LUNG CLEARANCE INDEX AS A MARKER OF VENTILATION INHOMOGENEITY IN EARLY-childhood with HEALTH and DISEASE

by

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

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Abstract

**Rationale:** Ventilation inhomogeneity (VI) may be an early sign of obstructive airway disease. The lung clearance index (LCI) has been suggested as a sensitive marker of VI, although it has not been well characterized in young children in health and in those with CF and asthma.

**Objective:** To determine if LCI can detect VI in asymptomatic infants and preschool-age subjects with CF or wheeze/asthma compared to healthy controls.

**Methods:** Sulphur hexafluoride (SF$_6$) multiple breath washout (MBW) testing was completed in all subjects.

**Results:** LCI was found to be dependent on age in a large healthy control cohort. Accounting for age, it was seen that LCI was significantly elevated in disease groups compared to healthy controls in early childhood, illustrating early presence of VI in wheezy infants and the progression of disease in CF. Furthermore, the effects of breathing pattern and the variability of MBW parameters showed positive associations with age and VI.
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List of Abbreviations

AHR: Airway Hyper-Responsiveness
ASL: Airway Surface Liquid
ATS: American Thoracic Society
CDI: Convection-Dependent Inhomogeneity
CEV: Cumulative expired volume of air
CF: Cystic Fibrosis
CFTR: Cystic fibrosis Trans-membrane Regulator
CHILD Study: Canadian Healthy Infant Longitudinal Development Study
DCDI: Diffusion and Convection-Dependent Inhomogeneity
eNO: Exhaled Nitric Oxide
ERS: European Respiratory Society
FEF_{25-75}: Forced expiratory flow between 25% and 75% of vital capacity
FEV_{0.5}: Forced expiratory volume in 0.5 seconds
FEV_{1}: Forced expiratory volume in 1 second
FOT: Forced Oscillation Technique
FRC: Functional Residual Capacity
FVC: Forced vital capacity
GINA: Global Initiative for Asthma
HRCT: High resolution computed tomography
HSC: Hospital for Sick Children (Toronto, Canada)
ICH: Institute of Child Health (London, UK)
IgE: Immunoglobulin E

IPFT: Infant Pulmonary Function Testing

LCI: Lung Clearance Index

MBW: Multiple Breath Washout

MR: Moment Ratio

PCL: Periciliary liquid

PET: Positron Emission Tomography

PEV: Peak Expiratory Flow

RBM: Reticular basement membrane

RR: Respiratory Rate

RV: Residual Volume

RVRTC: Raised Volume Rapid Thoraco-abdominal Compression

SBW: Single breath washout

SIII: Phase III Slope

SnIII: Normalized phase III slope

TLC: Total Lung Capacity

TO: Lung Turnover

τ: Time constant

ULN: Upper Limit of Normal

Vd: Deadspace Volume

VI: Ventilation Inhomogeneity

VT: Tidal Volume

WHO: World Health Organization
Chapter 1:
Introduction
1 Introduction

1.1 Asthma in Early Childhood

1.1.1 Prevalence and definitions

Asthma is one of the most common chronic conditions affecting both children and adults in the western world, and is the leading cause for hospitalization of children in North America [1]. The prevalence of diagnosed asthma in school-aged children varies by population from 5% to over 15%, and has shown a marked increase in many countries in the past several decades [2-7]. Defining asthma has traditionally been very difficult owing to the variability of the disease in its clinical presentation, etiologies, symptoms and outcomes [8]. According to the most recent document by Global Initiative for Asthma (GINA), asthma is currently defined as “a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness (AHR) that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early evening. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment” [9]. As no one objective test exists to determine a definite diagnosis of asthma, a combination of symptoms, lung function testing results, bronchial sensitivity as determined by metacholine challenge testing and assessment of airway inflammation where possible is used in combination to diagnose this disease.

Symptoms of asthma can vary from very mild and easily controlled by therapeutic methods to extremely severe and refractory to treatment. The classic “asthmatic attack” occurs during acute exacerbation of asthma symptoms caused by a variety of factors such as exposure to allergens, exercise, viral or bacterial infection, air pollution, emotional stress and anxiety, etc., [8]. The aim of treatment for most asthmatics is to control their symptoms to avoid exacerbations that may lead to a potentially fatal attack; however, symptoms of asthma tend to be only the “tip of the iceberg” [10]. It is well recognized that abnormalities of the airways can be present before a diagnosis of asthma is made and can continue to be present even when symptoms have disappeared either with treatment or spontaneously. This is particularly relevant to children younger than 6 years old for whom a definite diagnosis of asthma is difficult. Up to 50% of infants have wheezing before the age of 2 years, yet only 10-15% of these infants will go on to have persistent wheeze and be
classified as asthmatic [7, 11]. High prevalence of infant wheeze, coupled with the lack of routine lung function testing under the age of 6 years means that asthmatics may not be diagnosed until school-age, at which time irreversible pathological airway changes may have already occurred [12]. Recent research into infant and preschool wheeze has discovered histological features of airway smooth muscle hypertrophy, thickening of the basement membrane, and hypertrophy of goblet cells and submucosal glands, all of which are characteristic of the pathological airway changes referred to as airway remodeling [13, 14]. The presence of these changes at such a young age has led many authors to conclude that the pathological features of asthma that persist into childhood and adulthood develop between the ages of 1 and 3 years, making this a critical time for monitoring and treatment [15-18].

1.1.2 Asthma phenotypes in early childhood

While asthma symptoms can begin at any age, early childhood onset is most common. The phenotypes of wheeze observed during the first few years of life include transient wheezing, nonatopic wheezing, persistent wheezing and late-onset wheezing [19]. Transient wheezing is related to decreased airflows and symptoms associated with viral infections [20]. Transient wheezing tends to resolve by mid-childhood and has no long-lasting effects on pulmonary function [7]. In cases of persistent wheeze, the first symptoms of wheeze and atopy are generally experienced before the age of 3 years and are associated with an increased total serum content of immunoglobulin E (IgE) and specific IgE [21]. Symptoms of wheeze are experienced with viral infections and between such infections. This phenotype is linked to pulmonary function deficits that persist into childhood and adulthood [22]. Most infants with persistent wheeze will go on to be diagnosed with allergic asthma, which tends to lead to more severe symptoms in the long-term [23]. Less is known about the more recently described phenotypes of nonatopic wheeze and late-onset wheeze. Nonatopic wheeze may result in decreased pulmonary function and symptoms that continue into later life, although individuals with this phenotype tend to have no history of allergy. Late-onset wheeze is less well understood. It occurs later in childhood (after 42 months) where individuals may have atopy and go on to experience more persistent symptoms of asthma, although not in all cases [23, 24]. As pulmonary function outcomes seem to depend on asthma phenotype, there is a need to distinguish between those infants who will go on to develop persistent asthma and those who have transient wheeze.
1.1.3 Pathogenesis

1.3.3 i: Inflammation and Airway Remodeling

Airway inflammation is a hallmark of asthma; it is one of the few constant features found in the many variable asthma phenotypes. It has been found in both the large and peripheral airways of asthmatics during childhood, with a tendency to be more severe in the peripheral airways as demonstrated by postmortem lung studies, surgically resected lung tissue and bronchial biopsy from patients with moderate to severe asthma [25-28]. Features of acute inflammation include airway obstruction by mucus and airway mucosal edema, infiltration of activated T-lymphocytes, mast cells and eosinophils in the airway wall, increased bronchospasm (contraction of airway smooth muscle), and vasodilation [29, 30]. The cycle of inflammation characteristic of asthma is believed to begin with sensitization by the presence of allergens in the airway. Current evidence shows the presence of airway inflammation in infants and preschoolers with wheeze, demonstrating that initial sensitization of the immune system occurs during the first few years of life [31-33].

While acute inflammation of the airway can result in many of the symptoms of asthma, long-term changes in the structure of the airway have also been observed in the absence of symptoms. Shedding of the airway surface epithelium, subepithelial fibrosis due to the deposition of collagen fibers under the basement membrane, hypertrophy of submucosal glands, hyperplasia of mucus-secreting cells, reticular basement membrane thickening, and increased smooth muscle mass are common findings and can be found in the airway of children under the age of 6 years with asthma [9, 34]. These changes are referred to as airway remodeling. The classical paradigm of asthma pathogenesis describes airway remodeling as resulting from a repair process in response to airway damage caused by inflammation. While repair of acute inflammation may be a normal process, it becomes pathogenic when chronic repair results in altered tissue structure and function [30]. There have, however, been recent evidence and opinions put forth that question whether airway remodeling is a direct effect of inflammation, as previously believed. These studies come mostly from investigation of airway changes in infants and preschool-age children; the time when airway inflammation and remodeling are believed to initiate [35]. For example, bronchoscopy of children with difficult-to-control asthma found the presence of features consistent with airway remodeling in the absence of airway inflammation [13]. It is possible that a combination of
genetics and environmental factors may affect airway caliber prenatally, leading to some structural changes during infant and preschool life that have been wrongly assumed to be the result of airway remodeling [7, 36]. New models have therefore been suggested; some have described eosinophilic inflammation and airway remodeling as being parallel events caused by some underlying “asthma factor”[37]; others which see airway remodeling as representing a primary airway pathology to which inflammation is a secondary problem [35, 36, 38]. Although the exact mechanism is unknown, what is clear is that airway inflammation and airway remodeling are present in asthmatics over the age of 6 years and are the cause of airflow obstruction [15, 23, 29, 31, 33]. This suggests a critical window during the first few years of life where much of the pathological airway changes associated with asthma occurs, and where monitoring and treatment of the disease needs to be focused.

1.3.3 ii: Airway Hyperresponsiveness and Smooth Muscle Changes

The functional abnormality in all asthma phenotypes is airway narrowing and resulting airflow limitation in response to stimuli that would not cause such airway changes in a normal person. This phenomenon is called airway hyperresponsiveness (AHR), and is caused by contraction of the smooth muscle surrounding the airway in response to inflammatory mediators. This type of contraction commonly occurs in small airways, and this site is recognized to be the principal site of airflow obstruction in asthmatic patients, including young asthmatic patients under the age of 5 years [39, 40]. Airway narrowing caused by smooth muscle contraction is often amplified by airway wall thickening and mucus present in the airway lumen of asthmatic patients [9]. Therefore, inflammation and airway remodeling act in concert to increase not only the mean but the heterogeneity of constriction in the small airways of the lung [41]. Numerous imaging studies have demonstrated that this contraction is heterogenous within the lung, and can result in ventilation inhomogeneity (VI) that occurs both in the proximal small airways that are visible on scans and in the very distal airways that are often beyond the spatial resolution of even the most powerful scans [42, 43]. A recent study that used 3-dimensional functional modeling to match in vivo positron emission tomographic (PET) scans and data from the forced oscillation technique (FOT) that demonstrated ventilation and mechanical dysfunction from asthmatic patients found that heterogenous small airway closures, caused by muscle contraction or narrowing by inflammation, is necessary and sufficient for ventilation dysfunction and contributes to mechanical dysfunction [44]. Recent emphasis on treatment of peripheral AHR and inflammation demonstrates a growing interest in the small airway pathology of asthma [25, 39, 45-49].
Unfortunately, determining the function of the small airways by conventional lung function tests, especially in young children, remains difficult, as will be discussed in section 1.5.

1.2 Cystic Fibrosis

1.2.1 Prevalence, definitions and etiology

In addition to asthma, cystic fibrosis (CF) is one of the most common chronic obstructive lung diseases present in early childhood. CF is also one of the most common fatal, autosomal recessive genetic disease affecting Canadian children and young adults. In Caucasians worldwide, 1 infant in every 2500 live births will be born with CF [50]. The median survival age of individuals with CF in Canada is around 45 years, and has steadily been rising over the past few decades as a result of more effective therapies and available resources [51]. While it is a multi-organ disease, the effects of CF on the pulmonary system are particularly severe and life-threatening.

CF is caused by a genetic mutation in the cystic fibrosis trans-membrane regulator (CFTR) protein, which normally forms a chloride ion channel that is present on the apical surface of epithelium within the airways and in other organs including the pancreas, gastrointestinal tract, skin, liver and seminal ducts in males. There are over 1000 known mutations in the CFTR gene that can lead to dysfunction of the CFTR protein, although over 70% of the disease alleles worldwide are related to one specific amino acid deletion [52]. Normal CFTR function is required for the regulation of other chloride and sodium channels such as the outwardly rectifying chloride channel and the epithelial sodium channel. In combination, these ion channels work to control the salt content and height of the airway surface liquid (ASL) layer that lines all airways within the lung down to the acinar region. The ASL can be divided into two components; the periciliary liquid (PCL) layer that lines the luminal surface of the epithelium and supports cilia, and the mucus layer that resides atop the cilia [53]. Mechanical clearance of mucus by beating cilia which transport of the ASL layer along the airway is a primary means of defense against inhaled pathogens and is formally referred to as mucociliary clearance [53, 54]. Effective mucociliary
clearance is dependent on the height of the ASL, particularly the PCL layer, which is determined primarily by ion transport across airway epithelium. By controlling the salt content within the airway, normal airway epithelium is able to exert osmotic control of water movement across the membrane and maintain normal liquid layer heights [55, 56]. The loss of function of the CFTR protein caused by CF mutations leads to abnormal chloride ion conductance across the cell membrane and decreased water content of the ASL, leading to ciliary collapse and decreased mucociliary clearance [57]. The viscous mucus that remains in the airways becomes an incubator for the growth of pathogens which are not able to be cleared, leading to a cycle of infection and inflammation of the airway that eventually causes decreased lung function, impaired gas exchange and eventual lung failure.

Symptoms of cystic fibrosis appear early in life; most individuals are diagnosed before the age of 2 years [51]. Failure to thrive, constant productive cough, weight loss, bowel obstruction, frequent fatty stools, salty tasting skin and repeated, prolonged pulmonary infections are common manifestations of CF [58]. Diagnosis is usually confirmed with genetic screens for the most common CF mutations and sweat testing, where abnormal chloride levels in sweat points to CFTR dysfunction.

1.2.2 Pathogenesis of cystic fibrosis in early childhood

While infants are born with CF, the lungs of a CF newborn are essentially “normal”; this is mostly due to the presence of alternative chloride transport channels in the fetal lung to support tissue differentiation [59, 60]. Proper mucociliary clearance and regulation of the airway liquid ion composition becomes more important for air-breathing after birth, making newborns with CF immediately susceptible to problems with mucus clearance. Pathological structural changes and lung function decline occur within the first year of life for infants with CF [59, 61]. Small airway obstruction by abnormally viscous mucus is typically the earliest lesion that sets the stage for chronic infection to follow. It is therefore critical to detect pathological airway changes related to CF in children at an early age in order to begin therapeutic regimens that may maintain good lung function.

The two main components of CF airway disease are chronic infection and a particularly strong host inflammatory response to such infection. Pathogens typical in CF disease include
bacteria such as *Staphylococcus aureus, Haemophilus influenzae* and *Pseudomonas aeruginosa*. The inflammatory response within the airway is neutrophil-mediated. While neutrophils are normally a vital player in the innate immune response, their activation in CF airways is over-exaggerated and may work to maintain a state of chronic inflammation [62]. By interaction with bacteria and/or their products, local macrophages within the airway epithelia become activated and secrete an array of chemokine mediators that attract, activate and prolong the life of neutrophils [63]. An over-abundance of neutrophils and macrophages becomes present in the airways, releasing chemokines which continue the inflammatory response and oxidants and proteases that damage the airway epithelia [64]. Neutrophil elastase in particular is known to not only degrade elastin, but also to cleave immunoglobulins and components of the complement opsonization system used to clear bacteria. In this way, persistent infection is able to continue while an over-active neutrophil-mediated inflammatory response causes obstruction and damage of the airway [65, 66]. Dilation and inflammation of the bronchioles, a condition termed bronchiectasis, is common in CF, and causes the small airways to become easily collapsible and further decreases mucociliary clearance.

It has recently been suggested that inflammatory processes may occur in the airways of infants with CF without bacterial infection [67]. In this view, inflammation may arise from defects in the regulation of neutrophil recruitment by epithelial cells, causing an inappropriate recruitment of inflammatory mediators [68]. While some evidence contrary to this hypothesis has been reported [69], recent screens of CF infants have found structural changes and inflammation before bacterial infection has occurred [70-72].

While it is clear that the inflammatory process inherent in CF disease begins during infancy, clinical symptoms of CF may lag behind the establishment of airway inflammation and airway structural damage. Bronchoalveolar lavage and HRCT of infants under 1 year of age found that over 80% of infants who were tested were clinically asymptomatic and had evidence of bronchial wall thickening, neutrophil inflammation, pro-inflammatory cytokines, gas trapping and/or neutrophil elastase activity [70]. Clinical, non-invasive measures of lung function that are sensitive to early inflammation and obstruction are thus needed to monitor early changes in CF lung disease.
1.3 Structure and Function of Airways

Many of the pathophysiologic features present in chronic obstructive lung diseases, such as asthma and CF, are due to dysfunction of the airways. The primary function of the airways is to widely and efficiently distribute inspired, oxygen-rich air to millions of respiratory units called the alveoli. Not all airways, however, are the same. The bronchial tree can be roughly divided into large conducting airways and small peripheral airways, whose function and structure are quite different.

1.3.1 Bronchial Tree

In order to maximize the efficiency of gas exchange, inspired air must be widely distributed within the lung by airways to reach the large gas exchange surface area provided by alveoli. The conducting airways of the bronchial tree therefore develop as an extensive asymmetric branching system, starting at the bifurcation of the trachea into 2 primary bronchi and continuing through 23 successive branching generations until the distal gas exchange units are reached. The first 16-17 airway divisions contain conducting airways called bronchioles with the sole purpose of gas transport. After approximately 17 airway branching generations, alveoli become present along the airway and increase in density until the branching system terminates in alveolar saccules or ducts. These final airway generations distal to the most terminal bronchiole therefore participate in both gas transport and gas exchange, and form a respiratory unit called the acinus.

1.3.2 Large and Peripheral airways

With each branching generation the conducting airway becomes smaller in diameter. Large airways are generally defined as the first 7-8 generations of the bronchial tree. “Peripheral” or “small” airways are arbitrary terms generally referring to any airway smaller than 2mm in diameter, or approximately any airway distal to branching generation 8 [30, 48, 73]. It is important to note that the progression of the larger conducting airways to the smaller conducting airways and acinar regions is gradual and continuous, and occurs through a complex branching scheme that can make defining a boundary between small and large airways difficult, regardless of whether small airways be defined by size or function [74]. Despite the vagueness of the term, the peripheral
airways constitute a major portion of the bronchial tree and their function is necessary for effective ventilation of their subtended distal respiratory units [75].

1.3.3 Differences between peripheral and large airways

Peripheral airways differ from large airways both in structure and physiology. Structurally, large airways are lined by an epithelium consisting of ciliated and non-ciliated cells anchored to a reticular basement membrane (RBM). Non-ciliated cells are secretary cells with the capacity to secrete mucus if stimulated by inflammatory mediators [76]. Large airway walls are also supported by irregularly shaped cartilage and smooth muscle. The epithelium of peripheral airways is thinner, contains fewer ciliated cells with shorter cilia, and has secretory cells that do not have the ability to secrete mucus [77]. While peripheral airways are also lined with smooth muscle, cartilage disappears peripherally, and therefore most peripheral airways lack supporting cartilage. Once the acinus is reached, ciliated cells disappear and the airway wall is interrupted by alveoli. The thin wall of the alveolus (0.2µm or less) is crucial for efficient gas transport from the lumen to the capillary lining the wall of the alveolus [78]. In general, peripheral airways are composed of a wall that is thinner, less rigid, has fewer cell types, and is more susceptible to changes in diameter by external pressure forces compared to large airways.

Air flow dynamics within the airways is very much dependent on airway size and cross sectional area, and therefore differs between large and small airways. While a peripheral airway may be smaller in diameter than a large airway, the total airway cross sectional surface area exponentially increases with successive branching generations. As a result, resistance and linear velocity of gas transported within the airway both decrease peripherally. This allows peripheral airways to have laminar flow, while large airways tend to experience turbulent flow due to greater air velocities and a smaller total cross sectional area [75]. By the time inspired air reaches the most peripheral airways within the acini, forward velocity provided by bulk flow becomes so small that diffusion takes over as the primary mechanism of ventilation [74]. Due to the small size of the acinus, diffusion of gases is able to occur extremely quickly, making it an effective means of transport within the respiratory unit.

Another difference between peripheral and large airways is the elastic structure of the peripheral airway wall and its implications on airway patency. In the normal lung, breathing
mechanics (expansion and recoil of the lung) and radial traction on the airway by surrounding lung parenchyma aid to maintain the patency of the airway. As the walls of peripheral airways are elastic and possess no means of extra support such as the cartilage found in large airways, they can be easily deformed by surrounding pressures and are especially prone to collapse. Figure 1.1.1 demonstrates the balance of these forces acting outwardly to keep small airways open and those acting inwardly that tend to collapse them. Evidence that peripheral airway closure occurs in the normal lung was first described by Burger and Macklem, where it was seen that small airway closure occurred at the end of expiration as the lung approached residual volume [79]. The principal mechanical cause of such closure is surface tension at the interface between air in the airway and the liquid film lining the airways from the trachea down to the alveoli. This liquid lining acts as a protective barrier against infection, oxidation and dehydration of the airways, yet it adds considerable tension forces within the lung which are only limited by the presence of surfactant molecules [80]. As peripheral airways approach their smallest diameter during the end of expiration, it is possible for the liquid lining to block the airway, causing airway closure [80, 81]. Forces acting to open the airways during inspiration are usually enough to rupture the liquid blockage, although more permanent closures of peripheral airways are easily caused in pathologic conditions such as asthma and CF, where mucus, edema, structural deficiencies or inflammatory mediators may increase the tendency of small airways to collapse [82-84]. While these same surface tension forces may be present in large airways, the greater stiffness of these airways and the smaller curvature of the air-liquid interface on the inner wall of large airways reduces the compression forces acting inwards [80].
Figure 1.1.1. A model of an elastic small airway demonstrating those forces working to close the airway and those working to open the airway. The airway has a radius $r$, a thin liquid lining $h_0$, and wall thickness $h_W$ characterized by a stiffness represented by Young’s modulus $E$. Forces working to maintain the patency of such an airway include radial traction by surrounding lung parenchyma (modeled as springs with spring constant $k$) and the internal lumen pressure $p_{int}$. Forces acting inwardly which tend to collapse small airways include the surface tension caused by the air-liquid interface $\sigma$, and external pressures acting on the airway $p_{ext}$.

