for accuracy by leave one out cross-validation.

The developed models were then used to derive prediction estimates of annual adjusted averages for NO_2 and PM_{10} (Figure S1 in supplementary data) at the geocoded residential address of the participant. For temporal adjustment of modelled estimates, the DM data in the LUR-DM model was re-calibrated for each two-week period preceding the lung function test date, providing a temporally resolved two-week average per participant and per testing occasion.

Statistical Analysis

Descriptive data are presented as means and standard deviations (SD) of each measurement parameter and intra-subject variability as assessed by the coefficient of variation. Extreme values were identified and their removal from the analysis was considered. The normality of data was tested by Q-Q plots per outcome measure for each age group. Missing data patterns were further assessed and complete case analysis was used given the low rate of missing data (<10%).

To evaluate the time-varying effects of NO_2 and PM_{10} exposure on lung function outcomes among the various age groups and to account for the repeat measures, we used generalized linear mixed models following a sequential modelling approach. As a first step, we included important predictors likely to be associated with the outcome measures, and this included child sex, postnatal length gain and low birth weight. These were used as a start model for each measurement parameter. Dummy variables were used for each test age and were further included in interaction terms (pollutant*test age) per test occasion, to estimate the change in lung function over time. We included the dummy variables and interaction terms in the start model, and then added exposure variables: maternal smoking, maternal HIV status, housing type and primary heating source, NO_2 and PM_{10}. Thus, our final models are adjusted for infant anthropometry factors, maternal and environmental risk factors. We explored the interaction between the individual covariates (maternal smoking, maternal HIV, housing type and primary heating source) and the pollutant effect in each of the models. Data analyses were performed using STATA for Windows (STATA Corporation, College Station, TX, USA). Given the sequential

modelling approach, we used the Benjamini-Hochberg[32] approach to adjust for false discovery rates in the final models.

RESULTS

A total of 312 infant lung function tests were completed, of which 282 (90%) were considered acceptable based on visual quality and test attempts that satisfied the ERS/ATS validity criteria. Testing was performed at 6 weeks (n=70), 6 months (n=73), 12 months (n=66) and 24 months (n=73). In total there were 165 participants that had lung function testing, with 82 participants that had at least more than one test occasion.

Table 1: Participant characteristics

	Participants (N=165)	**6 weeks (N= 70)**	**6 months (N= 73)**	**12 months (N= 66)**	**24 months (N= 73)**
Female Sex, n (%)	83 (50.30)	35 (50)	43 (58.90)	33 (50)	37 (50.68)
Age at study date (months) mean (sd)	-	1.75 (0.46)	6.70 (1.02)	12.56 (1.06)	24.66 (1.46)
Weight at study date (kg), mean (sd)	-	4.68 (0.92)	8.17 (1.23)	10.54)1.64)	12.74 (1.92)
Length at study date (cm), mean (sd)	-	54.26 (4.18)	66.97 (3.73)	74.94 (4.10)	85.07 (4.05)
Low-birth weight[a], n (%)	18 (10.91)	11 (15.71)	9 (12.33)	8(12.12)	6 (8.22)
Maternal HIV, n (%)	55 (33.33)	25 (35.71)	25 (34.25)	18 (27.27)	23 (31.51)
Maternal smoking during pregnancy[b], n (%)	8 (4.85)	2 (2.87)	3 (4.11)	4 (6.06)	5 (6.58)
Air Pollution Exposure (2-week average)					
Nitrogen Dioxide (µg/m^3), mean (sd)[c]	-	16.31 (2.22)	16.42 (1.76)	17.09 (1.68)	17.35 (1.58)
Particulate Matter (µg/m^3), mean (sd)[c]	-	33.77 (7.73)	32.87 (7.14)	34.39 (11.01)	33.28 (8.43)

[a]Low birth weight is classified as birth weight<2.5kg as per the WHO guidelines[33].

[b]Maternal smoking during pregnancy is based on a "yes" response to the question "do you smoke now?".

[c]Air pollution estimates were derived from hybrid land use regression and dispersion modelling

Table adapted from unpublished work[34]

Most participants (94%) were from low-income households (<USD2000), with even gender distribution (50%). Among these, 10% resided in informal housing (classified as unplanned settlements and areas where housing is not in compliance with current planning and building regulations)[35], while <2% used gas/paraffin for domestic heating as opposed to electricity. There were 43 participants that resided in the less industrialized north Durban region and 122 participants residing in the highly industrialized south Durban region. The mean (SD) gestational age of infants was 38.9 weeks (1.78) with 6% born preterm. Approximately 30% of participants were HIV exposed. There was an overall low prevalence of maternal smoking among participants (<5%). Participant short-term exposure estimates to air pollutants NO_2 and PM_{10}, ranged from 16.31 – 17.35 $\mu g/m^3$ and 32.28 – 34.39 $\mu g/m^3$, respectively across age groups.

Descriptive data on lung function measurements are presented in Table S1 and described in detail elsewhere[34]. The key parameters showed expected age-related differences across the different age groupings (for example, higher tidal volumes and lower respiratory rates with the increasing age categories).

Table 2: Estimated change in lung function parameters per unit increase in NO_2 (μg/m³) and PM_{10} (μg/m³) across the test visits

	6 Weeks		6 Months		12 Months		24 months	
N= 282	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**
Tidal volume, mL/kg								
NO_2	1.1 (-1; 3.2)	0.317	1.4 (-0.9; 3.6)	0.226	-0.3 (-2.5; 2.0)	0.809	-1.3 (-3.1; 0.6)	0.174
PM_{10}	-0.4 (-0.9; 0.0)	0.065	**-0.5 (-1.0; 0.0)**	**0.046**	**-0.3 (-0.7; 0.0)**	**0.045**	**0.4 (0.1; 0.7)**	**0.013**
Respiratory rate, n/min								
NO_2	-0.6 (-2; 0.8)	0.420	-1.1 (-2.6; 0.5)	0.180	-0.3 (-1.9; 1.3)	0.707	0.7 (-0.5; 1.9)	0.249
PM_{10}	0.2 (-0.1; 0.5)	0.282	**0.4 (0; 0.7)**	**0.025**	0.1 (-0.1; 0.4)	0.266	-0.2 (-0.4; 0.0)	0.101
Minute ventilation, mL/min								
NO_2	-45.9 (-113.9; 22.1)	0.186	-58.9 (-132.4; 14.5)	0.116	-37.6 (-113.3; 38.2)	0.331	31.8 (-24.4; 87.9)	0.267
PM_{10}	6.0 (-9.0; 21.1)	0.432	**18.8 (3.2; 34.3)**	**0.018**	5.7 (-6.4; 17.8)	0.359	-7.6 (-17.8; 2.6)	0.143
Mean tidal inspiratory flow mL/s								
NO_2	-1.5 (-4.3; 1.3)	0.300	-1.6 (-4.6; 1.4)	0.291	-1.8 (-4.9; 1.3)	0.254	1.0 (-1.3; 3.4)	0.399
PM_{10}	0.3 (-0.3; 0.9)	0.293	**0.9 (0.2; 1.5)**	**0.008**	0.4 (-0.1; 0.9)	0.094	-0.4 (-0.9; 0)	0.046
Mean tidal expiratory flow mL/s								
NO_2	-1.3 (-3.8; 1.2)	0.305	-2.2 (-4.9; 0.5)	0.114	-1.0 (-3.8; 1.8)	0.499	1.0 (-1.0; 3.1)	0.328
PM_{10}	0.1 (-0.5; 0.6)	0.803	0.4 (-0.1; 1.0)	0.146	0.0 (-0.4; 0.5)	0.884	-0.1 (-0.5; 0.3)	0.554
tPTEF/tE %								
NO_2	0.1 (-2.0; 2.2)	0.933	1.2 (-1.0; 3.5)	0.283	-0.4 (-2.7; 1.9)	0.731	-0.2 (-1.9; 1.5)	0.830
PM_{10}	0.2 (-0.2; 0.7)	0.313	-0.3 (-0.8; 0.2)	0.231	0.2 (-0.2; 0.5)	0.276	-0.1 (-0.5; 0.2)	0.370
Functional residual capacity, mL/kg								
NO_2	4.2 (-1.5; 10)	0.150	**9.6 (3.5; 15.7)**	**0.002**	3.2 (-3.0; 9.4)	0.308	-4.7 (-9.6; 0.1)	0.057
PM_{10}	-0.4 (-1.6; 0.9)	0.581	-1.2 (-2.5; 0.1)	0.078	-0.2 (-1.1; 0.7)	0.689	0.4 (-0.5; 1.3)	0.344
Lung Clearance Index								
NO_2	-0.1 (-0.2; 0.0)	0.092	**-0.1 (-0.3; 0)**	**0.007**	-0.1 (-0.2; 0.1)	0.295	0.1 (0; 0.2)	0.077
PM_{10}	-0.0 (-0.04; 0.01)	0.171	0.0 (-0.03; 0.01)	0.418	-0.0 (-0.5; -0.0)	0.000	0.2 (0.0; 0.0)	0.015

