Longitudinal Measures of Lung Function in Healthy Infants

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Department of Physiology
University of Toronto

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Abstract

Effective treatment of paediatric pulmonary disease relies on early diagnostics, and thus requires pulmonary function testing. Before infant pulmonary function testing can be applied to detect disease, it must first be understood in health, especially in the context of the developing lung. Adult studies have suggested that the rapid expansion of alveoli leads to faster growth in the lung parenchyma with respect to the conducting airways. By longitudinally evaluating lung function parameters that approximate the size of the parenchyma and the airways in infants, it can be determined whether dysanapsis is present in this young age group. However, while we were unable to find functional dysanapsis in tidal breathing measures, it was found when comparing forced flow and volumetric measures. This indicates that while the airways are proportionally larger in youngest infants, rapid parenchymal expansion leads a decrease in the relative size of these airways compared to overall lung volume.
Acknowledgments

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<th>Description</th>
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<td>Pulmonary Function Test</td>
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<tr>
<td>IPFT</td>
<td>Infant Pulmonary Function Test</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>VT</td>
<td>Tidal Volume</td>
</tr>
<tr>
<td>RR</td>
<td>Respiratory Rate</td>
</tr>
<tr>
<td>VE</td>
<td>Minute Ventilation</td>
</tr>
<tr>
<td>MBW</td>
<td>Multiple Breath Washout</td>
</tr>
<tr>
<td>CEV</td>
<td>Cumulative Expired Volume</td>
</tr>
<tr>
<td>PNT</td>
<td>Pneumotachometer</td>
</tr>
<tr>
<td>RVRTC</td>
<td>Raised Volume Rapid Thoracoabdominal Compression</td>
</tr>
<tr>
<td>FRC&lt;sub&gt;MBW&lt;/sub&gt;</td>
<td>Functional Residual Capacity by Multiple Breath Washout</td>
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<tr>
<td>FRC&lt;sub&gt;PL&lt;/sub&gt;</td>
<td>Functional Residual Capacity by Body Plethysmography</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt;</td>
<td>Forced Expired Volume in 0.5 seconds</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
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Chapter 1
Introduction

1 Introduction

1.1 Relevance of Lung Function Testing

1.1.1 Utility of lung function testing in disease

Treatment of pulmonary disease requires insight into how the disease is manifesting in the body, and how it may be changing over time. Many techniques exist for evaluation of pulmonary diseases, including bronchoscopy, chest X-Ray, and computed tomography (CT) imaging. However, many of these are restricted in terms of the information they provide; pathology is cell-based and therefore cannot give a macroscopic image of the tissue, and imaging can be signal-restricted, offering only structural information. The true impact of disease as felt by the patient is not their tissue’s pathological state, but to what extent the organ is able to continue to perform its necessary function. Additionally, while anatomy can reflect functional ability, the influence of anatomical changes on pulmonary function is not always linear. Small changes in the tissue can result in large functional changes, and vice-versa. Furthermore, these tests only offer a static image of the lung, which due to its dynamic nature may perform differently at different times of its performance cycle. Therefore, dynamic tests that capture the spectrum of lung health and function are ideal for evaluating lung health, as they offer a more complete image of lung function.

In obstructive lung disease, improved patient outcomes are linked to earlier treatment. (1) Necessary to earlier treatment, is earlier diagnosis. However, this is reliant on better tools to track and identify deficiencies in lung health, as well as lung function. It would then be ideal to bring pulmonary function as early as possible into a patient’s care strategy, hence the particular interest in paediatric populations.
1.1.2 Lung function testing in paediatric populations

There is a large body of literature surrounding pulmonary function; however, the focus is on school-aged children, due to the effort-dependent nature of the tests. While this is useful for older populations, there still exists a need to bring pulmonary function testing to infants. It has been shown that even in infancy, obstructive lung diseases such as cystic fibrosis are already manifested through structural abnormalities. (2) However, structural changes do not necessarily reflect changes in function, and thus measuring the function itself is necessary in order to understand the effect of disease on the body.

In order to properly use lung function testing to monitor the subtle early changes associated with disease in infants, one must first have a critical, in depth understanding of normal, healthy lung function growth in infants. (3) While this understanding is important in any physiological system, it is especially important when tracking pulmonary health, as unlike most body systems, the respiratory system’s development is incomplete at birth, and continues to not only grow, but develop well into childhood. (4) Understanding pulmonary development is necessary to understand lung function in early life, as well as understanding how these changes are reflected in pulmonary function testing.

1.2 Lung Anatomy and Physiology

1.2.1 Layout of Respiratory Tree

The respiratory system, like the enteric system, is open to the exterior environment. The main function of the respiratory system is to bring oxygen to alveolar units, which can exchange this gas for a waste product produced by the body during cellular respiration. While not technically part of the bronchial tree, air from the environment first passes through the oropharynx, and into the first segment of the tree, the trachea. The trachea is the first of the conducting airways, and then begins the tracheobronchial tree with the first division into two bronchi. Including this first division, the tracheobronchial tree continues to branch and divide, until the terminal bronchioles lead to the acini. The tree divides in a dichotomous fashion, meaning that at the end of each branch, the original branch bifurcates into two daughter branches. Thus, assuming the trachea represents the zeroth generation, the number of branches at any given generation of airway can be described as $2^n$, where $n$ is the generation number. (5) Generally, each newer generation
results in two airway branches which are of smaller diameter than the parent branch, though the
two resulting daughter branches are not necessarily symmetrical in diameter. Indeed, it has been
shown that in later generations, the bifurcation is explicitly asymmetrical, with one daughter
branch being larger than the other. (6) While this is counterintuitive, it appears that an
asymmetric branching mechanism is most efficient for our respiratory system; some even
suggest that a symmetric tree would be dangerous, as it causes small geometrical changes to
result in large variations in airflow. (7)

1.2.2 The Conducting Airways

The conducting airways are made up of the airways from the trachea, to the terminal bronchioles.
As the name suggests, their main purpose is to transport air to the alveoli, and waste CO₂ from
the alveoli to the environment. As they make up the early generations of the airway, they are
significantly larger than anything found in the periphery, which will participate in gas exchange.
As these airways do not participate in gas exchange, these airways contribute to the dead space.
Thus, in order to ventilate the gas-exchanging units with fresh air, the dead space must first be
completely ventilated through, before any new gas can reach the alveolus. The relative
proportion of the volume of these airways compared to the tidal volume (VT) can affect tidal
breathing patterns, and as such the efficiency of breathing. (8) An in-depth discussion of dead
space, and how it may affect lung function measures is presented later on in Section 1.4.5.1.

The airways are wrapped with smooth muscle, which serve a structural purpose of keeping the
airways from collapsing, but can also constrict in conditions such as asthma. (9) The trachea and
bronchi are characterized by the presence of horseshoe-shaped hyaline cartilage rings to help
maintain the patency of these essential airways. (10) These cartilage rings disappear at the level
of the bronchioles, which compared to the earlier airways are much smaller in size, more pliable,
and as a result, prone to collapse under external pressure. During forced expirations, the pressure
exerted by the chest wall on these airways is enough to decrease the diameter of the airway. This
flow limitation is a limiting factor for the maximum amount of air that can be expired for a given
amount of time. This is the basis for spirometry, which is discussed further in Section 1.4.4.
1.2.3 The Peripheral Airways

The peripheral airways are made up of the smaller, gas-exchanging portions of the bronchial tree. They are sometimes termed the ‘distal airways’, as they are made up of the later generations of the tracheobronchial tree. The peripheral airways are much thinner than their conducting counterparts, out of necessity to facilitate the diffusion of gases between the air and the blood, through the cell epithelium. Furthermore, while these airways are significantly narrower on an individual level as the generation number increases, the total cross-sectional area of the airways is significantly larger as a result of the exponential increase in quantity of branches. (11) Therefore, these peripheral airways do not generally contribute significantly to the overall resistance of the respiratory system. (12, 13) However, some have suggested that this only becomes true during the preschool years, with a sharp rise in conductance occurring at around 5 years of age. (14)

Due to their function as gas exchanging units, the peripheral airways are mostly made up of epithelial cells. Therefore, the walls of these airways are generally thinner. The ciliated and secretory cells have disappeared, as has the large amount of smooth muscle and cartilaginous supports. This then causes the airways to be more pliable, and also susceptible to closure from external (thoracic) forces.

1.2.4 Physical Properties of Large and Small Airways

1.2.4.1 Fluid Dynamics

Due to the significantly different structure and size of the large conducting and small peripheral airways, fluids (including gases) behave differently in these two spaces. (11) The net flow across a generation of airways must remain the same. Thus the linear flow in a given airway is equal to the mass flow divided by the total cross sectional area of the airway at the given generation. As mentioned in Section 1.2.3, the total cross sectional area of the airways increases exponentially with increasing airway generation, despite smaller individual airway size. This increased cross sectional area thus results in very low flows in the peripheral airways. (15) However, due to the smaller cross sectional area of the airway lumen in these generations, air moving through the periphery exhibits laminar flow. In contrast, the air moving through the larger conducting airways moves much faster, and so exhibits a more turbulent flow.
1.2.4.2 Airway Patency and Surfactant

Alveoli represent the most peripheral airway structure, and are lined with fluid. This film of fluid creates a surface tension within the alveolus, due to the strong attractive intermolecular forces. This surface tension makes the alveolus prone to collapse. (16) The amount of pressure required to keep the alveolus open against this surface tension is described by the Laplace Equation (Equation 1). The pressure required to keep the alveolus open \( P \) is equivalent to 2 times the surface tension of the film \( \gamma \), divided by the radius of the space \( r \). This demonstrates that smaller airways and airspaces require a proportionally larger amount of pressure to maintain their patency. To alleviate this, the type II pneumocytes in the smallest airways produce surfactant, which acts as an emulsifier/detergent, creating small air bubbles in the fluid, which in turn reduce the tension \( \gamma \).

Equation 1. \( P = \frac{2\gamma}{r} \)

1.3 Pulmonary Development

1.3.1 Lung Structures and Functions

Structurally, the lungs can be divided into two major components: the parenchyma and the conducting airways. The parenchyma is composed of the smaller, peripheral airways (i.e. respiratory bronchioles and alveolar ducts), and alveoli, whereas the conducting airways are composed of the trachea, bronchi, and bronchioles. (5) As mentioned previously, the lungs are fairly unique among organs, as they are not fully developed at birth. (17) However, not all parts of the lung develop at the same time. At birth, the large, conducting airways are completely developed, and only need to grow in size. (18) In contrast, the alveoli and acinar units are only partially developed. In fact, these structures continue to develop morphologically, and physiologically postnatally.

1.3.2 Alveolar and Airway Development

The development of the mammalian lung proceeds through 5 stages: embryonic pseudoglandular, canalicular saccular, and alveolar. Like other organ systems, these stages all begin in utero. However, while the alveolar stage begins in utero, it terminates postnatally. The ranges of estimates for the timeframe of each of these stages is detailed below in Table 1. (19)
Table 1. Location and timeframe of stages of pulmonary development.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Region</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>Global</td>
<td>4-7 Weeks Gestation</td>
</tr>
<tr>
<td>Pseudoglandular</td>
<td>Global</td>
<td>7-17 Weeks Gestation</td>
</tr>
<tr>
<td>Canaliclar</td>
<td>Acinar</td>
<td>16-25 Weeks Gestation</td>
</tr>
<tr>
<td>Saccular</td>
<td>Acinar</td>
<td>24-38 Weeks Gestation</td>
</tr>
<tr>
<td>Alveolar</td>
<td>Acinar</td>
<td>38 Weeks Gestation → ???</td>
</tr>
</tbody>
</table>

The end of the pseudoglandular stage marks the end of the development of the conducting airways. While it will continue to grow and scale with the fetus/child, it will no longer differentiate. In contrast to this, the remaining stages of lung development – the canaliclar, saccular and alveolar stages – now serve to help establish the parenchyma/periphery of the lungs as functional units for gas exchange. As the canaliclar stage ends and the saccular stage begins, respiratory function would technically be possible. The ends of the airways made up of pneumocytes continue to form, creating ‘saccules’. The connective tissue between the saccules continues to thin, in order to facilitate easier gas exchange. The type II pneumocytes finally begin to secrete surfactant, which propagates throughout the respiratory system, to help in the expansion of the airspaces post-parturition.

The final stage of pulmonary development, the alveolar stage, begins just before birth and continues into childhood. Here, the previously formed alveolar ducts begin to divide in a process termed ‘septation’, in order to create more alveoli. Another process, ‘alveolarization’, also creates new alveoli, by the branching apart of existing septa into new alveoli. During this time, the double capillary network, which up to this point was perfusing the lung, switches to a single capillary at each alveolus in order to aid efficiency of the lung. While it is well known that the alveolar stage of lung development begins a few weeks before birth, the exact end point of alveolarization is under debate. Many agree that humans are only born with a small fraction of
their alveoli present at birth, with approximately 85% of the eventual alveoli being developed postnatally. However, estimates for the range of termination of alveolarization span from 2-4 years after birth, with more recent studies suggesting that it continues into the early teenage years. (17, 20-22)

1.3.3 Dysanaptic Growth of Airways and Alveoli

Though the airways and alveoli both grow during infancy and childhood, it is clear that they do not grow in the same fashion. The conducting airways are simply growing in size while the parenchyma of the lung continues both structural and functional development. Thus, it could be reasoned that the two lung structures grow independently and potentially at different rates. This phenomenon is termed ‘dysanapsis’. This was first suggested by Green et al. in 1974, but further examined in 1980 by Jere Mead. (23, 24) Mead showed that by comparing outcome measures sensitive to lung size and outcome measures sensitive to airway size, and observing their relative changes to one another, one can infer whether the airways and parenchyma of the lung are linked. The previous assumption was that larger lungs would necessitate larger airways. Mead demonstrated that, in fact, the ratio of airway measure relative to lung measure had a negative, non-linear relationship with lung volume measurement – thus those with higher lung volumes, had longer, but narrower airways. This suggests that there is dysanaptic growth between the airways and the parenchyma of the lung, as the airways were sometimes proportionally larger than the parenchyma in individuals with lower lung volumes. (24) However, this is only based on data obtained in adult subjects, after the growth of parenchyma and airways has already completed.