1.4 Development of the infant and preschool-age lung

In order to study functional deficits of airway changes related to asthma and CF in early childhood, developmental physiological changes during infancy and preschool-age must be taken into account. While lung function changes with growth throughout the pediatric age range and beyond, there are unique physiological elements present in early childhood due to the immaturity of the lung which may affect pulmonary function. This chapter discusses developmental elements of the infant and preschool-age lung.

1.4.1 The infant lung

At the time of birth, the infant lung is entering the final and longest stage of development; the alveolar stage. Table 1.2.1 outlines the stages of lung growth during gestation that precedes the alveolar stage. The switch from the intrauterine fluid environment to breathing air is dramatic, requiring the absorption of fetal lung fluid and the establishment of a functional residual capacity (FRC), the amount of air remaining in the lungs at the end of passive expiration, within the first few breaths [85]. As the basal metabolic rate of the infant at birth is almost double that of the adult, high oxygen requirements demand that the infant quickly adapt its underdeveloped lung to air-breathing [86]. Studies of human infant lung physiology over the past 4 decades have made it clear that the infant lung is not simply a miniature version of the adult lung, but is rather in a unique state of development. Table 1.2.2 provides a summary of the unique features of the infant lung to be discussed in this chapter.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Age Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>3-7 weeks</td>
<td>Out-pocket of primitive gut, formation of trachea, primary bronchi and segmental bronchi</td>
</tr>
<tr>
<td>Pseudoglandular</td>
<td>7-17 weeks</td>
<td>Differentiation of airway epithelial, cartilage, bronchial smooth muscle and pulmonary arteries and veins</td>
</tr>
<tr>
<td>Canaliclar</td>
<td>17-27 weeks</td>
<td>Formation of respiratory bronchioles, alveolar ducts and primitive alveoli</td>
</tr>
<tr>
<td>Saccular</td>
<td>28-36 weeks</td>
<td>Further development of alveolar cell types, synthesis of surfactant precursor molecules begins</td>
</tr>
<tr>
<td>Alveolar</td>
<td>36 weeks-2 years and older</td>
<td>Septation and multiplication of alveoli, growth of airway and lung size</td>
</tr>
</tbody>
</table>

## Table 1.2.2. Summary of Infant Lung Characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th>Anatomical or Physiological Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>High oxygen consumption</td>
<td>High basal metabolic rate</td>
</tr>
<tr>
<td>Dysanaptic lung growth</td>
<td>Uneven growth pattern between acinar region (substantial increases the size and number of alveoli) and airways (increases in size only)</td>
</tr>
<tr>
<td>Low lung volumes</td>
<td>Numbers of mature alveolar air spaces are low; less outward recoil of chest wall to balance inward collapse of lungs</td>
</tr>
<tr>
<td>High upper airway resistance</td>
<td>Small size of upper airway and obligate nasal breathing caused by structure of upper airway</td>
</tr>
<tr>
<td>Collapsible upper airway</td>
<td>Tissues of upper airway are soft and pharyngeal muscles are underdeveloped</td>
</tr>
<tr>
<td>High lower airway resistance and susceptibility to airway obstruction and collapse</td>
<td>Lower airways are small in absolute diameter and lack wall rigidity and radial traction from surrounding parenchyma</td>
</tr>
<tr>
<td>Proportionately large airways compared to lung volume</td>
<td>May minimize airway resistance caused by small size and high compliance of airways</td>
</tr>
<tr>
<td>Highly compliant chest wall</td>
<td>Soft bony structures and underdeveloped musculature of the thorax cause the infant chest wall to be ‘floppy’</td>
</tr>
<tr>
<td>Low lung compliance</td>
<td>Elastic nature of lung tissue is improved with alveolarization which is in early stages in the infant lung</td>
</tr>
</tbody>
</table>
1.2.2 i: Postnatal Lung Growth

The lungs increase in volume from approximately 80ml at birth to 6000 ml in the adult [10]. The vast majority of this development affects the acinar region due to the emphasis on alveolar growth in both number and size. Multiplication of alveoli occurs throughout the alveolar stage of development by the secondary septation of primary saccules, with the majority of new alveoli being formed before the age of 2 years [87, 88]. Enlargement in the size of alveoli continues until after adolescence [78, 89, 90]. A recent study assessing lung growth of children under the age of 3 years by multi-slice high-resolution computed tomography (HRCT) found that the air and tissue components of the lung parenchyma increased at a constant rate, suggesting that indeed lung volume primarily increases by alveolarization in this age group, and not by expansion of existing alveoli [91].

As the conducting airways are complete in number by the end of the canalicular stage during gestation, their growth during infancy is only in diameter and length. The extent of airway growth is dwarfed in comparison to the extent of alveolar growth [92, 93]. Recent techniques of measuring the number of terminal bronchiolar duct endings in newborns have shown that there is no significant increase in the number of these units after birth, adding support to earlier morphological studies that the bronchial tree develops before their subtended gas exchange units [94, 95]. In vivo studies have shown that airway conductance (the inverse of resistance) of both the large and small airways increases only slightly in the first 4 years of life, while lung compliance and volume increase greatly [90, 96-98]. Gerhardt et al. reported that lung compliance in healthy individuals increased approximately x25 by the age of five years [96]. This is suggestive of an uneven growth pattern between the airways and the acinar region in infants, where airways develop in size in a symmetrical manner with a constant relation to the rest of the lung, while acinar spaces increase in both size and number at a much faster rate, increasing compliance of the lung [99]. This uneven growth phenomenon has been termed ‘dysanaptic’ growth of the airways [10].

1.2.2 ii: Upper Airway

The anatomical configuration of the upper airways in infants is different than in older children and adults, causing infants to adopt breathing mechanisms that affect their respiratory physiology. For example, the epiglottis of the newborn is relatively large, floppy, and positioned
high in the pharynx so that its upper portion is in contact with the soft palate [86, 100]. Such a configuration, in addition to the relatively large size of the tongue compared to the mouth, strongly favours nasal breathing. The major consequence of obligate nasal breathing is the increase in resistance offered by the nasal passages, which can be 50% or more of the total airway resistance in the infant [101, 102]. The larynx and trachea are also less rigid in the newborn, and can easily be distended or collapsed. While the high position of the larynx and obligate nasal breathing is believed to provide benefits to the infant, such as the ability to breath during suckling and the dynamic breaking of expiration to artificially maintain a higher FRC, the greater resistance of the upper airway and its ability to collapse can also exacerbate any obstruction to the upper airway if it were to occur [86, 100]. Over the first few years of life, the larynx will move to a more posterior position and the epiglottis will assume a more rigid, smaller shape within the larynx, fostering the development of speech and easier mouth breathing [86]. Muscles responsible for maintaining upper airway patency will also develop over the course of infancy, overall improving the rigidity of upper airway structures.

1.2.2 iii: Lower Airways

As previously mentioned, the conducting airways are complete in number and demonstrate growth in size (diameter and length) that is proportionate with body length during infancy [91, 103]. In absolute terms, the airways within the infant lung are very small and may easily become obstructed simply due to their small size. Furthermore, developing tissues around the airways do not exert the same radial traction to maintain airway patency, causing further potential for obstruction due to airway closure and/or collapse [104, 105]. Compared to the adult lung, the infant lung is thus composed mostly of peripheral airways defined as those less than 2 mm in diameter, which provide greater airway resistance due to the lack of cross-sectional airway area in small infant lungs. Post-mortem studies, model analyses, and in vivo lung function tests have, however, demonstrated that the airways of infants are proportionately larger in size compared to their lung volume than in the adult. This finding was first reported in early lung function studies by Taussig et al.[106] and post-mortem examinations by Tepper et al.[107] and Horsfield et al.[103], and has since been supported by infant lung model studies [108]. Therefore, while the lower airways in the infant lung are indeed small in absolute terms, they are not simply scaled down versions of adult airways. Proportionately larger airways may aid infants in maintaining appropriate airflows and avoiding substantially increased airway resistance.
1.2.2 iv: Chest Wall, Lung Compliance and Breathing Mechanics

Breathing mechanics relies on a delicate balance of the forces acting to collapse the lungs and those acting to open them. In the adult, tidal breathing and the maintenance of FRC depends on the oppositely acting tendencies of the chest wall to recoil outwards and the lungs to recoil inwards. The movement of the chest wall and the lungs are coupled by a thin pleural fluid, forming a system that is powered by respiratory muscles, notably the diaphragm, and relies on pressure differences to open the lungs and then allow them to passively return to an equilibrium state (FRC). In the infant lung, breathing mechanics are similarly controlled, although there are some key differences in the characteristics of this system.

A unique feature of the infant pulmonary mechanics system is the high compliance of the chest wall. Compliance explains the ability of a tissue to stretch, or change volume, in response to a change in pressure. Infants have a less rigid bony structure and a very soft sternum which provides a poor base for rigid attachment of the ribs [85]. Furthermore, the intercostal muscles and the diaphragm of the infant are still developing strength, ultimately causing newborns and infants to have a floppy, or highly compliant, chest wall [109, 110]. Measures of chest wall compliance in infants under 1 year were found to be 1.4 times larger than those in infants older than 1 year, and correlated negatively with age in children 2 weeks to 3.5 years old [111].

The explosion of acinar lung growth within the first few years of life not only affects lung volume and gas exchange surface area, but it also changes the compliance the lung. Compliance of the lung is measured as the change in volume of the lung divided by a change in transpulmonary pressure, and is a characteristic of the lung tissue. Over the course of infancy, lung compliance increases, and is therefore low compared to a mature lung. Lung compliance measured immediately after birth increases substantially within the first minutes and hours of life [112]. This likely reflects changes in tissue elasticity brought on by the switch from the fluid intrauterine environment to external air at birth [85]. Over the course of infancy, lung compliance continues to increase along with lung volumes and alveolar multiplication [113]. Time constants (τ) of passively expired air, a measure of how low it takes for a percentage of an expired breath to leave the lungs, are calculated by multiplying compliance and resistance (C x R). It has been found that the τ of the respiratory system significantly increases during the first year of life due mostly to an increase in compliance and less to a decrease in resistance [114-116]. This is consistent with a dysanaptic infant lung growth; alveolar multiplication is dominant and improves the ability of the
lung to stretch with a given driving pressure, while airway growth in size is comparably modest leading to smaller decreases in resistance [117].

The effect of high chest wall compliance and low lung compliance is that the natural equilibrium position between the two, where the outward pull of the chest wall balances the inward pull of the lungs and where FRC is naturally established, is very low in infants. If infants were to expire until they reached this equilibrium position they would experience a large degree of airway collapse and ventilation-perfusion mismatching within the lung. Infants therefore artificially elevate their FRC through three mechanisms; i) a high respiratory rate that does not allow time for full expiration, ii) expiratory breaking by adduction of the larynx to increase resistance to airflow, and iii) post-inspiratory diaphragmatic activity [86]. Colin et al. determined that the respiratory pattern in infants was predominantly, but not universally, interrupted by respiratory breaking and post-inspiratory diaphragmatic activity below 6 months of age, and by the age of 1 year, FRC was generally maintained by passive respiratory mechanics [118]. Even with such corrective mechanisms, however, the infant lung is predisposed to higher levels of airway collapse and ventilation inhomogeneity caused by the imbalance between the compliances of the lung and chest wall [111, 119, 120].

1.4.2 The preschool-age lung

The alveolar stage of lung development ends around the age of 2-3 years completing the lengthy process of alveolarization by the formation of new alveoli. Until preschool-age, the volume of alveoli remains fairly constant, while the number of alveoli increases dramatically. After the age of 2-3 years, alveolar volume increases by the enlargement of alveoli [87]. The compliance of the chest wall is similar to the adult state, allowing the lung mechanics of preschoolers to be comparable to that of older children and adults [111]. Compliance of the lung has increased largely due to alveolarization during infancy, yet it will continue to improve with enlargement of acinar regions. Airway resistance increases throughout the preschool years, presumably with continued growth in the length of the airways [121]. The lung volume at which small airway and alveolar collapse occurs, known as the closing capacity, decreases during the preschool-age range [121]. This is further evidence that a combination of maturation of lung mechanics and increase in the rigidity of airways are acting to protect lung units from collapse. The normal preschool-age lung has presumably developed the functional units, structures and
characteristics of the mature lung; however, there is much growth that must now occur to match the degree of somatic growth that takes place in early childhood. Differences between lung volume and flow rates between boys and girls have been reported [87, 122, 123], with boys typically having larger lung volumes and higher forced expiratory airflows (L/sec) than girls, however more recent studies of preschool lung function have failed to find a gender bias [124]. In summary, the preschool age range represents a transition zone during which the lung moves from a state of immaturity during infancy to a state of functional maturity by the start of school-age.

1.5 Spirometry and the “silent years”

Measurement of lung function is important when monitoring early lung disease such as asthma and CF. The current “gold-standard” of clinical lung function testing is spirometry. Official guidelines for performing traditional (adult) spirometry are provided by joint task force documents by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) [125]. Spirometry measures maximal air flows (L/s) and volumes (L) at the mouth. Several forced flow and volume parameters can be calculated from spirometry and can be related to lung function (Table 1.5.2). By far, the most widely used parameter is forced expiratory volume in 1 second (FEV$_1$), which, when expressed as a percentage of the forced vital capacity (FVC) (FEV$_1$/FVC), is a global measure of airway obstruction [30, 126]. FEV$_1$ is strongly associated with mortality in the management of CF disease [127], and has been negatively correlated to levels of circulating eosinophils and symptom score in childhood asthma [128, 129]. Forced expiratory flow between 25% and 75% of the vital capacity (FEF$_{25-75}$) is regarded to be most representative of small airway function; it is often the first spirometric measure to decline in CF disease and it is found to be abnormal more often than FEV$_1$ in asthmatic children, although this measure is highly variable within individuals [130-132].
<table>
<thead>
<tr>
<th><strong>Measurement</strong></th>
<th><strong>Abbreviation</strong></th>
<th><strong>Explanation</strong></th>
</tr>
</thead>
</table>
| Forced Expiratory Volume in 1 Second                | FEV\(_1\)        | • Volume of air that can be forcibly expired in 1 second  
  • Overall measure of airway obstruction  
  • Typically used to report lung function in adults and older children |
| Forced Expiratory Volume in 0.5 Seconds             | FEV\(_{0.5}\)    | • Similar measure to FEV\(_1\)  
  • Usually reported in young children under the age of 5 years |
| Forced Vital Capacity                                | FVC              | • Volume of air that a subject can forcibly expire after full inspiration  
  • The ratio of forced vital capacity to forced expiratory volume in 1 second  
  • In healthy adults, this ratio is 80%, and tends to increase for younger children |
| FEV\(_1\)/FVC Ratio                                 | FEV\(_1\)\%      | • Measure of the mean flow between 25% and 75% of FVC  
  • Regarded to be more sensitive to small airway function  
  • The maximal flow achieved during a maximal expiratory effort |
| Forced Expiratory Flow between 25% and 75% of Forced Vital Capacity | FEF\(_{25-75}\)  | • Measure of the mean flow between 25% and 75% of FVC  
  • Regarded to be more sensitive to small airway function |
| Forced Expiratory Flow at 50% of Forced Vital Capacity | FEF\(_{50}\)    | • Measure of the mean flow at halfway of the flow-volume loop  
  • The volume of air inspired and expired during quiet breathing |
| Peak Expiratory Flow                                 | PEV              | • The volume of air inspired and expired during quiet breathing |
| Tidal Volume                                         | VT               | • The volume of air inspired and expired during quiet breathing |
1.5.1 Limitations of spirometry in children under 5 years of age

While spirometry is a very useful tool for aiding in diagnosis of lung disease and monitoring lung function over time in older children and adults, there are significant limitations to the use of traditional spirometry in children under the age of 6 years [133]. Infancy and preschool-age is often referred to as the “silent years” due to inability to monitor this age range using spirometry. The first major limitation of spirometry is that the test requires a high degree of subject cooperation and physical coordination to properly perform maximal exhalation maneuvers. For this reason, spirometry is often not attempted until a child has reached the age of 5 or 6 years old, at which time they are more likely to understand and perform the maneuvers required.

Secondly, forced flow and volume values measured by spirometry are essentially measurements of airway resistance at flow limitation. As the majority of overall airway resistance is offered by the larger airways, spirometry is relatively insensitive to small airway function and changes [134-136]. Macklem et al. observed that the complete obstruction of 50% of the small airways increased total airway resistance by only 10%, suggesting that spirometric measurements that detect elevated airway resistance would be of little use in quantifying small airway disease [75]. Furthermore, McNamara et al. have shown in excised canine lungs that distal heterogeneous airway emptying is masked by mechanisms present during forced expiration that tend to equilibrate different time constants of peripheral airway regions [137]. For children with asthma or CF, this shortcoming of spirometry is particularly relevant; early childhood (infancy and preschool-age) is the time in which the first disease lesions are present within the small airways. These early pathological changes do not affect the overall airway resistance of the lung, and therefore are often not detected by spirometry [138]. For this reason, the peripheral airways have often been called the “silent” or “quiet” zone [139]. It is well documented that school-age children with asthma and CF can have normal spirometry results despite evidence of substantial small airway inflammation [27, 35, 131, 140]. There is therefore a great need for an objective test of small airway function that can be used in young age groups to detect and monitor the earliest signs of chronic disease, before overall lung function has been affected.

1.5.1 ii: Preschool Spirometry and Raised Volume Rapid Thoracoabdominal Technique

In the past 2 decades, there has been interest in developing new techniques to gain spirometric-like measurements in children younger than 5 years. This has mainly been in an effort to solve the first limitation of spirometry mentioned previously; namely, the dependence of this
test on subject cooperation. For preschoolers aged 3-6 years, traditional spirometry has been adapted to suit the needs of very young children. High levels of coaching by the operator, incentives for the subject in the form of video games, and more lenient acceptability and repeatability criteria have made preschool spirometry feasible in a high proportion (over 80%) of children tested, including healthy subjects and those with disease [130, 141-143]. Official guidelines have since been published emphasizing the importance of pulmonary function testing in this age group [144]. While these results are encouraging, preschool spirometry is not practiced regularly in most centers. This type of testing requires skilled operators and different testing criteria than adult spirometry. Furthermore, while preschool spirometry may aid the first limitation of traditional spirometry, it does not address that fact that spirometry is a measure of airway resistance and is insensitive to small airway changes. For these reasons, it may not be a suitable clinical tool for regularly assessing disease in young children.

In the case of infants, traditional spirometry is not possible as it depends on some level of cooperation from the subject. New techniques, such as the raised volume rapid thoracoabdominal compression (RVRTC) technique, have therefore been developed to gain forced flow and volume measurements from sleeping infants. During testing, infants are sedated and fitted with a facemask while sleeping in the supine position. An inflatable jacket is placed loosely around the infants’ abdomen and chest. A pneumotachometer is fitted to the facemask to measure flow and volume (Figure 1.5.1). During quiet breathing, a series of rapid lung inflations are produced by administering positive pressure at the airway opening to raise lung volume to near total lung capacity (TLC). The jacket is then inflated to produce a forced expiration that continues until near residual volume (RV). Guidelines for safety, protocol and data analysis were last published by the ATS/ERS in 2005 [145], and studies by Lum et al. have done much to fine tune protocol standards [146, 147].

Forced flow measurements and lung volumes can be obtained from infants using the RVRTC technique that are comparable to the measurements gained using traditional spirometry, although physiological differences between infants and older children are evident. For example, as infants have much smaller lung volumes and are able to empty their lungs more quickly compared to older children, FEV in 0.5 seconds (FEV0.5) is usually reported instead of FEV1 [148]. The descending part of the flow/volume loop also tends to be more convex in infants and young children, representing larger airway size relative to lung volume in these young subjects which allows for greater flows [149]. Despite these differences in infants, it has been found that lung
function parameters measured by RVRTC are reduced in clinically stable infants with CF and are able to distinguish these infants from healthy controls [150, 151]. As it is known that early inflammation occurs in the small airways of CF infants, it may be that RVRTC provides more information about the small airways than traditional spirometry in older children due to the unique physiology of the infant lung; namely, the greater tendency for airway closure/collapse in infants [152]. A major limitation of this test is its feasibility as a clinical tool. Expensive equipment and highly skilled personnel are required to carry out RVRTC testing at a given center. Furthermore, infants must be sedated during the test, which may be a deterrent for parents and research ethics boards alike, especially in the case of healthy controls.

**Figure 1.5.1.** Schematic illustration of the equipment set-up for the raised volume rapid thoracoabdominal compression (RVRTC) technique. Exp = expiratory, insp = inspiratory. Reproduced from Lum et al.[145]
1.6 Gas Washout Techniques

The limitations of spirometry and RVRTC in measuring early airway pathology in CF and asthma often preclude the use of this test as a marker of disease in young children. A different approach must be taken in early childhood that allows for feasible testing in this young age group and that is able to detect small changes in airway function. The ability to detect and monitor early disease changes would allow for earlier intervention and may help to maintain long-term lung function [71, 153]. Gas washout techniques may be such an alternative technique. This technique includes the single and multiple breath washouts (SBW and MBW). Both tests involve the analysis of how a detectable resident gas of the lung (i.e. nitrogen) or an inert gas that is previously inhaled (i.e. helium or sulfur hexafluoride) changes in concentration as it is exhaled from the lung during a single breath or over the course of multiple breaths. Washout tests measure ventilation inhomogeneity (VI), which describes how efficiently inhaled gas mixes with the resident gas of the lung (see section 1.7). While VI is present even in healthy lungs, it may be increased in obstructive lung disease due to blockage of peripheral airways, which is the principal site of gas mixing [154]. Blockage/obstruction of airways has been shown to be distributed heterogeneously throughout the lung in imaging and post-mortem studies of both CF and asthma [155-157]. Importantly, ventilation defects identified in these imaging studies of children with asthma or CF were observed in the presence of normal spirometry in these subjects, demonstrating that measurements of forced flow are not able to adequately quantify levels of VI.