NO_2 linear mixed effects models adjusted for test age, child sex, postnatal length gain, low birth weight, NO_2 and interaction terms (NO_2*6week, NO_2*6 months, NO_2*12 months) per outcome measure

PM_{10} linear mixed effects models adjusted for test age, child sex, postnatal length gain, low birth weight, PM_{10} and interaction terms (PM_{10}*6week, PM_{10}*6 months, PM_{10}*12 months) per outcome measure

p<0.05 in bold

Table 2 shows the air pollutant-related change in lung function parameters per age group, adjusted for the key covariates from the linear mixed models. Increased exposure to PM_{10} resulted in a decline in tidal volume at 6 weeks, 6 and 12 months. Respiratory rate, minute ventilation, mean tidal inspiratory

and mean tidal expiratory flow increased per unit increase in PM_{10} exposure. Exposure to NO_2 resulted in a decline in tidal volume and functional residual capacity at the 24-month age group, though only statistically significant for certain age groups. Exposure to NO_2 resulted in an increase in functional residual capacity and a decline in the lung clearance index at 6 months. NO_2 appeared to show inconsistent changes for the other parameters assessed, with no apparent trends observed.

The effects of selected environmental factors (maternal smoking, HIV exposure, housing type and primary heating source) were explored in the linear mixed models (Table S2 in the supplementary data), with maternal smoking emerging as a significant predictor. Maternal smoking showed statistically significant effects ($p<0.05$), with increases observed for minute ventilation and mean tidal inspiratory flow, this was similarly observed for exposure to PM_{10}, while in both models these exposures results in a lower tPTEF/tE. There were no significant effects observed for the respective outcome measures for those infants with HIV exposure. Exposure to NO_2 and living in informal housing showed a reduction in tPTEF/tE.

DISCUSSION

In this study, we assessed lung function among infants from a low-socioeconomic setting, in a previously poorly studied population. Short-term exposure effects of nitrogen dioxide (NO_2) and particulate matter (PM_{10}) on lung function were demonstrated among infants over the first two years of life. Exposure to PM_{10} showed effects on tidal volume and mean tidal inspiratory flow, while the effects of NO_2 were less substantial. The effects of PM_{10} and NO_2 on lung function and respiratory health is well documented in older children and adults[36,37] but there are limited reports in the infant age group. This study represents one of only two infant lung function studies conducted in Sub-Saharan Africa.

The parameters of lung function in infants are a reflection of lung maturation and are influenced by altered growth and development, which must be considered when interpreting any observed lung function changes[38]. In our linear mixed effects models, in addition to short-term exposure to air pollution, we adjusted for the age of the child, sex, postnatal length gain and low birth weight, which are considered as primary determinants of physiological changes[39,40].

Exposure to PM_{10} resulted in increased minute ventilation and respiratory rate from the age of 6 weeks to 12 months, while a tendency for a decline in functional residual capacity was observed for the same age groups. These findings are supported by a plausible clinical theory of compensation for the structural restriction in airflow. An increase in pre-natal PM_{10} exposure resulted in higher minute ventilation as reported for 5-week old infants in the Bern Infant Lung Development (BILD) study[20] (24.9 mL/min per $\mu g/m^3$ PM_{10}). In our study, we found a 6.0 mL/min increase in minute ventilation per $\mu g/m^3$ PM_{10}, however this was based on short-term postnatal exposure estimates. The consistent PM_{10} related findings across both studies could imply pathophysiological exposure-related effects occurring at the level of gas exchange. The higher respiratory rate and minute ventilation observed in infants suggests an increased oxygen demand for rapid lung and somatic growth (increased work of breathing for a damaged lung from pollutant exposure). The pollutant-related increase in these parameters further suggests that there is increased oxygen demand.

There are no other studies that assessed lung function in infants without sedation in a repeated cross-sectional design, thus data comparison is a challenge. However, we assessed the observed outcome measures in relation to their known physiological influence. For example, although tidal breathing is reported to remain invariant per increase in body weight[3], we noted a pollutant-related decline from 6 weeks to 6 months for PM_{10}, while NO_2 related changes were less evident. Studies have shown that changes in tidal volume may be attributed to airway obstruction[41 38,42], for example, in one study tidal breathing parameters changed with increasing severity in bronchopulmonary dysplasia (BPD)[43]. It was also suggested that such morphological changes have functional consequences on lung volume,

ventilation homogeneity, mechanics of the respiratory system and control of breathing[38]. In addition, young infants, particularly those born prematurely, are prone to airway narrowing and closure during tidal breathing, and thus have increased vulnerability to wheezing disorders[42].

The observed findings of pollutant related effects on lung function parameters may imply current impaired lung physiology or indicate the likelihood of the development of airway disease. Though minute ventilation, respiratory rate and tidal volume are considered the most robust and meaningful measurement parameters of tidal breathing, quantitative separation of their individual role, together with a meaningful interpretation of their relative role within the tidal breathing process, remains a significant challenge, especially in infancy[44]. Furthermore, functional residual capacity is critical for peripheral airway function, thus an observed decline is likely due to reduced opening of the airways, compromising gas exchange[45,46]. These pollutant-related pathophysiological responses may also increase the risk of airway obstruction and the development of wheezing disorders[46]. Such observations were reported in the BILD cohort, in which it was reported that infants that subsequently developed wheeze had a lower respiratory rate but higher tidal volume[47]. While minute ventilation was significantly increased in infants with CF (n=47) compared to healthy infants (n=95)[48].

In this study, we investigated short-term exposure effects using a two-week average of air pollutant concentrations. This was achieved by developing models to precisely define exposure at the residence of the participants. Ambient air pollutants are known to be spatially and temporally heterogenous[49], thus we accounted for this variability by including known meteorological and topographical differences in our modelling approach. Furthermore, the incorporation of dispersion modelled estimates in our modelling framework allowed for temporal adjustment of the modelled estimates. The refined exposure assessment by hybrid modelling, which is data- and computationally-intense, was a significant strength of this study.

Maternal smoking was significantly associated with increased minute ventilation, while a decline in tPTEF/tE, a marker of airway obstruction, was observed in our sample. Smoke exposed infants in the Drakenstein Child Lung Health Study (DCHLS) cohort were reported to have smaller lung volumes (β: -4.7mL/kg; 95%: -8.23; -1.12; p= 0.010), indicative of early structural impairment. Recent work by the DCLHS cohort further indicates that in-utero exposure to HIV resulted in lower tidal volume in HIV exposed infants (n=175) in comparison to unexposed infants (n=682) (mean difference of 1.13 mL (CI: 0.02–2.23), p<0.045) between exposure groups at 6 weeks, though the observed effect was not consistent through follow-up at two years of age. We were unable to replicate such findings in our cohort. This may be because HIV effects are counteracted by the maternal use of antiretroviral treatment. Infants exposed to both pollutants and living in informal housing had lower tPTEF/tE by approximately 5%. Reduced tPTEF/tE in early infancy has been identified to be an important predictor

of later life wheezing[50,51]. For those infants that were exposed to household gas/paraffin use, increased mean tidal inspiratory flows were observed for both pollutant models (β: 22mL/s (95%CI: 6.2; 37.8); p=0.006 for NO_2) and (β: 21.4mL/s (95%CI: 6; 36.9), p=0.007 for PM_{10})[4].