In infancy, anatomical studies suggest that the conducting airways in infants are actually disproportionally large for their lung size. (25) This is not surprising, as the conducting airways are fully developed at birth, whereas the parenchyma is not. (17) However, the parenchyma will continue to rapidly grow and develop, and has been shown to increase significantly in volume in the first year of life, due to alveolar multiplication. (17) Studies have reported lung function measures such as maximum flow (Vmax), and Vmax/FRC (functional residual capacity) throughout infancy, but there has yet to be a study to report multiple functional measures throughout infancy in a single study population. (26, 27)
1.4 Pulmonary Function Testing

1.4.1 Rationale for Infant Pulmonary Function Testing

While lung function testing is not a recent concept, there is interest in moving lung function testing into early childhood and infancy. (28) This has resulted in many studies; however, more work is required before it is adopted for routine clinical use.

There is increasing evidence that respiratory disease begins to manifest itself in early life. This has led to earlier interventional treatments, and as a result, improved patient outcomes. (29) By introducing pulmonary function testing into the care plan of an infant with respiratory disease, health care providers would be better able to monitor and track the state of their disease. By better understanding the state of disease, therapies can be introduced sooner. Furthermore, while these treatments are a result of advances in cell biology and molecular genetics, their efficacy ultimately needs to be measured in the relevant patient population, in order to ensure that the correct course of therapy is being pursued. Infant pulmonary function testing in combination with other means of evaluating lung health such as imaging (X-rays, CT scans, MRI) is able to provide the care team with more information regarding lung health, taking into account measurable differences in structure and changes in lung function.

On a more epidemiological level, chronic lung disease persists despite advancements in molecular therapies. As more therapies are identified, sensitive tools such as lung function measurements will be required in order to evaluate treatment efficacy, before they can be evaluated for their impact on the population.

1.4.1.1 Differences between adult and infant lung function

While infants produce significantly lower flows and volumes than their adult counterparts, similar testing equipment can still be used, with minor modifications to accommodate for patient size. However, there are unique considerations that need to be applied both when considering the procedure of lung function testing, and the interpretation of the results. (30)

Firstly, the largest consideration when comparing infant and adult lung function is that infants cannot cooperate in the same fashion as adults. Thus, forced flow maneuvers such as spirometry, which require a high degree of coordination, are not feasible if regular adult or older paediatric
guidelines are followed. This would restrict infant pulmonary function testing to only those tests that can be modified to accommodate a lack of involvement from the patient, or those that are performed during regular tidal breathing.

Another major difference between infant and adult lung function, is that infants fundamentally have different breathing mechanics due to their unique anatomy. Notably, the mechanism by which infants and adults set their FRC is different. The FRC in adults is static; it is set as the point of equilibrium between the recoil of the chest wall, and the compliance of the lung tissue. However, the chest wall in infants is quite pliable due to incomplete ossification, and thus the FRC must be set actively, via expiratory braking. (31) The infant thus ends its expiratory effort before the equilibrium of forces in the thorax is met. This prevents the FRC from being too low, and prevents atelectasis. This also causes a positive pressure to build inside the alveolar spaces at the end of expiration, also known as positive end-expiratory pressure (PEEP). In addition to secretion of surfactant, this expiratory braking helps to maintain the patency of the smaller airways and alveoli. This is necessary in order to keep pliable structures open, while the chest wall is not pliable enough to combat the elastic recoil of the lung tissue.

This elevation in FRC would reduce minute ventilation. To compensate, tidal volume would also need to increase. To prevent over inflation, infants employ the ‘Hering-Breuer Reflex’. This reflex is a vagally mediated response to pulmonary stretch receptors, which during large inspiratory efforts, stalls inspiration, and promotes expiration. (31) This leads to smaller tidal volumes (VTs), with a relatively larger FRC.

By engaging active expiratory muscle braking and limiting VT in an effort to maintain ventilation, respiratory rate (RR) must increase. A further side effect of this process is decreased expiratory time relative to inspiration, since expiration is being actively terminated. In contrast, deflation of the lung in infants results in a triggering of an inflationary reflex – that is, at low lung volumes, compression of the chest results in a subsequent strong inspiratory effort. (32) This is further evidence that the infant has respiratory mechanisms in place to maintain their FRC, beyond that of a mechanical equilibrium of the overly compliant chest wall and lung tissue, as is the case in fully developed/adult lungs. This is not only necessary in order to maintain efficient ventilation (by minimizing airway opening pressure), but also to prevent atelectasis.
Even during tidal breathing, the lack of infant cooperation requires special consideration. In order to minimize variability in the outcomes of a tidal breathing maneuver, the tidal breathing must be very regular with regards to both tidal volume and respiratory rate; which normally occurs during non-REM natural sleep. (33, 34) Therefore, infant pulmonary function relies on mildly sedating the infants in order to minimize both the variability arising from dynamic infant breathing, as well as from infant movement during testing. While some studies have suggested that lung function may be affected by sedation due to a change in the control of respiratory muscles as compared to natural sleep, this has yet to be fully explored in healthy infants. (35, 36)

1.4.2 Multiple Breath Washout

Multiple breath washout (MBW) is a pulmonary function test that was initially explored in the early 1950’s, but has only recently regained popularity due to advances in computing methods. (37) MBW has utility in infant pulmonary function testing, as it only requires quiet breathing, rather than coordinated, forced maneuvers.

The MBW test procedure results in multiple outcome measures. However, the general purpose of the test is to measure the efficiency (or inefficiency, in the case of disease) of ventilation in the lungs. This occurs via the continuous measurement of an inert gas as it is washed out of the lungs, from a known concentration to a target concentration. The inert gases used are either nitrogen (N2), or sulfur hexafluoride (SF6).

The procedure for MBW utilizes four main components: an inert tracer gas, a gas analyzer, a flow analyzer and a computer to integrate the signals. The test begins by “washing” the inert gas into the lungs to a stable concentration. This is accomplished via a constant, bias flow that flows from the gas source, past the subject through tubing. Attached to the tubing, a T-piece allows the subject to aspirate the gas from the bias flow system into the lungs. The flow sensors and gas analyzer port are also located in this path from the bias flow through to the patient. The gas analyzers either use a combination of a capnogram, oximeter, and subtraction to determine the amount of tracer gas (N2 based washouts), or a mass spectrometer to identify and quantify the gases based on molar mass (SF6 based washouts). The apparatus is sealed to the patient using either a mouthpiece with a tight lip-formed seal, or a silicone mask filled with therapeutic putty for younger populations. (Figure 1)
Figure 1. Example of an SF6 based MBW apparatus during washin phase of testing. Flexible tubing (a) provides a bias flow of tracer gas from which the subject breathes. The subject then breathes the gas through a pneumotachometer (PNT) (b). The PNT measures flow using a computer, not pictured, to integrate pressure differences. The PNT also contains a capillary sampling line (c) which aspirates gas to a mass spectrometer, not pictured, to determine gas composition and concentration. The PNT is also attached to a silicone facemask (d) filled with putty, in order to achieve a good seal and minimize dead space. Adapted from Benseler et al, 2015. (38)

After the subject has breathed in the inert gas to a constant concentration, the supply of the tracer gas is cut off at the end of an inspiration, marking the start of the washout. The subject then continues to breathe room air normally, with each breath mixing, diluting, and washing out the tracer gas. (Figure 2) Once the tracer gas reaches a pre-defined endpoint (traditionally 1/40th or 2.5% of initial starting concentration), the test is concluded.
MBW was initially developed using nitrogen as the washout gas, however many studies used sulfur hexafluoride (SF₆) as the inert gas of choice, as it is not present inside the body in any quantity (unlike N₂) or generally in a testing environment (unlike N₂). This permits for more accurate and stable testing, as there is a smaller chance of gases from the exterior environment affecting results. Furthermore, as mass spectrometry relies on molar mass and charge as a means of identifying molecular compounds, the relatively high molar mass of SF₆ (146.06 g/mol) makes it easy to distinguish from other physiologic gases such as N₂ (28 g/mol), O₂ (32 g/mol), and CO₂ (44.01 g/mol). Unfortunately, due to the environmental impact of SF₆, many centers are moving towards N₂ washouts as certain governments (namely the USA) have outlawed its use for medical purposes. Furthermore, the N₂ washouts utilize specialized sensors to detect O₂ and CO₂, and thus have no need for a mass spectrometer. This dramatically lowers the equipment cost of the test, making it more accessible to research centers and clinics. However, the hyperoxic environment that is introduced during a N₂ washout test has been shown to depress RR in infants, and also to cause the breathing pattern to be more erratic. (40) For this reason,
infant studies continue to use SF6 as the inert gas, despite many centers moving to N2 for preschool, older paediatric, and adult populations.

1.4.2.1 Outcome Measures of Multiple Breath Washout

The primary outcome measure of MBW is the Lung Clearance Index (LCI). The LCI is thought to remain relatively stable with age. It is obtained by taking the quotient of the Cumulative Expired Volume (CEV) divided by the Functional Residual Capacity (FRC). The equation for LCI is described below (Equation 2).

**Equation 2.** $\text{LCI} = \frac{\text{CEV}}{\text{FRC}}$

The CEV is simply the integral of the flow and gas concentrations throughout the duration of the test. The FRC however requires a more complicated equation, and is defined below as the quotient of the total volume of expired tracer gas, divided by the difference in initial ($C_{ETGi}$) and final concentration ($C_{ETGf}$) of the tracer gas (Equation 3).

**Equation 3.** $\text{FRC} = \frac{\text{CEV}_{TG}}{C_{ETGi} - C_{ETGf}}$

In these equations, the calculation takes place after the subject has washed out the tracer gas to a concentration of 2.5% of the initial starting concentration. While it may appear initially that older, and larger subjects would have larger LCI values as the washout would be longer due to increased size and complexity of the lungs, this is not the case. The denominator in the equation is FRC, taking the larger lung into account. Thus, in health, LCI should remain fairly stable throughout life.

In addition to observing the LCI, the CEV and the FRC can also be interpreted for lung function information. CEV is a more direct measure of lung ventilation efficiency than LCI, but it does not correct for size, and thus cannot be realistically tracked longitudinally. Therefore, it is not traditionally used in the clinical setting. It may still be useful to track, in order to better understand the longitudinal change in LCI.

FRC, on the other hand, is a measure of lung volume. While technically only representing the amount of air that is left in the lungs at the end of expiration, it can be used as a reasonable
estimate for total lung size in healthy subjects. This may not be the case in obstructed or fibrotic patients as they may be overinflated or experience decreased vital capacity, respectively. In healthy subjects, where the relationship of the recoil of the chest wall and compliance of the lung is normal, FRC ought to be able to represent lung size, and thus growth longitudinally. Since the bulk of the growth of the lung postnatally is made up of the parenchyma, these changes in FRC can be mostly attributed to changes in the lungs parenchyma and increases in alveolar volume.

It is important to note that the FRC value calculated by the MBW test is not a complete measurement of FRC. The calculation performed relies on gas dilution and mixing. This implies that the FRC is limited to the communicating airways. Any airways that may be blocked with mucus or collapsed from pleural pressures will not be measured by MBW, despite technically being a part of the FRC.

1.4.3 Body Plethysmography

Body plethysmography is a classic pulmonary function test (PFT), having been used for many years. Unlike MBW, body plethysmography measures all compressible gas in the thorax. This is measured by enclosing the patient in a sealed box containing a pneumotachometer to measure flow, which is can be completely occluded with the help of an electronic relay. The subject breathes tidally through the box to an exterior air supply, and at the end of expiration, the relay occludes the airway. As the patient attempts to breathe against the occluded air supply, the pressure changes are measured within the box, and changes in thoracic volume are inferred. This then gives a measurement of FRC.

FRC as measured by MBW differs in comparison to FRC measured by plethysmography. While plethysmography measures more volume than MBW, it can also in fact overestimate the FRC if there is gas trapping. In disease, FRC can be tracked longitudinally to determine the state of the alveoli, and to determine the stiffness of the lungs; clinically, it is more so an indicator of lung growth and alveolar volume.
1.4.4 Forced Flow Function Tests – Raised Volume Rapid Thoracoabdominal Compression

1.4.4.1 Forced Flow Outcome Measures

Raised volume rapid thoracoabdominal compression (RVRTC) relies on the same concepts as spirometry in adults. One can evaluate the relative health and function of the airways by measuring the amount of volume that can be expired by a subject for a given amount of time, given a maximum effort. In adults, this is performed by having the subject inhale to total lung capacity (TLC) and then forcefully expire for as long as they can. The amount of gas that is expired in the first second is the Forced Expired Volume in one second (FEV1). The test operates on the premise that during a forceful expiration, the positive pressure that is exerted by the chest wall is transduced by the pleura onto the thoracic space and the subsequent airways. This causes a positive pressure to be exerted on the airway wall itself, causing a restriction of flow through the airway. As effort and subsequent pressure increases in an attempt to increase flow, more pressure is exerted on the airway, further restricting its diameter. Therefore, increased effort, does not lead to more flow after a certain point. This is called ‘flow limitation’. Since increased effort does not improve this PFT after flow limitation has been achieved, the intrinsic state and health of the airways can be inferred from the FEV1 value, usually reported as a percentage of predicted value based on population studies. As the forced expiration progresses, the choke point where the limitation is occurring moves towards the periphery, as the pressure differential decreases between the alveoli and the environment.