Gas washout techniques were first described in the 1950s due to the development of the first fast-responding gas analyzers [158, 159]. These classical studies were interested in developing a test that could investigate the normal physiology of lung ventilation and gas mixing and that could potentially differentiate between normal and diseased subjects [160-162]. By the late 1960s, gas washout techniques had been extended to use in the pediatric population [163]. The techniques seemed promising, yet they were limited by a lack of data processing technologies and complicated, unfocused analytical techniques. A second wave of research interest in these techniques occurred with the advent of the personal computer which allowed for real-time data recording, and online data storage and analysis [164]. This subsequent development of the gas washout tests, particularly the MBW, has propelled research into washout tests and their parameters, especially in the pediatric age range.
1.6.1 The Single Breath Washout

The SBW was first described by Fowler in 1949 following the development of fast-responding gas analyzers that allowed the concentration of a marker gas, such as nitrogen (N\(_2\)) to be measured during a single expiration [165]. In this classical definition, the SBW requires the subject to exhale to RV, followed by inhalation of 100% oxygen to TLC, then exhalation from TLC back down to RV [164]. This breathing maneuver is done at a low constant flow, and the concentration of N\(_2\) during the expiration is plotted against expired volume (Figure 1.6.1). Four distinct phases can be described in this expirogram that are the result of the anatomy of the bronchiole tree and the sequential arrival of air fronts from different parts of this tree; Phase I represents the absolute dead space usually contributed by equipment set-up; Phase II, the bronchial phase, produced by the sequential arrival of alveolar gas fronts from larger lung units; Phase III, the alveolar phase, which represents gas coming from the alveoli; and Phase IV, the closing volume phase where closure of some small airways at residual volumes causes an increase in N\(_2\) concentration. Variations to this procedure, including the inhalation of inert gas mixtures of 4% He and 4% sulfur hexafluoride (SF6) to a volume below TLC are thought to better reflect peripheral airway function at tidal volumes. The slope of the Phase III section of the washout curve is an indicator of gas mixing efficiency within small lung units, with an increased slope representing intraregional acinar VI over a range of lung volumes [166]. While this test was historically important during the first investigations into ventilation inequalities within the lung and continues to be useful for phase III slope analysis [167], it requires a level of coordination to perform breathing maneuvers and is not performed in young children.
Figure 1.6.1. Expirogram from a vital capacity single breath washout in a normal subject. Variations during the alveolar phase represent cardiogenic oscillations. VC: Vital Capacity. Reproduced from Robinson, P. D., M. D. Goldman, et al. (2009).[164]

1.6.2 The Multiple Breath Washout

The MBW was first described in 1950 by Robertson et al. as a method of assessing the “distribution of tidal breath into the deeper pulmonary structures”, and as a method of measuring FRC [168]. The major benefit of this test compared to the SBW is that it is completed during tidal breathing, requiring little cooperation from the subject and making it applicable to all age groups [169-171]. Original MBW tests used resident lung N₂ as a marker gas and a respiratory mass spectrometer as a gas analyzer. The subject would be required to inhale 100% oxygen, and over a series of multiple breaths, they would “washout” the resident N₂ in the lungs until the concentration of gas was 1/40th of its initial concentration at the start of the washout, or around 2%. This cut-off was historically the point at which the earliest N₂ gas analyzers lost resolution and accuracy, and is still the end-of-test criteria used today for MBW tests.

More recently, inert gas mixtures have been substituted for N₂ for a few key reasons. Firstly, breathing 100% oxygen has been reported to alter the breathing pattern of some subjects, particularly in infants [172, 173]. Secondly, excretion of small amounts of N₂ dissolved in the
tissues of the lung meant that additional correction factors had to be applied to attain accurate volume measurements when using N2 washouts; this could be avoided by using inert gases [174]. Lastly, by using an inert gas mixture of 2 gases with different densities and which equilibrate at slightly different airway generations within the airways, investigators could compare the washout curves of both gases to better pinpoint the location of VI within the lung. For these reasons, inert gas washouts are potentially more useful, especially in young children. As this is also the primary method used in this thesis, “MBW” will hereafter refer to SF6/He inert gas MBW unless specified otherwise.

Figure 1.6.2 illustrates the procedural components of an MBW test. A wash-in phase is required when performing the MBW, during which a subject inhales the gas mixture, typically containing 4% He and 4% SF6, until the concentration is stable within the lung. At this point, the subject is switched to breathing room air by disconnecting the bias flow during inspiration. The inert gas mixture is washed out of the lung over a series of multiple breaths until the end-tidal concentration of the gases is 1/40th of the initial concentration. Flow is recorded by a pneumotachometer, while He and SF6 concentrations are recorded by a mass spectrometer in real time by continuous sampling of breath and plotted against gas sampling time. Each MBW trial typically takes 5-7 minutes to complete, and at least 2 technically reproducible trials should be attained. Using a fast-responding respiratory mass spectrometer detector is considered the “gold standard” for the MBW technique, although other detectors have been used and may prove useful after validation [175, 176]. While the wash-in phase makes the MBW a more lengthy procedure, it provides more information on lung VI than SBW and can be used in all age-ranges [177].
Figure 1.6.2. Illustration of the multiple breath washout test procedure. A: Wash-in phase. Arrows demonstrate the direction of inert gas mixture flow; B: Washout phase during exhalation. The inert gas bias flow has been disconnected and the subject is breathing room air.
1.6.2 i: Parameters of the MBW Technique

Figure 1.6.3.A shows a typical MBW test recording from a 4 year old boy. Two types of parameters of VI can be estimated from the MBW during analysis. The first type includes parameters that reflect global VI within the lung, derived from characteristics of the washout curve. Although many such parameters have been described over the past 50 years, two parameters in particular have come to be most commonly reported. These include the lung clearance index (LCI) and moment ratios (MR).

The MR is calculated from the ratio between the actual and the estimated ideal number of breaths needed to lower the end-tidal gas concentration to $1/40^{th}$ of the initial value. In moment analysis, the progression of end-tidal gas concentration over the washout is plotted against lung volume turnovers (TO), which are calculated by the cumulative expired volume of each breath (CEV) divided by FRC. The area under this curve is called the $0^{th}$ moment. The first and second moments multiply the end-tidal gas concentration by the TO ($1^{st}$ moment) or the TO squared ($2^{nd}$ moment), which gives progressively more weight to the latter portion of the washout curve. The ratios of between the $1^{st}$ and the $0^{th}$ moment, or the $2^{nd}$ and the $0^{th}$ moment are then calculated. A higher ratio signifies that a greater portion of the lung is ventilated slowly, leading to higher gas concentrations at higher TOs [30]. Typically, moment analysis only includes the washout curve up until 8 TOs, allowing the calculated MR to be compared between subjects.

In the past decade, the LCI has become the dominant parameter reported for MBW studies (see section 1.8). While the LCI is theoretically similar to the MR and provides comparable information, it is conceptually easier to understand and calculate [154, 178, 179]. The LCI is calculated as the number of lung volume turnovers (TO) needed to washout the inert gas to $1/40^{th}$ of its original concentration. It is therefore calculated by dividing the cumulative expired air volume (CEV), or the summation of tidal breaths over the course of the washout, by the FRC (Equation 1). Lung volume after a passive expiration, or FRC, can be calculated by the MBW by dividing the net volume of inert gas exhaled over the washout by the difference in end-tidal gas concentration at the start ($C_{et_{in}}$) and end ($C_{et_{fin}}$) of the washout (Equation 2). The higher the LCI value, the more TOs are required to washout the tracer gas, which reflects a decrease in mixing efficiency within and between lung units likely caused by mechanisms of VI within the lung. Mechanisms underlying increased VI which may lead to increased LCI values are discussed in section 1.8.
The second type of parameter that can be gained from the MBW is attained by the analysis of the phase III slope (SIII), or alveolar phase, of each breath within the washout (Figure 1.6.3B). These parameters convey more mechanistic information about small airway VI by providing more detail on the origin of obstruction within the airways [167]. This type of analysis can be thought of as many SBW analyses; each breath is isolated and analyzed separately using custom computer software. Figure 1.6.3B shows a typical SF6 tracing from a single breath of the MBW. The same gas washout phases that were described for the SBW are present except for Phase IV, the closed volume phase, which rarely occurs within the tidal breathing range of an MBW in a healthy subject. The phase III, or alveolar phase, represents the average of many gas concentration fronts arriving from the alveoli during expiration. An increased Phase III slope (SIII) is caused by differences in gas concentration between lung regions that have gas fronts that arrive at the mouth at different times due to sequential emptying of better-ventilated regions first. By plotting how the SIII changes with each breath of the MBW, indices of inhomogeneity resulting from convection-dependent regions of the conducting airways and diffusion-convection-dependent regions of acinar zones can be calculated [180, 181]. A review of the calculations of these parameters is beyond the scope of this thesis; however, the mechanisms of VI that increase the SIII slope also work to increase LCI, and thus will be discussed in section 1.8. While SIII analysis is a powerful tool to interpret the MBW, its use in young children is not clear. Infants and many preschool-aged children do not generate sufficient tidal volumes to consistently identify a clear phase III slope for each breath, and in infants this problem is confounded by a very short phase III slope resulting from large airways relative to lung size and small acinar spaces [154]. Due to these difficulties in analyzing the progression of SIII in young children, it will not be a focus of this thesis.
Figure 1.6.3. Gas concentration tracings from a multiple breath SF₆ inert gas washout. A) MBW washout curve showing SF6 concentration and flow tracings from a healthy 4 year old boy. Wash-in phase is complete when the SF6 concentration reaches equilibrium at 4%. Progressive decline in SF6 concentration follows disconnection from the gas supply which marks the beginning of the wash-out phase (arrow). Green line: SF6 concentration; Black line: Flow. B) SF6 concentration and volume tracing from first expired breath of the wash-out phase from MBW recording in A), showing Phase I: absolute dead space; Phase II: bronchial phase; Phase III: alveolar phase. Black line: SF6 tracing; Blue and red dotted lines: artificial volume and concentration lines added by analysis software; Red solid line: phase III slope.
1.7 Ventilation Inhomogeneity

VI has been recognized as an element of airway disease for decades. Early studies in the 1940s of nitrogen elimination from the lung during high-oxygen breathing made simple observations that the amount of resident nitrogen in the exhaled breath of patients with emphysema lung disease was higher compared to healthy subjects after a given time of breathing 100% oxygen [182]. Since adequate distribution of inhaled oxygen to the pulmonary airspaces is required for the displacement of resident nitrogen gas, quantifying the washout of nitrogen was therefore described as a means of demonstrating the effectiveness of the distribution of tidal air to acinar zones [182, 183]. Rate of emptying of inert nitrogen from the lung during pure oxygen breathing was seen to be dependent on the following factors; the volume of resident air in the lung, the tidal volume, rate of respiration, and the adequacy of distribution of each tidal breath to deep pulmonary spaces [184, 185]. These simple early assertions laid the foundation of the development of gas washout techniques, and remain important considerations in the interpretation of gas washout tests today.

1.7.1 Mechanisms of Ventilation Inhomogeneity

Gas mixing within the lungs occurs through two mechanisms; convective gas transport (bulk flow) and diffusive gas transport (molecular diffusion, or Brownian movement). Convective flow is responsible for moving gas through the large airways down to the terminal bronchioles, at which point forward velocity becomes so small that diffusion takes over within the acinus. Effective gas mixing relies on these two mechanisms to move inhaled air into all regions of the lung in order to mix with resident air remaining in lung spaces. In the idealized symmetrical lung with homogenous compliant and resistive characteristics, inspired air would be distributed evenly to all lung regions. In reality, a degree of VI exists even in healthy lungs due to gravitational effects on ventilation, asymmetry of the bronchial tree and small regional differences in mechanical properties of the lung. VI is made worse in disease when inflammation, structural airway changes, and mechanical changes in lung tissue cause asymmetric ventilation distribution. Two types of VI have been identified based on the gas transport mechanism prevailing at the site of VI; convection-dependent inhomogeneity (CDI) and to diffusion and convection-dependent inhomogeneity (DCDI). Table 1.7.1 summarizes these two mechanisms of VI.
Table 1.7.1. Summary of the types of ventilation inhomogeneity

<table>
<thead>
<tr>
<th>Type of VI</th>
<th>Description</th>
<th>Causes</th>
</tr>
</thead>
</table>
| Convection dependent inhomogeneity (CDI)       | VI resulting from differences in specific ventilation of lung compartments that are large enough and sufficiently far apart for diffusion to be negligible | • Gravity Independent: Differences in mechanical properties and size of lung units  
• Gravity Dependent: Better ventilation of dependent lung regions |
| Diffusion and Convection-dependent Inhomogeneity (DCDI) | VI present within distal small lung regions close to and within the acinus where both diffusion and convective gas transport dominate | • Asymmetric size/relative expansion of acinar regions  
• Differences in airway cross-sectional area at the entrance of one acinus compared to another |

1.7.1 i: Convection-dependent Inhomogeneity

CDI explains much of the classical models used to explain uneven ventilation distribution, whereby parallel peripheral lung ‘compartments’, or units with similar gas concentrations that may share a branch-point, are ventilated with unequal volumes. Over the course of an MBW, lung compartments that are better ventilated will empty of inert gas first, while poorly ventilated regions will take longer to empty and prolong the washout. If the lung compartments are large enough and sufficiently far apart, diffusive equilibration of gas between the units during a breath cycle will be negligible, and therefore the inhomogeneity thus produced is due to differences in convection (bulk flow) only [186].

CDI can generally be attributed to gravity-dependent and gravity-independent causes. Gravity affects regional differences in pleural pressure [187], and will cause lung units that exist topographically in line with gravity to be ventilated unevenly. In the upright position, basal lung units tend to be compressed during passive expiration due the weight of the lung acting
downwards due to gravity. During inspiration, these basal compartments are preferentially ventilated as the change in specific ventilation, the volume per unit of volume ($\Delta V/V$), is greater compared to apical lung units. This uneven ventilation will cause basal compartments to washout faster, a phenomena present even in healthy lungs. Gravity-independent CDI has also been identified through experiments of ventilation distribution in zero-gravity environments. This type of CDI is caused by different $\tau$ existing among lung compartments existing closer together and not differentially affected by gravity [188]. In this case, small changes in either compliance, resistance, or both in one compartment relative to another will cause differences in specific ventilation, allowing one compartment to empty or fill faster than the other [189-192]. Furthermore, gravity-independent CDI can also result from asymmetries in the bronchial tree, whereby a smaller lung compartment will be relatively better ventilated compared to a larger lung compartment if both were to receive the same volume of inspired air. Increased CDI caused by any one of these mechanisms can result in regional disparities in ventilation and may prolong an MBW, increasing LCI.

1.7.1 ii: Diffusion and Convection-dependent Inhomogeneity

DCDI dominates in the distal lung periphery at the entrance to the acinus where convective gas transport and diffusive gas transport are of a similar magnitude. Theoretical model analyses using a two-trumpet or multi-branch-point model have demonstrated that asymmetry in the size or relative expansion of branches within the acinus, or inequality of airway cross section between branches within the acinus will result in different gas concentrations among these small parallel units [193-196]. While acinar branching asymmetry is commonly present in the lung, mucous plugging, inflammation and structural changes in these most peripheral airways can enhance this asymmetry. During the first inspiration of the washout phase of an MBW, when room air lacking the inert gas is inhaled, the acinar branch with the smaller volume or with the larger airway cross section will receive more room air per unit volume. This will cause the concentration of inert gas in this branch to be less (more diluted) at the end of inspiration compared to the larger branch or the branch with a smaller airway cross section. During expiration, diffusive forces will tend to cause the inert gas to move from the branch with high concentration of the gas to the branch with low concentration of gas. This diffusive movement of gas back and forth between parallel branches is called the “diffusive pendelluft” phenomenon, and serves to actually enhance mixing within the acinus [196, 197]. It is for this reason that DCDI contributes increasingly to
overall VI during the first few breaths of a MBW, but will soon reach a maximum plateau. Further increase in VI beyond around the fifth breath reflects CDI only [181].

### 1.7.1 iii: Lung Clearance Index and Mechanisms of Ventilation Inhomogeneity

LCI is a global measurement of overall lung VI, calculated by a simple ratio of culmulative expired air (sum of tidal volumes) during the washout and the calculated FRC. The higher the LCI value, the more lung volume turnovers are required by a given subject to washout the inert tracer gas to 1/40th of its initial concentration. LCI is increased if there is sequential emptying of lung units or if lung units are ventilated unevenly resulting in different concentrations of inert gas in one compartment relative to another. Either or both of the two types of VI discussed previously, CDI or DCDI, can potentially cause an increase in LCI by decreasing gas mixing efficiency. Due to the simplicity of the measure, the LCI does not provide information on the specific contribution of each type of VI to the overall measure, but rather this parameter provides a quantification of the overall effect of VI such as CDI and DCDI. The pathological mechanisms underlying an increased LCI have been studied in an array of age groups and lung diseases, and are discussed in section 1.8.

### 1.7.2 Other Factors Affecting the Lung Clearance Index

One of the principal difficulties in interpreting an LCI value is discriminating between the effects of increased VI due to disease and the effects of enlarged respiratory dead space, apparatus dead space, the effect of changes in tidal volume ($V_T$) on VI, or changes in FRC, all of which may alter LCI. By the nature of its calculation, LCI is inherently dependent on breathing pattern. By changing frequency of breathing or $V_T$, it is likely that emptying of inert gas from lung units may change due to greater/less expansion of airways, greater/less convective airflow to lung units, or changes in airway closure. For example, it has been shown in infant subjects that compliance of the respiratory system increases during breathing at volumes above $V_T$, demonstrating a recruitment of lung units that were closed in the $V_T$ range [198]. It would be likely that markers of VI would also change in response to changes in compliance and the number of lung units being relatively better ventilated when breathing pattern and $V_T$ are altered. Likewise, increases in deadspace volume ($V_D$) will likely decrease the proportion of $V_T$ reaching gas mixing units in the lung, thereby increasing VI.
A handful of studies have shown that varying $V_T$, $V_D$ and FRC factors can have an effect on LCI. Larsson et al. demonstrated in a one-compartment lung model that increasing $V_D$ while maintaining the same $V_T$ increased LCI [199]. The same study also showed that in mechanically ventilated adults, LCI decreased significantly with a change from small to large $V_T$. Larger $V_T$ will likely recruit lung units and improve the conductance of airways which were narrowed during small $V_T$, increasing the efficiency of filling and emptying of these units. The effect of altering the $V_D/V_T$ ratio on ventilation inhomogeneity has also been studied by Damato et al.[200] and Crawford et al.[201]. It was seen that while increasing $V_T$ will act to slightly increase both series and alveolar dead space, the proportion of dead space within each breath diminishes with a greater $V_T$, with a net effect of increasing alveolar ventilation. Furthermore, indices of CDI and DCDI calculated from nitrogen washout revealed that by increasing $V_T$, DCDI is reduced while CDI increases. Gronkvist et al. found similar results in a study of nitrogen washouts in healthy adult males, where it was found that greater $V_T$ improved overall VI as defined as the sum of CDI and DCDI, particularly in the supine position compared to standing [202]. In a study looking at the effect of altering $V_D/V_T$ and $V_T/FRC$ ratios in newborn artificially ventilated piglets, Schmalisch et al.[203] demonstrated that LCI significantly decreased from baseline after increasing peak inspiratory pressure (PIP). PIP increased $V_T$ and FRC, yet since the effect on $V_T$ was greater than on FRC, the net effect was an increase in the $V_T/FRC$ ratio and a concomitant decrease in $V_D/V_T$, which improved LCI. In summary, the effect of breathing pattern and changes in FRC on LCI are complicated. The previously mentioned studies have illustrated how LCI is sensitive to changes in breathing pattern, although none of these studies have looked into the effect of variations in breathing pattern within the tidal volume range on LCI. Since the MBW is a tidal breathing test, such studies are needed in order to distinguish the degree of variability in LCI that may be attributable to breathing pattern variability.

Body position during testing may be another factor that could change $V_D/V_T$ and $V_T/FRC$ relationships and could change CDI. There is evidence that ventilation distribution and pulmonary function can be affected by body position and is generally improved in the standing or seated position compared to supine [187, 202]. Gustafsson et al. has shown that testing in the supine versus standing position in healthy adults did not affect overall VI, the supine position did increase gas trapping in asthmatic children and adolescents, while the change in body position had no effect on LCI in healthy controls or the asthmatic group [204]. In infants, a meta-analysis of literature concerning body position and its effects on oxygenation and ventilation in mechanically
ventilated infants found no significant differences between the prone and supine position [205]. Studies examining the effect of body position on LCI in infants during sleep are lacking. In summary, breathing pattern and body position and their concomitant effects on $V_T$, $V_D$, and FRC, as well as apparatus dead space can affect LCI and are important considerations when investigating this measure.
1.8 Previous Studies Reporting the Lung Clearance Index

The MBW technique has emerged in recent years as a promising tool for measuring VI in pediatric populations with health and respiratory disease. The LCI has likewise become a popular reporting parameter of global lung VI. The vast majority of pediatric MBW studies reporting LCI have focused on CF, yet the potential of LCI as a sensitive measure of VI as demonstrated in these CF studies has spurred the expansion of this research into other disease cohorts.

1.8.1 Normative LCI data

Normal LCI values from large, representative groups of healthy subjects throughout the pediatric age range must be established to characterize LCI in health in order to subsequently define LCI in disease. There is currently a lack of such data in the MBW literature. Table 1.8.1 summarizes pediatric normative LCI data that is available to date using SF\textsubscript{6} mass-spectrometry MBW. These studies include small cohorts of healthy children that are limited to small age ranges. Nonetheless, MBW testing in healthy subjects at all ages has been highly feasible, with 70-100% of preschool aged subjects 4-6 years old or older being able to successfully complete 2-3 MBW trials [206, 207]. One of the most popular aspects of the LCI that has emerged from the current normative data is the apparent consistency of LCI across school-age ranges (over 5 years) and between research centers [179]. By using FRC as a component in the calculation of the LCI, there is an intrinsic correction for variations in lung size due to age, height, weight and gender. In young children under the age of 6 years, however, there may be some dependence of LCI on age. A comparison of published mean LCI values for groups of healthy controls in infancy, preschool-age and older demonstrates that LCI tends to be higher in younger children (Table 1.8.1). Surprisingly, there are currently no published studies that have investigated this apparent trend in LCI in young children. The age ranges of the healthy cohorts currently published are too small with too few subjects to see any relationship with age and LCI; it is only when means are compared between studies that a trend is observed. If LCI is dependent on age in early childhood and not in older children, this age-dependency must be taken into account when investigating LCI in disease. Furthermore, such a trend may suggest that LCI could provide novel physiological information on
gas mixing efficiency in early childhood, at a time when the lung is relatively immature compared to older childhood and adulthood.