We find confidence in our descriptive lung function findings, which compared well with that reported in the other South African cohort, the DCLHS. The values reported in the BILD and the European/Australian multicentre study were slightly higher for certain parameters such as tidal volume and functional residual capacity, while lung clearance index and respiratory rate were within $\pm$1SD across studies and minute ventilation was higher in South African infants[52] from both cohorts in comparison to the European and multicentre studies[53,54]. These differences are likely attributed to the study sample, variability in population characteristics and anthropometric differences between studies.

While other studies have reported on air pollutant related effects on repeated lung function measures in older children[55], similar research among infants is lacking. Our study suggests that continued exposure to air pollutants throughout infancy is likely to result in adverse effects among some of the lung function parameters, leading to compromised lung health as the child ages. This is justified by studies showing alterations in early lung function that track into adulthood[13,14]. For example, studies have shown that measures of tPTEF/tE are diminished in infants who subsequently wheeze[50,51], with further suggestion that lower airway function early in life may be a predisposing factor for recurrent wheezing or respiratory illness starting in the first year of life[51,56]. It has also been shown that reduced tPTEF/tE was related to an increased risk of asthma by ten years of age[13]. In general air pollutant exposure in early life has been related to an increased risk of asthma development as the child ages[57,58]. Studies that provide evidence for repeat airway insults resulting in airway anatomical modification[59] lends support to our assertion that repeat pollutant related effects in infancy compromises lung development. The epidemiological implications of these findings are important particularly for increasing the knowledge about effects on infants residing in polluted environments. However, challenges still remain in inferring a cause-effect association with the outcome measures assessed as normative or reference values are lacking, particularly as the age groups vary. In the past lung function without sedation was a challenge hence most of the published data were spirometry measures on older children[60]. Furthermore, reference equations for African populations are still sparse[61].

The findings from our study should however be interpreted with caution, given the limitations. Key among these are the sample size and distribution of sample numbers across age groups. This is reflected in the wide confidence intervals, several including the null for various parameters. Despite this, the consistency of our findings when compared to other studies provides support for the validity of the estimates. In addition, the findings of statistically significant effects for certain parameters provide evidence of pollutant-related effects, though with variability observed in others. A further limitation to

assessing acute effects of air pollutant exposure on infant lung function outcomes was characterising exposure. The uncertainties about which is the most critical exposure period that will influence lung function in an infant is tremendous and likely to be a combination of time points[62]. In utero exposure, neonatal and early infancy may contribute a longer-term exposure effect, while current and short-term lags (past 24 hours, previous days or an average of the past few days) could result in acute effects at the time of the test.

Among the strengths of this study was the use of standardized methods and techniques for infant lung function testing as per the ERS/ATS guidelines[6,25]. Furthermore the repeated measures study design for the assessment of population-level variability in air pollution exposure-related lung function outcomes was an important advantage. We were also were able to achieve a 90% success rate on infant lung function testing without sedation, with consistent quality control during testing and analysis in adherence to published guidelines, further implementing blind analyses by two independent researchers for validation of test results.

The findings of this study are novel as there have been no prior studies to demonstrate time-varying effects of air pollutant exposure on change in lung function parameters of tidal breath and multiple breath washout measures in the infant age group. We were able to show acute lung function responses to short-term air pollutant exposure. Our study findings provide further support to existing literature on the causal role of exposure to air pollution on respiratory health. Though the observed effect estimates may be considered marginal, they may still have an important public health impact, particularly in low-middle income settings. In epidemiological studies exposure to ambient air pollutants have been shown to be independent indicators of all-cause mortality, although lung function markers alone are not the only criterion to determine lung function abnormalities, they nonetheless provide important insight on growth and development. Comprehending the time-varying effects of air pollutant exposure on lung function outcomes remains a complex task.

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MANUSCRIPT III – SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY INFORMATION

Effect of short-term exposure to ambient nitrogen dioxide and particulate matter on repeated lung function measures in early childhood: A South African birth cohort

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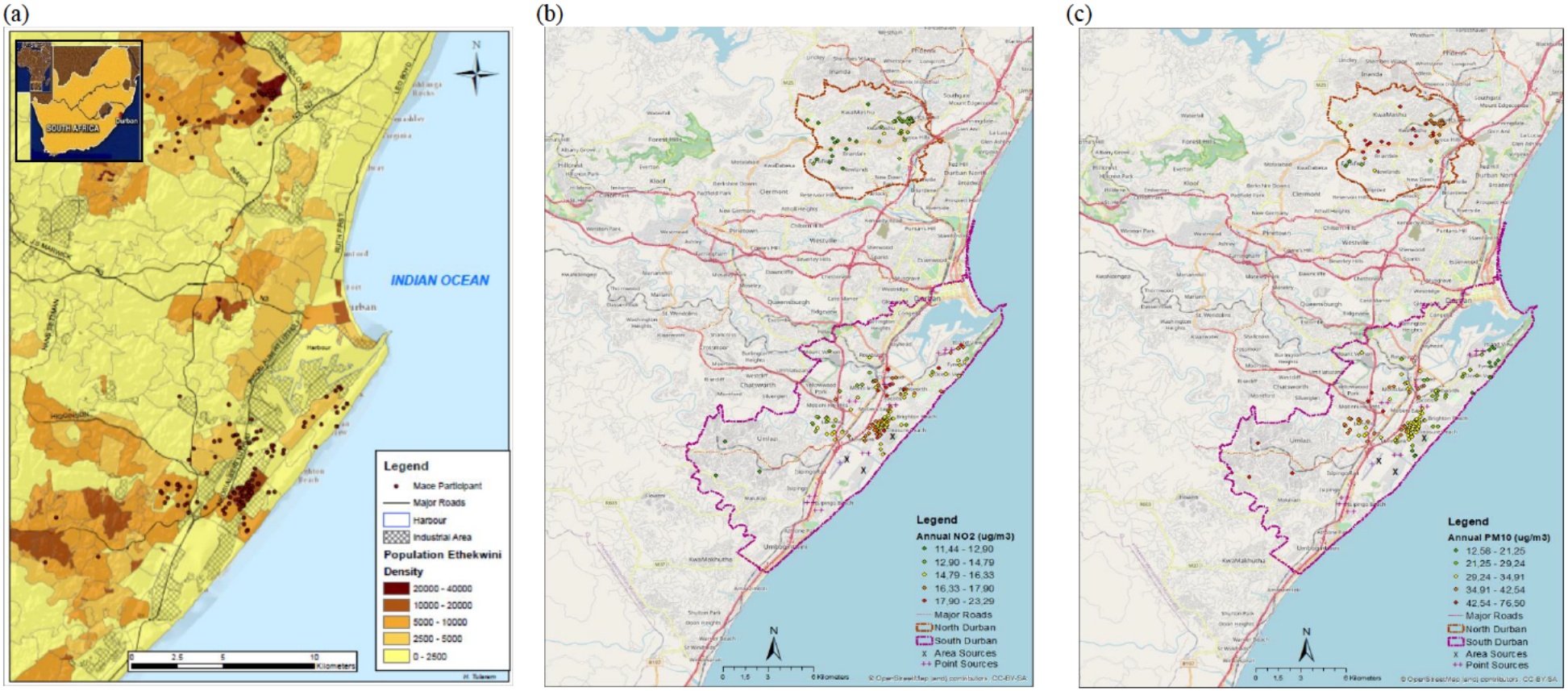

Figure S1: (a) Distribution of participant address points with urban and population characteristics; (b) Concentration mapping of annual NO_2 (ug/m^3) over the study domain; (c) Concentration mapping of annual PM_{10} (ug/m^3) over the study domain.