In addition to FEV1, measuring a non-limited forced expiration is also useful. By following a similar procedure of inhalation to TLC followed by a forced expiration, the Forced Vital Capacity (FVC) can also be measured. The advantage of FVC is that it allows for the measurement of flow from the slower ventilating units of the periphery, which may be missed during the FEV1 measurement. Furthermore, while FEV1 is useful for cases of obstruction (where the parenchyma has lost its elasticity due to alveolar damage), FVC is useful for detecting restriction. Those with fibrotic lungs may actually have a higher FEV1 due to the lack of compliance of their lungs, but will have a decreased FVC. FVC is also usually reported as a percentage of a predicted value, with a threshold for normalcy.
While percentage predicted is useful for individual patients, it is difficult to use these values when looking at populations in order to track longitudinal change. This is especially true in paediatric populations, since as the subject grows, their FEV1 and FVC will naturally increase, as their lungs get larger. Thus, it is useful to observe the FEV1/FVC ratio. This examines FEV1 in the context of the overall lung volume. In healthy older subjects, this ought to remain fairly constant, at approximately 0.8. Any changes can then be attributed to changes in airway health. In paediatric applications however, it may reflect changes in lung/airway size that are related to growth and development.

1.4.4.2 Forced Flow Adoptions to Infants – Raised Volume Rapid Thoracoabdominal Compression

As spirometry requires a coordinated effort from the patient, it cannot be completed in the infant population in the same manner as it would be performed in adults. To compensate for this, the Raised Volume Rapid Thoracoabdominal Compression technique was developed. (41) In lieu of having the infant perform such a conscious, coordinated maneuver, the maneuver is essentially recreated using pneumatic equipment. The infant is sedated, and lying supine in a box, whilst wearing an inflatable vest. The infant continues to breathe tidally through a t-piece connector via a putty-filled facemask similar to the equipment setup for MBW as described in the previous section. There is a bias flow of medical air passing through the T-piece, and at the beginning of inspiration, the open end of the t-piece is occluded through the aid of a pneumatically controlled balloon, resulting in a positive pressure being introduced into the infant lung, which leads to an inspiration that is beyond their normal tidal volume. This simulates the adult subject inhaling to TLC in regular spirometry. At the end of the inspiration, the pneumatic vest which the infant subject is wearing rapidly inflates, up to a pre-selected cm of H2O pressure on the chest wall of the subject, emulating a ‘hug’. This simulates a forced expiration that would normally be elicited through contraction of the intercostal and accessory muscles in an adult patient. This procedure is repeated multiple times, with varying pressures being applied through the vest in order to achieve flow limitation (the key feature of the RVRTC), and other forced flow procedures.

RVRTC is a relatively good analogue for forced flow maneuvers in the place of spirometry. Furthermore, the FVC outcome measure is still applicable, as are both the FEV and the
FEV/FVC ratio. However, while in adult populations, the time limitation for the forced expiration is 1 second, this is too long a timeframe for infants. Due to their relatively low lung volume (compared to airway), 1 second of forced expiration would prove too long in order to ensure constant flow limitation through the suggested period. As such, infant forced flow procedures are reported at time 0.5 seconds of expiration. Likewise, the FEV/FVC ratio utilizes FEV0.5 rather than FEV1.

1.4.5 Technical Considerations of Pulmonary Function Testing

In addition to the differences in the lungs themselves, there are technical differences in infant testing that need to be considered. In order to maintain stability, infants are tested supine, which changes the structure of the alveoli due to the effects of gravity. This has been shown to have a significant impact on lung function testing, and outcome measures of MBW in adults. (42) Therefore, data from adults and older paediatric subjects cannot necessarily be applied to infants due to the difference in testing posture.

1.4.5.1 Dead Space

As previously mentioned, portions of the lung which do not participate in gas exchange make up the dead space of the lung. However, when extra equipment is added during pulmonary function testing, the volume of conducting airspace between the gas source and site of gas exchange is extended, thus creating an added, ‘equipment’ dead space. This equipment dead space acts as an extension of the anatomical dead space, and thus will also impact washout measures.

Dead space has been shown to have a significant effect on MBW measures. (38) Dead space must be ventilated through before any fresh air reaches the gas exchanging alveoli. At the end of an expiration, the dead space is full of expired gas; at the beginning of inspiration, fresh gas will be pulled through said dead space, and cause gas mixing – resulting in an inhaled gas that is not equivalent in makeup and concentration as the inspired environment, or medical gas. Therefore, increased dead space results in less efficient washouts, as the gas that is being used to dilute the existing tracer gas, will already contain some of the tracer gas from the previous breath. This becomes increasingly relevant in smaller lung volumes, as the equipment dead space is then relatively larger, and so less of a tidal breath actually consists of fresh gas.
Infants have a proportionally smaller VT compared to the equipment dead space, which results in less efficient washouts, and is reported to lead to higher LCI values. As the lung grows, this effect is diminished, since the equipment dead space stays the same. Furthermore, in the youngest infants, as the alveoli are not completely developed, the proportion of conducting airway to ventilating airspace is even larger, and thus again, increased equipment dead space must be considered when evaluating outcomes of lung function measurements.

In the setup for MBW, the gas and flow sampling points lie in the middle of the pneumotachometer, and thus divide the equipment dead space in two – the pre-sampling dead space, and the post-sampling dead space. These two volumes can affect the measurement differently. Pre-sampling dead space acts as an extension of the anatomical dead space, thus increasing gas mixing, and is included when dead space is computed by the software by Fowler’s method. (8) However, due to the use of a facemask with an infant, it is extremely difficult to accurately measure the volume of space in a facemask that is not occupied by the infant’s face, thus pre-sampling dead space is usually computed, but not corrected for in the software algorithm. Post-sampling dead space however, can be easily measured using water-filling measurements. Thus, while the post-sampling dead space still acts as source of gas mixing (as it is between the alveoli and the air source), it can be corrected for by the software. Both of these volumes will contribute to gas mixing during a washout, and need to be considered for younger populations such as infants, where the amount of relative dead space is large enough to impact washout outcomes.

1.5 Pulmonary Function Testing and Development

For many of the adult measures listed above, there exist multiple population studies which resulted in relatively standardized normal values, given the patient’s anthropometric characteristics. (43-45) However, this is only possible because the organs are no longer developing. Conversely, in infancy the lungs continue to change. Therefore, studying the longitudinal change of various lung function measurements is an excellent way to gain a more thorough understanding of how these tests can be used for both clinical and research purposes in a pediatric population. However, if the same PFTs are to be applied for infant studies and infant treatment plans, the trajectories of lung function measures in infants must be studied as well.
1.5.1 Basic Breathing Parameters – Tidal Volume

In 1987, Gerhardt et al. performed a study whose main goal was to further understand pulmonary mechanics in children. The study also involved the measurement of VT, and RR in the study population (40 children between birth and age 5). (46) It was found that while VT increases with age and size, correction of VT for subject bodyweight (VT/kg) yielded a stable VT/kg measure across the wide range of infants in this age range. In general, RR decreases with lung size, and this is observed throughout the mammalian class. (47) However, it is more difficult to directly correct RR for body size, and thus RR as a raw measure of breathing frequency did indeed change with age, and was found to decrease with time. This is likely due to the fact that for a set amount of minute ventilation ($V_e$), a lower RR matched with a larger VT requires less work from the respiratory muscles than a high RR and lower $V_e$. As the infant ages, the cartilaginous sections of the chest wall begin to ossify, and thus become more resistant to movement. (48) This likely results in a shift to a more efficient breathing pattern, with deeper and less frequent breaths, which reduces the workload on the respiratory muscles.

1.5.2 Changes in Functional Residual Capacity

As previously mentioned, FRC is a good estimate for lung volume in the healthy population, and thus is useful to track over time. Traditionally obtained via body plethysmography, there are many studies which have evaluated how FRC changes in young subjects, and how it changes with anthropometric correction. However, it was also initially studied using nitrogen dilution, which operates on a similar principle as multiple breath washout.

An early study from 1963 by Nelson et al. used both plethysmography and nitrogen dilution in order to understand their differences in infancy. (49) Both methods of FRC measurement were performed in 23 infants. They found that the raw value of both measurements increased with age. However, both FRC values corrected for body weight decreased with increasing body weight. This indicates relatively lower FRC values in heavier subjects. The extra weight on the rib cage may prove to serve as a compressive force in infants with relatively pliable chest wall. Furthermore, FRC as measured by plethysmography was higher in 10 of 23 subjects. This is due to the fact that the nitrogen dilution method only measures communicating lung units. Since these are healthy infants, they should not have mucus plugging, as is typical of patients suffering from cystic fibrosis. Differences between plethysmographic and dilution-based FRC
measurements in healthy subjects are more likely due to measurement of gastric gas by plethysmography, or due to incomplete wash-in of the tracer gas in MBW measurements.

A more recent study which was performed in 1993 by Gappa et al. examined similar concepts of measuring FRC using both methodologies. (50) The sample size was reduced in this study, using only 11 infants, however, paired measurements strengthened the power of the study. With both protocols, Gappa et al. found that the expected FRC was relatively well matched with the measured FRC values. The expected FRC was based on a modeled equation where the main predictive variable is length. This supports the notion of FRC increasing with age and size. Length is used as a predictor in the equation, as it is a better estimate for lung size, as opposed to weight.

Interestingly, while length is used as a predictor for FRC, subject weight is used for estimating VT. Gappa et al. reported higher VT/kg for the subjects performing plethysmographic measurements, though this could be due to the extra dead space of the plethysmographic equipment. Nevertheless, as mentioned earlier, modification of the VT can influence FRC measurements. Similar to the study by Nelson et al, Gappa et al. found that the FRC values calculated by plethysmography had consistently higher values than those obtained by nitrogen washout. This again supports the notion that there are units in the healthy infant lung which are not consistently communicating. This is further corroborated by the fact that despite a larger reported VT/kg (which would actually decrease FRC), there was no correlation found between the VT/kg and difference in FRC values.

It can then be concluded that FRC in general will increase as the infant ages, with a dependence on the length of the subject. Furthermore, plethysmographic measurements will likely be higher than those performed by a gas dilution technique, such as MBW.

1.5.3 Changes in Forced Flow Maneuvers

Spirometry is one of the most pervasive clinical pulmonary function tests, and as such has been well characterized in the literature regarding its interpretation and predicted ranges for various patients. Nonetheless, as it has been previously mentioned, infants are not capable of performing spirometry, and thus forced flow maneuvers are restricted to RVRTC maneuvers. RVRTC is a more recent test than classic spirometry, and thus lacks the large base of data that spirometry has.
Nevertheless, RVRTC has still been studied sufficiently to obtain reference values in infants. A study published in 2000 by Jones et al. established the evolution of RVRTC measures in infants, constructing a set of normalized data. (43) 155 healthy infants performed RVRTC to obtain reference values for future studies. In addition to this goal, the study examined the changes in this measure over time and with growth. FEV$_{0.5}$ continued to correlate with age despite length correction, indicating that airway maturity increases forced flows and FEV$_{0.5}$, and not simply the physical length of the airways. Forced Expiratory Flow (FEF) measurements, such as FEF (50%, 75%, 100%) also correlated with length. Interestingly, when observing the FEV$_{0.5}$/FVC ratio, it was demonstrated that as lung volume increased, FEV$_{0.5}$/FVC decreased, indicating that smaller subjects actually empty their lungs faster than their larger counterparts. While this study from 2000 provided early reference values, a more recent study developed new reference values, demonstrating that different equipment setups will affect the outcomes. (51) This provides more evidence that the amount of equipment dead space likely plays a significant role in pulmonary function measure outcomes.

1.5.4 Changes in Multiple Breath Washout Measures

As previously mentioned, the Lung Clearance Index (LCI) is the primary outcome of MBW. One of the two components, FRC has already been outlined above with regards to how it changes over time. The Cumulative Expired Volume (CEV) is generally not reported individually, as it requires a parameter of size to contextualize it, hence the utility of correcting it with FRC, – which results in the LCI. LCI has been studied in older paediatric subjects, but few studies have been performed evaluating its change in early infancy.

Because LCI is an index that accounts for lung size, it is generally thought to be relatively stable with age. As CEV increases with increasing lung size and complexity, so should FRC, thus leaving the LCI to remain stable. However, Lum et al. demonstrated in 2012 that while LCI remains relatively stable in later paediatric years (starting from around age 5) into early adulthood, the LCI is actually elevated in infancy and early preschool. (52) Furthermore, it was demonstrated that there is indeed a height and age-dependence of LCI, but it is mostly only seen in those subjects younger than 5 years of age. This may be due to the increased equipment dead space relative to tidal volume in these younger subjects. As previously mentioned, dead space leads to gas mixing, and thus a less efficient washout, and a subsequently higher LCI. The
relative amount of equipment dead space decreases as the lung volumes of the subjects’ increase with age and height, and thus the degree of gas mixing would be decreasing as these volumes decrease. The impact of equipment dead space is very small in older subjects, and so this effect is mostly only seen in young children and infants. Another potential explanation is that ventilation homogeneity is actually improving in young subjects. Increasing ventilation homogeneity has been reported in older age groups, and may be even more relevant in infancy due to the large degree of change that is occurring in infancy in terms of lung growth. (53, 54) Since gas mixing decreases homogeneity, and gas mixing increases with dead space, the rapidly expanding parenchyma of the lung in infancy may cause the decrease in LCI, as the relative amount of dead space becomes smaller. This may be due not only to equipment differences, but also to the relative size of the conducting airways compared to the gas-exchanging parenchyma. As the latter is rapidly expanding, the impact of the anatomical dead space may decrease, thereby increasing homogeneity, as well as LCI.