Knowledge about the normal variability of LCI in healthy subjects is also needed in order to determine the stability of the test in health. For example, one of the major limitations of spirometry in young children is that many spirometric measures are highly variable within an individual subject. As an individual LCI value is typically the average of 2-3 technically acceptable washout trials, the variability in the LCI measure between these trials (within-test variability) can be calculated. Reported coefficients of variation, the ratio of the standard deviation of the measure to the mean, range from 4-6% in health [154]. In disease, within-test variability of LCI has also been reported to be just as small [179]. Studies investigating repeatability of LCI over longer periods of time are few. Fuchs et al. have recently investigated the repeatability of LCI calculated using a sidestream molar mass and ultrasonic flowmeter MBW technique, where they found that there was no significant difference in LCI at baseline compared to 1 hour later and 6 months later in health [178]. Similar studies are needed for MBW techniques using a mass spectrometer in order to comment on the natural variability the LCI as a measure. Furthermore, within-test variability needs to be correlated with elevated LCI in disease in order to determine if LCI and other MBW parameters are more variable in those individuals with high VI compared to healthy controls. This is an important consideration when defining quality control criteria.
Table 1.8.1. Summary of LCI data from SF6 mass spectrometry MBW in healthy controls and subjects with CF or asthma

<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>N</th>
<th>Mean LCI (SD)</th>
<th>Within-test Variability (CV%)</th>
<th>Disease</th>
<th>N</th>
<th>Mean LCI (SD)</th>
<th>Within-test Variability (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lum, S. et al. (2007) [147]</td>
<td>2 months-2 yrs</td>
<td>21</td>
<td>7.2 (0.3)</td>
<td>NR</td>
<td>CF</td>
<td>39</td>
<td>8.4 (1.5)</td>
<td>NR</td>
</tr>
<tr>
<td>Aurora, P. et al. (2005) [208]</td>
<td>2-5 yrs</td>
<td>30</td>
<td>6.89 (0.44)</td>
<td>5.2</td>
<td>CF</td>
<td>30</td>
<td>9.61 (2.19)</td>
<td>7.8</td>
</tr>
<tr>
<td>Gustafsson, P. et al. (2003) [209]</td>
<td>4-18 yrs</td>
<td>28</td>
<td>6.33 (0.43)</td>
<td>NR</td>
<td>CF</td>
<td>43</td>
<td>8.33 (2.5)</td>
<td>NR</td>
</tr>
<tr>
<td>Aurora, P. et al. (2004) [179]</td>
<td>6-16 yrs</td>
<td>33</td>
<td>6.45 (0.49)</td>
<td>5.2</td>
<td>CF</td>
<td>22</td>
<td>11.5 (2.9)</td>
<td>6.2</td>
</tr>
<tr>
<td>Macleod, K.A. et al. (2009) [206]</td>
<td>5-16 yrs</td>
<td>29</td>
<td>6.24 (0.47)</td>
<td>4.8</td>
<td>Asthma</td>
<td>31</td>
<td>6.7 (0.9)</td>
<td>4.2</td>
</tr>
<tr>
<td>Sonnappa, S. et al. (2010) [210]</td>
<td>5-6 yrs</td>
<td>72</td>
<td>6.6 (6.5-6.7)*</td>
<td>NR</td>
<td>Asthma</td>
<td>62</td>
<td>Viral wheezers: 6.7 (6.5-6.9)<em>&lt;br&gt;Persistent wheezers: 7.4 (7.1-7.8)</em></td>
<td>NR</td>
</tr>
</tbody>
</table>

SD: Standard Deviation; CV%: Percent Coefficient of Variation; NR: Not Reported
*Interquartile range
1.8.2 LCI in respiratory disease

1.8.2 i: Cystic Fibrosis

Table 1.8.1 summarizes key published studies investigating LCI gained from SF₆ MBW to date. One of the first studies to reignite the current research interest in LCI was published by Gustafsson et al. in 2003 [209], which demonstrated that LCI was not only elevated in CF children compared to healthy controls, a finding that had been established by various studies in the 1980s and 1990s [211-214], but that LCI was more sensitive than spirometry in detecting CF lung disease. This finding meshed well with a growing appreciation that current clinical lung function tests—namely spirometry—were perhaps not sensitive enough to monitor early disease changes; by the time spirometric indices declined in chronic respiratory disease such as CF, permanent inflammatory and structural changes may have already occurred. Aurora et al. followed-up these results with two studies investigating LCI compared to spirometry in British children with CF compared to healthy controls. The first paper in 2004 investigated school-aged children where LCI was again found to be a better indicator of CF lung disease than spirometry, reproducing Gustafsson’s results [179]. The second paper in 2005 made the same comparisons in preschool-aged children, finding again that LCI is elevated in CF, particularly those individuals infected with Pseudomonas aeruginosa, and that it was more sensitive and easier to perform in this population than spirometry or plethysmography [208]. A longitudinal study by Kraemer et al. that performed annual lung function testing including nitrogen MBW on a cohort of CF children ranging from 6-20 years of age found that LCI was the first index to decline and was the strongest predictive measure of the progression of CF disease [215]. Although the MBW has been used to investigate gas mixing efficiency in premature neonates [216, 217], the only study looking at LCI in infants with CF is by Lum et al., where LCI was found to be elevated in CF subjects compared to healthy infants [207]. Interestingly, when LCI was compared to RVRTC, both tests detected airway dysfunction in a similar proportion of CF infants that were not always the same individuals. This suggests that the MBW and RVRTC technique may be providing different information on airway and lung function.

One of the limitations of the aforementioned studies of LCI detecting CF disease in children was that there was no way to correlate an elevated LCI value with structural disease changes. This important question was recently addressed in a study by Gustafsson et al. which compared LCI to HRCT scan scores of abnormal lung structure in a cohort of CF children aged 5-
These results have been mirrored in a more recent study comparing LCI to lower-resolution CT. The sensitivity to detect abnormal lung structure was 85-94% in LCI, and LCI was found to be a more sensitive indicator of structural CF disease than any spirometric measure.

With the establishment of LCI as a sensitive marker of CF lung disease, most recent work has now focused on its response to treatment methods and its ability to predict progression of disease. While most studies show a mean improvement in LCI after treatment interventions, a heterogeneous individual response has often been documented. These results further highlights the need to more carefully consider the variability of LCI both within and between tests in order to remove possible confounding variables of breathing pattern and age in the response. Physiotherapy, a method used to improve mucus clearance, was found to have no short-term effect on LCI in a cohort of children tested using a sidestream-ultrasonic flowmeter MBW technique. It may be that improvement in LCI after intervention is not immediate, and therefore more longitudinal studies following CF children at multiple time points after therapy are needed. Importantly, recent evidence shows that elevated LCI values at the age of 4 years in CF patients predict subsequent abnormal spirometry during school age, whereas a normal LCI in preschool years tends to remain normal as the child grows older. Therefore, early elevation of LCI seems to predict future pulmonary function outcomes, making LCI an important outcome measure of therapeutic intervention and highlighting the importance of early MBW testing in CF disease.

Indeed, LCI has become well-established as a sensitive marker of CF in preschool and school-aged children, with a trend of increasing rates of abnormal LCI with increasing age in CF children. It is now necessary to more carefully define LCI in CF disease, including investigation into trends of how LCI varies with age and breathing pattern, how within-test variability of LCI may relate to CF disease, and how LCI in CF compares to LCI in other disease groups.

1.8.2 ii: Asthma

Asthma is well-known to involve pathological small airway changes, however the episodic nature of the disease means that lung function measured by conventional testing is not consistent. As a more sensitive marker of subtle VI changes, the MBW is a potentially useful tool to improve detection of asthma disease. Original N₂ MBW studies found MR parameters were elevated in asthmatic patients compared to healthy controls, and that these indices correlated with FEV1,
but not with impedance parameters gained from FOT [224, 225]. More recent studies investigating LCI in adults have found a significant increase in asthmatic patients which decreases significantly with inhaled corticosteroid treatment, although residual ventilation inhomogeneity post-treatment is present [226]. In children with asthma, VI in both the conductive and acinar zones is present, much of which remains after bronchodilator treatment, particularly in the conducting airways [227, 228]. LCI is significantly elevated in asthmatic teenagers compared to healthy controls, and shows significant decrease after bronchodilator treatment which brought mean LCI to a value similar to healthy controls [229]. Studies investigating LCI in young children with asthma/wheeze are few. Using a cohort of 31 well-controlled asthmatics aged 5-15 years, Macleod et al. found that mean LCI was significantly elevated in this group compared to age-matched healthy subjects. Furthermore, LCI did not significantly change, but remained elevated in this group after bronchodilator treatment. The authors thus suggested the presence of residual VI in asthmatics that did not reflect airway inflammation, as indices of exhaled nitric oxide in these subjects were normal [206]. Recently, Sonnappa et al., divided a large preschool asthmatic group into persistent wheezers and viral wheezers and compared MBW results from these two phenotypes [210]. It was found that LCI was significantly elevated only in persistent wheezers compared to healthy controls and viral wheezers, regardless of the presence or absence of atopic status or symptoms. In addition, MBW indices of VI, including LCI, were more sensitive than any other pulmonary function measure at detecting airway dysfunction in these children, including spirometry, exhaled nitric oxide, and specific airway resistance. This study therefore suggests a potential for LCI to discriminate between asthma phenotypes in early childhood. While the MBW literature is sparse for asthma compared to CF, it is clear that LCI can detect group differences between asthmatic children and healthy controls. The next steps are to investigate whether this difference is also present in infancy, to study trends in LCI elevation with age during early childhood, to determine how LCI relates to breathing pattern, and to compare LCI trends in asthma with those in CF.
Chapter 2:

Rationale & Objectives
2 Rationale and Objectives

2.1 Rationale

The peripheral airways are the principal site of inflammation, mucus plugging and structural changes inherent in obstructive lung diseases such as asthma and CF [25, 45, 68, 230]. It is now accepted that these conditions affect the lung in a heterogeneous manner, causing overall VI [231, 232]. These pathological changes are recognized to begin early in life, may occur before the onset of clinical symptoms, and may persist even in the absence of symptoms [35, 68, 233]. An objective measure of peripheral airway function that is sensitive to these early changes and feasible in young children is needed to help monitor disease in children under the age of six years. The LCI, gained from the MBW technique, is an appealing candidate for such a test due to the ease with which it can be measured in young children and due to its sensitivity to changes in ventilation inhomogeneity related to small airway disease [209, 219, 234]. Its use in both infancy and preschool-age in asthmatic and CF children has not been investigated concurrently. Large healthy control populations that span larger pediatric age ranges are also needed in order to better distinguish changes in VI due to growth and lung development. Furthermore, LCI must be related to other important parameters, such as breathing pattern, FRC and age group in both health and disease in order to more carefully characterize this measure. The hypothesis for this project is that LCI will be a sensitive index of VI in infants and preschoolers, and will detect asthmatic/wheezy and CF individuals with increased VI compared to healthy controls.

2.1.1 Research Question

Can the LCI derived from the MBW technique detect increased VI in asthmatic/wheezy and CF infants and preschoolers tested in the absence of acute symptoms compared to healthy controls?
2.2 Objectives

The primary aim of this project is to determine if increased VI indicated by elevated LCI values is able to distinguish asthmatic/wheezy and CF infants and preschoolers tested in the absence of clinical symptoms from healthy controls. This aim will be investigated through the following three objectives:

1) To determine the LCI in healthy subjects. In order to determine if LCI is elevated in disease, we must first examine LCI in healthy children to determine a baseline. Previously reported LCI values from small cohorts of healthy infants [207], healthy preschool-age subjects [208] and healthy school-age subjects [179, 209] have demonstrated that mean LCI values are higher in younger age ranges (Table 1.8.1). To investigate these differences in LCI between age groups, we combined all infant, preschool-age and school-age MBW data collected at the Hospital for Sick Children (HSC) in order to investigate how LCI is related to childhood development.

2) To determine whether LCI is significantly elevated in infants and preschoolers with disease compared to healthy subjects. Breathing pattern and changes in FRC will also be taken into account to understand the change in LCI seen in disease groups.

3) To describe the within-test variability of LCI in health and disease during early childhood. Low within-test variability will illustrate the stability of the test, and comparison between health and disease will demonstrate if disease is related to higher variability.
2.3 Hypotheses

As airway changes related to the chronic disease processes of both CF and asthma are known to initiate within the first few years of life, we believe that measurements of VI will be particularly sensitive to these changes in infancy and preschool-age. Therefore, we hypothesize that LCI will be able to detect VI in infants and preschoolers with asthma/wheeze and CF compared to healthy controls. More specifically, the following hypotheses address the three objectives of this project previously stated:

1) LCI will show a negative age dependency in young children.
2) LCI will be significantly elevated in subjects with disease compared to healthy subjects.
3) LCI will have low variability in health and in disease.

2.4 Clinical Relevance

Lung function testing in infants and preschool-aged children remains difficult due to issues of subject cooperation and insensitive measures of small airway function. The ability to better monitor children with disease starting in infancy could hasten the application of therapeutic interventions that can prolong lung function and improve disease outcomes. The MBW is a potentially useful clinical tool as it can be performed in all age groups with a high degree of feasibility and may detect pathological VI that is missed by conventional lung function tests. The establishment of the LCI as a sensitive marker of airway disease in infants and preschoolers in the absence of clinical symptoms is important in the development of this test as a potentially useful clinical tool, and may provide more information on airway physiology during the early years of life. Also, a more careful analysis of how LCI changes with development in health and disease, and how it is related to breathing pattern parameters and within test variability is necessary to better understanding the measure and for defining quality control criteria for future clinical use.
Chapter 3:

Methods
3 Methods

3.1 Research Setting

All pulmonary function tests were completed at the Infant Pulmonary Function Testing Laboratory and/or the Pediatric Pulmonary Function Testing Laboratory, Division of Respiratory Medicine at the Hospital for Sick Children (HSC), Toronto, Canada. Recruitment and testing of the subjects in this study took place from May 2009 to February 2011.

3.2 Ethics

Due to the young age of subjects, written informed consent was obtained by the parents or guardians of study subjects. All studies of subjects included in this thesis were approved by the Research Ethics Board at HSC.

3.3 Subject Recruitment

Table 3.3.1 outlines general inclusion and exclusion criteria used for all study participants. Participants were only included in this project if they met inclusion criteria and if they did not meet any of the exclusion criteria. The following sections provide further detail on how each age group and diagnosis cohort was recruited and any specific entry criteria that applied.
Table 3.3.1. General inclusion and exclusion criteria for all study participants undergoing pulmonary function testing

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clinically stable/healthy at time of PFT&lt;br&gt;• Within age range</td>
<td>• History of prematurity (premature birth ≤ 37 weeks gestation, low birth weight)&lt;br&gt;• Respiratory infection currently or within 2-3 weeks prior to testing&lt;br&gt;• Other chronic lung disease other than CF or asthma&lt;br&gt;• Supplemental oxygen requirements&lt;br&gt;• Cardiac disease, neurological disorders, bone disease&lt;br&gt;• Contraindications for sedation (for infants only)</td>
</tr>
</tbody>
</table>

3.3.1 Healthy Control Subjects

3.3.1 i: Healthy Infants

Infants were recruited as part of the Canadian Healthy Infant Longitudinal Development (CHILD) Study, a national birth cohort study recruiting 5000 children in 5 centers across Canada. The CHILD Study is investigating the environmental, host, psychosocial and genetic origins of childhood asthma and allergy by recruiting mothers during pregnancy and following their child from birth to 5 years of age. At the Toronto site, CHILD Study personnel recruited pregnant mothers from the general population at obstetrical clinics at Mount Sinai Hospital. As part of the consent, participants at the Toronto site were given the option of participating in the infant pulmonary function testing (IPFT) arm of the study. These children completed standard IPFT protocol (see section 3.5) including eNO, MBW, plethysmography, RVRTC, and bronchodilator response at the age of 3 months, and were invited to return for follow-up testing at 1 year and 18 months. All infants were healthy at the time of testing and had no history of prematurity or assisted ventilation. Those infants who had a history of respiratory disease diagnosis, a history of wheeze or respiratory illness requiring pharmacological treatment, an emergency room visit and/or hospitalization were excluded. Therefore, all infants included in this thesis from the CHILD Study met our definition of health at the time of testing.
3.3.1 ii: Healthy Preschool-age and School-age Subjects

Healthy siblings and friends of the CF and asthmatic patients attending the Respiratory Medicine clinic at HSC, and family of staff members at HSC, were invited to complete MBW testing. We aimed to recruit a large healthy cohort including 50 children from 3-18 years of age. These children completed MBW and spirometry testing on one test occasion. For the recruitment of this large healthy cohort, we aimed to include 10 subjects in each of 5 age ranges (3-5, 6-8, 9-11, 12-14, 15-18), allowing for an equal distribution of subjects from 3-18 years. Parents/guardians of healthy subjects were approached by study personnel who explained the study protocol and obtained consent. Subjects were then asked to complete PFT on the day of enrollment or returned for a subsequent visit to the HSC for testing. All subjects were healthy at the time of testing and had no acute illness three weeks prior to testing. Subjects were excluded if they had a history of prematurity, chronic productive cough, recurrent wheezing or shortness of breath, physician diagnosed chronic lung disease, congenital heart disease, neuromuscular disorder, bone disease or any previous hospitalization for a respiratory condition.

3.3.2 Wheezy/Asthmatic Subjects

A history of wheeze during infancy and preschool age is the earliest symptom of chronic asthma, although not all individuals who experience wheeze will have this diagnosis [9]. Infants between the ages of 2 months—3 years with a history of recurrent severe wheezing requiring a visit to the Emergency Department, a hospital admission, or follow-up at the Respiratory Medicine Department at HSC were invited to complete MBW testing in addition to standard IPFT. These infants were invited to complete IPFT within 3 months of their wheezing episode during a period of clinical stability. Preschool-age patients with diagnosed asthma attending the Respiratory Medicine Department at HSC who were clinically stable completed MBW testing in addition to spirometry. All infants and preschool-age subjects were not acutely exacerbating the time of testing. Subjects were encouraged to continue their normal medication regimen, if applicable, on the day of testing, but were asked to avoid bronchodilator medication 12 hours before testing. Any subjects with a history of prematurity, other chronic lung diseases (bronchopulmonary dysplasia, CF, etc), cardiac disease, or a history of respiratory illness in the 4 weeks prior to testing were excluded from testing.
3.3.3 CF Subjects

Infants and preschool-age patients with CF attending the Respiratory Medicine Department at HSC were invited to complete baseline MBW testing. Many of these subjects (n=22) were part of the Infant Study of Inhaled Saline (ISIS) Study, registered with clinicaltrial.gov as NCT00709280. ISIS subjects completed MBW as an add-on test to the pulmonary function testing they completed as part of the ISIS protocol. Diagnosis of CF was confirmed in all subjects by one or more clinical features of CF and a documented sweat chloride ≥ 60 mEq/L by quantitative pilocarpine iontophoresis test or genotyping showing two disease causing mutations. All subjects were clinically stable at the time of testing, and any subject requiring supplemental oxygen or with respiratory infection, viral illness or wheezing onset less than 2 weeks before testing were excluded.

3.4 Equipment and lung function measurements

3.4.1 Medications

Infants were mildly sedated with chloral hydrate (80 mg/kg). Preschool-aged children were tested while awake and therefore did not receive chloral hydrate. For those infant and asthmatic preshooler subjects that continued post-baseline lung function testing, inhaled bronchodilator medication was administered (100 µg/puff; 4 puffs) (Novo Salbutamol HFA Inhaler, Novopharm) using a spacer (AeroVent, Collapsible Holding Chamber, Monaghan Medical Corporation, Plattsburgh, NY) and lung function testing was repeated.

3.4.2 The Multiple Breath Washout

Figure 3.4.1 provides an illustration of the MBW equipment set-up. Infants were tested while sleeping in the supine position within the infant plethysmograph (IPL) box wearing a facemask (Silkomed, Rendell Baker Masks sizes 2 and 3, Rusch Canada Inc., Benson Medical Industries, Markham, Ontario) sealed with therapeutic putty (Air Putty, Sammons Preston Canada
Inc., Mississauga, Ontario) to reduced dead space within the mask and avoid air leaks. Preschool-aged children over the age of 3 years were tested while awake in the seated position wearing a facemask, again sealed with therapeutic putty. Flow was measured using a heated pneumotachometer, with infants aged 0-4 months using a smaller model (3500B) and children older than 4 months using a larger model (3700B) (Rudolph Linear Pneumotach, Hans Rudolph, Shawnee, KS, USA). Gas was sampled from the breath by aspiration through an inlet capillary line affixed in the pneumotachometer which led to a fast-responding respiratory mass spectrometer (AMIS 2000; Innovision A/S, Odense, Denmark). The inert tracer gas mixture consisting of 4% He, 4 % SF\textsubscript{6}, 21% O\textsubscript{2} and balance nitrogen was delivered to the subject from a pressurized gas tank (Praxair Products Inc., H/K, Brampton, Ontario). An open-circuit inert gas delivery system was created using 2 lengths of corrugated flexible tubing (CareFusion, Yorba Linda, CA, USA) attached in line with each other to a plastic T-connector that could be attached and easily detached from the pneumotachometer. Flow and gas concentration data was recorded and displayed in real time using a laptop computer and custom acquisition software, and was stored digitally for later analysis. The apparatus deadspace was divided into 2 components, the first being pre-capillary deadspace defined as the deadspace between the infant’s lips and the inlet capillary, and the second being the post-capillary deadspace defined as the deadspace between the inlet capillary and the end of expiratory port of the pneumotachometer. Estimating the deadspace within the facemask can be difficult, as it will change with the amount of putty used and with the shape and size of the child’s face. We therefore only calculated the apparatus deadspace that will not change (the volume within the pneumotachometer and end expiratory port). This deadspace was calculated as 15.4mL for the larger pneumotachometer (3700 series) and 10.4mL for the smaller pneumotachometer (3500 series). These volumes were subtracted from each breath during analysis to calculate cumulative expired volume (CEV). All MBW parameters were reported from the washout and analysis of SF\textsubscript{6} gas.

The MBW procedure has been described previously in section 1.6.2. Preschool-aged children were distracted during testing by watching a DVD in order to obtain relaxed tidal breathing. All subjects completed at least 2 technically acceptable trials. Subjective quality control criteria employed during testing included; encouraging quiet breathing by coaching for preschoolers or waiting for normal breathing to be established in infants; full wash-in of inert gas to 4% represented by a stable plateau in gas concentration before disconnect; a disconnect during expiration as seen by a clean drop in inert gas concentration to 0%. Any trials where sighs, long
pauses, or irregular breathing patterns were observed were dropped from analysis. All technically acceptable trials were analyzed in order to determine the variability of parameters.