Table S1: Lung function outcome measures in study participants

	1,5 Months (N=70)	6 Months (N=73)	12 Months (N=66)	24 Months (N=73)
	Mean (SD); Range	**Mean (SD); Range**	**Mean (SD); Range**	**Mean (SD); Range**
Tidal Breathing				
Tidal volume, mL	33.69 (7.24); 16.00–56.00	69.54(11.97); 38 – 93	101.92 (14.68); 68 – 133.5	131.71 (18.35); 85 – 182
Respiratory rate, n/min	47.13 (12.64); 24.90–77.70	33.46(7.95); 21.6 – 74.1	28.88 (5.83); 21 – 60.5	24.39 (3.89); 18.5 – 36.35
Minute ventilation, mL/min	1517.62 (277.53); 796 - 2274	2237.1 (506.94); 3.04 – 3370	2679.14 (891.4); 2.43 – 4338	2989.8 (826.73); 2.92 – 4077
Mean tidal inspiratory flow mL/s	55.57 (11.82); 30.00–89.00	87.25 (17.85); 30 – 89	112.71 (17.24); 82 – 168	126.34 (19.83); 93 – 179
Mean tidal expiratory flow mL/s	47.73 (10.19); 22.00–78.00	68.08 (14.89); 36 – 119	85.06 (16.95); 50 – 155	91.6 (14.05); 60 – 124
t_{PTEF}/t_E %	43.08 (14.24); 11.29–89.85	30.8 (10.77); 12.29 – 68.57	30.06 (11.85); 14.82 – 76.01	28.29 (11.21); 7.41 – 64.17
Multiple Breath Washout				
Functional Residual Capacity, mL	84.2 (18.11); 50.33–128.95	146.33 (33.27); 72.53 – 229	201.8 (29.87); 139.15 – 283.33	248.97 (49.18); 152.17 – 435.93
Lung Clearance Index	6.91 (0.43); 6.03–8.63	7.11(0.6); 5.89 – 9.71	7.4 (0.56); 6.17 – 8.74	7.4 (0.64); 6.06 – 9.1

**Table adapted from unpublished work[1]*

[1] Muttoo S, Jeena P, Röösli M, et al. Low Birth Weight and Maternal Smoking as Predictors of Infant Lung Function from a South African Birth Cohort within Low-Socioeconomic Communities [Unpublished Manuscript]. University of KwaZulu-Natal; 2021.

Table S2: The effect of environmental factors on lung function outcomes

	Maternal Smoking (active smokers)[a]		HIV Exposure[b]		Housing Type (informal housing)[c]		Primary Heating Source (gas/paraffin use)[d]	
Nitrogen Dioxide (µg/m^3)	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**
Tidal volume, mL	3.7 (-4.5; 12)	0.375	-1.9 (-5.9; 2.2)	0.364	4 (-2.2; 10.1)	0.209	17.2 (3.6; 30.9)	0.014
Respiratory rate, n/min	2.8 (-2.1; 7.6)	0.262	0.3 (-2; 2.5)	0.807	-1.2 (-4.7; 2.4)	0.523	1.6 (-5.9; 9)	0.684
Minute ventilation, mL/min	**259.2 (33.9; 484.5)**	**0.024**	13 (-92.9; 118.8)	0.810	52.3 (-116.2; 220.9)	0.543	312.4 (-36.7; 661.4)	0.079
Mean tidal inspiratory flow mL/s	**13.3 (3.4; 23.2)**	**0.008**	0.3 (-4.5; 5)	0.907	1.9 (-5.6; 9.4)	0.613	**22 (6.2; 37.8)**	**0.006**
Mean tidal expiratory flow mL/s	4.7 (-3.4; 12.9)	0.256	1.3 (-2.5; 5.1)	0.499	1.1 (-4.9; 7.2)	0.718	3.8 (-8.7; 16.3)	0.554
tPTEF/tE %	**-7.3 (-14.6; 0)**	**0.050**	1.3 (-2.1; 4.8)	0.445	**-5.7 (-11.1; -0.3)**	**0.038**	-7.2 (-18.8; 4.5)	0.226
Functional residual capacity, mL	-1.9 (-23.4; 19.6)	0.861	-0.9 (-11.1; 9.3)	0.865	-4.8 (-20.8; 11.1)	0.552	-7.9 (-43; 27.2)	0.660
Lung clearance index	0.2 (-0.1; 0.5)	0.248	-0.1 (-0.2; 0.1)	0.375	0.1 (-0.2; 0.3)	0.491	**0.7 (0.2; 1.2)**	**0.005**
Particulate Matter (µg/m^3)								
Tidal volume, ml	2.4 (-5.7; 10.5)	0.558	-1.3 (-5.3; 2.6)	0.509	4 (-1.9; 9.9)	0.182	16 (2.7; 29.4)	0.019
Respiratory rate, n/min	3.1 (-1.6; 7.9)	0.198	0.1 (-2.2; 2.3)	0.960	-1.3 (-4.7; 2.2)	0.471	2 (-5.4; 9.3)	0.601
Minute ventilation, ml/min	**259.9 (38.9; 480.9)**	**0.021**	-0.5 (-105.3; 104.4)	0.993	55.7 (-106; 217.4)	0.500	308 (-36; 652)	0.079
Mean tidal inspiratory flow mL/s	**13.4 (3.7; 23)**	**0.007**	-0.3 (-5; 4.4)	0.893	2.6 (-4.6; 9.7)	0.480	**21.4 (6; 36.9)**	**0.007**
Mean tidal expiratory flow mL/s	4.8 (-3.3; 12.8)	0.248	1 (-2.8; 4.7)	0.620	0.9 (-4.9; 6.8)	0.750	3.8 (-8.5; 16.2)	0.543
tPTEF/tE %	**-7 (-14.2; 0.2)**	**0.056**	1.3 (-2.2; 4.7)	0.470	-4.9 (-10.1; 0.3)	0.063	-7.4 (-18.9; 4.1)	0.207
Functional residual capacity, mL	-3.1 (-24.7; 18.5)	0.776	0.5 (-9.9; 10.9)	0.919	-2.4 (-18.1; 13.4)	0.766	-9.5 (-45.1; 26)	0.600
Lung clearance index	0.2 (-0.1; 0.5)	0.256	-0.1 (-0.2; 0.1)	0.355	0 (-0.2; 0.2)	0.993	**0.7 (0.2; 1.2)**	**0.005**

[a]Model adjusted for test age, child sex, length/height at test date, low birth weight, **maternal smoking**, pollutant and interaction terms (pollutant*6week, pollutant*6 months, pollutant*12 months)

[b]Model adjusted for test age, child sex, length/height at test date, low birth weight, **HIV exposure**, pollutant and interaction terms (pollutant*6week, pollutant*6 months, pollutant*12 months)

[c]Model adjusted for test age, child sex, length/height at test date, low birth weight, **housing type**, pollutant and interaction terms (pollutant*6week, pollutant*6 months, pollutant*12 months)

[d]Model adjusted for test age, child sex, length/height at test date, low birth weight, **primary heating source**, pollutant and interaction terms (pollutant*6week, pollutant*6 months, pollutant*12 months)

CHAPTER 5

5.1. Discussion

This work investigated the effect of nitrogen dioxide and particulate matter exposure on infant lung function. Comprehensive measures of lung function were established and performed at six weeks, six, twelve and twenty-four months. We were able to undertake lung function testing by multiple breath washout and tidal breathing in the infant age group without sedation. We further present data until two years of age, of which published lung function measures among this age group is limited.