1.6 Sex Differences in Pulmonary Development and Function

It is well documented that males and females do not present with pulmonary diseases equally. (55) Indeed, there are marked differences in rates of susceptibility and disease risk between men and women. (56, 57) For example, wheezing appears to manifest (as early childhood asthma) in young boys more-so than girls, however asthma is more common in adult women than it is in men. It is possible that these differences in pulmonary disease between the sexes may have an underlying basis in differences of early pulmonary development.

1.6.1 Structural Differences Between Sexes

Early morphometric studies performed on young cadavers demonstrated that there are some inherent sex differences between the sizes of various structural elements of the lungs themselves. Most notably, but not entirely unexpectedly, females tend to have smaller lungs than males. (17) In addition, the total alveolar volume of the female lung is smaller than that of a male. However, the size of the alveoli was found to be the same between the sexes. This suggested, and was later confirmed, that indeed there is a greater number of alveolar units in males. In addition to this increasing the overall volume in males as compared with females, this also increased the overall surface area of the alveoli. Furthermore, male subjects also tended to have more respiratory
bronchioles than their female counterparts. These differences remained true after both correcting for size of the subject, as well as the age. (17)

1.6.1.1 Differences in Structural Growth Between Sexes

In addition to changes in alveolar volume, studies have suggested that there are inherent differences between males and females in the way the parenchyma and airways grow. In the same study where Mead demonstrated dysanaptic growth between the parenchyma and the airways, Mead found that the dysanaptic growth was not presenting in the same fashion between the sexes. The data demonstrated that for all the females in the study, the ratio of airway to lung volume functional measurements was lower for a given lung volume than for the male subjects. Data from boys aged 13-18 also had lower ratios than their adult male counterparts. (24) This then suggests that the airways are not growing in females as much as they would in males, for a given lung volume.

However, these results do not match more recent studies, where it was suggested that females exhibit faster growth of the airways than males. Using a similar study design to Mead, Hoffstein correlated tracheal cross-sectional area with lung volume. (58) Dysanapsis was first demonstrated between the sexes, showing that while males had a decreasing relationship of lung volume to tracheal cross-sectional area, females had an increasing relationship. In addition, Hoffstein concluded that the female parenchyma grew faster than female airways; however, this was not the case in males. Thus, not only was there different, uncoupled growth between the parenchyma of the lung and the airway, but also different, or dysanaptic growth between the sexes.

The two studies outlined above agree on the concept of dysanapsis – that the airways and parenchyma grow independent of each other. However, the two studies do not agree on the respective degree, and skew of the dysanapsis occurring. It is important to note that these studies were performed in adult humans, with data being retrospectively extrapolated to imply growth of features that took place during early childhood. Thus, while interesting and informative, more studies are required in paediatric populations in order to truly discern sex differences during the periods of lung growth.
1.7 Limitations of Current Literature

To date, there has not yet been a longitudinal study in which multiple pulmonary function tests are compared in order to understand the functional growth of lung structures in health during infancy. There are multiple studies that have performed some sort of pulmonary function testing in healthy infants; none of them are able to adequately evaluate multiple parameters of lung growth, while minimizing variability by tracking subjects (and their outcomes) longitudinally throughout infancy.

While many of the studies listed in Section 1.5 address one or more of the outcomes used in infant pulmonary function testing, none of them are able to evaluate all of the parameters (e.g. LCI, FEV, FRC etc.) in the same study population. This is necessary, as the comparison between multiple parameters within the same subject is essential in order to understand relative differences in functional growth between lung structures. The comparison of these measures in different subjects is not possible, as too much variation would be present in order to isolate measurable change in the lung. Furthermore, the time frame between these studies sometimes spans several decades, and sometimes continents, which adds another dimension of variability; different environments may prove especially important given that environmental exposure is known to have an impact on pulmonary function. (59-61) Thus, a consistent population, as well as standardized equipment setups and protocols are required in order to minimize any external variability.

Many of the early studies were cross-sectional, using unique infants for each time point of the study. This introduces variability; as individual differences may skew certain measures at certain time points. Longitudinal studies are required in order to track the same subjects through time, minimizing inter-subject variability, and better isolating the factors that are driving the differences in lung function through time.

This study seeks to eliminate the above pitfalls, by performing all testing at a single center, with a large number of subjects, who perform multiple lung function measurements that can be tracked longitudinally throughout infancy. This will help to discern how the healthy lung grows and develops functionally. It also and aids in the understanding of the different rates at which pulmonary structures develop.
Chapter 2
Rationale and Objectives

2 Rationale and Objectives

2.1 Rationale

Pulmonary obstructive diseases such as cystic fibrosis can induce abnormalities and damage before overt symptoms begin to develop. (2, 62) As such, clinical focus should be on early detection and prevention of developing pathologies, as well as treating existing disease. (1) While prevention is not always possible, early detection and subsequent treatment is, and has been shown to be effective in improving long-term patient outcomes. (1, 63, 64) It would thus be ideal to move pulmonary function testing into the infant population. Indeed, there are now well-defined guidelines outlining proper infant pulmonary function testing equipment and test analysis. (65, 66) While pulmonary function testing is useful in differentiating patients with disease from healthy controls when studied cross-sectionally, the longitudinal tracking of lung growth over time may hold additional value in predicting the development of lung disease. (67-69) In order to accurately track the onset of disease, an understanding of longitudinal lung function growth in healthy individuals must be elucidated. Due to the unique, postnatal development of the respiratory system, different PFT’s outcomes measures may change at different rates, thereby indicating how respiratory structures grow postnatally. (17) By comparing the changes in lung function parameters that measure lung volumes (surrogate for parenchymal growth) and those that measure airway flow (surrogate for airway size); it is possible to determine the extent of dysanaptic growth between these two pulmonary features. There has yet to be a published study that examines multiple longitudinal lung function parameters in a large population.

2.1.1 Research Question

How does dysanaptic growth between the conducting airways and the acini of the lung affect the longitudinal measures of lung function in infant populations?
2.2 Objectives

This study’s objectives are to first, characterize the changes in the lung clearance index, lung volumes and infant spirometry as a reflection of the growth of the alveoli and airways during infancy. In addition, it seeks to determine whether these growth patterns differ between male and female infants. These objectives will be addressed through the following specific aims:

1. Longitudinally evaluate ventilation efficiency of the lung as measured by the lung clearance index

2. Longitudinally evaluate growth of the lung parenchyma by measurement of lung volumes, using different methodologies (MBW, RVRTC and plethysmography) and examine how these measurements change relative to each other

3. Longitudinally evaluate forced flow maneuvers as a surrogate measure of airway caliber, and evaluate relative change in airway caliber as compared with parenchymal growth.

4. Determine whether lung volume or airway growth differs between the sexes by evaluating potential differences in their lung function

2.3 Hypotheses

Due to continued postnatal acinar development, as well as growth, lung volume measures such as FRC will increase significantly despite correction for body length

Furthermore, the following will be observed:

1. Forced flow measures will also increase despite length correction

2. Lung volume measurements will increase proportionally faster than forced flow measurements

2.4 Clinical Relevance

As mentioned previously, lung function testing has utility in the diagnosis and management of pulmonary diseases. However, the evolution of normal, healthy lung function must first be understood in order to assess whether lung function changes reflect health or disease. Once this
is understood, physicians and other health care providers will be better able to assess the
effectiveness of their patient’s treatment plans. This will further strengthen the use of
personalized therapy to treat individual deficits. This study will further the application of these
lung function tests, some of which are still novel, bringing them closer to full clinical integration
and application. Furthermore, lung function testing is relevant in that it directly reflects the
performance of the respiratory system. This will impact how it may be affecting other bodily
systems, which is especially important in non-communicating patient groups such as infants.
Chapter 3
Methods

3 Methods

3.1 Research Setting
All pulmonary function testing took place at The Hospital for Sick Children’s Infant Pulmonary Function Laboratory, in the Division of Respiratory Medicine, Department of Paediatrics, in Toronto, Canada.

3.2 Ethics
All studies were performed following approval by the Research Ethics Board at the Hospital for Sick Children. Informed, written consent for all subjects was provided by the parents of the subjects on behalf of their children, due to their young age.

3.3 Study Population
Subjects were recruited from the Canadian Healthy Infant Longitudinal Development (CHILD) Study. The CHILD Study is a nation-wide study that recruited 3500 families from 4 sites across Canada. The study aims to explore the genetic and environmental factors that influence children’s development, particularly in the area of allergy and asthma. The participants for this study were recruited from an infant pulmonary function sub-study of the CHILD study. This group was recruited as part of the overall CHILD study but was invited to also participate in an intensive pulmonary function phenotyping arm, and as such underwent repeated lung function testing from infancy.

3.3.1 Subject Recruitment
For the CHILD study, mothers were recruited antenatally during pregnancy from obstetrical units. (70) Infants were enrolled into the overall CHILD study if they met the study inclusion criteria and none of the exclusion criteria (Table 2). Of particular relevance, only full-term infants (>35 weeks) were recruited. For this particular longitudinal evaluation study, we further only evaluated the longitudinal lung function in those infants who were healthy.
Table 2. Inclusion and exclusion criteria for the participation in the general CHILD Study.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
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<tbody>
<tr>
<td>• Pregnant woman aged 18, with residential proximity to study center (50 km)</td>
<td>• Infant born with major congenital abnormality or respiratory distress syndrome</td>
</tr>
<tr>
<td>• Ability to read, write and speak English</td>
<td>• Expectation of moving away from study within 1 year</td>
</tr>
<tr>
<td>• Willing to donate cord blood</td>
<td>• Birth of multiple children</td>
</tr>
<tr>
<td>• Planning delivery at recruitment center hospitals</td>
<td>• Birth as a result of in vitro fertilization</td>
</tr>
<tr>
<td>• Infant delivered at 35+ weeks gestation</td>
<td>• Child not living at index home minimum 80% of the time</td>
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</table>

3.3.1.1 Healthy Subjects

In order to avoid the introduction of any variance resulting from a diseased state, or from subjects who may be exposed to factors affecting the function of the respiratory system, only subjects who are likely to have uncompromised respiratory health and/or function were included from the larger Toronto CHILD study population. Factors that would cause exclusion consisted of history of respiratory illness, hospitalization for respiratory illness, exposure to cigarette smoking in the home or during pregnancy, and any chronic illness.

3.4 Pulmonary Function Testing

3.4.1 Infant Sedation

Due to the sensitive nature of the PFTs performed, regular tidal breathing is required in order to maintain test accuracy and reproducibility. Due to the infant subjects’ young age, mild sedation
was employed in order to ensure quiet tidal breathing throughout the testing procedure. Chloral hydrate (prepared by the Department of Pharmacy at the Hospital for Sick Children) was administered orally via syringe at a dose of 80 mg/kg. Infant pulmonary function was performed in all subjects by a registered respiratory therapist. Vital signs such as respiratory rate, heart rate, and blood oxygen saturation levels were continuously monitored and recorded throughout testing to monitor for signs of distress.

3.4.2 Pulmonary Function Testing Procedure

All PFTs were performed in the same chronological order in sedated, supine infants; MBW measurement, followed by plethysmography, and then RVRTC. This order was chosen to ensure that the forced flow maneuver (RVRTC) did not impact the tidal breathing maneuvers (MBW). The IPFT protocol of the CHILD study included administration of salbutamol, and a repeat of all 3 PFTs in the same order. The data from these test conditions are not examined or reported in this thesis. Testing concluded when all PFTs were completed to standard or when the infant was aroused from sleep. (72)

3.4.3 PFT Equipment and Setup

3.4.3.1 Multiple Breath Washout

MBW was performed via a mass spectrometer system. The inert gas used was sulfur hexafluoride (SF₆) (PRAXAIR, Mississauga, ON, Canada). The delivery gas was composed of 4% SF₆, 4% He, 21% O₂, and 71% N₂. The gas was directed from the cylinder to a gas valve regulator, then through a high purity flexible hose to a standard plastic fitting. A fixed length of disposable, highly pliable clear plastic tubing then went from the plastic fitting, to a plastic, three-way T-piece, along the longer side. Opposite the end from the cylinder side, an equal piece of the clear plastic tubing served as an exhaust for the gases; the gas to the patient entered/exited through the center piece, perpendicular to the supply and exhaust ports of the T-piece. This creates a bias flow, with equal pressure along the supply and exhaust. Directly fitting into the center of the T-piece is the pneumotachometer, measuring gas flow to/from the patient. The pneumotachometer used was either a 3500 or 3700 series (Hans-Rudolph, Shawnee, KS, USA), depending on the size of the infant tested (<10 kg infants used 3500, while >10 kg used 3700).
Mass spectrometry served to detect the concentration of gases. A metal-tipped, flexible capillary line was fitted into a small opening in the pneumotachometer, near the pressure-sensitive screens, on the patient side. This capillary line aspirated the gas at a constant rate, and carried the gas to an AMIS 2000 mass spectrometer running proprietary analytical software. (Innovision ApS, Odense, Denmark). The patient end of the pneumotachometer fitted into a silicone facemask (Silkomed, Rendell Baker Masks sizes 2 and 3, Rusch Canada, Inc., Benson Medical Industries, Markham, Ontario) that contours to the patient’s face. Empty space in the facemask was filled with therapeutic putty (Achieva Air-Putty, North Shore Medical Inc., Gilroy, CA, USA) in order to reduce dead space, as well as to ameliorate the seal.