Figure 3.4.1. Multiple breath washout equipment set-up during wash-in phase. A: corrugated tubing delivering inert gas; B: plastic T-connector; C: pneumotachometer; D: flow lines leading to flow analyzer; E: inlet capillary line leading to mass spectrometer; F: facemask with therapeutic putty. Arrows represent direction of inert gas bias flow.
3.4.3 Raised volume rapid-thoracoabdominal compression technique and infant plethysmography

RVRTC technique and infant plethysmography was performed according to the American Thoracic Society and European Respiratory Society guidelines for infants [145] using the Ferraris Respiratory Infant Plethysmograph (IPL) system (nSpire Health Inc., Louisville, CO USA). While data from this testing is not presented in this thesis, a short description of the equipment set-up is provided here as this testing was part of the infant lung function testing protocol along with the MBW. The IPL system includes the plethysmograph, all equipment for the RVRTC technique as described previously [235], and a computer with software for data acquisition and storage. Heart rate and blood oxygen saturation were continuously monitored using pulse oximetry (Radical-7, Masimo Canada ULC, Saint-Laurent, Quebec). End-tidal CO$_2$ concentration was monitored using a capnograph as a safety precaution to detect any fall in PaCO$_2$ that may be associated with lung inflations. Infants were sedated with chloral hydrate (80 mg/kg) administered orally by syringe. Infants were tested while sleeping in the supine position within the infant plethysmographic box wearing a facemask lined with therapeutic putty to reduce dead space within the mask and avoid air leaks. Flow and volume was measured using a heated pneumotachometer (Hans Rudolph Linear Pneumotach, Hans Rudolph, Shawnee, KS, USA).

During plethysmography, the infant was enclosed in the IPL box but breathed room air from a bias flow passed into the box and across the facemask using a T-connector. During quiet breathing, occlusion of the airway is made and the infant is allowed to perform 2-3 inspiratory efforts against the resistance. Each measure of FRC for an occlusion trial is the average of 2-3 inspiratory efforts.
3.5 Lung function testing procedure

3.5.1 Infants

All infants attempted the same lung function testing procedure which is outlined in Table 3.5.1. For this thesis, only baseline MBW results were used in the analysis. In general, the full testing procedure includes the exhaled nitric oxide (eNO) testing, MBW testing, the RVRTC technique, and plethysmography, which have been described in section 3.4. This testing procedure was completed during one visit to HSC. MBW testing was repeated after RVRTC and plethysmography to determine any response in LCI due to forced flow maneuvers. MBW, RVRTC and plethysmography were repeated after the administration of inhaled bronchodilator medication (BD) to investigate a BD-related response in lung function parameters. Since infants must be asleep during all tests, the number of tests completed depended on the length of time that the infant slept. All infants were mildly sedated with the same dose of oral chloral hydrate (80 mg/kg) administered using a syringe. Once asleep, the infant was placed in the supine position within the IPL box and fitted with a facemask. All IPFTs can be completed from this supine position within the IPL box without waking and/or moving the infant. Testing commenced in the order which appears in Figure 3.5.1. Testing was paused if the infant stirred from sleep or showed any signs of distress, and testing was stopped if the infant woke up or if there were contraindications to continuing testing as judged by the registered respiratory therapist (i.e. blood oxygen desaturations, snoring, apneas, etc.). Infants were continually monitored throughout testing by recording heart rate and blood oxygen saturation. During the visit, parents were asked to fill out general health questionnaires specific to the study the infant was enrolled in, from which basic medical history, including a history of wheeze, pulmonary infection, past hospitalizations, and ER visits was acquired.
Table 3.5.1. General pulmonary function testing procedure for infants

<table>
<thead>
<tr>
<th>Order</th>
<th>Lung Function Test/Intervention</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>eNO (baseline)</td>
<td>Tidal breathing, 3 reproducible trials</td>
</tr>
<tr>
<td>2</td>
<td>MBW (baseline)</td>
<td>3 technically acceptable trials</td>
</tr>
<tr>
<td>3</td>
<td>RVRTC (baseline)</td>
<td>3 technically acceptable flow-volume loops</td>
</tr>
<tr>
<td>4</td>
<td>Plethysmography (baseline)</td>
<td>3 technically acceptable occlusion trials</td>
</tr>
<tr>
<td>5</td>
<td>MBW (post-RVRTC)</td>
<td>2 technically acceptable trials</td>
</tr>
<tr>
<td>6</td>
<td><em>Bronchodilator Administration</em></td>
<td>4 puffs of <em>Ventolin</em>, wait 15 minutes</td>
</tr>
<tr>
<td>7</td>
<td>MBW (post-BD)</td>
<td>2 technically acceptable trials</td>
</tr>
<tr>
<td>8</td>
<td>RVRTC (post-BD)</td>
<td>3 technically acceptable flow-volume loops</td>
</tr>
<tr>
<td>9</td>
<td>Plethysmography (post-BD)</td>
<td>3 technically acceptable occlusion trials</td>
</tr>
</tbody>
</table>

3.5.2 Preschool-age subjects

All preschool-aged children attempted the same baseline pulmonary function testing protocol, outlined in Table 3.5.2. Only baseline MBW data was used in the analysis for this thesis. Each successive test in the PFT protocol was attempted as long as the child and parents were willing and able to continue. This procedure included baseline MBW testing to achieve 3 technically reproducible trials, followed by preschool spriometry to achieve 3 technically reproducible flow-volume loops. If baseline testing was successful and both the parents and child were willing, MBW was repeated post-spirometry to determine if forced flow maneuvers changed any of the MBW parameters. For preschoolers within the asthmatic group, 2 puffs of inhaled bronchodilator (BD) (*Ventolin*) were administered after post-spirometry MBW testing, and repeat MBW testing and preschool spirometry were attempted to determine the effects of BD on lung function parameters. All pulmonary function tests were completed during one visit to HSC. During the visit, parents were asked to fill out general health questionnaires specific to the study the infant
was enrolled in, from which basic medical history, including a history of wheeze, pulmonary infection, past hospitalizations, and ER visits was acquired.

**Table 3.5.2.** General pulmonary function testing protocol for preschool-aged subjects

<table>
<thead>
<tr>
<th>Order</th>
<th>Lung Function Test/Intervention</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MBW (baseline)</td>
<td>3 technically acceptable trials</td>
</tr>
<tr>
<td>2</td>
<td>Preschool Spirometry (baseline)</td>
<td>3 technically acceptable flow-volume loops</td>
</tr>
<tr>
<td>3</td>
<td>MBW (post-RVRTC)</td>
<td>2 technically acceptable trials</td>
</tr>
<tr>
<td>4</td>
<td><em>Bronchodilator Administration</em></td>
<td>4 puffs of <em>Ventolin</em>, wait 20 minutes</td>
</tr>
<tr>
<td>5</td>
<td>MBW (post-BD)</td>
<td>2 technically acceptable trials</td>
</tr>
<tr>
<td>6</td>
<td>Preschool Spirometry (post-BD)</td>
<td>3 technically acceptable flow-volume loops</td>
</tr>
</tbody>
</table>

3.6 Data analysis

3.6.1 Statistical analysis software

Statistical tests and the creation of all graphs and figures were carried out using SPSS Student Version 16.0 and GraphPad Prism 5.04 for Windows. Fractional polynomial regression analysis to compute centiles for LCI in healthy controls was calculated using the statistical program R (version 2.13; R Foundation) available from www.r-project.org.

3.6.2 Data presentation

All subjects were described based on demographic data such as gender, height, weight and age. All demographic and MBW group results were presented as mean (SD) unless otherwise stated. Z-scores were calculated for height-for-age, weight-for-age and BMI-for-age for subjects using World Health Organization (WHO) predicted values (WHO Anthro for personal computers,
version 3.2.2, 2011: Software for assessing growth and development of the world's children. Geneva: WHO, 2011, http://www.who.int/childgrowth/en/). Nonparametric tests were used unless otherwise stated. All statistical tests used a two-sided p value of 0.05 to be significant.

3.6.3 Data and statistics used to define LCI in Healthy Subjects (section 4.1)

Our first objective was to establish and describe a range of normal LCI values based on results from our infant, preschool-age and school-age subjects. Z-scores were calculated for respiratory rate (RR) and tidal volume (Vₜ) by linear regression including significant factors (height and age). MBW parameters were compared between infants, preschoolers and school-age subjects using Kruskal-Wallis ANOVA with Dunn’s comparison post-hoc tests. Investigation into whether LCI in health was correlated with growth parameters (age, height, weight) or gender was performed by Spearman correlations for all healthy subjects combined, and within each age group separately. Spearman correlations were also calculated to determine if LCI was associated with RR, Vₜ or FRC for all healthy controls combined and within each age group separately. We modeled LCI based on factors that were highly associated with LCI in health using fractional polynomial regression, which is an extension of simple regression analysis that allows for modeling of non-linear relationships (249). This type of regression allowed for the determination of significant predictor variables for LCI in health, and provided a measure of the relative goodness of fit of the model called the Akaike Information Criterion (AIC). The AIC is a relative measure of the information lost when a given model is used to describe the LCI, and therefore a lower AIC number represents a better model fit. Using this model, we added in breathing pattern parameters (RR and Vₜ), FRC and age group to determine if the inclusion of these factors improved the model of LCI in health.

3.6.4 Data and statistics used to determine if LCI is elevated in infants and preschoolers with disease compared to healthy controls (section 4.2)

Mean values of MBW parameters from wheezy/asthmatic subjects and from CF subjects were compared to healthy controls using Mann-U Whitney t-tests. Additionally, we added each respective disease diagnosis (wheeze/asthma or CF), and significant breathing pattern parameters (RR, Vₜ) to our regression model of LCI to determine if a disease diagnosis or breathing pattern
caused LCI to be significantly different in disease groups compared to healthy controls. In order to investigate the change in LCI within each disease group between infancy and preschool-age, we compared LCI using a two-way ANOVA with Bonferroni post-hoc tests. We defined an upper limit of normal for LCI using the upper 95% confidence interval for LCI from our fractional polynomial regression of LCI in health. Differences in the proportion of disease subjects with LCI values falling above this limit compared to healthy controls was calculated using Friedman exact tests.

3.6.5 Data and statistics used to describe the within-test variability of LCI in health and disease (section 4.3)

Differences in the within-test percent coefficient of variation (WTCV%) between disease subjects and healthy controls was calculated using Kruskall-Wallis one-way ANOVA. Association between WTCV% for LCI and age, MBW parameters and MBW parameter variability was completed using Spearman correlations in infants and preschoolers desperately. Association between LCI and MBW parameter variability was completed using Spearman correlations for infants and preschoolers separately.

3.6.6 Sample size calculations

Previous work has demonstrated a mean difference in LCI between asthmatic preschoolers and healthy controls of 0.5 (SD: 0.4) [206], while a typical mean difference of 2.7(SD: 0.4) exists between CF preschoolers and healthy controls [207]. With a power of 80% at the 0.05 significance level, a sample size of 10 children per group should be sufficient to detect a significant difference of LCI in each respective age group.

There is currently a lack of LCI data from published large healthy control cohorts. We therefore aimed to recruit a large number of healthy controls. In the infant age group, we aimed to include at least 10 subjects from each IPFT visit at 3-months, 1 year and 18-months. For our older healthy cohort, we aimed to recruit at least 10 subjects in each of 5 age groups (3-5, 6-8, 9-11, 12-14, 15-18 years). Therefore, a total of at least 80 healthy controls over the entire pediatric cohort, and at least 40 healthy controls under the age of 6 years, would be recruited. We recruited healthy
controls between May 2009 to February 2011, and included all subjects that met our inclusion/exclusion criteria and completed technically acceptable trials.
Chapter 4:

Results
4 Results

4.1 LCI in Healthy Subjects

Our first objective was to establish and describe a range of normal LCI values from a large healthy control cohort. As mentioned in the Rationale and Objectives (section 2.2), the mean LCI values from small published healthy control cohorts tend to be higher in younger ages (Table 1.8.1). We therefore compared MBW results from a large group of healthy controls that included subjects from ages 3 months to 18 years. It should be noted that the inclusion of the school-age healthy control cohort with our infant and preschool healthy controls was strictly for the purpose of better defining LCI. This older healthy control cohort is not included in any further analyses in subsequent sections of this thesis.

4.1.1 Outline of Healthy Controls

Pulmonary function testing was attempted in 145 healthy subjects. These subjects consisted of 86 infants (aged 0-3 years), 10 preschoolers (aged 3-6 years) and 49 school-aged subjects (aged 6-18 years). After further evaluation, 5 healthy infants were excluded due to history of wheeze requiring ER visits and/or pharmaceutical treatment, while an additional 12 infants did not complete any test due to failed sedation. Furthermore, 8 of the school-aged subjects were dropped from analysis due to erratic breathing patterns, failure to complete any trials, or technical difficulties on the day of testing. Therefore, 120 healthy subjects completed MBW testing, consisting of 69 infants, 10 preschoolers and 41 school-aged subjects. Figure 4.1.1 shows a breakdown summary of healthy subject recruitment.
Table 4.1.1 summarizes demographic characteristics for healthy control subjects categorized by age group (infant, preschool, school-age) and for all healthy subjects combined. Subjects were grouped separately according to age group due to differences in testing procedure and equipment set-up: infants were tested while asleep in the supine position wearing a facemask; preschoolers were tested while awake seated in a chair and wearing a facemask; school-aged children were tested while awake seated in a chair wearing a mouthpiece and noseclips. For children older than the age of 10 years (n= 29), BMI-for-age z-score have been calculated instead of weight-for-age z-scores due to the inability of weight z-scores to properly assess normal weight during the pubescent period.
### Table 4.1.1. Demographic characteristics for all healthy control subjects compared by age group

<table>
<thead>
<tr>
<th></th>
<th>Infants</th>
<th>Preschoolers</th>
<th>School-Age</th>
<th>All Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>69</td>
<td>10</td>
<td>41</td>
<td>120</td>
</tr>
<tr>
<td>% Male</td>
<td>57.4 (0.06)</td>
<td>60.0 (0.15)</td>
<td>57.4 (0.08)</td>
<td>54.2 (0.05)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.71 (0.51)</td>
<td>4.81 (0.82)</td>
<td>12.47 (3.65)</td>
<td>5.07 (5.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>69.5 (7.97)</td>
<td>110.8 (8.9)</td>
<td>152.4 (18.6)</td>
<td>101.3 (40.6)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.1 (1.08)</td>
<td>0.5 (1.1)</td>
<td>0.5 (0.95)</td>
<td>0.3 (1.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.2 (2.10)</td>
<td>19.9 (4.0)</td>
<td>50.3 (21.4)</td>
<td>23.6 (23.2)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.04 (0.9)</td>
<td>0.7 (1.0)</td>
<td>0.6 (0.95) †</td>
<td>0.2 (0.93)</td>
</tr>
<tr>
<td>BMI (kgm⁻²)</td>
<td>16.75 (1.53)</td>
<td>16.1 (0.9)</td>
<td>20.8 (6.0)</td>
<td>18.1 (4.16)</td>
</tr>
<tr>
<td>BMI-for-age z-score</td>
<td>-0.05 (1.1)</td>
<td>0.67 (0.6)</td>
<td>0.6 (1.3)</td>
<td>0.23 (1.18)</td>
</tr>
</tbody>
</table>

† Weight z-scores were only calculated for children under the age of 10 years (n = 12)

### 4.1.2 MBW Parameters for Healthy Controls

#### 4.1.2 i: Calculation of z-scores for respiratory rate (RR) and tidal volume (VT)

Tidal volume (VT) and respiratory rate (RR) were calculated during the MBW for all healthy subjects. VT was positively correlated to age and height (p<0.001), while RR was negatively correlated to height (p<0.001) for all healthy controls. Figure 4.1.2 illustrates the relationship between VT and age and between VT and height. Figure 4.1.3 illustrates the relationship between RR and age. Each of these figures includes the coefficient of determination (R²) of the linear relationship between axes variables, which describes the proportion of data variability that is accounted for by the linear relationship. In order to compare VT and RR between groups, linear regression was performed to correct for significant factors in the model. Table 4.2.3 shows the linear regression equations used to generate predicted values for VT and RR. Z-scores were then calculated using the following equation: (value-predicted value)/Residual Standard Deviation (RSD).
Figure 4.1.2. Relationship between tidal volume ($V_T$) and height and between $V_T$ and age for all healthy control subjects. $R^2$: Coefficient of determination.

Figure 4.1.3. Relationship between respiratory rate (RR) and height for all healthy control subjects. $R^2$: Coefficient of determination.
Table 4.1.2. Linear regression equations to calculate predictive values for tidal volume and respiratory rate based on significant factors

<table>
<thead>
<tr>
<th>Slope (Coefficient B)</th>
<th>Y-intercept</th>
<th>$r^2$</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Height (cm)</td>
<td>$r^2$</td>
<td>RSD</td>
</tr>
<tr>
<td>$V_T$</td>
<td>18.17</td>
<td>1.64</td>
<td>-53.79</td>
</tr>
<tr>
<td>RR</td>
<td>na</td>
<td>-0.12</td>
<td>38.22</td>
</tr>
</tbody>
</table>

Predicted value for $V_T = (\text{age value} \times B) + (\text{height value} \times B) + \text{Y-intercept}$

Predicted value for $RR = (\text{age value} \times B) + \text{Y-intercept}$

$V_T$: tidal volume; $RR$: respiratory rate; RSD: Residual standard deviation; na: not applicable

4.1.2. ii: Comparing MBW parameters between healthy control age groups

Table 4.1.3 presents MBW parameters for all healthy controls. Mean LCI for healthy infants was significantly higher than LCI in preschool-aged children and in school-aged children, with no significant difference in LCI existing between our preschool-age and school-age children. There were significant differences in FRC between age groups, which was expected since FRC is highly dependent on age ($r = -0.5$, $p=0.001$). In an effort to illustrate that FRC trends were not significantly different between infants, preschool-age and school-age subjects, FRC values have been plotted against age for all healthy controls in Figure 4.1.4. It can be seen from the $R^2$ value that the curvilinear relationship between FRC and age accounts for a high proportion of variability in FRC values for all healthy control subjects. Since FRC is a component of the LCI calculation ($LCI = CEV/FRC$), it is unlikely that the variability in FRC is responsible for the high variability in mean LCI observed in infants compared to older subjects.
Table 4.1.3. MBW parameters for all healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Infants (n=69)</th>
<th>Preschool-age (n=10)</th>
<th>School-aged (n=41)</th>
<th>Kruskal-Wallis P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC (L)</td>
<td>0.18 (0.04)</td>
<td>0.67 (0.204)†</td>
<td>1.74 (0.79)†*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LCI</td>
<td>6.95 (0.61)</td>
<td>6.21 (0.42)†</td>
<td>6.23 (0.42)†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RR (per minute)</td>
<td>30.2 (5.5)</td>
<td>21.2 (2.8)</td>
<td>20.7 (5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RR z-score</td>
<td>-0.01 (1.13)</td>
<td>-0.72 (0.66)</td>
<td>0.11 (1.03)*</td>
<td>0.04</td>
</tr>
<tr>
<td>VT (ml)</td>
<td>70.3 (21.6)</td>
<td>254.3 (124.1)</td>
<td>434.8 (192.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VT z-score</td>
<td>-0.04 (1.45)</td>
<td>0.10 (0.64)</td>
<td>-1.02 (1.5)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

†Significantly different from infants; *Significantly different from preschoolers

Figure 4.1.4. Relationship between FRC (L) and age for all healthy controls. Variability of LCI during infancy is relatively small compared to older children and follows a quadratic trend with age. $R^2$: Coefficient of determination.
4.1.3 Correlation of LCI with growth parameters, age, and other MBW parameters

Investigation into whether LCI in health was associated with any demographic characteristics (age, sex, height z-scores, and weight z-score) or with other MBW parameters ($V_T$, RR and FRC) was carried out by Spearman correlations for all healthy controls combined (Table 4.1.4). LCI was significantly negatively correlated with age, while no association was found between LCI and growth demographics (height z-score, weight z-score or BMI z-score), or between LCI and breathing pattern parameters (RR z-score or $V_T$ z-score). There was also no significant difference in LCI between males and females (Mann-U Whitney Z score = -0.23, p =0.82).

There was a significant negative association between LCI and FRC ($r = -0.5$, $p=0.001$). It is not surprising that LCI correlated well with FRC, since FRC is used in the calculation of LCI ($LCI=CEV/FRC$). Figure 4.1.5 plots LCI against FRC for all healthy controls. This figure illustrates the higher mean and range of LCI values in infants compared to older subjects, and shows how LCI decreases as FRC values increase with growth.

![Figure 4.1.5. LCI plotted against FRC values in liters (L) for all healthy controls (n=120).](image)

Figure 4.1.5. LCI plotted against FRC values in liters (L) for all healthy controls (n=120).
To investigate whether LCI was differentially dependent on demographic and MBW parameters depending on the age group of healthy subjects, we correlated LCI with these parameters within each age group separately (Table 4.1.4). Correlations between LCI and weight z-scores existed in healthy infants and in school-age groups, although the direction of the correlation was positive in infants and negative in school-age. In the preschool age group, there was a negative correlation between LCI and FRC values; however this association is driven by 2 older subjects within this small sample group with relatively low LCI values.

Table 4.1.4. Correlations between LCI and demographic/MBW parameters for healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Infants (n=69)</th>
<th>Preschoolers (n=10)</th>
<th>School-age (n=41)</th>
<th>All subjects (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.374 (0.002)*</td>
<td>-0.25 (0.5)</td>
<td>0.134 (0.4)</td>
<td>-0.385 (0.001)*</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.025 (0.8)</td>
<td>-0.03 (0.9)</td>
<td>-0.032 (0.8)</td>
<td>-0.128 (0.2)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.337 (0.005)*</td>
<td>-0.24 (0.5)</td>
<td>-0.65 (0.015)*</td>
<td>0.038 (0.7)</td>
</tr>
<tr>
<td>BMI-for-age z-score</td>
<td>n/a</td>
<td>n/a</td>
<td>-0.31 (0.08)</td>
<td>-0.12 (0.2)</td>
</tr>
<tr>
<td><strong>MBW Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR z-score</td>
<td>0.001 (0.9)</td>
<td>-0.467 (0.2)</td>
<td>-0.16 (0.3)</td>
<td>-0.065 (0.5)</td>
</tr>
<tr>
<td>VT z-score</td>
<td>0.162 (0.2)</td>
<td>-0.18 (0.6)</td>
<td>-0.21 (0.2)</td>
<td>0.074 (0.4)</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>0.041 (0.7)</td>
<td>-0.69 (0.03)*</td>
<td>0.02 (0.9)</td>
<td>-0.502 (0.001)*</td>
</tr>
</tbody>
</table>

Values are presented as Spearman correlation coefficient (p-value). *Bolded = significant p-value
4.1.4 LCI is dependent on age

Figure 4.1.6 plots LCI and age for all healthy controls. As can be surmised from this figure, the relationship between LCI and age is not linear one; LCI decreases with age in a curvilinear fashion. Table 4.1.4 also demonstrated that age is negatively correlated with LCI. We therefore determined the 50th centile (predicted median), and the 97.5th centile and 2.5th centile (95% confidence interval) of LCI with age using fractional polynomial regression, which is an extension of simple regression analysis that allows for modeling of non-linear relationships [236]. We were therefore able to plot a continuous curvilinear model for LCI based on age that spanned the entire pediatric age range, based on healthy LCI data from our center. The equation for the predicted median curve that was calculated using fractional polynomial regression can also be found in Figure 4.1.6.