In our preliminary analysis (Manuscript I), antenatal and post-natal risk factors were found to be associated with lung function measures in infancy, some of which have been previously described and some that were novel. In general, anthropometric measures were linearly associated with measures of lung growth and development including tidal breathing indices and functional residual capacity. Notable findings were the emergence of low-birth weight and maternal smoking as predictors of early-life lung function. Our findings of low birth weight resulting in lower functional residual capacity as well as tidal volume, minute ventilation and tidal flows (MTEF and MTIF) at 6 weeks, 6 and 12 months aligns with previous reports that have shown that infant size at birth is an important predictor of lung growth and development as the child ages[1]. Effects of reduced functional residual capacity in low-birth-weight infants, who are subject to increased risk for structural changes and premorbid lung function, further supports this. This novel finding suggests that infants with low birth weight are likely to have compromised respiratory function during the first-year months of life; predisposing them to more severe consequences of acute respiratory infections. Maternal smoking did not emerge as a consistent predictor of low lung function in our sample unlike other studies[2-5], but this is likely due to the low prevalence of maternal smoking during pregnancy (4.9%) in our study, and a lack of variability across the different age groups and outcomes. However, maternal smoking was associated with lower tPTEF/tE, a measure of airway conductance[6], across age groups[7-9]. The significance of this finding is that it highlights the impact of in-utero exposures on the mechanisms of lung growth and development. We did find that effects among the 24-month age group were less substantial for the respective predictors, and this may likely be attributed to maturity in development by the age of two years. Studies investigating the association between birth weight and lung function tend to adjust for maternal smoking during pregnancy, however indications that birth weight maybe mediating the effects of maternal smoking on lung function outcomes and potentially underestimating this effect, is an important consideration. The use of path analysis in the statistical approach has been recommended to elucidate these complex relationships[10].

In subsequent analysis (Manuscript III) we investigated the relation between short-term exposure to air pollutants (NO_2 and PM_{10}) and acute response in lung function measures during these age-sensitive windows. Particulate matter and nitrogen dioxide are known markers for industrial and traffic emissions, and have been associated with acute respiratory effects and morbidity. Though in the larger MACE cohort $PM_{2.5}$ and SO_2 were additionally modelled, the models did not perform well with low predictive capabilities ($R^2<0.50$)[11] hence they were not included in our analyses. The predictive hybrid (land use regression and dispersion) models used in this study account for 60% (cross validation; $R^2=0.40$) and 85% (cross validation; $R^2=0.69$)) of the observed annual average concentrations in NO_2 and PM_{10} respectively[11]. To determine short-term exposure these models were temporally calibrated to obtain two-week average estimates preceding the lung function test date, which were used in our analyses. Air pollution exposure has also been shown to demonstrate a linear association with lung function and this has been affirmed by longitudinal analyses in large cohorts[12,13]. Our research findings show that short-term exposure to PM_{10} resulted in an increase in minute ventilation and respiratory rate from the age of 6 weeks to 12 months, while a reduction in functional residual capacity was observed for the same age groups, after adjustment for covariates including low birth weight and maternal smoking. Several pathophysiological pathways provide biological plausibility for this observation. Notably increased minute ventilation is reported to be associated with higher respiratory needs. Increased minute ventilation in infants, is required when there is an increased demand for oxygen, and this is achieved primarily through an increased respiratory rate while weight-adjusted tidal volume remains invariant. The rapid lung and somatic growth that occurs during the first year of life is also accompanied by major developmental changes in respiratory physiology, particularly with respect to the influence of the upper airways, the highly compliant chest wall and dynamic elevation of functional residual capacity[14,15]. In the infant, although lung recoil is similar to that in an adult, the chest wall is extremely compliant, resulting in minimal outward recoil with which is needed to keep the lungs and airways distended. This results in variability of functional residual capacity and a tendency for peripheral airway closure during tidal breathing. The latter not only impairs gas exchange and ventilation-perfusion balance, particularly in the dependent parts of the lung, but together with the small absolute size of the airways, renders the infant and young child particularly susceptible to airway obstruction and wheezing disorders[15]. Thus, our findings suggest early life sensitivity to short-term exposure to ambient particulate matter, particularly among measures of tidal breathing and functional residual capacity. The observed changes may also be important predictors of wheezing in early life attributed to factors that impede gas exchange and ventilatory characteristics of the lungs. We did not find any significant findings to report on with respect to short-term exposure to nitrogen dioxide and lung function measures. This may be attributed to the source characteristics of air pollutant emissions. Particulate matter accounts for a mixture of solid and liquid particles and is known to be a common proxy indicator of air pollution. It is also reported to affect more people than any other pollutant[16].

The lack of standardised normative or reference data for healthy infants makes it difficult to distinguish between health and abnormalities[17], thus we used previously published reports as inferences for comparison. We found comparison with the Drakenstein Child Lung Health Study (DCHLS)[2], to be particularly useful, attributed to similar population characteristics, however, this comparison was limited to certain age groups. We made further comparisons in the six-week age group to two European-based studies, the Bern Infant Lung Development (BILD)[18] study, and a European/ Australian multicentre study[19]. Similar to other studies, we report on known anthropometric variables to be associated with lung function (age, weight and height)[20], consistent with growth and development. However, we also report on novel risk factors including HIV status, household primary heating source and housing type that were associated with adverse lung function outcomes. These are also notable proxy markers of socioeconomic status (SES), which suggests an underlying effect of SES on lung function outcomes.

We acknowledge that this study had some limitations. The small sample size and distribution of the sample across the different age groups warrants cautionary interpretation of the results. We do however note that despite this limitation our results were comparable to prior studies of selected age groups, with the multiple linear regression models yielding moderate adjusted R^2 values comparable with previous reports. Even with larger sample numbers other studies still had weaker multivariable linear regression models, suggesting that there are unaccounted factors in explaining the observed variability in measures of tidal breathing and multiple breath washout, and this was more so the case for the 24-month age group in our study, which overall showed poor adjusted R^2 values (<1%). Our observations of less substantial findings among the 24-month age group, may be attributed to lung maturity in this age group. It is also likely that the complex interactions between several of the lung function parameters may not be adequately captured by linear modelling. Thus, there is a need to not only understand lung function in this age group but to also improve our understanding of the interaction between these parameters and their effects on lung growth and development.

We were also unable to show which age-adjusted time lag was the most sensitive to air pollutant exposure, and much like pregnancy studies, difficulties in distinguishing time-variant (e.g., trimesters of pregnancy) health responses persist. However, this may likely be addressed by improved accuracy in modelling. Furthermore, consideration of an adequate control population of non-exposed individuals may better demonstrate the effects of exposure on lung health. Though the modelling structure presented may be robust it could be that input data into this model were less robust. For example, dispersion modelling requires validation by continuous motoring from an air quality monitoring station, which in the Durban region has not been optimally operational. Furthermore missing data in source and emission factors may also be a contributing factor, however, this model was extensively validated as described elsewhere[11].

Among the strengths of this study was the use of an integrated hybrid modelling approach that was able to capture meteorological, topographical and geographical attributes of air pollutant dispersion, further incorporating detailed emissions factors and source characteristics. The refined nature of the modelling structure addresses such limitations as the inability to undertake personal monitoring as well as the lack of reliable continuous monitoring data. Exposure characterisation with time integration has been a significant challenge in epidemiological studies, as most report on the use of annual adjusted averages when assessing exposure-outcome relationships, but this may not always be in line with the study design and objectives. In this study, our novel approach of assessing two-week averages of exposure preceding the lung function test provides a snapshot of short-term exposure in accounting for acute lung function responses. However, the significance of temporality and its link between exposure and the onset of respiratory responses is still an area of uncertainty. A further challenge is the correlation of pollutant concentrations between timepoints, however this may be addressed by improving on the granularity and variability of the input data to mitigate the potential for correlation. In the modelling framework used in this study such challenges were limited to the government level datasets, of which such factors can not necessarily be controlled given that the data is lacking, however it can be compensated for by ensuring that the models are validated for their prediction accuracy. Questions remain on how variable exposure is over a short time frame and how long does it take to trigger an acute respiratory response, particularly given the sensitivity of infants. In utero exposure, neonatal and early infancy may contribute a longer-term exposure effect, while current and short-term lags (past 24 hours, previous days or an average of the past few days) could result in acute effects at the time of the test. Though it is acknowledged that several factors play a role in the dose-response effects, quantification of temporal variation in exposure may help better explain exposure-induced changes that occur at the physiological level of the lung.

In a critical review of several exposure assessment methods (Manuscript II) used in the evaluation of pregnancy and early childhood air pollutant related effects, hybrid models were highlighted to be a significant advancement in the field, with the strengths of individual approaches amplified when applied as integrated approach combining two or more methods. Children are notably more vulnerable to air pollution insults as their lungs are still developing at birth[21], with physiological and structural changes still occurring within the first five years of life after birth[22]. Thus their susceptibility to adverse respiratory health outcomes in early childhood[23] is influenced by factors that occur-in utero[24], perinatally[25] and in the early post-natal[26] developmental stages. Capturing exposure variation during these windows of susceptibility is critical to understanding the role air pollutants play on the maturation and development of the growing lung.