Flow and concentration were then sent via USB to a laptop computer running custom written software based on Test Point (P. Gustafsson, Measuring Computing Corporation, Norton, MA, USA). Data during MBW was captured using this software, and subsequently analyzed post-hoc using a different package of the same software.

The testing procedure generally followed ATS/ERS guidelines. (72) As mentioned previously, sedation and quiet tidal breathing was achieved with sedation by administration of oral chloral hydrate. After opening the gas source, the facemask was placed on the subject, and the gas was permitted to wash in into the subject’s lungs to a steady concentration of 4%. Once the gas concentration was steady and non-variable between breathing phases, the gas source was manually disconnected from the pneumotachometer and shut off (to avoid accidental inhalation of exogenous gas). The subject then continues regular tidal breathing, and in the process diluting and exhaling the SF6 from their lungs. This washout of the inert gas is continuously measured by the gas analyzer and computer. Once the gas concentration reaches 1/40th of its initial starting concentration (0.025 * 4% = 0.1%), the washout and trial is deemed complete and the mask is removed from the infants face. The procedure was attempted enough times to achieve 3 acceptable trials per test condition, pending patient and equipment cooperation.

3.4.3.2 Body Plethysmography

Body plethysmography was performed using a commercially available system, the Infant Pulmonary Lab System (nSpire Health Inc., Longmont CO, USA). The IPL system consisted on a plexiglass airtight box that could be opened, by sliding the cover off a bed (in which the infant lays). This box contained the pneumotachometer and shutter valves necessary for
plethysmography, complete with airtight connections. The infant was placed inside the box, with the same mask fitted with therapeutic putty (as described) used during MBW connecting the system to the infant. The mask connected to a plastic T-piece connector that then was connected to a bias flow system connected to medical air. The box was sealed to ensure an isovolumetric environment. The sedated infants continued to perform tidal breathing through the system, and then performed 2-3 respiratory efforts against the system, which was electronically occluded. Flow and volumes were measured using a similar heated pneumotachometer setup as described above for MBW.

3.4.3.3 Raised-Volume Rapid Thoracoabdominal Compression

Raised-volume rapid thoracoabdominal compression (RVRTC) was completed using the same IPL box as described in Section 3.4.3.2. The infant is placed inside the box, but the box remains open for RVRTC (as opposed to plethysmography). The infant is fitted with an inflatable, pneumatically controlled compressible vest. Flexible plastic tubing leading from a medical air source, to the IPL box, to a pneumotachometer, to a silicone mask, with the PNT measuring the flows and volumes of the infant during tidal breathing. At the beginning of inspiration, a positive pressure from the system is introduced to the airway, in order to help the infant achieve their total lung capacity. When the positive pressure ceases, the pneumatic vest compresses the chest of the infant, simulating a forced expiration. This process was performed 5-10 times with a variety of pressures administered to the vest, in order to identify the optimal pressure to achieve flow limitation.

3.5 Data Analysis

3.5.1 Collection Software

Collection and analysis of data from each PFT was performed via proprietary software. MBW was collected and analyzed via custom-made, Test Point-based software (P. Gustafsson, Measurement Computing Corporation, Norton, MA, USA). RVRTC and plethysmography data was collected using nSpire software.

Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA), while graphics were created using both SAS 9.3 and R 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria)
3.5.2 Data Analysis and Interpretation

MBW data was compiled, and analyzed by Krzysztof Kowalik, using the aforementioned Test-Point based software (P. Gustafsson). Analysis was performed to ensure adequate test quality for inclusion of a subject’s data into the dataset. Trial inclusion was based on ATS/ERS criteria for infant pulmonary function testing. Trials were performed in triplicate, but test occasions with 2 or more acceptable trials were included in the dataset. Quality control of a trial included the evaluation of five critical elements. First off, the trial had to have a stable wash-in of SF$_6$ to a steady concentration of 4%. The trial also had to have a clean disconnection of the SF$_6$ gas source from the subject, resulting in the first breath post-disconnection having a concentration of SF$_6$ of 0%. A normal and representative pre-washout breath (used to determine FRC) was also evaluated for each trial. Lastly, a stable breathing pattern comprised of both a regular and appropriate respiratory rate and tidal volume was also evaluated for each trial.

Registered Respiratory Therapists collected and analyzed RVRTC, and plethysmography tests to ensure the quality of data. Statistical models were constructed by a team statistician (Winnie Shen), and subsequently interpreted by Krzysztof Kowalik.

3.5.3 Statistical Analyses

In order to determine longitudinal changes in the variables, mixed effect models (utilizing both fixed effects and random effects) were constructed for each variable, taking into account repeated measures, age, sex, weight, and length (where appropriate).

In order to determine relative changes during infancy, percent changes were calculated between 0-12 months of age, and 12-24 months of age based on estimated values from a mixed effects model that was constructed for each subject using the random effects. This ensured that multiple test occasions were taken into account, as well as individual measures. The data was analyzed longitudinally, and using individual data points to strengthen the model. Comparison between two parameters was performed using ANOVA, as well as a Tukey Correction Comparison in order to determine statistical significance.
Chapter 4
Results

4 Results

4.1 Study Population

4.1.1 Recruitment and Retention

768 subjects were enrolled in the CHILD Study at the Toronto site, of which 500 were interested in participating in infant pulmonary function testing (IPFT) testing. 231 unique subjects came in for testing. 72 subjects were subsequently excluded from this study’s analysis as they met the exclusion criteria as described in Section 3.3.1.1. This left 159 healthy subjects to come in for the 3 proposed visit times. There were 68 infants attended an early-infancy visit, 121 a mid-infancy visit, and 48 infants attended a late-infancy visit. (Figure 3). Furthermore, 11 subjects were able to complete testing at all 3 visit points, 56 at two of the visit points, and 92 subjects completed one visit.

Figure 3. Consort diagram describing study population recruitment and retention.
4.1.2 Subject Demographics

In order to determine if our IPFT cohort is in-line with the rest of the CHILD Study subjects in Toronto, we investigated the demographics at the mid-infancy visit. We compared the demographics between those who participate in the IPFT portion of their mid-infancy visit, to those who did not. (Table 3) The cohort is generally very similar, though the IPFT cohort has slightly more non-Caucasians, and is generally smaller.

<table>
<thead>
<tr>
<th></th>
<th>IPFT (n=202)</th>
<th>Non-IPFT (n=472)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>13.32 (2.4)</td>
<td>13.2 (2.88)</td>
<td>0.51</td>
</tr>
<tr>
<td>Sex</td>
<td>1.4 (0.49)</td>
<td>1.47 (0.50)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>0.53 (0.5)</td>
<td>0.628 (0.48)</td>
<td>0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>9.8 (1.22)</td>
<td>9.91 (1.27)</td>
<td>0.27</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>75.52 (3.82)</td>
<td>76.11 (3.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.08 (1.00)</td>
<td>0.25 (0.94)</td>
<td>0.04</td>
</tr>
<tr>
<td>Length z-score</td>
<td>-0.38 (1.30)</td>
<td>-0.039 (1.21)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3. Comparison of IPFT vs. non-IPFT cohort at the mid-infancy visit. Values expressed as mean (SD).
Table 4 below describes the anthropometric characteristics of the healthy patient population, separated by visit. Table 5 describes baseline results for IPFT outcomes for each visit group, and Table 5 includes those outcomes as corrected for subject size.

**Table 4. Subject demographics. Values expressed as number of subjects (SD, range) unless noted otherwise.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early Infancy (N)</th>
<th>Early Infancy IPFT</th>
<th>Mid Infancy (N)</th>
<th>Mid Infancy IPFT</th>
<th>Late Infancy (N)</th>
<th>Late Infancy IPFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity (Caucasian)*</td>
<td>68</td>
<td>36 (52.94%)</td>
<td>121</td>
<td>65 (53.72%)</td>
<td>48</td>
<td>27 (56.25%)</td>
</tr>
<tr>
<td>Gender (Female)*</td>
<td>68</td>
<td>28 (41.18%)</td>
<td>121</td>
<td>52 (42.98%)</td>
<td>48</td>
<td>21 (43.75%)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>68</td>
<td>6.02 (1.77, 2.76 to 9.10)</td>
<td>121</td>
<td>12.99 (2.08, 8.15 to 23.62)</td>
<td>48</td>
<td>19.48 (1.78, 16.43 to 24.05)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68</td>
<td>7.70 (1.31, 5.20 to 12.10)</td>
<td>121</td>
<td>9.73 (1.27, 7.20 to 13.90)</td>
<td>48</td>
<td>11.08 (1.31, 8.50 to 14.00)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>68</td>
<td>0.06 (1.04, -2.26 to 3.26)</td>
<td>121</td>
<td>0.10 (1.02, -2.18 to 3.37)</td>
<td>48</td>
<td>0.06 (0.90, -1.85 to 1.65)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>68</td>
<td>66.54 (3.35, 60.00 to 73.00)</td>
<td>121</td>
<td>75.17 (3.56, 64.30 to 90.00)</td>
<td>48</td>
<td>82.01 (3.08, 73.00 to 90.00)</td>
</tr>
<tr>
<td>Length z-score</td>
<td>68</td>
<td>-0.02 (1.08, -2.66 to 2.06)</td>
<td>121</td>
<td>-0.37 (1.12, -3.87 to 2.76)</td>
<td>48</td>
<td>-0.34 (0.90, -3.24 to 1.27)</td>
</tr>
</tbody>
</table>

*Expressed as % Caucasian, or % Female*
Table 5. Characterization of the mean outcomes of all performed pulmonary function tests, separated by visit. Values expressed as number of subjects (SD, range).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early Infancy (N)</th>
<th>Early Infancy IPFT</th>
<th>Mid Infancy (N)</th>
<th>Mid Infancy IPFT</th>
<th>Late Infancy (N)</th>
<th>Late Infancy IPFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (mL)</td>
<td>68</td>
<td>66.0 (12.1, 39.7 to 95.1)</td>
<td>121</td>
<td>90.4 (13.7, 63.7 to 144.2)</td>
<td>48</td>
<td>107.6 (16.2, 74.0 to 145.3)</td>
</tr>
<tr>
<td>RR (br/min)</td>
<td>68</td>
<td>30.6 (5.2, 22.0 to 45.0)</td>
<td>121</td>
<td>28.3 (4.7, 18.3 to 41.2)</td>
<td>48</td>
<td>24.5 (4.6, 16.6 to 43.5)</td>
</tr>
<tr>
<td>Dead Space (mL)</td>
<td>68</td>
<td>19.7 (5.5, 10.3 to 31.81)</td>
<td>121</td>
<td>25.7 (5.7, 12.2 to 40.7)</td>
<td>48</td>
<td>29.6 (7.00, 13.7 to 46.8)</td>
</tr>
<tr>
<td>LCI</td>
<td>68</td>
<td>7.0 (0.8, 5.8 to 10.6)</td>
<td>121</td>
<td>6.8 (0.5, 5.4 to 8.5)</td>
<td>48</td>
<td>6.7 (0.6, 5.8 to 8.6)</td>
</tr>
<tr>
<td>FRC_{MBW} (mL)</td>
<td>68</td>
<td>158.8 (25.8, 102.5 to 225.9)</td>
<td>121</td>
<td>208.1 (38.1, 109.7 to 318.9)</td>
<td>48</td>
<td>251.6 (38.3, 154.0 to 334.2)</td>
</tr>
<tr>
<td>FRC_{PL} (mL)</td>
<td>43</td>
<td>178.9 (33.9, 110.6 to 286.7)</td>
<td>96</td>
<td>228.9 (40.9, 149.3 to 340.7)</td>
<td>29</td>
<td>279.9 (51.5, 192.8 to 417.7)</td>
</tr>
<tr>
<td>ΔFRC (mL)</td>
<td>43</td>
<td>16.6 (25.1, -59.0 to 64.2)</td>
<td>96</td>
<td>22.6 (34.3, -105.9 to 118.3)</td>
<td>29</td>
<td>30.0 (56.4, -50.7 to 196.9)</td>
</tr>
<tr>
<td>CEV (L)</td>
<td>68</td>
<td>1.1 (0.2, 0.7 to 1.7)</td>
<td>121</td>
<td>1.4 (0.3, 0.8 to 2.0)</td>
<td>48</td>
<td>1.68 (0.2, 1.0 to 2.4)</td>
</tr>
<tr>
<td>FEV05 (mL)</td>
<td>33</td>
<td>243.8 (47.6, 138.0 to 371.0)</td>
<td>70</td>
<td>312.1 (51.1, 220.0 to 466.0)</td>
<td>22</td>
<td>390.1 (51.7, 299.0 to 498.0)</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>33</td>
<td>302.7 (62.2, 162.0 to 447.0)</td>
<td>70</td>
<td>416.5 (78.1, 280.0 to 607.0)</td>
<td>22</td>
<td>543.5 (80.4, 382.0 to 678.0)</td>
</tr>
</tbody>
</table>