Figure 4.1.6. Relationship between LCI and age for all healthy control subjects
4.1.4 i. Addition of MBW parameters to regression model of LCI based on age for all healthy controls

In order to confirm results presented in Table 4.1.4 which demonstrated that LCI is not associated within changes in breathing pattern variables yet is associated with FRC, we added RR, $V_T$ and FRC parameters to the fractional polynomial regression of LCI based on age (Table 4.1.5). As suspected, RR and $V_T$ were not significant factors in the model. By comparing the akaike information criterion (AIC), a measure of the relative goodness of fit of our model to LCI results, it can be seen that addition of RR, $V_T$ and FRC either separately or in combination did not improve the fit of the regression line as demonstrated by an increase in the AIC value. Interestingly, the addition of FRC was not significantly predictive in our model of LCI based on age, despite the strong negative correlation seen between LCI and FRC in Table 4.1.4. This demonstrates that there is no correlation between LCI and FRC independent of age.

The addition of age group to our model of LCI allowed us to examine whether our predicted regression line differed in subjects who were designated as “infants” compared to those designated as “preschool or older”. Adding the age group of the subject as a factor improved the predictive power ($p<0.001$) and fit of our model as illustrated by the decrease in the AIC value. It can be seen that the slope estimate of LCI is lower in the preschool age group (-1.33) compared to the infant age group (-1.06), demonstrating that independent of age in years, preschooler healthy controls have lower LCI values compared to infants.
Table 4.1.5. Addition of RR, VT and FRC variables to our fractional polynomial regression model for LCI

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Coefficients</th>
<th>Slope</th>
<th>95% CI</th>
<th>p Value</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>(age)(^{0.5})</td>
<td>-0.236</td>
<td>-0.31 to -0.162</td>
<td>&lt;0.001</td>
<td>2.06</td>
</tr>
<tr>
<td>+ RR</td>
<td>(RR)</td>
<td>-0.006</td>
<td>-0.03 to 0.01</td>
<td>0.6</td>
<td>2.08</td>
</tr>
<tr>
<td>+ VT</td>
<td>(VT)</td>
<td>-0.0007</td>
<td>-0.002 to 0.0006</td>
<td>0.3</td>
<td>2.07</td>
</tr>
<tr>
<td>+ RR + VT</td>
<td>(RR)</td>
<td>-0.008</td>
<td>-0.03 to 0.01</td>
<td>0.4</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>(VT)</td>
<td>-0.0008</td>
<td>-0.002 to 0.0005</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>+ Age Group</td>
<td>(infant)</td>
<td>-1.06</td>
<td>-1.5 to -0.6</td>
<td>&lt;0.001</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>(preschool)</td>
<td>-1.33</td>
<td>-2.0 to -0.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+ FRC</td>
<td>(FRC)</td>
<td>-0.06</td>
<td>-0.3 to 0.2</td>
<td>0.7</td>
<td>2.07</td>
</tr>
<tr>
<td>+ FRC + RR</td>
<td>(FRC)</td>
<td>-0.05</td>
<td>-0.3 to 0.2</td>
<td>0.7</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>(RR)</td>
<td>-0.006</td>
<td>-0.03 to 0.01</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>+ FRC + VT</td>
<td>(FRC)</td>
<td>-0.018</td>
<td>-0.3 to 0.3</td>
<td>0.9</td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td>(VT)</td>
<td>-0.0007</td>
<td>-0.0007 to 0.0007</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>+ FRC + RR + VT</td>
<td>(FRC)</td>
<td>-0.004</td>
<td>-0.3 to 0.3</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>(RR)</td>
<td>-0.008</td>
<td>-0.03 to 0.01</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(VT)</td>
<td>-0.0008</td>
<td>-0.002 to 0.0006</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

RR: Respiratory Rate; VT: Tidal Volume; FRC: Functional Residual Capacity; CI: Confidence Interval; AIC: Akaike Information Criterion.
4.1.5 Comparison of healthy MBW results obtained at HSC with those from other centers

Table 4.1.6 provides a comparison of healthy control MBW results obtained at our center to those previously published by the Institute for Child Health (ICH) in London, UK and the Department of Pediatrics in Skövde, Sweden. A strength of this study was the large number of healthy control subjects tested; we were therefore interested in the level of agreement between our data and those from similar but smaller populations at other centers that used the same equipment set-up. Infants studied at our center were similar to those at ICH with respect to age, height, weight and LCI values; however, mean FRC values were significantly lower at ICH compared to our infant subjects (p<0.001). The variability of LCI mean values represented by LCI standard deviation (SD) within our infant subjects was greater than that of ICH. LCI was significantly higher for preschool-aged children at ICH compared to our center, despite the study samples being comparable in age, height and weight. School-aged children at our center were on average approximately 1 year older than school-age study populations at ICH and Sweden, and also significantly heavier (p<0.001). LCI was lower in our school-aged subjects compared to those at ICH and Sweden, with a statistically significant difference between our center and ICH (p<0.05).
Table 4.1.6. Comparison of results obtained from the Hospital for Sick Children, Toronto, Canada with those previously reported from the Institute of Child Health, London, UK and the Department of Pediatrics, Skövde, Sweden

### INFANTS

<table>
<thead>
<tr>
<th></th>
<th>Canada</th>
<th>UK †</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>68</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>0.69 (0.5)</td>
<td>0.71 (0.29)</td>
<td>-0.02 (-0.24 to 0.2)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>8.1 (2.04)</td>
<td>8.5 (1.2)</td>
<td>-0.36 (-1.3 to 0.6)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>69.2 (7.61)</td>
<td>72.2 (4.9)</td>
<td>-3.0 (-6.5 to 0.51)</td>
</tr>
<tr>
<td><strong>LCI</strong></td>
<td>7.0 (0.61)</td>
<td>7.2 (0.3)</td>
<td>-0.25 (-0.53 to 0.03)</td>
</tr>
<tr>
<td><strong>FRC (mL)</strong></td>
<td>180 (40)</td>
<td>156 (38)</td>
<td>24 (4.4 to 43.6)</td>
</tr>
</tbody>
</table>

### PRESCHOOL-AGE

<table>
<thead>
<tr>
<th></th>
<th>Canada</th>
<th>UK §</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>4.85 (0.71)</td>
<td>4.31 (0.84)</td>
<td>0.34 (-0.3 to 0.94)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>19.1 (4.02)</td>
<td>18.8 (3.4)</td>
<td>0.3 (-2.3 to 2.9)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>109.2 (8.9)</td>
<td>105.3 (7.7)</td>
<td>3.85 (-2.1 to 9.8)</td>
</tr>
<tr>
<td><strong>LCI</strong></td>
<td>6.2 (0.42)</td>
<td>6.9 (0.44)</td>
<td>-0.57 (-0.9 to -0.25)*</td>
</tr>
<tr>
<td><strong>FRC (L)</strong></td>
<td>0.67 (0.204)</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

### SCHOOL-AGE

<table>
<thead>
<tr>
<th></th>
<th>Canada</th>
<th>UK ‡</th>
<th>Sweden‡</th>
<th>Kruskal Wallis P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>41</td>
<td>33</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>12.47 (3.65)</td>
<td>11.3 (3.2)</td>
<td>11.4 (3.2)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>73.3 (49.0)</td>
<td>41.1 (14.7)*</td>
<td>40.3 (11.7)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>152.4 (18.5)</td>
<td>147.4 (18.3)</td>
<td>148.6 (13.0)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>LCI</strong></td>
<td>6.2 (0.42)</td>
<td>6.5 (0.49)*</td>
<td>6.33 (0.43)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>FRC (L)</strong></td>
<td>1.74 (0.79)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as mean (SD). LCI: Lung clearance index; FRC: Functional Residual Capacity; ULN: Upper Limit of Normal; NR: Not reported. Difference calculated as Canada-UK.

*Significantly different from Canada
† Lum et al.[207]; § Aurora et al.[208]; ‡ Aurora et al.[179]; ‡ Gustafsson et al.[209].
4.2 LCI in Diseased Cohorts Compared to Healthy Subjects

This section investigates whether LCI is elevated in infants and preschool-aged subjects with disease (CF or wheezy/asthmatic) compared to healthy infants and preschool-aged children, how elevated LCI values relate to other MBW parameters, and compares the proportion of those subjects who fall above our upper limit of normal for LCI based on age.

4.2.1 Description of Infant and Preschool-age Subjects

We attempted pulmonary function testing in 178 subjects between the ages of 3 months to 6 years. Of these children, 35 were CF subjects, (19 infants, 16 preschoolers), 47 were wheezy/asthmatic subjects (30 wheezy infants, 17 asthmatic preschoolers), and 96 were healthy controls (86 infants, 10 preschoolers). In our CF diagnosis group, 1 preschool-age subject was unable to complete any testing, while 2 other preschool-age subjects had to be excluded due to equipment problems on the day of testing. In addition, 4 of our wheezy infants did not complete any testing due to failed sedation. Thus, successful MBW testing was obtained in 33 subjects with CF (19 infants, 13 preschoolers), and in 43 wheezy/asthmatic subjects (26 infants, 17 preschoolers).
Figure 4.2.1. Summary of subject recruitment for all infants and preschool-age subjects

As explained in the previous section for healthy controls, subjects were grouped separately based on the type of testing they completed; either infant testing requiring sedation, or preschool testing while awake. These groups generally corresponded to age, with infants aged 0-3 years and preschoolers aged 3-6 years. The only exception to this age categorization was 2 CF subjects who were both 2 years old and completed preschool testing (no sedation). Once separated into age groups, subjects were compared by diagnosis group (healthy, wheezy/asthmatic, or CF).
4.2.2 Demographics of infant and preschool subjects

Table 4.2.1 compares the demographic characteristics between healthy, wheeze/asthmatic, and CF subjects for infant and preschool-age groups separately. For the infant group, there was a significant overall age difference between the disease cohorts. Post-hoc comparison revealed that wheezy infants were significantly older than healthy controls; no significant difference was found for age between healthy controls and infants with CF. As such, discrepancies in height and weight values were also found between the disease cohorts. After adjusting for age and sex, infants with CF were found to be shorter than healthy controls (p<0.05). There was no significant difference in weight corrected for age between the disease cohorts during infancy. BMI was not calculated for the infant group as it is less useful as a measure of body mass during this age group compared to older children [237].

For the preschool group, there was only a significant age difference between CF and asthma cohorts (p<0.05). No significant difference in the comparison of age was seen between healthy controls and CF subjects or between healthy controls and asthma subjects. Healthy controls were found to be heavier and taller than children with CF or asthma, with a significant difference in height and weight z-score existing between healthy and CF preschoolers (p<0.05).
Table 4.2.1. Demographic characteristics of diagnosis groups for infants and preschoolers

<table>
<thead>
<tr>
<th></th>
<th>INFANTS</th>
<th>PRESCHOOLERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>Wheezy</td>
</tr>
<tr>
<td>N</td>
<td>69</td>
<td>26</td>
</tr>
<tr>
<td>% Male</td>
<td>57.4 (0.06)</td>
<td>80.8 (0.08)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.71 (0.51)</td>
<td><strong>1.7 (0.59)</strong></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>69.5 (7.97)</td>
<td>82.5 (6.74)</td>
</tr>
<tr>
<td>Height-for-age (z-score)</td>
<td>0.1 (1.08)</td>
<td>-0.4 (1.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.2 (2.10)</td>
<td>11.4 (1.77)</td>
</tr>
<tr>
<td>Weight-for-age (z-score)</td>
<td>0.04 (0.9)</td>
<td>0.03 (1.13)</td>
</tr>
<tr>
<td>BMI</td>
<td>16.1 (0.9)</td>
<td>15.9 (0.97)</td>
</tr>
<tr>
<td>BMI-for-age (z-score)</td>
<td>0.7 (0.6)</td>
<td>0.4 (0.7)</td>
</tr>
</tbody>
</table>

*Significant difference compared to Healthy Controls; †Significant difference compared to Asthma group
4.2.3 MBW parameters in wheeze/asthma compared to healthy controls

Table 4.2.2 demonstrates the differences in age and MBW parameters between wheezy infants and healthy infants, and between asthmatic preschoolers and healthy preschoolers. Mean LCI was elevated in infants with a history of wheeze and in preschoolers with asthma compared to healthy controls. Since there was also a significant age difference between wheezy infants and healthy controls, we investigated whether a diagnosis of wheeze/asthma caused LCI to be higher than healthy controls using the fractional polynomial regression model of LCI corrected for age, by adding diagnosis (healthy control or wheeze/asthmatic) as a factor in the model. The slope estimate of LCI in wheezy infants and asthmatic preschoolers was on average 0.72 units (95% CI: 0.42 to 1.03) greater than that of healthy controls (p<0.001).

FRC values were also elevated in infants with a history of wheeze compared to healthy controls, but not in preschoolers. This finding is likely due to the significant age difference (p<0.001) between infants with a history of wheeze and healthy controls, as FRC is dependent on age (r = 0.905, p<0.01). Figure 4.2.3 plots the relationship between FRC and age for all diagnosis cohorts (healthy, asthmatic, CF) in both infants and preschoolers, demonstrating that there are no differences in trends for FRC between the different diagnosis cohorts.

After correction for age and length, RR z-scores were found to be significantly lower in infants with a history of wheeze compared to healthy controls (p=0.03), although only 2 (7.7%) infants with wheeze were found to have RR z-scores below the normal range (<1.96 SD). There was no significant correlation between LCI and RR z-score in infants with wheeze (r = -0.13, p=0.5), yet there was a positive correlation between LCI and RR z-score in asthmatic preschoolers (r= 0.61, p=0.01). Figure 4.2.2 demonstrates the relationship between RR z-score and LCI. We investigated the interaction between RR and age group in our fractional polynomial regression model of LCI based on age in order to determine if RR had a different impact on the regression model of LCI in infancy compared to preschool-age. We observed a slope of 0.002 in infants with wheeze compared to a slope of 0.1 in preschoolers with asthma (p<0.001). These results suggest that after accounting for age effects, LCI increases with increasing RR in preschool asthmatics but no such trend is seen in infants with a history of wheeze.

$V_T$ z-scores were lower in infants with a history of wheeze and preschool asthmatics compared to healthy controls, although these differences were not significant in either age group. LCI positively correlated with $V_T$ z-score corrected for age and height in infants with a history of wheeze.
wheeze (r = 0.5, p=0.01), yet no such correlation was found in asthmatic preschoolers (r =-0.4, p =0.12). We studied the interaction between Vₜ and age group in our fractional polynomial regression model of LCI and age, and found no significance (p=0.07). We therefore saw no significant trends between Vₜ and LCI in wheezy/asthmatic subjects.

Table 4.2.2. MBW results for infants with a history of wheeze (Wheezy) and asthmatic preschoolers compared to healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Infants</th>
<th>Preschoolers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Controls (n=69)</td>
<td>Asthma (n=17)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.7 (0.5)</td>
<td>4.8 (0.81)</td>
</tr>
<tr>
<td></td>
<td>1.68 (0.59)</td>
<td>4.7 (1.1)</td>
</tr>
<tr>
<td></td>
<td>p Value</td>
<td>p Value</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>0.2 (0.04)</td>
<td>0.7 (0.21)</td>
</tr>
<tr>
<td></td>
<td>0.27 (0.06)</td>
<td>0.6 (0.17)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.3</td>
</tr>
<tr>
<td>LCI</td>
<td>7.0 (0.61)</td>
<td>6.2 (0.41)</td>
</tr>
<tr>
<td></td>
<td>7.4 (0.8)</td>
<td>7.1 (1.34)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>RR (per minute)</td>
<td>30.2 (5.5)</td>
<td>21.22 (2.8)</td>
</tr>
<tr>
<td></td>
<td>26.0 (6.7)</td>
<td>24.8 (7.4)</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.2</td>
</tr>
<tr>
<td>RR z-score</td>
<td>-0.01 (1.13)</td>
<td>-0.64 (0.6)</td>
</tr>
<tr>
<td></td>
<td>-0.7 (1.58)</td>
<td>-0.1 (1.47)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>VT (ml)</td>
<td>71.0 (21.6)</td>
<td>254.2 (124.0)</td>
</tr>
<tr>
<td></td>
<td>104.4 (19.7)</td>
<td>210 (39.0)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.2</td>
</tr>
<tr>
<td>VT z-score</td>
<td>-0.6 (0.144)</td>
<td>0.1 (1.22)</td>
</tr>
<tr>
<td></td>
<td>-0.8 (0.4)</td>
<td>-0.7 (0.5)</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Bolded = significant p value (p<0.05).

Figure 4.2.2. Relationship between LCI and RR z-score for asthmatic preschoolers.
4.2.4 MBW Parameters in CF compared to healthy controls

Table 4.2.3 compares age and MBW parameters between CF infants and preschoolers and healthy controls. In subjects with CF, mean LCI was significantly elevated compared to healthy controls in preschool-aged children, but not in infants. Using the fractional polynomial regression model of LCI corrected for age, we examined the effect of diagnosis (CF vs. healthy controls). LCI in CF infants and preschoolers was on average 1.46 units greater than that of healthy controls, (p<0.001). There was, however, a significant difference in the amount of elevation of LCI above healthy controls between infants and preschool-aged subjects with CF. While LCI in CF infants was on average 0.4 units higher than healthy control infants, this difference increased to 4.0 units in preschool-age (p<0.001).

FRC values on average were significantly higher in infants with CF compared to healthy controls (p=0.04), while they were significantly lower in preschoolers with CF (p=0.01). Similar to the wheezy/asthmatic cohort, this difference in FRC compared to healthy controls is likely due to significant age differences between healthy and CF subjects in both age groups. The relationship with FRC and age for all subjects is illustrated in Figure 4.2.3, where it can be seen that subjects with disease (history of wheeze/asthma or CF) show the same trend in FRC with age as healthy subject. Furthermore, no significant correlation was found between FRC and LCI in infants (r = -0.17, p=0.5) or preschoolers (r=0.19, p=0.5) with CF.

Subjects with CF had a greater mean RR compared to healthy controls during preschool-aged years (p=0.003). RR z-scores corrected for height were also significantly elevated in preschool aged subjects with CF (p=0.05). LCI did not correlate significantly with RR z-scores for CF subjects during infancy (r = -0.26, p=0.3), or during preschool years (r = -0.03, p=0.9). The slight negative trend between LCI and RR illustrated in these correlations, however, is also reflected when RR is added as a factor to our fractional polynomial regression model of LCI corrected for age and diagnosis. The addition of RR contributes a negative slope estimate of -0.005 to the model, yet this addition is not significant. By investigating the interaction between RR and age group, we observed a slope of -0.02 in infants compared with 0.1 in preschoolers. These results suggest that after accounting for age effects, LCI increases slightly with increasing RR in preschoolers, but not in infants with CF (p=0.04), although a strong correlation between LCI and RR is missing in CF subjects.
Mean $V_T$ values did not differ between healthy controls and CF children during infancy or during preschool-age after correction for height and age, and no significant correlation between LCI and $V_T$ was seen in infants with CF ($r=0.02$, $p=0.9$), or in preschoolers with CF ($r=0.3$, $p=0.3$). There was therefore no association between LCI and $V_T$ in any of the CF subjects.

Table 4.2.3. MBW results for infants and preschoolers with CF compared to healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Infants</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Controls (n=69)</td>
<td>CF (n=19)</td>
<td>p Value</td>
<td>Healthy Controls (n=10)</td>
<td>CF (n=13)</td>
<td>p Value</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.7 (0.5)</td>
<td>0.9 (0.35)</td>
<td><strong>0.005</strong></td>
<td>4.8 (0.81)</td>
<td>3.7 (0.77)</td>
<td><strong>0.004</strong></td>
<td></td>
</tr>
<tr>
<td>FRC (L)</td>
<td>0.18 (0.04)</td>
<td>0.20 (0.07)</td>
<td><strong>0.04</strong></td>
<td>0.67 (0.21)</td>
<td>0.49 (0.11)</td>
<td><strong>0.01</strong></td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>7.0 (0.61)</td>
<td>7.4 (0.91)</td>
<td>0.15</td>
<td>6.2 (0.41)</td>
<td>9.5 (2.63)</td>
<td><strong>&lt;0.001</strong></td>
<td></td>
</tr>
<tr>
<td>RR (per minute)</td>
<td>30.2 (5.5)</td>
<td>33.1 (7.4)</td>
<td><strong>0.09</strong></td>
<td>21.2 (2.8)</td>
<td>27.4 (6.4)</td>
<td><strong>0.001</strong></td>
<td></td>
</tr>
<tr>
<td>RR z-score</td>
<td>-0.01 (1.13)</td>
<td>0.17 (1.32)</td>
<td>0.5</td>
<td>-0.64 (0.6)</td>
<td>0.19 (1.11)</td>
<td><strong>0.05</strong></td>
<td></td>
</tr>
<tr>
<td>VT (ml)</td>
<td>71.0 (21.6)</td>
<td>80.5 (24.6)</td>
<td>0.1</td>
<td>254.2 (124.0)</td>
<td>188.7 (52.4)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>VT z-score</td>
<td>-0.6 (0.144)</td>
<td>-0.7 (0.32)</td>
<td>0.7</td>
<td>0.1 (1.22)</td>
<td>-0.5 (0.65)</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

**Bolded** = significant p value (p<0.05).
4.2.5 Change in LCI between age groups

In order to investigate whether LCI elevation reflects disease progression, we compared LCI in infancy and in preschool-age for healthy controls, wheezy/asthmatics, and CF subjects respectively (Figure 4.2.4). There was a significant increase in mean LCI in preschool-age children with CF compared to infants with CF (p<0.0001). There was no change in LCI between age groups for healthy children or for children with a history of wheeze or asthma after post-hoc corrections. These results were confirmed by testing the interaction effect of age group and diagnosis cohort in our fractional polynomial regression of LCI and age for all infants and preschoolers. For wheezy subjects, LCI corrected for age was on average 0.68 units higher for preschoolers compared to infants; a difference which did not reach statistical significance (p=0.55). In contrast, the slope estimate for LCI was increased by 3.57 units in CF preschoolers compared to healthy controls, while LCI was only 0.4 units CF higher than healthy controls in infants when age group was added to the model (p<0.0001).
**Figure 4.2.4.** Mean LCI difference in infants and preschoolers compared by diagnosis group. Error bars represent SD. *p<0.0001.