The strengths of using multiple breath washout testing in lung function assessment, include its non-invasive approach, the availability of commercial equipment and standardised techniques of testing and

analysis, was reassuring in the results obtained. Additionally, the use of a relatively heavy gas, with low diffusivity (SF_6) has the advantage of being less susceptible to leaks than other inert gases[27]. Potential disadvantages of the gas dilution/washout technique are the requirement of infant sleep state with non-rapid eye movement. Furthermore, the validation of equipment and techniques for infant's lung function testing may be difficult due to the limited duration of sleep epochs in certain cases, thus adherence to published guidelines is critical in achieving comparable results[27]. In addition, errors arising from miscalculated dead space, a difficulty imposed by the use of circuitry and software for gas/dilution or washout is the lack of quality control measures to ascertain the stability of breathing or to identify the phase of respiration[27]. We extensively addressed equipment calibration and standardisation in analyses, by strict adherence to published guidelines as well as the manufacturers' standard operating procedures.

5.2.Conclusion

This study was able to demonstrate an association of effect between ambient air pollutant exposure (nitrogen oxides and particulate matter) and measures of lung growth and maturation, after adjustment for maternal, infant and environmental risk factors. We demonstrated the feasibility and utility of multiple breath washout testing without sedation among infants from a low-socioeconomic setting. The exposure assessment methods developed and utilised in this study demonstrate the efficacy of modelling strategies in the absence of personal monitoring or continuously monitored data. Exposure to nitrogen dioxide and particulate matter is synonymous with urban development, but may be significantly exacerbated in areas with heavy industry and major road networks such as the south Durban region. The hybrid modelling framework within the MACE cohort was able to capture spatio-temporal contrasts in the observed measures. The findings of this work show that even in a small sub-sample an association of effect was observed, however, we further acknowledge that complex interpretation and interaction of the identified measures, merits further investigation in epidemiological studies, particularly in low-income settings which are subject to increased vulnerability from SES-related factors.

Summary of Study Findings:

- The findings of this study suggest early life sensitivity to short-term exposure to ambient particulate matter, particularly among measures of tidal breathing and functional residual capacity.
- Low birth weight was found to be an important predictor for tidal volume and minute ventilation, this was expected as lung size increases proportionally with body weight.

- Effects of pollutant exposure were noted for PM_{10} particularly for tidal breathing outcomes; however, these effects were less substantial for NO_2.

Future Research

- As lung function tracks into adulthood, longitudinal follow should be considered to improve our understanding of the determinants of long-term respiratory health in a low-socioeconomic setting.
- Multi-ethnic reference equations by the Global Lung Initiative were shown to have poor fit for African regional populations, thus highlighting an urgent need for standardised normative or reference data for African infants.
- Several proxy measures of indoor air quality emerged as risk factors of adverse lung function, as this is the immediate environment in which infants spend the majority of their time, assessment of indoor air quality and indoor/outdoor infiltration ratios maybe of significance in exposure assessment.
- The above further has increased relevance for those living in informal housing and using gas/paraffin as their primary heating source, which directly influences exposure. Clean fuel alternatives for heating should be encouraged among these communities.
- Microenvironmental exposures are considered important with respect to the above, and maybe particularly relevant among low-income communities who are likely to use public transport and work in uncontrolled environments, thus exposure assessment methods that are able to incorporate such mobility patterns, through time activity diaries or daily logs, may better account for the spatial variability in observed estimates.

5.3.Recommendations

- Maternal smoking was shown to be associated with lung function reductions as observed for several measurement parameters. Cigarette smoke is known to contain harmful chemicals with carcinogenic properties and should be eliminated as a potential risk factor through health intervention at primary healthcare clinics and assessment of maternal lifestyle and behavioural characteristics during clinical visits.
- Evidence of a high incidence of low birth weight in this population warrants early intervention in maternal health care during pregnancy. Equitable and accessible prenatal health care services

are critical to reducing the burden of low-birth-weight incidence, in addition the provision of prenatal vitamins, nutrition and weight monitoring of the pregnant mother, as well as physician advice on prenatal care at home may be of further benefit.

- Informal housing was further identified as a risk factor for poor lung health. Though we report on low-income communities, with a low prevalence of informal housing, it is likely that indirect factors such as overcrowding and indoor heating practices (biomass fuel combustion) may be a contributing factor. The observed effects are thus thought to be a proxy for indoor air quality, resulting from a lack of ventilation, moulding or exposure to makeshift building materials.
- Primary heating source further emerged as a risk factor for adverse lung health in our study, though only a few participants reported on the use of gas/paraffin, this is similarly observed to be a proxy for indoor air quality. The potential use of gas/paraffin is further exacerbated by intermittent interruptions in the supply of electricity by the national service provider, which makes control and intervention more complex. However, as this may be an inherent challenge among low-income communities, it should be encouraged to do so within well-ventilated areas or outdoors, if possible, to avoid immediate exposure and subsequent respiratory health effects. Community-level engagement on safe heating practices in well-ventilated spaces as well as the use of clean fuel alternatives should be encouraged.
- To address limitations in obtaining accurate air quality data from routine monitoring networks, incorporating computational and algorithmic modelling such as those described in our narrative review, may greatly improve monitoring networks. For example, regional dispersion modelling and satellite-based air pollutant emissions data may be used synergistically with measured data to provide air pollutant estimates near real-time data through web-based online applications with fine-scale estimation at varying spatial resolutions.
- The lack of reference equations for these age groups among this population makes it difficult to distinguish between health and abnormalities. This challenge was further highlighted by the Global Lung Initiative, with multi-ethnic reference equations having shown poot fit for African regional populations[28], thus exemplifying an urgent need for standardised normative or reference data for African infants.

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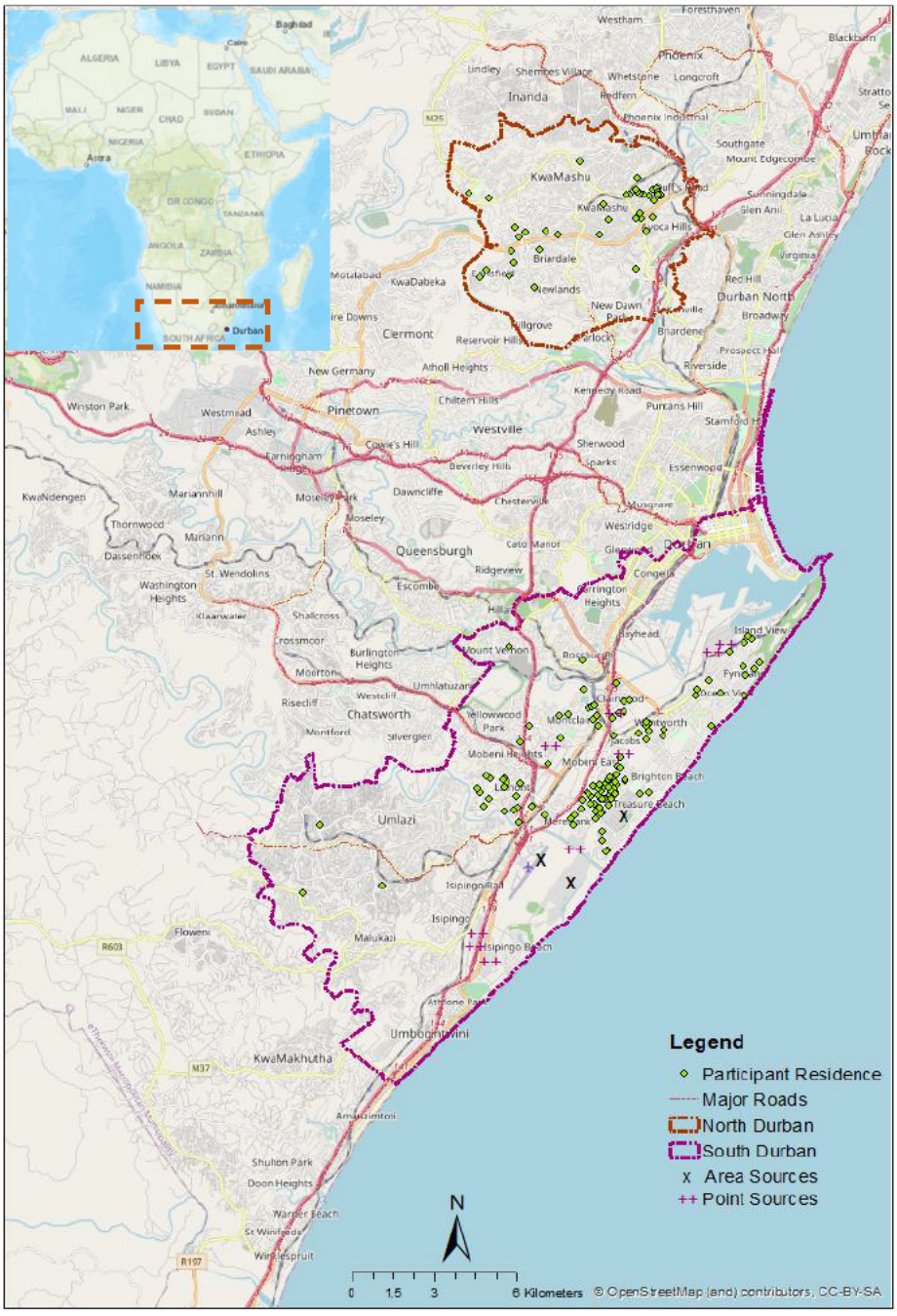
APPENDIX A: Study Area