In order to properly evaluate the longitudinal change over time of the above variables, many of them required correction for lung size, either estimated by VT, subject length, or FRC. This standardizes the measures by accounting for increasing lung size as the subject grows. The resulting corrected parameters (by dividing for subject length) are described below in Table 4.
Table 6. Characterization of the mean outcomes of all performed pulmonary function tests with appropriate size corrections, separated by visit. Values expressed as number of subjects (SD, range).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early Infancy (N)</th>
<th>Early Infancy IPFT</th>
<th>Mid Infancy (N)</th>
<th>Mid Infancy IPFT</th>
<th>Late Infancy (N)</th>
<th>Late Infancy IPFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT/Length</td>
<td>68</td>
<td>0.99 (0.15, 0.64 to 1.38)</td>
<td>121</td>
<td>1.20 (0.16, 0.87 to 1.87)</td>
<td>48</td>
<td>1.31 (0.18, 0.90 to 1.74)</td>
</tr>
<tr>
<td>VD/Length</td>
<td>68</td>
<td>0.29 (0.07, 0.17 to 0.46)</td>
<td>121</td>
<td>0.34 (0.07, 0.17 to 0.53)</td>
<td>48</td>
<td>0.36 (0.08, 0.16 to 0.56)</td>
</tr>
<tr>
<td>FRCMBW/Length</td>
<td>68</td>
<td>2.38 (0.34, 1.65 to 3.37)</td>
<td>121</td>
<td>2.77 (0.48, 1.50 to 4.31)</td>
<td>48</td>
<td>3.06 (0.43, 2.05 to 3.92)</td>
</tr>
<tr>
<td>FRCPL/Length</td>
<td>43</td>
<td>2.67 (0.45, 1.81 to 4.15)</td>
<td>96</td>
<td>3.05 (0.50, 2.04 to 4.33)</td>
<td>29</td>
<td>3.42 (0.56, 2.41 to 4.86)</td>
</tr>
<tr>
<td>CEV/Length</td>
<td>68</td>
<td>0.02 (0.00, 0.01 to 0.02)</td>
<td>121</td>
<td>0.02 (0.00, 0.01 to 0.03)</td>
<td>48</td>
<td>0.02 (0.00, 0.01 to 0.03)</td>
</tr>
<tr>
<td>FEV05/Length</td>
<td>33</td>
<td>3.62 (0.59, 2.26 to 5.08)</td>
<td>70</td>
<td>4.16 (0.61, 2.97 to 5.68)</td>
<td>22</td>
<td>4.78 (0.61, 3.48 to 6.00)</td>
</tr>
<tr>
<td>FVC/Length</td>
<td>33</td>
<td>4.50 (0.78, 2.66 to 6.12)</td>
<td>70</td>
<td>5.54 (0.91, 3.73 to 7.47)</td>
<td>22</td>
<td>6.64 (0.84, 5.00 to 8.17)</td>
</tr>
<tr>
<td>VD/VT</td>
<td>68</td>
<td>0.30 (0.05, 0.21 to 0.46)</td>
<td>121</td>
<td>0.28 (0.05, 0.17 to 0.40)</td>
<td>48</td>
<td>0.28 (0.06, 0.13 to 0.40)</td>
</tr>
<tr>
<td>FRCPL-FRCMBW</td>
<td>43</td>
<td>0.24 (0.38, -0.91 to 0.93)</td>
<td>96</td>
<td>0.30 (0.46, -1.43 to 1.56)</td>
<td>29</td>
<td>0.37 (0.68, -0.61 to 2.29)</td>
</tr>
<tr>
<td>FEV/FRCMBW</td>
<td>33</td>
<td>1.51 (0.28, 1.04 to 2.29)</td>
<td>70</td>
<td>1.57 (0.30, 0.85 to 2.39)</td>
<td>22</td>
<td>1.59 (0.27, 1.07 to 2.12)</td>
</tr>
<tr>
<td>FEV/FRCPL</td>
<td>31</td>
<td>1.39 (0.27, 1.01 to 2.24)</td>
<td>67</td>
<td>1.40 (0.29, 0.86 to 2.23)</td>
<td>18</td>
<td>1.44 (0.36, 0.72 to 2.23)</td>
</tr>
<tr>
<td>FEV/FVC</td>
<td>33</td>
<td>0.81 (0.05, 0.70 to 0.90)</td>
<td>70</td>
<td>0.76 (0.07, 0.58 to 0.89)</td>
<td>22</td>
<td>0.73 (0.09, 0.48 to 0.85)</td>
</tr>
</tbody>
</table>
4.2 Descriptive Variables

4.2.1 Tidal Volume & Respiratory Rate

As expected, VT increased relatively linearly with age, while RR decreases. This is summarized in Table 7 below.

Table 7. Changes in tidal volume and respiratory rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth Rate</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal Volume / Length</td>
<td>0.01554 mL/cm/month</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Respiratory Rate</td>
<td>- 0.2347 br/min/month</td>
<td>0.1071</td>
</tr>
</tbody>
</table>

To adjust for increased subject size, VT was divided by length as a proxy for lung volume. Despite the general correction for body length, VT continued to increase. Length-corrected VT was increasing at a rate of 0.01554 mL/cm/month, which was statistically significant (p = < 0.0001).

A decrease in respiratory rate is observed, indicating a negative relationship of RR and age. A non-significant trend that demonstrates RR decreases with age throughout infancy. RR is decreasing at a rate of 0.2347 breaths/min/month (p = 0.1071).

4.3 Lung Clearance Index

As previously mentioned in Section 1.4.2.1, the LCI is already corrected for length. When fitted to a linear model, the LCI decreased at a rate of 0.045 units/month (Figure 4), which was statistically significant (p = 0.0021).
4.4 Lung Volume Measurements

Since the LCI was decreasing, it was appropriate to further examine the two components of the LCI measurement: the CEV, and FRC, with the results below in Table 7.

4.4.1 Cumulative Expired Volume

There is a clear, positive relationship between CEV and the age of the subject. When correcting for subject length in order to estimate changes in lung size, the relationship persisted. CEV corrected for subject length increased at 0.209 mL/cm/month (p < 0.0001).
4.4.2 Functional Residual Capacity

The other component of the LCI is the FRC. As previously mentioned, the FRC serves as a relatively reliable estimate of lung size, and thereby lung volume. It can be calculated using two different protocols – via MBW and body plethysmography. As each measure lends unique information regarding the size and function of the lung (Section 1.4.3), both were studied, and are described below.

4.4.2.1 FRC via MBW

We found that the FRC increased with length. Once corrected for subject length, the positive relationship persisted. With the model applied, the FRC changed at a rate of 0.0464 mL/cm/month. This change was statistically significant (p < 0.0001).

4.4.2.2 FRC by Body Plethysmography

Similar to FRC as measured by MBW, we found that FRC from body plethysmography increased with age. With the statistical model applied, the FRC changed at a rate of 0.0393 mL/cm/month. This was found to be significant with p= 0.0006.

Table 8. Measure of growth in CEV, and FRC by MBW and plethysmography.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth Rate</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEV / Length</td>
<td>0.209 mL/cm/month</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FRC by MBW</td>
<td>0.0464 mL/cm/month</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FRC by Plethysmography</td>
<td>0.0393 mL/cm/month</td>
<td>0.0006</td>
</tr>
</tbody>
</table>
4.4.2.3 Difference in FRC – Measured by Body Plethysmography and Multiple Breath Washout

In general, it was found that FRC as measured by plethysmography was higher than FRC as measured by MBW. This increase in FRC seems to be constant over age (p = 0.8039). This relationship is exhibited in Figure 5.

![FRC_{MBW} and FRC_{Pleth} vs. Age](image)

Figure 5. FRC_{MBW} and FRC_{Pleth} plotted concurrently against age in months. Both FRC measures are increasing with age, however FRC_{Pleth} remains consistently higher than FRC_{MBW}.

4.5 Forced Flow Measurements

The FRC, and CEV measurements focus on lung volume, and give a reasonably good estimate of growth of the lung parenchyma. In contrast, section 4.5 presents results from measures that
capture details about the large, conducting airways. These measurements are $\text{FEV}_{0.5}$, FVC, and FEV/FVC. RVRTC.

### 4.5.1 $\text{FEV}_{0.5}$ & FVC

In this sub-cohort, $\text{FEV}_{0.5}$ increased with age. Length correction of this measure did not change the relationship. $\text{FEV}_{0.5}$ had a change of 0.0773 mL/cm/month ($p < 0.0001$). (Table 8)

FVC followed a similar pattern as $\text{FEV}_{0.5}$, demonstrating a near-linear positive relationship between FVC and age of the subject. Again, similar to $\text{FEV}_{0.5}$, length correction did not change this relationship. The amount of increase of FVC per cm of growth per month was 0.1414 mL/cm/month, and was statistically significant ($p < 0.0001$). (Table 8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth Rate</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{FEV}_{0.5}$</td>
<td>0.0773 mL/cm/month</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FVC</td>
<td>0.1414 mL/cm/month</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### 4.5.2 FEV/FVC Ratio

Plotting the $\text{FEV}_{0.5}$/FVC ratio over the age in months, as shown in Figure 6, shows a negative relationship. This decrease can be ascribed to the faster increase in FVC relative to $\text{FEV}_{0.5}$, and occurs at a rate of -0.00496 mL/cm/month, and was statistically significant ($p=0.0075$).
Figure 6. Relationship of FEV/FVC and age in months. FEV/FVC decreases significantly with age throughout infancy.

4.6 Sex Differences in Pulmonary Function

In all of the above analyses (Section 4.2- Section 4.5.3), a separate analysis was performed by sex, in order to determine if there were any differences. No differences were found between sexes in any of the pulmonary function outcomes listed below.

4.7 Relative Rate Changes

Several of the relationships observed above, it was found that the relationships were described in sections 4.2 – 4.7 have non-linear associations. In light of this, it would be inaccurate to model the relationships using a single linear regression. As seen in many of the relationships, the non-linearity arises from a shift in the model around the 12-month age point. This indicates that there may be a change occurring in the development of the respiratory system, and that the rate of development differs between early and late infancy. To confirm this, analysis should be performed separately for the PFTs occurring at this break. Therefore, we created a linear model
using the data from the 2 to 12-month age points, and the 12-24 age points. From this point forward, the data will be split into these two categories, early infancy, and late infancy.

Slopes were calculated for each of the lung function parameters. We report the relative change for each slope between early and late infancy below. It is described as a ratio of early infancy to late infancy (Table 9). In general, the data in the table demonstrates that the most rapid growth occurs in early infancy (< 12 months), and slows significantly afterwards.

Table 10. Relative rates of change in early and late infancy, and degree of change.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early Infancy Rate (/month)</th>
<th>Late Infancy Rate (/month)</th>
<th>Δ in Rate (/month)</th>
<th>Proportion of Early Infanty</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT*</td>
<td>0.034 mL/cm</td>
<td>0.016 mL/cm</td>
<td>-0.018 mL/cm</td>
<td>2.12</td>
</tr>
<tr>
<td>FRC_{MBW}*</td>
<td>0.049 mL/cm</td>
<td>0.027 mL/cm</td>
<td>-0.022 mL/cm</td>
<td>1.79</td>
</tr>
<tr>
<td>FRC_{PL}*</td>
<td>0.085 mL/cm</td>
<td>0.048 mL/cm</td>
<td>-0.037 mL/cm</td>
<td>1.78</td>
</tr>
<tr>
<td>CEV*</td>
<td>0.44 mL/cm</td>
<td>0.16 mL/cm</td>
<td>-0.28 mL/cm</td>
<td>2.81</td>
</tr>
<tr>
<td>FEV_{0.5}*</td>
<td>0.11 mL/cm</td>
<td>0.030 mL/cm</td>
<td>-0.082 mL/cm</td>
<td>1.36</td>
</tr>
<tr>
<td>FVC*</td>
<td>0.20 mL/cm</td>
<td>0.037 mL/cm</td>
<td>-0.016 mL/cm</td>
<td>1.23</td>
</tr>
<tr>
<td>FEV/FVC</td>
<td>-0.0076</td>
<td>-0.0056</td>
<td>0.0020</td>
<td>1.36</td>
</tr>
</tbody>
</table>

* Denotes variable divided by length in order to account for body size

4.8 Relative Inter-Variable Growth Rates

Given that intra-variable percentile changes were different between early infancy and later infancy in all of the variables (Table 9). Rates between variables must also be compared between early and late infancy.
4.8.1 Lung Volumes

As previously mentioned, the FRC is a good approximation of lung volume, and thus parenchymal growth. Because there are two different protocols for measuring FRC (by body plethysmography and by MBW), it is useful to compare how the two FRC measures change over time. In early infancy, FRC\textsubscript{MBW} increased 24.21%, and similarly FRC\textsubscript{PL} increased 23.95%. In later infancy, both rates slowed significantly, FRC\textsubscript{MBW} increases 10.09%, and FRC\textsubscript{PL} increases 11.75%. Thus, FRC\textsubscript{MBW} slowed more than FRC\textsubscript{PL}, leaving FRC\textsubscript{PL} to increase at a slightly faster rate. While the rates are not drastically different, these two rates are statistically different (p < 0.05). Therefore, while the two FRC protocols appear to grow at similar rates in early infancy, FRC\textsubscript{PL} appears to begin to grow faster than FRC\textsubscript{MBW} in later infancy. However, there is little discrepancy between the two measures, as FRC\textsubscript{PL} slowed by 1.781-fold, and FRC\textsubscript{MBW} slowed by 1.789-fold.

4.8.2 Components of LCI

The LCI is composed of the FRC and CEV. Because there is a decrease in the LCI over time, it can be potentially explained by evaluating the components individually. As previously mentioned, FRC\textsubscript{MBW} increases 24.21% in early infancy. The CEV on the other hand increases only 18.86% in early infancy. Thus, the FRC\textsubscript{MBW} is increasing faster than the CEV (p < 0.05). This relationship is maintained in later infancy, as FRC\textsubscript{MBW} increases 10.09%, and CEV increases 8.12% (p < 0.05). Thus, the FRC\textsubscript{MBW} continuously increases faster than CEV throughout infancy. Similarly, the CEV slows more than the FRC\textsubscript{MBW}, with the measures decreasing by factors of 2.81 and 1.79 respectively.