### 4.2.6 Discrimination between health and disease by ULN

We have investigated healthy LCI values from the full pediatric age range and have demonstrated that LCI is not independent of age. It is therefore difficult to use the upper limit of normal (ULN) for LCI as traditionally defined as 1.96SD above the mean LCI of a healthy control population of a specific age group. Alternatively, we have used the 97.5th centile (that is the same as 1.96SD) calculated by fractional polynomial regression of our full pediatric healthy control cohort (ages 3 months to 18 years) to define a continuous ULN for LCI values for our center (see section 4.2c). Any LCI value falling on or above this ULN is considered to be abnormally elevated compared to our healthy cohort. This limit is plotted for all diagnosis cohorts in the infant and preschool age groups in Figure 4.2.5.
Figure 4.2.5. LCI plotted against age for healthy controls, CF and wheezy/asthmatic subjects in the infant and preschool age groups. ULN: Upper limit of normal for an LCI measurement as calculated by regression of all healthy controls at our center (ages 3 months to 18 years).
A summary of the proportion of subjects falling on or above our calculated ULN for LCI is demonstrated in Table 4.2.4. A total of 29 subjects had LCI values equal to or greater than our ULN. Of these 29 subjects, 14 were CF subjects, (44% of total CF population; n=32), 11 were wheezy/asthmatic subjects (26% of total wheezy/asthmatic population; n=46), and 4 were healthy controls (5% of total healthy control population; n=79). The proportion of wheezy infants with elevated LCI values was significantly higher than healthy controls, yet the proportion of CF infants with elevated LCI values was not significantly different from healthy controls (p=0.06). In the preschool-age group, the proportion of CF and asthmatic preschoolers detected as having abnormally high LCI values was significantly greater than healthy controls.

Table. 4.2.4. Proportion of subjects with LCI values greater than or equal to our upper limit of normal for an LCI measure

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>Wheezy/Asthmatic</th>
<th>CF</th>
<th>Wheezy/Asthmatic vs. Healthy Controls p-value</th>
<th>CF vs. Healthy Controls p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>5.8% (4/69)</td>
<td>27.0% (7/26)</td>
<td>21.1% (4/19)</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Preschool-age</td>
<td>0% (0/10)</td>
<td>23.5 % (4/17)</td>
<td>77% (10/13)</td>
<td>0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are presented as percent proportions (number of individuals with elevated LCI/n for group)
4.3 Variability of LCI in Health and Disease Groups

The LCI and other MBW parameters are calculated by averaging the values of 2-3 technically acceptable MBW trials completed by each subject. Knowledge of the variability of repeat measures of MBW parameters and how such variability is related to LCI is necessary in order to define the stability of the test within individuals. This knowledge is also required for established quality control criteria during analysis of MBW trials. The aim of this section is to illustrate the within-test variability of MBW parameters and correlate this variability to potentially influencing factors in an effort to better characterize the LCI in health and disease.

4.3.1 Within-test Variability of MBW Parameters

The percent coefficient of variation (CV%) was used to define the variability of MBW parameters between the 2-3 technically acceptable MBW trials completed by each subject. Within-test CV% (WTCV%) is a common form of assessing the repeatability and precision of data and the methods used to obtain data by calculating the percent ratio of the standard deviation (SD) to the mean [238]. A smaller ratio demonstrates lower variability amongst measurements. Table 4.3.1 shows WTCV% results for LCI, FRC, VT, and RR. WTCV% is on average low for all MBW parameters, regardless of age group or diagnosis group. The only significant difference in the variability of an MBW parameter between diagnosis groups was for RR in infants. Healthy infants had a significantly higher RR WTCV% compared to wheezy infants (p<0.05), although no significant difference was found between healthy controls and CF infants.

Variability in MBW parameters was generally higher in preschool-aged children compared to infants for healthy controls and wheezy/asthmatic subjects, but no such difference was found in CF subjects. For healthy controls, FRC and VT had variability that was significantly higher in preschoolers compared to infants (p values = 0.003 for both). For wheezy/asthmatic subjects, all parameters except for FRC had variability that were significantly higher in preschool aged subjects (LCI p-value = 0.012, VT p-value = 0.04, RR p-value = 0.0004). For subjects with CF, there was surprisingly no significant difference in the variability of any MBW parameter investigated between infants and preschoolers.
Table 4.3.1. Percent coefficient of variation for MBW parameters

<table>
<thead>
<tr>
<th>WTCV%</th>
<th>ALL SUBJECTS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>INFANTS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>PRESCHOOLERS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Controls (n=79)</td>
<td>Wheezy (n=43)</td>
<td>CF (n=32)</td>
<td>Kruskall-Wallis P Value</td>
<td>Healthy Controls (n=69)</td>
<td>Wheezy (n=26)</td>
<td>CF (n=19)</td>
<td>Kruskall-Wallis P Value</td>
<td>Healthy Controls (n=10)</td>
<td>Wheezy (n=17)</td>
<td>CF (n=13)</td>
<td>Kruskall-Wallis P Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>4.7 (3.7)</td>
<td>5.0 (3.1)</td>
<td>6.7 (2.2)</td>
<td>0.6</td>
<td>4.5 (3.9)</td>
<td>4.0 (2.8)</td>
<td>4.9 (4.1)</td>
<td>0.8</td>
<td>5.8 (3.1)</td>
<td>6.4 (3.1)</td>
<td>5.7 (3.1)</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRC</td>
<td>4.0 (3.6)</td>
<td>5.6 (4.4)</td>
<td>2.4 (4.0)</td>
<td>0.6</td>
<td>4.5 (3.6)</td>
<td>5.26 (4.8)</td>
<td>6.46 (5.6)</td>
<td>0.4</td>
<td>8.4 (4.7)</td>
<td>6.1 (3.7)</td>
<td>5.6 (4.8)</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>5.3 (4.2)</td>
<td>7.0 (8.4)</td>
<td>6.2 (5.9)</td>
<td>0.7</td>
<td>4.6 (3.3)</td>
<td>5.36 (3.4)</td>
<td>6.4 (4.0)</td>
<td>0.2</td>
<td>9.6 (7.0)</td>
<td>10.5 (11.3)</td>
<td>7.9 (9.5)</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>6.8 (12.7)</td>
<td>7.4 (7.9)</td>
<td>6.8 (6.7)</td>
<td>0.5</td>
<td>6.5 (13.6)</td>
<td>3.5 (3.5)*</td>
<td>5.4 (4.5)</td>
<td>0.04</td>
<td>9.3 (3.6)</td>
<td>12.2 (10.7)</td>
<td>7.2 (7.5)</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CV%: coefficient of variation; VT: tidal volume; RR: Respiratory rate; FRC: Functional Residual Capacity; LCI: Lung Clearance Index. *Significant compared to healthy controls
4.3.2 Associations between percent coefficient of variation of LCI with age, MBW parameters and MBW parameter variation

We investigated whether the within-test variability of LCI correlated with age, MBW parameters and the variance of MBW parameters. In concordance with generally higher variability in MBW parameters in the preschool group as seen in Table 4.3.1, there was a significant correlation between the WTCV% of LCI and age when all infant and preschool subjects were combined ($r = 0.225$, $p = 0.005$). Figure 4.3.1 plots the WTCV% of LCI against age for all diagnosis cohorts, with a linear regression line demonstrating a mean increase in LCI with age when all subjects are combined.

![Figure 4.3.1](image)

**Figure 4.3.1.** LCI within-test percent coefficient of variation (WTCV%) plotted against age (years) for all diagnosis cohorts. Line shows increase in mean LCI WTCV% values with age.
4.3.2. Associations between LCI WTCV% and age, MBW parameters, and variation of MBW parameters in infants

Table 4.3.2 shows the correlations between LCI WTCV% and age, MBW parameters and the variability of MBW parameters for the infant age group. Variability of LCI positively correlated with age and FRC in infants with CF. In wheezy infants, LCI WTCV% correlated with LCI, demonstrating that those wheezy infants with high LCI values also had more variability in LCI between trials.

Table 4.3.2. Correlations between LCI within-test percent coefficient of variation and age, MBW parameters and coefficients of variation of MBW parameters in infant subjects divided by diagnosis group

<table>
<thead>
<tr>
<th>LCI WTCV%</th>
<th>Healthy controls (n=69)</th>
<th>Wheezy/Asthmatic (n=26)</th>
<th>CF(n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p Value</td>
<td>r</td>
</tr>
<tr>
<td>Age</td>
<td>0.04</td>
<td>0.7</td>
<td>-0.17</td>
</tr>
<tr>
<td>LCI</td>
<td>-0.02</td>
<td>0.9</td>
<td>0.43</td>
</tr>
<tr>
<td>FRC</td>
<td>-0.13</td>
<td>0.3</td>
<td>-0.26</td>
</tr>
<tr>
<td>VT z-score</td>
<td>-0.09</td>
<td>0.4</td>
<td>0.21</td>
</tr>
<tr>
<td>RR z-score</td>
<td>-0.21</td>
<td>0.1</td>
<td>-0.08</td>
</tr>
<tr>
<td>FRC WTCV%</td>
<td>0.38</td>
<td><strong>0.002</strong>*</td>
<td>0.26</td>
</tr>
<tr>
<td>VT WTCV%</td>
<td>0.01</td>
<td>0.9</td>
<td>0.10</td>
</tr>
<tr>
<td>RR WTCV%</td>
<td>-0.14</td>
<td>0.3</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

WTCV%: Within-test percent coefficient of variation; r: Spearman correlation coefficient; * Significant p Value (p<0.05)
4.3.2 ii. Associations between LCI WTCV% and age, MBW parameters, and variation of MBW parameters in preschoolers

Table 4.3.3 shows the correlation results between LCI WTCV% and age, MBW parameters and the variation of those MBW parameters for preschool age subjects divided by diagnosis cohort. Interestingly, no significant associations were found between the variation of LCI and any of the parameters investigated in healthy controls and asthmatic preschoolers. In preschool-age subjects with CF, the variability of LCI was significantly positively correlated with LCI values, FRC WTCV%, RR WTCV% and V_TCV%.

Table 4.3.3. Correlations between LCI within-test percent coefficient of variation and age, MBW parameters and coefficients of variation for MBW parameters in preschool-aged subjects divided by diagnosis cohort

<table>
<thead>
<tr>
<th>LCI WTCV%</th>
<th>Healthy controls (n=10)</th>
<th>Wheezy/Asthmatic (n=17)</th>
<th>CF(n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p Value</td>
<td>r</td>
</tr>
<tr>
<td>Age</td>
<td>-0.479</td>
<td>0.2</td>
<td>0.071</td>
</tr>
<tr>
<td>LCI</td>
<td>0.03</td>
<td>0.9</td>
<td>-0.13</td>
</tr>
<tr>
<td>FRC</td>
<td>0.03</td>
<td>0.9</td>
<td>-0.027</td>
</tr>
<tr>
<td>VT z-score</td>
<td>0.5</td>
<td>0.2</td>
<td>0.105</td>
</tr>
<tr>
<td>RR z-score</td>
<td>-0.382</td>
<td>0.3</td>
<td>-0.301</td>
</tr>
<tr>
<td>FRC WTCV%</td>
<td>-0.006</td>
<td>0.9</td>
<td>-0.196</td>
</tr>
<tr>
<td>VT WTCV%</td>
<td>0.261</td>
<td>0.5</td>
<td>-0.01</td>
</tr>
<tr>
<td>RR WTCV%</td>
<td>0.006</td>
<td>0.9</td>
<td>-0.199</td>
</tr>
</tbody>
</table>

r = Spearman correlation coefficient; *Significant p Value

4.4.2 iii. Associations between LCI variability and FRC variability are not consistent

While strict quality control guidelines for MBW testing are currently lacking in LCI literature, it has been suggested that the 2-3 technically acceptable trials used for the average calculation of LCI and other parameters should have FRC values within 10% of each other [154]. This approximately corresponds to a WTCV% for FRC of no more than 5%. This criterion is based on the assumption that since FRC is used in the calculation of LCI, high variability of FRC measurements may increase variability of LCI, thereby diminishing the repeatability of the test.
Interestingly, it can be seen in Table 4.3.2 for infants and in Table 4.3.3 for preschool-age subjects that WTCV\% of LCI does not always positively or significantly correlate with WTCV\% of FRC. Significant correlations were only found in healthy infants and in preschoolers with CF. Figure 4.3.2 demonstrates the relationship between LCI WTCV\% and FRC WTCV\% for all subjects marked by diagnosis group. It can be seen that while the majority of subjects have FRC WTCV\% below 10\%, those individuals that do have variability greater than 10\% for FRC do not consistently have greater LCI variability.

**Figure 4.3.2.** LCI WTCV\% plotted against FRC WTCV\% for all diagnosis cohorts
4.3.3 Associations between LCI and MBW parameter variability

We investigated any possible association between LCI values and the WTCV% of FRC and of breathing pattern parameters (RR and VT) in an effort to understand the influence of variability on mean LCI. Table 4.3.4 demonstrates correlation results between LCI and WTCV% of FRC, VT and RR for all infants and preschoolers. Significant associations were only observed in preschool diseased groups, where LCI was positively related to VT variability in CF subjects, and to both VT and RR in asthmatic subjects. Instability of breathing pattern parameters between trials is thus related to preschool subjects with elevated VI. LCI did not correlate with variability of FRC values in any age group, regardless of diagnosis cohort.

**Table 4.3.4.** Correlations between mean LCI values and the within-test percent coefficient of variation for MBW parameters for infant and preschool groups

<table>
<thead>
<tr>
<th></th>
<th>INFANTS</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>INFANTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRESCHOOLERS</td>
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<tr>
<td></td>
<td></td>
<td>LCI</td>
<td></td>
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<td></td>
<td></td>
<td>LCI</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>All Infants</td>
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<td></td>
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<td></td>
<td></td>
<td>Healthy controls (n=69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=69)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wheezy/Asthmatic (n=26)</td>
<td></td>
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<td></td>
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<td>CF (n=19)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>r</td>
<td>p Value</td>
</tr>
<tr>
<td>FRC WTCV%</td>
<td>-0.028</td>
<td>0.8</td>
<td>-0.093</td>
<td>0.5</td>
<td>-0.14</td>
<td>0.5</td>
<td>0.221</td>
<td>0.4</td>
</tr>
<tr>
<td>VT WTCV%</td>
<td>-0.005</td>
<td>0.9</td>
<td>-0.048</td>
<td>0.7</td>
<td>-0.135</td>
<td>0.5</td>
<td>0.217</td>
<td>0.4</td>
</tr>
<tr>
<td>RR WTCV%</td>
<td>-0.088</td>
<td>0.4</td>
<td>-0.073</td>
<td>0.6</td>
<td>-0.086</td>
<td>0.7</td>
<td>0.32</td>
<td>0.2</td>
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<td></td>
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<td></td>
<td></td>
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<td>All Preschoolers (n =40)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy controls (n=10)</td>
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<td></td>
<td></td>
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<td>(n=10)</td>
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<td></td>
<td></td>
<td></td>
<td>Wheezy/Asthmatic (n=17)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=17)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CF (n=13)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>r</td>
<td>p Value</td>
</tr>
<tr>
<td>FRC WTCV%</td>
<td>-0.001</td>
<td>0.9</td>
<td>-0.515</td>
<td>0.1</td>
<td>0.39</td>
<td>0.1</td>
<td>0.527</td>
<td>0.06</td>
</tr>
<tr>
<td>VT WTCV%</td>
<td>0.329</td>
<td>0.04*</td>
<td>0.091</td>
<td>0.8</td>
<td>0.735</td>
<td>0.001*</td>
<td>0.604</td>
<td>0.03*</td>
</tr>
<tr>
<td>RR WTCV%</td>
<td>0.172</td>
<td>0.3</td>
<td>0.103</td>
<td>0.8</td>
<td>0.635</td>
<td>0.01*</td>
<td>0.412</td>
<td>0.2</td>
</tr>
</tbody>
</table>

WTCV%: Within-test percent coefficient of variation; r = Spearman’s correlation coefficient; *Significant p Value
Chapter 5:

Discussion
5 Discussion

This thesis has investigated LCI derived from SF₆ mass spectrometry MBW in the largest cohort of healthy infants and school-age children to date. Based on this healthy cohort, we have established that LCI is negatively correlated with age, and have therefore modeled a continuous upper limit of normal for LCI that spans the entire pediatric age range. Taking this important age dependency into account, this thesis has also concurrently investigated how LCI is affected in two common chronic obstructive airway diseases in early childhood; asthma and CF. LCI is significantly elevated in young children with wheeze/asthma and CF compared to healthy subjects, yet shows different patterns of elevation with growth between these two disease groups. In CF subjects, mean LCI values are higher in preschool-age children compared to infants, which may reflect disease progression. In wheeze/asthma, LCI was significantly elevated compared to healthy controls in infancy, however, progressive increases in VI as measured by LCI under the age of 6 years were not present in the asthmatic subjects studied. We have also systematically correlated LCI with breathing pattern parameters (RR and Vₜ), and have shown that while these factors have no association with LCI in health, RR becomes positively associated with LCI in disease cohorts in preschool-age, particularly in asthmatic subjects. Lastly, it has also been shown that increased VI is related to greater instability of MBW measures and of breathing pattern parameters in diseased cohorts.

5.1 LCI is dependent on age in health

Contrary to published opinion [177, 209, 222, 239], we have found that LCI in health is dependent on age. When LCI values from healthy subjects ranging in age from 3 months to 18 years old were correlated with demographic and MBW parameters, LCI was significantly and negatively associated with age and FRC. No other parameters correlated with LCI when all healthy controls were combined. In an effort to model the curvilinear relationship evident between LCI and age over the entire pediatric age range, we used fractional polynomial regression to determine a median line and 95% confidence intervals (CI) for LCI in health.
Despite the significant negative correlation found between FRC and LCI, this association was lost when we included both age and FRC in our model of LCI, demonstrating that changes in FRC were not consistently related to changes in LCI independent of age.

5.1.1 A Continuous Upper Limit of Normal (ULN)

Previously published studies including healthy control subjects have traditionally defined the upper limit of normal (ULN) for an LCI measurement as being a discreet cut-off calculated as +1.96 standard deviations (SD) above the healthy control mean LCI [179, 207-209, 218]. Any LCI value falling above the ULN is considered to be abnormally elevated, allowing for the identification of high LCI values related to respiratory disease. This traditional definition of the ULN has been based on the finding that LCI was independent of age in all previous studies which investigated relatively narrow pediatric age ranges. Our study has demonstrated a significant age dependence of LCI between infancy and older healthy controls. We have described a continuous ULN that was calculated as the 97th centile of fractional polynomial regression of LCI and age based on our healthy control subjects. Figure 5.1.1 plots our healthy control LCI data against age, and compares our continuous ULN as calculated by regression to those published ULNs from the Institute of Child health (ICH) in the UK and from the Department of Pediatrics in Skövde, Sweden, which are calculated as +1.96SD above healthy subjects within a narrow age range. Demographics and mean LCI and FRC values of the study population used by these studies to compute the ULN can be found in Table 4.1.6. The current published ULN for infants is 7.8 [207]; for preschoolers the ULN is 7.77 [208]; for school-aged children it has been reported as 7.4 [179] in the UK and 7.17 in Sweden [209].

While our continuous ULN generally agrees with the published ULNs from ICH and Sweden in school-aged children, there are some marked differences in infants and preschoolers. As noted in Table 4.1.6, the LCI values for our preschool healthy control subjects was significantly lower on average than LCI values of a comparable population of children from the UK. Our calculated ULN was therefore lower throughout the preschool age range than the ULN published by Aurora et al [208]. Although mean LCI values were similar for our healthy control infants compared to a comparable population in the UK, the variability of LCI values within our
infant population was much higher than the UK, resulting in an overall higher ULN during the infant period compared to the ULN published by Lum et al [207].

**Figure 5.1.1.** LCI plotted against age, comparing upper limits of normal from our center (HSC), the Institute of Child Health (ICH) in London, UK, and from the Department of Pediatrics, Skövde, Sweden.
This thesis is the first time that the separate age ranges studied in previous published work have been considered together as one continuous pediatric age range. A continuous ULN for LCI is able to illustrate changes in LCI that may be due to growth. This will be critical in longitudinal studies in order to discriminate changes in LCI due to growth and changes due to disease. The greatest discrepancies between our continuous ULN and discreet limits that have been published were within the infant and preschool age ranges, where our limit was higher for very young infants and lower in preschool-aged children compared to what has been reported. Due to the large size of our infant healthy control group, it seems likely that variability in LCI has been underestimated in previous published studies of smaller cohorts. The preschool-age cohort was relatively small in this project, and therefore it is likely that we have underestimated the variability in this age group. There is a need for comparison of LCI across age groups in an even larger, representative healthy control sample in order to define the variability of the measure in health.

5.1.2 LCI is significantly elevated and more variable in healthy infants

Much of the curvilinear nature of our plotted model of LCI based on age is due to the elevated and more variable mean LCI of infants compared to older children. Mean LCI was significantly elevated in infants compared to the older groups (p<0.01). The SD of the mean LCI was also larger in the infant group compared to older cohorts (0.6 vs. 0.4). Furthermore, our results demonstrated that subjects with the age categorization of “preschool or older” had significantly lower LCI values independent of age compared to the categorization of “infant” when this age grouping was added to our regression model of LCI in health (Table 4.1.5.). This suggests that apart from age, additional factors may be causing LCI to be higher in infants.

In an effort to explain the elevated mean LCI values and variability of infants compared to older children, we correlated LCI with MBW parameters such as $V_T$ and RR in an attempt to discern trends between breathing pattern or lung volume and LCI. LCI did not correlate with any of these parameters for infants, preschoolers, or school-aged children, nor did these parameters add any predictive value when added to our regression of LCI based on age for all healthy controls combined (p>0.05 for all factors). Furthermore, average RR and $V_T$ z-scores corrected
for age and height in healthy infants did not differ significantly from those of older healthy children. FRC values for our healthy control infants had a low variability as expressed by the FRC standard deviation, were not correlated with LCI, and followed the generally linear trend based on age for all healthy controls as demonstrated by Figure 4.1.4.

In light of the stability of FRC measurements in our infants, the cumulative expired volume (CEV) must be variable in order to produce the variable results we have observed for LCI. While small variations in RR and VT between infants are likely responsible for some of the variation in CEV in infants as they are in older children and adults [200], the lack of correlation between LCI and breathing pattern and our previous assertions that RR and VT z-score mean and variability are similar amongst the age groups suggest that one or more additional factors must be working to proportionately increase the values of CEV and the variability of mean CEV in infants compared to older children. Such factors may be related to differences in lung physiology between healthy infants and older children, differences in testing conditions for infants and older children, or more likely, a combination of both.