APPENDIX B: Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

15 January 2018

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PROTOCOL: The impact of Nitrogen Oxides & Particulate Matter Exposure on Infant Lung Function Development
Degree: PhD
BREC Ref No: BE431/17

EXPEDITED APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 07 July 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 15 December 2017 to BREC correspondence dated 14 September 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 15 January 2018.

This approval is valid for one year from **15 January 2018**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on 13 February 2018.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

Prof Joyce Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: naidoon@ukzn.ac.za
cc postgraduate administrator: nyelam@ukzn.ac.za

UNIVERSITY OF
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30 July 2019

Ms S Muttoo
Discipline of Occupational and Environmental Health
School of Nursing and Public Health
College of Health Sciences
sheena.muttoo@gmail.com

Dear Ms Muttoo

PROTOCOL: The Impact of Nitrogen Oxides & Particulate Matter Exposure on Infant Lung Function Development
Degree: PhD
BREC Ref No: BE431/17

We wish to advise you that your response dated 14 March 2019 to BREC letter dated 13 March 2019 has been noted by a subcommittee of the Biomedical Research Ethics Committee.
Your application for amendments received on 22 January 2019 to include the 2 year age into the above study has now been approved by a subcommittee of the Biomedical Research Ethics Committee.

The committee will be advised of the above at its next meeting to be held on 10 September 2019.

Yours sincerely

[redacted]

Prof V Ramdhith
Chair: Biomedical Research Ethics Committee

APPENDIX C: Participant Consent

The Mother and child in the Environment (MACE cohort)
Consent Form

1. Date:____/____/____ Day Month Year	2. Mother Identification No. ___ - ____ - ____
Gestational Week _____	

1. **Title of research project**
Mother and Child in the Environment

2. **Purpose of the research**
The University of KwaZulu-Natal, Department of Occupational and Environmental Health is conducting this study of the health effects of air pollution in the eThekwini municipality. In this study we want to learn whether pollution has an effect on the unborn child, and whether these effects are likely to create health problems for the child has he or she grows. The study has the support of the local industry, community groups concerned about these sorts of health problems, and the City Health Department. We are studying this community because of its location near sources of air pollutants such as oil refineries and because of health concerns expressed by teachers and students at schools and by the larger community. The purpose of the study is to figure out whether any health problems are being caused by air pollution in the community, its effect on pregnant mothers and the unborn child, and, if so, to make recommendations to improve the situation.

3. **Description of the research project**
If you agree to participate, you will be interviewed during your pregnancy, and we will ask you to provide us with blood samples while you are pregnant. We will also ask you to allow us to take a sample of blood from the birth cord after the delivery of your child, and for permission to take a spot of blood from your newborn baby and follow-up the baby until 5 years old.

Interviews: You will be interviewed by a trained interviewer from our research team. This interview will be done at the clinic, in the complete privacy of one of the consulting rooms at the clinic. You can choose the language in which you will prefer to be interviewed. During the course of this interview you will be asked many questions about your pregnancy, diet, work exposures etc. You may refuse to answer any questions that you feel uncomfortable about answering. If during the course of the interview, you wish to stop and continue at a later stage, you may do so.

Blood tests: Trained technicians will take samples of blood from you. This blood will be analysed for your body's reaction to external environmental factors (referred to as "oxidative stress"), assessing your allergy status and potential, vitamin analysis and genetic analysis will be done on the blood prenatally. 4 Tablespoons of blood will be taken at recruitment. A home visit to assess indoor air quality will be done twice during the study period. You will be informed prior to these visits. Similarly, a sample of blood will be taken from the umbilical cord, once this is removed after the birth of your child. These tests will then be repeated. Only these specified tests will be conducted on this blood sample. We will store a sample of your DNA so that we can assess for new genes including but limited the current spectrum of genes might make your child more likely to develop lung conditions such as asthma. The total amount of blood taken will not exceed more than four tablespoons. YOU WILL NOT BE INJECTED WITH ANY SUBSTANCE/MEDICATION.

During the 5 year follow-up period we will assess your child at different time points where **lung functions tests** will be performed and blood samples will be taken. However you will be informed in advance when this will occur.

4. **Confidentiality of information collected**
The interview and blood test information we collect about you is completely confidential and will never be seen by anyone other than the personnel conducting the study without your written consent.

The results of the overall study, which will be made available to the local government and the community, will presented so as to protect the identity of individual participants.

5. **Risks and discomforts of the research**
There are no risks from the interviews and the blood tests.

6. **Expected benefits to you and to others**
You will be given a written copy of all your test results along with an explanation of what they mean. You may wish to show these to your doctor if you are having any problems. If problems are found in the community, we may be able to make specific recommendations about air pollution to improve the situation. What we learn from this study may help to protect people in South Africa and other parts of the world from problems caused by air pollution.

7. **Costs to you resulting from participation in the study**
The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

8. **Voluntary nature of participation**
You are free to decline to participate or to withdraw from the study at any time without suffering any penalty or disadvantage.

9. **Contact person**.
You may contact **DR. RAJEN NAIDOO** (telephone no: **031 260 4385**) for answers to further questions about the research. For reporting of complaints/problems, you may contact the Biomedical Research Ethics Committee, whose details are shown below:

Contact details of BREC Administrator or Chair –

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

University of KwaZulu-Natal

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001, Durban, 4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2604769 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

10. **Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information, Dr./Mr./Ms. _____

has offered to answer any questions concerning the study. By signing this form, I hereby consent to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

11. **Documentation of the consent**

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

_____	_____
Printed name of participant	Signature, Mark, or Thumb Print
_____	_____
Interviewer's name (Print)	Signature

DATE: _____

APPENDIX D: Participant Information Sheet

Date

Dear Participant

Re: **Name** DOB: **X**

Thank you for your consent and your ongoing participation in the MACE infant lung function component of the cohort study. Your valued participation is important and the results from these lung tests will enable us to further understand how air pollution impacts lung function in early childhood.

Name attended the King Edward VIII Hospital on **Date** as part of this study for their scheduled **X** week/year lung function test.

On the day of the test, **Name** had a length of **X** cm and a weight of **X** kg.