4.8.3 Parenchymal Volume Growth vs. Airway Growth

As FRC can be used to estimate parenchymal size, FEV is a representation of the airway diameter. Comparing the relative growths of these two measures should offer insight into the growth of the parenchyma and the airway, and thus the degree of dysanapsis that is occurring.

FRC\textsubscript{MBW} in early infancy increases by 24.21%, and FEV0.5 increased 23.1%, suggesting that the two measures are similar (p > 0.05). In later infancy, the rate of FRC\textsubscript{MBW} increase slows to 10.09%, and FEV increase slows to 12.98%. While this suggests that FEV0.5 is then growing faster in later infancy, the two measures are still growing at relatively similar rates.
When performing a similar analysis with FVC instead of FEV0.5, it is found that while FRC_{MBW} in early infancy increases 24.21%, FVC increased 40.81%, which is statistically significant (p < 0.05), and also represents the largest change in all of the lung function parameters collected. The trend continues in late infancy, with a 10.09% increase in FRC_{MBW}, but a 18.89% increase in FVC (p < 0.05). This demonstrates that FVC is increasing significantly faster than the FEV0.5 throughout infancy.
Chapter 5
Discussion

5 Discussion

In this study, we have demonstrated that despite rapid parenchymal growth, we were unable to detect functional evidence of dysanapsis in tidal pulmonary function measurements. Instead of lung volume measurements increasing at a faster rate than measurements of airway caliber, we found that in infancy, lung volume by MBW and airway diameter estimated by spirometry increase at similar rates. What we did find, however, is that changes in FEV/FVC seem to indicate that the functional dysanapsis is present during forced expiratory maneuvers. That is, the forced volumes do not increase at the same rate as forced flow measures of airway diameter. The rapidly growing lung parenchyma may not be fully utilized during tidal breathing. It may only demonstrate its larger size and rapid growth when the infant is forced to maximize its respiratory system’s capacity.

5.1 Parenchyma and Airways Grow at Similar Rates

Previous studies have suggested a dysanapsis between the growth of the lung parenchyma and the conducting airways. (24) However, all the studies that supported this theory were performed in adults. Thus, the dysanapsis may become more apparent later on in life.

By observing the relative changes of certain parameters that we have collected, it is possible to infer the relative growth of different parts of the lung. The FRC, because it is a resting lung volume, can be used as a reasonably good estimate for the size of the lung. As the majority of the volume of the lung is made up of the parenchymal tissue, changes in parenchymal volume and growth can be roughly approximated by changes in FRC. Similarly, FEV$_{0.5}$ can be used to approximate airway caliber (however, the models upon which this is based, were constructed in adults). Observing longitudinal changes in FEV$_{0.5}$ can offer information regarding the growth of the conducting airways during infancy. It is essential to compare the height-corrected variants of these measures, in order to account for individual size variation as much as possible. Comparing the height corrected relative changes in FRC and FEV$_{0.5}$ can offer insight as to whether the lung parenchyma and conducting airways are growing in tandem in infancy, or if dysanapsis is occurring.
In both early and late infancy, FEV and FRC are increasing in similar amounts (though the degree of growth slows in both measures). It had been hypothesized that FRC would increase faster than FEV$_{0.5}$ due to the well-known expansion of alveoli during this time. (17) The difference in rates is statistically significant, however it is a small functional difference, and not what was initially expected. This suggests that despite the continuous expansion and growth of the alveoli, the conducting airways grow at a rate that functionally matches the rapid growth of the lung parenchyma.

While this may be counterintuitive, given that the alveoli are increasing in both number and size, Zeltner et al. also found that there is an overproportioned growth of the ‘air-carrying structures’ of the lung, compared to the parenchyma in the first 18 months of life, as performed via morphometric, cadaveric studies. (25) Thus the rapid division of the alveoli may rapidly match the size of the airways in very early infancy, and subsequently grow together to match their functional performance.

Other studies have also attempted to quantify the amount of growth that occurs in the airways. Hislop et al. described the change in the diameter of the main bronchus (left) as $D(\text{mm}) = 0.67 + 0.043 \times (\text{post-conceptual age in weeks})$. (73) This allows for estimation of airway diameter at a given age. By observing changes in flow, changes in airway size can be inferred. In addition, changes in flow can be approximated using Pouseille’s Law (Equation 4). This allows the determination of whether the flows measured in this study accurately reflect airway growth.

**Equation 4.** $F \propto \frac{\Delta P \cdot r^4}{\eta \cdot L}$

Assuming ages of 3, 12, and 24 months (53, 92, and 144 weeks’ post-conception), we can obtain estimated bronchial diameters of 2.95mm, 4.63mm, and 6.86mm respectively. Assuming that the pressure difference ($\Delta P$), and density ($\eta$) as constant, and taking into account that our percent changes reported consider subject length (thus need to divide by length twice, using US CDC 2000 50th percentile for age), we can approximate the expected differences in flow. From 3-12 months, there is an expected increase of 288% and from 12-18 months an expected increase of 258%. Because these calculations include many assumptions and approximations, these numbers are very far removed from our actual data. Furthermore, these are approximations of the flows in a single main bronchus, and not the collective flow of the respiratory system.
Nevertheless, it demonstrates that the earlier changes in airway diameter cause higher increases in flow than are seen in later infancy, due to the expected linear nature of airway diameter growth and the exponential impact of radius on flow. This also demonstrates how large changes in function can occur, despite relatively smaller morphometric changes in the airway.

This contrasts the results in which $FRC_{pl}$ was increasing faster than $FRC_{MBW}$, which suggested that the parenchyma might be growing faster than the conducting airways. However, in tidal maneuvers such as MBW, the flow rates may not have been large enough to facilitate the movement of gas into and around the periphery, thus not permitting SF6 to ventilate into some regions. This would lead to lower $FRC_{MBW}$ values – as was found in our dataset.

Another explanation for matched increase in flows compared to parenchymal measures is that alveolar multiplication may be filling the thoracic cavity with more alveoli, which form attachments to the conducting airways. (74) These alveoli with their airway attachments may form a sort of scaffold, holding the airway open despite the elastic recoil of the airway wall. (74) These tethers may not only hold the airway open to a larger diameter, but may also hold them open longer. Furthermore, as more alveolar units form, the new pulmonary arteries and capillaries attach their adventitia to that of the alveoli and airways (in order to facilitate gas exchange). Due to the turgidity of these vessels, they also serve to maintain airway patency. (22) These scaffolds and attachment points may have a significant effect on infant lung function, as studies have shown that in those with decreased lung function due to maternal smoking, there is a significant reduction in the amount of these attachments. (75)

5.1.1 Large Increase in Forced Vital Capacity

The largest amount of growth was seen in the FVC throughout infancy, and thus also supports the theory of alveolar scaffolding and stenting. The FVC measure is not as focused on the caliber of the large airways as FEV0.5, since FEV0.5 relies heavily on flow limitation for both consistencies of the test and information regarding the lung itself. The more the flow is limited; the less gas will be measured during the designated time frame of 0.5 seconds. However, assuming that the rest of the lung is in good health and is able to clear gas from ventilating units, flow limitation will not affect the FVC measurement, as there is no time restriction. Therefore, FVC is both a measure of airways diameter as well as overall parenchymal volume. Hence, it is not surprising that the FVC, even when correcting for subject length, has the largest relative
percent change in both early infancy and late infancy. FVC increases due to both the increased overall volume of the lungs from the increase in parenchyma and the increase in the diameter of the conducting airways. The sharp increase in FVC in early infancy further reinforces the notion that the largest expansion of the parenchyma and the greatest change in airway growth occurs in early infancy.

The large increase in FVC is not entirely explained by increases in parenchymal volume alone, as the FRC measures did not change as drastically as FVC measures did. Thus, the sharp increase must also include changes occurring within the airways. A possible explanation is that with increased cartilage in the larger conducting airways, the equal pressure point where airway closure occurs would be shifted to a higher pressure than is exerted by the chest wall, and thus would permit more complete emptying. By having the airways open for longer, more air is permitted to flow through before the airway lumen begins to decrease in diameter as a result of exterior pressure. This represents a shift from early infancy; whereas in early infancy, the larger airways may be more susceptible to closure from the pressure of the chest wall as the point of closure moves down the bronchial tree into the smaller airways. In early infancy, the FEV/FVC ratio is closer to 1, as the large airway closure causes most of the flow to be limited. As the infant ages, the larger airways are open for longer during a forced expiratory procedure, allowing the increased parenchymal volumes to contribute to the flows. This is especially important in the small, slowly ventilating airways (those with large time constants), which may contribute during an unlimited expiration such as FVC, but not in FEV1. The rapid rise in FVC suggests that these slow, peripheral units are not contributing to flow measurements in early life. This may also be occurring during tidal breathing maneuvers, such as MBW, creating a less efficient washout, and higher LCI in early life.

Another potential reason as to why FVC is increasing faster than FEV, while FRC is not, is that the newly grown parenchyma and alveoli may not be necessarily ventilated well at tidal volumes. In the tidal maneuvers, the flow rates may not have been large enough to facilitate the movement of gas into and around the periphery, thus not permitting SF₆ to ventilate into some regions. However, because RVRTC employs a raised volume maneuver, it is possible that these peripheral units became ventilated, and thus participated in the FEV0.5 and FVC measurements, even though they would not have been measured by MBW. (71)
Furthermore, while the FEV_{0.5}/FVC ratio is decreasing in infancy, it is known to rebound to ~ 1 around 3 years of age. This is due to the shift from using FEV_{0.5} to FEV_{1}. In infancy, expiration is complete in less than 1 second, and thus FEV_{1} is an inappropriate measure. Initially, FEV_{0.5} takes approximately 50% of the time that it would take for a complete emptying of the lung to RV. However, as the infant ages, the lung parenchyma will continue to increase, and be increasingly limited by smaller airways leading to the newly formed alveoli. Increasing pressure generated by the infant upon forced expiration will lead to flow limitation, and it will take longer for the infant to expire fully. As such, the amount of gas measured by FEV0.5, compared to the overall lung volume, will decrease, lowering the FEV0.5/FVC ratio. However, once the infant is large enough that it can sustain a forced, flow-limited expiration beyond 1 second; FEV_{1} becomes a more appropriate measure of flow limitation. With the use of FEV_{1}, as opposed to FEV0.5, more of the lungs volume empties during the flow-limited expiration, and thus the ratio of FEV/FVC, returns closer to 1.

5.1.2 Decreasing LCI Due to Increased Ventilation Efficiency

We initially began to look at LCI in this study due to the previously reported decrease in LCI in early childhood. (52) Due to the reported rapid alveolar growth in infancy, we thought that perhaps the increased alveolar growth would improve ventilation efficiency. (17) Thus, we expected to see a decrease in LCI throughout infancy. The LCI does decrease very subtly, but systematically and significantly in normal healthy infants. This indicates that lung ventilation is becoming more efficient with age. However, the subtlety of this decrease suggests that the decrease previously seen in LCI likely occurs between infancy and preschool age, rather than during infancy itself.

Nevertheless, as the infant ages, both components of LCI, the CEV and FRC_{MBW}, increase. This is not surprising, as the lung is increasing in size as well as complexity, as new alveolar units are added to the tracheobronchial tree, thereby increasing both FRC_{MBW} and CEV. However, if the CEV increases faster, it will cause an increase in LCI. Conversely, an increase in FRC will result in a decreasing LCI.
The latter situation is what was found in this data, FRC seems to increase at a significantly faster rate than CEV, indicating that the lung is becoming more efficient with age.

This could be due to either a decreasing proportion of dead space, or an increase in the efficiency of ventilation caused by increased patency of the conducting airways. The decrease in LCI also further supports the hypothesis of alveolar structure supporting the patency of the conducting airways. As the parenchyma develops more alveolar units, these alveolar sacs may develop attachments to the conducting airways, which would help to maintain alveolar and airway patency. This may prove especially important in the smaller airways such as the bronchioles, where there is an increased susceptibility to airway closure due to thoracic pressure. This increased ventilation efficiency caused by increased alveolar patency could also be responsible for the slower increase in CEV in relation to FRC. Both the decrease in relative dead space, and increase in ventilation efficiency could prove responsible for the decrease in LCI in infancy.

5.2 Differences in FRC between MBW and Plethysmography

Both MBW and plethysmography are used to measure FRC, which can be used as an analogue for the size (and growth) of the parenchyma. While the two FRC measures are both exhibiting a similar trend of increasing throughout time, they are not measuring the same thing (though there is some overlap). As previously mentioned, MBW only measures communicating airways whereas plethysmography measures all compressible gas. Therefore, plethysmography will capture the information offered by MBW, as well as any gas that may be trapped by plugged or collapsed airspaces. Our measurements support this, as it appears that FRC_{PL} is continuously higher than FRC_{MBW}. This indicates the presence of non-communicating gas in the thorax.