The measurements of infant lung function performed successfully on the day included tidal breathing and multiple breath wash out. The results from recordings are briefly discussed below:

1. Tidal Breathing

This test is used to determine the average amount of air breathed in and out of the lungs with each breath. **Name** had an average tidal volume of **X** ml and a respiratory rate of **X** breaths/min. For a **X** week/year old infant the normal range for tidal volume is **A** to **B** mLs and for respiratory rate is **C-D** breaths/minute. Your child's tidal breathing results are normal.

2. Multiple Breath Washout

The multiple breath washout test involves breathing a special gas mixture to measure your child's lung volume. We attempted to obtain 3 good recordings and successfully managed to get **X** good readings.

The normal range for lung volume in a **X** week/year infant is **E-F** mL/kg. **Name's** lung volume results are **X** ml and were within normal limits. The Lung Clearance Index normal values are **G-H**. Your child has a Lung Clearance Index of **X** and is normal.

Should you have any questions regarding the lung function tests or any other aspect of the study, please feel free to contact the MACE Study office on 031 260 4523.

Once again, thank you for your participation in the MACE Cohort Study.

Kind Regards,

The MACE Team:
Mrs Kareshma Asharam (Project Manager)
Prof Prakash Jeena (Peadiatric Pulmonologist)
Prof Rajen Naidoo (Medical Specialist)

APPENDIX E: Infant Lung Function Testing

Participants aged 6 weeks, 6, 12 and 24 months were tested by multiple breath washout and tidal breath assessment. We conducted testing in non-sedated infants, by complying with published guidelines and recommendations, below is a short summary of the conditions of testing adhered to:

i. Sleep state – participants were required to be in a natural quiet sleep state with non-rapid eye movement[1].
ii. Posture during testing – testing was conducted on infants in a supine position, as this influences the position of the diaphragm, lung mechanics and distribution of ventilation[2].
iii. Duration of testing – total testing time included explanation of the procedure to parents, clinical examination (where required), equipment preparation / disinfection at commencement and completion of test, as well as interval until the onset of sleep, amounting to 3 – 4 hours inclusive of preparatory measures and 30-60 minutes of actual testing[2].
iv. Participant safety – testing was conducted by trained competent testing technicians in a clinical setting with physicians available and appropriate resuscitation equipment, in the event of an emergency[2].
v. Anthropometric measures – for accuracy of measurements a stadiometer was used for measurement of length, which is critical during infancy due to rapid growth[2].
vi. Equipment requirements – standardised measurement and analysis software were used following guidelines such as those published by the European Respiratory Society (ERS)/ American Thoracic Society (ATS)[3].
vii. Minimizing leaks and dead space – the use of transparent masks instead of the traditional mouthpiece, may introduce physiological problems, due to relatively large apparatus dead space, and technical problems, as it is difficult to estimate the effect of dead space on the outcome measures, additionally leaks may occur around the mask which affects tests quality and subsequent outcome measures, thus some studies have reported the use of therapeutic putty to facilitate and airtight seal between the face and the mask[2].

Testing Procedure:

After several breaths of room air, the wash-in phase begins, the patient then breaths the tracer gas mixture (sulphur hexafluoride) until equilibrium is reached with a plateau, with washout beginning at the end of the plateau and breathing of room air commences. Multiple breath washout measurements are commonly performed as three consecutive tests, taking approximately thirty minutes to perform, depending on lung volume and presence or severity of disease[4].

The calculation of parameters derived from multiple breath washout is based on breath by breath analysis of gas concentrations and volumes. Functional residual capacity is calculated from the cumulative volume of expired gas divided by the difference between end-tidal gas concentration at the start and end of the washout. The cumulative expired volume is the cumulative volume of expired air during the washout phase that is corrected for apparatus dead space[5].

References:

1. Prechtl HF. The behavioural states of the newborn infant (a review). Brain Res 1974;76:185-212.
2. Stocks J. Pulmonary function testing in infants. In: Laurent GJ, Shapiro SD, eds. Encyclopedia of Respiratory Medicine. Oxford: Academic Press; 2006:564-77.

3. Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. European Respiratory Journal 2013;41:507-22.
4. Fuchs SI, Gappa M. Lung clearance index: clinical and research applications in children. Paediatric Respiratory Reviews 2011;12:264-70.
5. Horsley A. Lung clearance index in the assessment of airways disease. Respiratory Medicine 2009;103:793-9.

APPENDIX F: MACE Study Background

Study Title: The Adverse Effects of Ambient and Indoor Pollution on Childhood Respiratory Development

Study Description: The Mother and Child in The Environment (MACE) Cohort Study

Study Aim:

To determine the effect of ambient and environmental pollution exposure on childhood respiratory development, and the modification of effect by birth factors, genetic predisposition, epigenetic changes and oxidative stress, and to test the effectiveness of dietary intervention in reducing the risk for adverse respiratory outcomes.

Study Objectives:

To characterise environmental exposure patterns among a cohort of pregnant women and subsequently, their offspring among communities with documented high levels of industrial exposure and a comparison, communities without such exposures;

To describe perinatal risk factors including exposure to ambient and indoor pollutants, maternal smoking, or maternal exposure to passive smoking, allergen exposure (ingestion of peanuts, egg protein etc) and atopy status, diet generally and specifically dietary intake of antioxidants, HIV and anti-retroviral treatment status and other maternal stressors likely to impact on the foetus;

To describe neonatal, infant and early childhood risk factors, such as adverse birth outcomes (low birth weight, intra-uterine growth retardation and preterm deliveries), dietary factors (breastfeeding or formula feeding; intake of antioxidants etc), exposure to allergens (aerosol or dietary) and atopy status, exposure to environmental pollutants, ambient and indoor;

To assess genetic status of these children with respect to a set of polymorphisms in specific genes associated with response to oxidative stress and respiratory biomarkers, cellular changes from environmental pollutants, and to response to therapeutic interventions (dietary supplementation with vitamin C and vitamin E.) These genes include GSTM1, GSTP1, and TNF.

To investigate the genome wide DNA methylation status of the neonate using umbilical cord blood;

To investigate the relationship between genome wide DNA methylation status and prenatal environmental exposures;

To investigate the relationship between genome wide DNA methylation status and respiratory outcomes in children.

Cohort Background

In the initial cohort 1099 pregnant women were recruited. Over the period April 2013 through to March 2018. The cohort size at each trimester of pregnancy were 987 (1st trimester), 932 (2nd trimester), 869 (3rd trimester), and 760 (at delivery). Relocation of participants outside the study area and choosing to use clinics closer to their new homes was the single largest reason for the loss to follow-up (n = 257). Participants (n = 82) who experienced miscarriages and stillbirths or terminating their pregnancy were subsequently removed from the cohort.

Selection of communities, study population and study sample

Pregnant women attending the public sector ante-natal clinics in Durban and surrounding areas (Lamontville, Merebank, Bluff, Austerville, Wentworth, KwaMashu, Newlands East and Newlands West), and who met the inclusion criteria were invited into the cohort. The objective of the MACE study was to determine the risk of environmental pollutant exposure commencing in utero on long term respiratory health of children to 6 years of age and specific outcomes such as asthma.

The selection criteria for subjects to enter this study were as follows:

a. The recruited participants had to be residing in the geographical area within which the clinic is located, and had to live in this area for the full duration of the pregnancy and follow-up period of up to 5–6 years. The children had to be resident in the communities for the duration of follow-up (to monitor the health of the child from birth up to 6 years of age, and to keep track of their health by gathering information from their Road to Health charts).
b. Pregnant females had to preferably be less than 20 weeks of gestational age on entry although those presenting before the onset of the 3rd trimester were not excluded.

Context of the current research within the larger study:

This research aimed to establish how the identified risk factors, during pregnancy and early childhood, influence the growth and maturation of the infant's lungs. Particular focus is on exposure to ambient air pollutants nitrogen dioxide and particulate matter and acute effects on tidal volume and multiple breath washout measures. This study addresses the growing need to understand the early life determinants that impede respiratory growth and development, particularly as there is increasing evidence showing that diminished lung function early on has implications for long term respiratory health, with structural and physiological changes occurring at the onset of exposure. Moreover, low-income communities have been shown to be at an increased risk, particularly among HIV exposed infants and those exposed to maternal smoking, as demonstrated in recent research.