In observing the difference between the two FRC measures, it is possible to calculate the volume of non-communicating gas. When the trend is observed over time, a stable positive value is observed. Therefore, it appears that the two measures are rising in tandem. It had previously been thought that the early elevated LCI may be caused by weak and small infant airways collapsing, causing minor amounts of gas trapping. Yet, this data demonstrates that this is not the case, as the degree of trapped gas remains fairly constant with increasing age. Furthermore, other studies comparing FRC between plethysmographic and dilution procedures were unable to find consistent gas trapping in all infants, further supporting our evidence that healthy infants do not have a significant degree of airway closure or plugging. (49)
While the degree of gas trapping remains stable throughout infancy, there is a statistically significant difference in the rate of increase of FRC between the two measures. While both $FRC_{MBW}$ and $FRC_{PL}$ increase with age at what appears to be a stable rate, the growth rate actually differs between the two during late infancy. While the rates are similar enough to be non-significant in early infancy, $FRC_{PL}$ appears to be growing significantly faster in later infancy, despite a small difference in actual growth rate. This small difference may be an early sign of a developing trend. As the $FRC_{PL}$ measurement includes all compressible gas, this implies that there is unmeasured gas in the MBW model in late infancy. This may be caused by a change in communicating alveoli, as SF6 that was washed in to an area of the lung at the beginning of the washout has ceased to communicate. However, this is unlikely in healthy infant lungs. Alternatively, there may be areas of the lung into which SF6 has not yet washed in. This may be due to an expanded lung volume due to parenchymal growth, and which SF6 was not able to wash into units that have slow time constants, due to small peripheral airways. It is these SF6-free lung volumes that are being measured by plethysmography, but not MBW. This would suggest faster growth of the parenchyma as compared to the airways, however this difference is minor.

### 5.3 Longitudinal Change of Individual Parameters

As a result of attempting to understand parenchymal and airway growth, many of the PFTs performed have secondary outcome measures which are able to solidify previous work. This study adds unique longitudinal information for many of the published parameters.

Most of the parameters ($VT$, $FRC_{PL}$, $FRC_{MBW}$, $FEV0.5$) increase over time (despite length correction) – with the exception of RR which decreases. These variables continued to change despite length correction, which ought to compensate for increasing lung with age. $VT$ and RR change reciprocally in order to minimize the work of breathing, that is to increase $VT$ while decreasing RR. This is due to the stiffening of the chest wall, but also an expansion of the lung parenchyma. Similarly, the FRC also increases with time during infancy. This is true in both MBW, and plethysmographic protocols.
5.3.1 Functional Residual Capacity

FRC as measured by MBW maintains all the general characteristics of FRC that were described above. However, due to its use of gas dilution, the measured FRC only takes into account the areas and units of the lung that are communicating. This will thus include newly formed alveoli. \( \text{FRC}_{\text{MBW}} \) increases through time, both in early and in late infancy. Similar to other volume-based PFTs, FRC has a strong relationship with lung size, and thus was also corrected for by patient length. Similar to VT and CEV, the increasing relationship between \( \text{FRC}_{\text{MBW}} \) and age persisted, despite correcting for subject length.

While the conducting airways are also growing in size during this time, they only make up a small fraction (volumetrically) of the lung itself. Therefore, it is reasonable to assume that the increase in FRC is largely due to increases in the lung parenchyma. Analysis of the rate of increase of \( \text{FRC}_{\text{MBW}} \) (after correction for subject length) suggests that there is much more growth occurring in the first year of infancy, as compared to the second year. Many studies report that alveolarization likely goes on past the second year of life; however, this study suggests that the largest increase is occurring within the first year, and while it continues beyond this point, it does slow considerably. (17, 20) This may be due to more rapid alveolarization in the first year, rather than growth and expansion of existing alveoli. This would cause a greater increase in volume, as opposed to simple growth in length. The slowing of the rate of increase of FRC may be due to a shift in the pattern of alveolar growth. With the bulk of alveolarization occurring within the first year of life, there may be a shift to alveolar expansion with novel alveolarization taking a minor role. Alternatively, the difference in rates may be an artefact of the change in equipment dead space that usually occurs between the two time points. This could influence VT, and as a result the FRC as well. However, the slowing in rate is only seen once the FRC is corrected for height, and thus it is less likely to be an artefact of dead space.

\( \text{FRC}_{\text{PL}} \) is expected to increase with age, in a similar fashion as \( \text{FRC}_{\text{MBW}} \). Indeed, similar to \( \text{FRC}_{\text{MBW}} \), \( \text{FRC}_{\text{PL}} \) appears to increase near linearly with age, and this relationship is not eliminated by a length correction. Hence, this corroborates the data from the \( \text{FRC}_{\text{MBW}} \) dataset, as now two different measurement techniques are demonstrating that there is an increase in parenchymal/alveolar volume, despite the length correction.
5.3.2 Forced Expired Volume

The forced expired volume in 0.5 seconds (FEV$_{0.5}$) is a measure of larger airway function. As it is a good estimate for airway caliber, it is unsurprising to have found that the FEV$_{0.5}$ is increasing with age. Because airway caliber is correlated to lung size, FEV$_{0.5}$ was divided by length (similar to many other lung function parameters), and continued to increase despite the length correction. Unlike the other lung function parameters, the large airways measured by FEV$_{0.5}$ are no longer dividing or changing in morphology, but rather only increasing in size. This indicates a relatively fast degree of growth occurring in the airways. This may be driven by an attempt to compensate for the increasing amount of air available for ventilation due to the large increases in lung parenchyma. In fact, there is no significant difference between the rate of growth of FRC (length corrected), and FEV$_{0.5}$ (length corrected) in early infancy. This supports the notion that the airways are rapidly growing in order to compensate for the need established by the larger parenchyma.

5.4 No Gender Differences in Pulmonary Function Test Outcomes, and Rates of Change

Despite studies in adults demonstrating the existence of dysanapsis between genders, we did not find any significant differences in rates of change in pulmonary function outcomes between the two sexes. (24) We also were unable to find any meaningful differences in any of the individual measures of pulmonary function tests between the two sexes. This is in line with previous literature, which could not demonstrate any biochemical differences in infant sheep models, or any differences in terms of lung morphology. (76)

However, other studies did find differing lung function between males and females, with the dysanapsis being demonstrated in adult subjects. (24) Therefore, there is a chance that these differences in lung function require more time to develop. This is supported by the fact that in Mead’s study, dysanapsis was found between men and women, as well as between men and boys, while boys and women had similar ratios of lung volume to airway size. (24) This suggests that the dysanaptic difference between the sexes occurs later in development. This may be due to a combination of genetic predisposition and long-term environmental and hormonal exposure, which have not had enough time to manifest, and thus were not significantly detectable in our young, infant population. While there were no differences between the sexes, sex proved to be a
helpful variable in the modeling of predicted lung function and anthropometric measurements, and thus still ought to be considered in future studies.

5.5 Limitations and Considerations

While this is a longitudinal study, one of the major limitations of this study is that we were unable to complete longitudinal analysis of all subjects. Ideally, all recruited patients would have come in for testing at the early, mid, and later infancy visits. However, due to scheduling conflicts with families, most patients were not able to come in for all three visits. Indeed, most were only able to come for two visits, with a large portion of subjects coming in for only one visit. While these individual subjects were able to assist with the construction of the models used to predict values, they could not be used for the evaluation of relative rate changes, as they had no longitudinal data to offer. Furthermore, there was a high skew of subjects around the 12-month time point. Ideally, subjects would be more spread around all three points to ensure data accuracy throughout the range of time studied.

In addition to subjects not being able to attend all of the planned study visits, not all infants were able to complete all three stages of testing (MBW, plethysmography and RVRTC). Because the infants required sedation to complete the testing, those infants who woke up prematurely were not able to complete the testing. Infants were given weight-appropriate doses of sedative, however as each infant’s metabolism differs, and since not all infants were able to retain all the sedative (spitting out sedative), some infants awoke prematurely, thus ending their visit early.

Thus, due to the difficulty of having subjects come in for all scheduled visits, as well as the feasibility of completing all three PFTs per visit, overall there are less visits in the dataset than was intended. This may have influenced the power of the study. With increased power (by increasing subject/visit number), there is potential for detecting differences in measures and or populations that were not found in this study.

There were also technical factors that impacted this study. Due to the extremely small tidal breathing of the youngest infants, a different, smaller pneumotachometer was used in the smallest of the infant population, generally at the early infancy visit. This different pneumotachometer has a different dead space (0.01014 L vs. 0.0154 L) than that used for the larger subjects, and thus the equipment is not standardized across the visits, introducing a
variable that would ideally be taken into account upon interpretation of this data. Unfortunately, due to this change in equipment, longitudinal change in dead space (and its impact) was not able to be evaluated in this study. Furthermore, the subjects were lying supine and sedated during testing. This inherently has an impact on respiratory function (due to the effects of the sedative on breathing patterns, as well as the effects of gravity on the structures of the lung). As these conditions are not represented well in older pediatric or adult studies, this must be taken into account when comparing this study to those performed in older subjects.

Due to the young age of this population, it must also be considered that the modifications to spirometry that permit its use in this infant populations. Traditional spirometry relies on the subject being able to inhale to vital capacity, and then forcefully expire. This is then taken as a physiological measure as it relies on the natural abilities of the muscles, but most notably recoil of the chest wall and the elasticity of the lung to perform this action. This then gives an insight into the state of these structures, and thereby their relative functions. In infancy however, this is not possible, and thus RVRTC is employed. This simulates the inspiration by adding a positive pressure to the airway, and simulates expiration by compressing the thorax using a pneumatically controlled vest. As these maneuvers are not naturally performed by the infant, the resulting outcome measures are not truly physiological, as they rely partially on the efficiency of the technique, not just the function of the tissues. By adding this positive pressure into the airway, it is stacking a secondary “breath” onto the infants natural inspiration, which could be opening and ventilating lung units, which would not normally be used during a normal expiration, adding to the volume measured during this technique.

In addition, while it is interesting that FEV$_{0.5}$/FVC is decreasing over time, it cannot be solely ascribed to changes in the lung parenchyma. Indeed, while the lung is developing in infancy, the chest wall is also stiffening, as it becomes ossified. Due to the increased stiffness, the relative amount of transpulmonary pressure that can be applied by the jacket during RVRTC is decreased, potentially limiting the driving force of this forced expiration. That is, increasing jacket pressure is no longer proportionally increasing the transpulmonary pressure, as a result of the stiffer chest wall. Thus, RVRTC may not be simulating a forced expiration as well in older infants.
Finally, in this study, we aimed to use a healthy control population in order to better understand trajectories of lung function parameters in health, which subsequently helps to better identify trajectories of disease. This then required the elimination of subjects who have history of wheeze, respiratory conditions, or environmental exposure to factors that have been shown to impact early lung health and development, such as exposure to either pre or post-natal smoking. (63) While this is helpful in studying the physiology of the developing lung; and identifying healthy trajectories, the nature of IPFTs makes it limited in terms of applicability to most clinicians. Infant pulmonary function testing is intensive, as it requires specialized, expensive equipment that requires highly trained and qualified personnel to operate. As such, it is not feasible for physicians outside large academic medical centers to utilize IPFT to detect routine respiratory conditions in their healthy population. However, IPFT can be used in conjunction with these healthy trajectories in those more intensive centers, for patients with serious obstructive pulmonary disease. This would be useful in order to detect the subtle differences that are seen in infant disease, and to better understand the treatment effect of various therapies.

5.6 Conclusions and Relevance

This study addressed the longitudinal change in many popular lung function parameters, including LCI, a parameter that is gaining popularity due to its sensitivity in young, early disease populations. It also sought to determine the presence and degree of dysanaptic growth between various pulmonary structures, and whether there were any differences between the sexes in this regard.

The study demonstrated that LCI is higher in young infant populations and slowly begins to decrease over time into later infancy (and onwards into preschool years) – however the largest decrease in LCI is suspected to occur not during infancy, but in the transition from infancy to preschool age. This may to be due to an improvement in the efficiency of the lung potentially due to parenchymal tissue support of the airways. Furthermore, many of the lung function parameters that were evaluated were determined to change with age, despite correcting for size.

The study also demonstrated that despite the large degree of alveolar multiplication and growth, the conducting airways continue to grow along with the parenchyma in order to match the
increasing parenchymal volume throughout infancy during tidal breathing. However, forced
maneuvers require extra alveolar recruitment, and thus demonstrate increasing relative size of the
parenchyma as compared to the airways. Finally, no differences between the sexes were found
between any parameters, or in their rates of change or degree of dysanaptic growth.

This study is important, as it is the first to comprehensively evaluate multiple lung function
parameters longitudinally throughout infancy. This permits a more accurate image than
individual cross-sectional studies with different study populations, eliminating multiple sources
of variability. It also better helps characterize these pulmonary function tests, by demonstrating
which variables are the driving factors behind its change. This will help with the interpretation
of these tests for both research, and potential future clinical applications.

5.7 Future Directions

Many of these tests provide approximations and estimates with regards to what is actually
occurring inside the lung. Without simultaneous imaging, it is impossible to confirm whether the
changes seen in this study are truly due to differences in development and anatomy, or artefacts
of technical factors such as equipment dead space. Performing these pulmonary function tests in
conjunction with imaging studies, which help image the structure of the airways and their
ventilation, (ex: MRI using hyperpolarized Xe\textsuperscript{131}) has the potential to further solidify the findings
made in this study.

Furthermore, this study sought to understand the changes that occur in healthy individuals, in
order to better understand the changes that are seen in disease. Therefore, a similar study that
would include a population of children with disease such as cystic fibrosis and asthma in
conjunction with these healthy controls would be ideal in order to determine how these healthy
changes may go awry in a diseased state. This would also help to determine which of the infant
PFTs is best for the detection of disease, as there would be a direct comparison for expected
change in health vs. actual change in disease for each individual PFT.

Finally, this study uses lung function outcome measures in order to infer changes in anatomy and
function of tissue. However, a deeper understanding of what is occurring could be achieved by
performing a series of experiments that address more core physiological measurements, such as
pressure changes in various airways and across different spaces, such as esophageal or gastric
manometry. This would bring out a deeper understanding from what has been demonstrated in this study, as we would be able to identify what are the driving factors for the lung function changes that have been presented here.
References