5.1.2 i. Possible effects of lung physiology on infant LCI in health

Section 1.4.1 of this thesis provides an in-depth review of the unique physiological differences in the infant lung compared to the mature lung. During the first few years of life, the lung is still within its final stage of development characterized by dysynaptic growth dominated by alveolarization, immature lung mechanics caused by a highly compliant chest wall that is less able to balance the inward recoil of a less compliant lung, and small airways that are more prone to partial closure or collapse. Lung volumes tend to be less after expiration, further increasing the likelihood of partial airway closure or collapse as the outward pull on airways by surrounding parenchyma decreases at lower lung volumes. In addition, series dead space is proportionately larger in infants compared to older children due to fewer and smaller alveolar volumes and proportionately larger airways compared to lung volume [203]. It has been reported that increasing the VD/VT ratio will increase LCI [200, 203], and it is likely that a larger deadspace coupled with the small tidal volume and quick respiratory rate characteristic of infants would result in an increase VD/VT ratio. It may be that this unique and often unstable lung physiology causes a relative degree of ineffective gas mixing in infancy compared to older children. As
mentioned previously in section 1.7.1, a degree of VI is present even in healthy lungs due to the
effects of gravity and asymmetries naturally present in bronchial tree. In infants, there is an
additional tendency for airway closure due to functional immaturities of the lung. Gas mixing
efficiency in newborns studied by N₂ MBW has been reported to be low due to a high turnover
rate in infants [240], which may increase VI dependent on diffusion. In summary, it may be that
in health, infants have LCI values that are on average higher and more variable than older
children due to additional and more variable VI caused by lung immaturity.

5.1.2 ii. Possible effects of testing conditions on infant LCI

One of the main reasons for comparing infants and older children separately in this
thesis was due to the different MBW testing protocols completed by each age group. Infants
completed the MBW test while asleep under sedation in the supine position and wearing a
facemask. Children over the age of 3 years completed MBW testing while awake and seated in a
chair. Furthermore, the apparatus dead space including the facemask, pneumotachometer and T-
piece is proportionately larger in infants compared to their lung size than for older children. In
the presence of an already proportionately larger anatomical deadspace in infants, it may be more
likely that the breathing pattern in infants may be altered in order to offset increases in
deadspace, potentially affecting LCI. While no correlation between LCI and breathing pattern
was observed in our healthy infants, it is possible that breathing pattern was uniformly altered
due to testing conditions.

The effect of body posture on LCI in infants is unknown. A recent study of ventilation
distribution by electrical impedance tomography demonstrated that prone versus supine position
did little to change ventilation distribution in healthy infants [241]. Evidence from studies in
older children and adults have shown that VI and general pulmonary function can be improved in
the standing or seated position compared to the supine position, although such improvements are
more often found in subjects with respiratory disease [202, 204, 242, 243]. Therefore, while
previous work suggests that the effect of body posture is minimal in health, even in infants, it
cannot be ruled out in this study as a possible explanatory variable in the elevation of LCI in
infancy.
Although chloral hydrate is a common sedative used in the pediatric population due to the low depressive effects it has on respiration [244], the effects of this sedation on breathing pattern were not studied in our subjects. Sedation can cause greater relaxation of the diaphragm and of intercostal muscles, thereby lowering FRC and potentially leading to dependent lung region collapse [241]. It is logical that depending on the degree of FRC decrease and the maturity of the lung, different degrees of airway collapse may occur in different infants. In very young infants, when small airways are especially prone to collapse and in whom resting lung volumes approach closing capacity, sedation may be more likely to cause total collapse of dependent lung regions, eliminating them from gas exchange and therefore having little effect on VI. In older infants, in whom FRC may be better maintained by lung mechanics and in whom small airway collapse is relatively less likely, sedation may cause only partial closure of dependent lung regions, which would increase VI by increasing the variability in regional time constants and prolong the washout. This may in fact be a possible underlying mechanism to explain why LCI correlated positively with age within the infant group. Comparison of FRC measured by MBW and FRC measured by plethysmography could potentially investigate this theory to assess levels of gas trapping in healthy infants. Again, it could be expected that the degree of this type of partial closure would vary from infant to infant, possibly leading to the highly variable CEV observed in our infant cohort. Recent studies by Schrieber et al. measuring SF₆ LCI using an ultrasonic flowmeter in small numbers of healthy infants during natural sleep observed mean LCI values of 6.5 [245] and 6.2 [246]. While these study population differed in age and demographics compared to ours, the lower mean LCI demonstrated in these studies may suggest a functional difference between testing under sedation and under natural sleep. Future work should include a comparison of LCI from infants under natural sleep and under sedation, as well as a comparison of FRC measured by MBW with FRC measured by plethysmography to determine the presence of gas trapping in healthy infants that may be due to sedation.

Unfortunately, it is difficult to discern if and to what extent the previously mentioned testing conditions had on LCI, and more specifically, CEV, in healthy infants. It is therefore suggested that while VI is likely elevated in healthy infants compared to older children due to gas mixing inefficiencies related to lung immaturity, it may be that these elevated indices are made more variable by infant testing conditions. Until the individual effects of breathing pattern, body posture, sedation, and dead space on LCI and CEV are elucidated in infants, they must all
be considered concurrently as possible explanatory factors for the elevated and more variable CEV observed in healthy infants. It is thus suggested that while physiological elements of the infant lung may be responsible for the overall elevation of LCI in these subjects compared to older children, increased variability of the infant group is likely attributable to testing conditions.

5.2 LCI is elevated in disease

5.2.1 Trends in LCI elevation in disease change with age and diagnosis group

With the establishment of our healthy control cohort, we were able to directly compare the LCI results from asymptomatic disease groups of infants and preschoolers with a history of wheeze/asthma and CF to assess if LCI was significantly elevated in these children compared to healthy controls. We found that not only were LCI values on average elevated in disease groups compared to healthy controls, but the trend in average LCI elevation changes with age group (infants vs. preschoolers).

5.2.1. i: LCI elevation in CF subjects

In CF subjects, mean LCI was on average 1.46 units higher than that of healthy controls (p<0.001), however, when we studied the interaction of CF diagnosis with age group, we found that while infant CF subjects have LCI values that are on average 0.37 units higher than healthy controls, preschooler CF subjects have LCI values that are 3.94 units higher (p<0.001). This difference between infancy and preschool-age is again illustrated in the comparison of mean LCI compared to healthy controls, where CF preschoolers had significantly higher LCI values (p<0.01), while there was no statistically significant difference between CF infants and healthy controls (p=0.15). Furthermore, 21.2% of CF infants had LCI values that fell on or above our ULN as calculated by our regression model of LCI and age in healthy controls, while 77% of preschool-aged CF subjects had LCI values that were elevated above this limit. It seems likely that LCI is reflective of the progression of disease from infancy to preschool years in CF, especially in light of evidence of a strong correlation between elevated LCI and structural
lung disease pathology measured by HRCT [218]. Other studies of older CF subjects have similarly noted that LCI is a better marker of disease severity than FEV1 [215]. Our results indicate that there may be a limited time period during infancy and before preschool-age where LCI related to CF disease is not significantly different from healthy controls.

5.2.1. ii: LCI elevation in wheezy/asthmatic subjects

We observed a different trend in LCI elevation from that seen in CF subjects in our wheezy/asthmatic cohort compared to healthy controls. In the regression model of LCI based on age, LCI was on average 0.73 units higher than that of healthy controls (p<0.001). When we investigated the effect of age group in this model, we found that wheezy infants had LCI values which were on average 0.45 units higher than healthy controls, while asthmatic preschooler values were 1.13 units higher than healthy controls. This change in average LCI between infancy and preschool-age did not meet statistical significant (p=0.055). Likewise, when we compared the mean LCI values of wheezy infants and asthmatic preschoolers to healthy controls, we found that while both were significantly elevated (p=0.01, p=0.04, respectively), mean LCI was higher in wheezy infants compared to asthmatic preschoolers (LCI in infancy=7.41; in preschoolers = 7.14). There was no significant difference between the mean LCI of wheezy infants and asthmatic preschoolers, nor was there any difference in the proportion of wheezy infants compared to asthmatic preschoolers with LCI values above the ULN for LCI based on age. These results demonstrate that LCI is elevated in wheeze/asthma starting in infancy, yet shows no progression in the degree of elevation with age.

It cannot be distinguished if LCI is detecting VI abnormalities related to transient wheeze, future persistent wheeze, or both phenotypes in infancy from this project. It has been suggested that transient wheeze during infancy may be due to a physiological susceptibility to VI due to particularly small airways in these infants, which may be detected by LCI [247]. Sonnappa et al. have demonstrated that LCI may be more reflective of chronic airway changes related to persistent wheeze (asthma) by preschool age [222]. Sonnappa et al. found that mean LCI was not different from healthy controls in episodic viral wheezers, while multiple-trigger wheezers (persistent wheezers with symptoms in between viral infections) had elevated LCI on average compared to healthy controls. This thesis did not discriminate between different wheezy
phenotypes; it may be that wheezy infants with elevated LCI values above healthy controls are a mixture of both transient and future persistent wheezers. Our results warrant more careful distinction between wheezy phenotypes in the future. Longitudinal studies following infant wheezers into preschool-age and older are needed in order to retrospectively comment on how LCI tracks from infancy in order to determine if all or only a portion of those infants with elevated LCI in infancy will develop persistent wheeze.

5.2.2 Elevated LCI values are unrelated to changes in FRC or VT, yet show an association with RR in preschoolers

Since FRC as calculated by the MBW is used in the calculation of LCI, it was important to investigate whether changes in FRC affected LCI measures. Hyperinflation, or gas trapping, is a common characteristic of obstructive small airway disease [248, 249]. This phenomena occurs when small airways totally collapse, trapping air in distal lung units and removing them from participating in gas exchange. Since the MBW only calculates lung volumes in communication with the mouth, it is not possible to quantify the amount of gas trapping occurring in the lung with this test. However, if hyperinflation was present in diseased individuals, it may be expected that their FRC measurements from the MBW may be different from healthy controls of the same age. If this were to occur, LCI measurements could be affected simply due to an underestimation of FRC. Mean FRC values were elevated in infants with a history of wheeze and in CF compared to healthy controls (p<0.001 for wheeze, p=0.04 for CF), while FRC was lower in disease groups compared to healthy controls during preschool age. These differences in FRC values between health and disease, however, may be due to age differences also present between these groups. Unfortunately, there are no reference equations currently in existence for correcting FRC measured by SF6 MBW for significant growth factors. In an effort to investigate whether FRC trends with age changes significantly with disease, we plotted FRC and age for all infants and preschoolers (Figure 4.3.1). No observable differences in FRC with age were seen. Furthermore, there was no correlation between LCI and FRC in any of our disease groups. Our results suggest that elevated LCI values are not the result of differences in FRC for these subjects compared to healthy controls. It is therefore likely that relative increases in CEV are driving increases in LCI. While VI related to disease processes (mucous plugging, airway obstruction, airway closure) can
alone affect CEV by delaying the washout of inert gas from poorly ventilated regions of the lung, it is also possible that RR or $V_T$ in disease may be increasing VI and thus affecting CEV.

Changes in $V_T$ can alter $V_T/VD$ ratios which can affect VI; therefore, we investigated whether $V_T$ was altered in disease and its effect on LCI. $V_T$ z-scores did not differ significantly between healthy controls and disease groups for infants or preschoolers. $V_T$ was only found to be positively correlated with LCI in asthmatic preschoolers ($r=0.5, p=0.01$), although all 4 asthmatic subjects detected as having abnormally high LCI values had $V_T$ z-scores within normal limits. There was also a small trend in CF subjects, where LCI was positively associated with greater $V_T$ z-scores in preschoolers, although the strength of this association is difficult to determine. In the disease cohorts studied here, therefore, small changes in $V_T$ between subjects had little impact on LCI. Future studies that specifically investigate changes in LCI with larger changes in $V_T$ are needed to determine if our subjects simply had variation in $V_T$ that was too small to affect LCI.

While FRC and $V_T$ showed little association with LCI, there were some interesting trends between LCI and RR in diseased subjects. RR z-scores correlated significantly and positively with LCI in asthmatic preschoolers ($r=0.61, p=0.01$), while there was no significant association between RR and LCI in wheezy infants. In contrast, no significant correlation between LCI and RR were seen in CF subjects, and surprisingly the relationship between changes in LCI and RR were slightly negative (for CF infants: $r = -0.26, p=0.3$; for CF preschoolers: $r = -0.03, p=0.9$). There were significant interactions between RR and age group in our regression model of LCI based on age for both disease cohorts (p<0.001 for both), demonstrating that RR is more positively related to LCI in preschool-age subjects with CF compared to infants with CF.

Increased RR in obstructive lung diseases has been linked to severity of airway obstruction as measured by spirometry, arterial oxygenation, and hyperinflation, yet the mechanisms for why RR is elevated in disease are not well understood [250]. It has been suggested by some studies that increased RR in subjects with obstructive lung disease may represent an increased need for ventilation in the presence of reduced gas exchange [250, 251]. Ranganathan et al. reported that RR was particularly elevated in CF subjects with a history of lower respiratory infection. These authors suggested that increased RR may be attributed to VI that was not detectable by RVRTC, as no correlation was found between RR and FEV0.4 in
these CF infants [251]. It may also be possible that increased inflammation and airway obstruction may be stimulating pulmonary sensory receptors in our CF subjects to cause an increase in RR [252, 253]. Another possible alternative is that RR is not directly influenced by VI, but is rather the result of increased work of breathing, increased metabolic rate, or increased surface-to-volume ratio of gas exchange due to the smaller size of our CF preschoolers compared to healthy controls [251]. Regardless of the mechanism, it is evident that mean increases in RR are associated with CF disease in preschool-aged subjects. In the case the CF subjects presented here, however, particularly high RR was not consistently related to particularly high LCI values in this group. Our results are therefore in agreement with Lum et al., who suggested that RR is influenced by factors related to CF lung disease other than VI as detected by LCI [207].

In asthma, there was a positive association between RR and elevated LCI values. RR z-scores were higher in asthmatic preschoolers on average compared to healthy controls, but this difference was not significant. It is interesting that the two asthmatic preschoolers with the highest LCI values also had the highest RR z-scores (Figure 4.2.2). The correlation observed between LCI and RR in preschool asthmatics was dependent on these two individuals and therefore is difficult to interpret. We are not able to determine from this project whether both measures are related to similar disease processes in asthma and thus trend together, or if elevation in one measure is causing elevation in the other. It is possible that highly elevated RR values may increase VI dependent on diffusion regardless of the presence of disease in these two subjects. It may also be possible that both LCI and RR are elevated due to the presence of airway dysfunction related to asthma in these two individuals. It seems likely that LCI is overestimated in these two asthmatic subjects due to elevation of RR, but this does not mean that these individuals would have had normal LCI values if their RR values were lower. The only way to distinguish between these underlying mechanisms is to quantify the effect of controlled changes in RR in these subjects on LCI in both healthy and asthmatic subjects, and include this effect in the interpretation of LCI in asthmatic individuals. It is at least suggested here that LCI and RR do demonstrate a positive relationship, and the cause of this relationship must be further investigated, especially in asthmatics.
5.3 Within-test Variability of LCI

5.3.1 Within-test variability of LCI is low and related to age and disease

As with any physiological measurement, knowledge of the variability of the LCI are necessary components in the interpretation of data from healthy and diseased subjects. We aimed to study the within-test variability of LCI and other MBW parameters as measured by the coefficient of variation (WTCV%) in order to comment on the stability of these parameters between successive trials and determine if changes in variability are related to disease status.

Our data demonstrate that WTCV% for LCI and FRC are small on average for all subjects, regardless of age or diagnosis, with mean values ranging from 4-8%, which is similar to other published data [178, 206]. In comparison with traditional lung function testing, where the common variability of forced expiratory volumes in preschool children ranges from 2-4% and forced expiratory flows range from 7-12% [130], the stability of MBW trials is comparable, if not greater. This suggests that the LCI is an extremely stable test within-subject, even during early childhood. Within age groups, there was no significant difference in mean WTCV% between diagnosis cohorts. As a measure of the average stability of breathing pattern parameters, we also studied the WTCV% of RR and VT for all subjects. These values were also very low, suggesting that our subjects were generally successful at maintaining a stable breathing pattern during each trial. Despite generally low variability in all parameters across subjects, preschool-aged subjects were noted to have higher variability on average compared to infant subjects, with significant differences existing for FRC and VT in healthy infants (p=0.003 for both), and for LCI, VT and RR in wheezy infants (p<0.05). The effect of age on MBW parameter variability is most likely a result of testing differences between infants and preschoolers. Since preschool-aged children are tested while awake, they are more likely to change breathing patterns, shift position, take longer pauses between trials, etc., all of which can act to increase variability of parameters compared to infants who complete trials while asleep.

Despite the stability of all MBW parameters, we discovered that increased within-test variability of parameters correlated positively with elevated LCI. In CF, increased WTCV% of
LCI was significantly associated with increased WTCV% in FRC, VT and RR in CF preschoolers (Table 4.3.3). Furthermore, increased WTCV% of LCI is also positively associated with LCI values in preschool CF subjects. While these associations were lacking in CF infants, it was observed that variability in LCI increased with greater age and FRC values. This is suggestive that as CF infants grow older, disease progression may first cause LCI values to become more variable before they increase on average compared to healthy controls. By the time CF subjects reach preschool-age, disease changes have progressed to a point where high VI not only causes LCI to be elevated on average, but these changes also causes LCI and breathing pattern parameters (RR and VT) to be become more variable between trials. It may be expected that more poorly ventilated lung units would have emptying times that are more variable between trials, causing a higher WTCV% of LCI. Shifts in mucus plugging or areas of collapse between trials may also cause more variability in LCI and FRC in CF disease. It is therefore suggested that VI caused by CF lung disease progression is not only associated with high mean LCI values, but also with higher variability of LCI and other MBW parameters between trials.

In the wheezy/asthmatic group, the WTCV% of LCI was only associated with high mean values of LCI in wheezy infants; it is important to note that wheezy infants also had higher LCI values on average compared to asthmatic preschoolers. Presumably, this association is perhaps similar to the one seen in CF preschoolers, where increased VI related to airway dysfunction may cause emptying times in various lung units to not only be greater but also more variable between trials. It is interesting that the same association was not observed in asthmatic preschoolers, yet there was a strong association between mean LCI values and the variability of breathing pattern parameters (RR and VT). It should also be noted that mean values of WTCV% of RR and VT were the highest in preschool asthmatics of all subjects studied, although no significant difference between these subjects and healthy controls existed (Table 4.3.1). It is difficult to distinguish whether WTCV% of RR and VT and mean LCI values are separately related to VI and therefore trend together, or if higher variability in breathing pattern parameters may be causally increasing LCI in preschool-age asthmatics. We have already suggested that at least in some preschool asthmatics, elevated RR may be responsible for overestimating LCI related to disease processes. Considering, however, that there were no significant differences in the average within-test variability of RR or VT in disease groups compared to healthy controls, and that variability was relatively low in all subjects, it is unlikely that the within-test variability seen
in this project is causing the elevated LCI values observed in some asthmatics. It is instead proposed that increased VI related to asthma is associated with increased breathing pattern instability. It must therefore be expected that children with more severe airway changes related to obstructive lung disease will have more variable MBW results. This is an important implication which needs to be incorporated into quality control criteria.

5.3.2 Quality control definitions based on variability of FRC are flawed

Quality control protocols for MBW trial acceptability are currently not standard and vary from center to center. Most centers tend to accept only those MBW trials that have FRC values within 10% of each other for a given subject, which generally converts to a WTCV% for FRC of about 5% [154]. It is logical that high variability of FRC measurements may in fact cause high variability of LCI, thus reducing the stability of the measure. Our results demonstrate that within-test variability of LCI does not consistently correlate with within-test variability for FRC for all subjects, regardless of diagnosis cohort. Significant associations were found between LCI and FRC variability in healthy infants, and in preschool-age subjects with CF. We have suggested in the previous section that increased variability is associated with increased VI. Both healthy infants and CF preschool-age subjects have on average been shown to have elevated LCI values compared to older healthy controls likely due to lung immaturity and testing conditions in healthy infants and due to small airway disease in the case of CF subjects. It may be that the presence of VI is independently increasing variability of LCI and FRC in these groups, rather than the variability of FRC causing an increase in the variability of LCI. In other words, changes in FRC values may not affect LCI in a predictable way if changes in CEV are also occurring; likewise, LCI measurements may be variable due to CEV variability and not due to variability in FRC. Figure 4.3.2 demonstrates that while the majority of all subjects have within-test variability measures within 5-10% for both FRC and LCI, a high variability of FRC does not always correlate with a high LCI variability, or vice-versa. While more testing with larger sample sizes is needed in order to come to confident quality control protocols, we suggest that more lenient variability definitions for acceptable trials may need to be applied to subjects where it is expected that VI will be higher.
5.4 Conclusions and Relevance

We have determined that LCI is dependent on age in healthy controls, especially in young children under the age of 6 years. Taking this age dependence into account, we have shown that LCI is significantly elevated in asymptomatic infants and preschool-aged children with CF or a history of wheeze/asthma compared to healthy controls. Trends between how LCI changes with age in chronic disease differ between CF and wheeze/asthma; in CF, LCI seems to represent disease progression with age. In wheeze/asthma, LCI is elevated in infancy, yet does not show progression in elevation with age. Increased RR is associated with disease and elevated LCI values, indicating a potentially important factor in the interpretation of LCI which needs to be investigated further in future studies. Furthermore, while the variability of MBW parameters are low in our subjects, quality control measures must take into account that some parameters are more variable in subjects with disease and high LCI values, and that FRC variability does not consistently predict LCI variability. In conclusion, both wheeze/asthma and CF disease groups can be distinguished from health in early childhood by LCI, making this a potentially useful marker of VI related to early obstructive disease.

5.5 Limitations and Considerations

I acknowledge that there may be several pitfalls and specific considerations in this study, which I will address here. The first limitation of this study was small sample sizes in some of our disease cohorts and also few numbers of healthy preschool subjects. Larger sample sizes not only lead to a more representative sample to study, but also generally increase the precision and statistical power of estimates.

There may have been unknown and uncontrollable biases in our subject sample due to methods of recruitment. Our subjects were recruited from our clinic in the case of subjects with asthma or CF, from obstetrical clinics at Mount Sinai Hospital in the case of healthy infants, or from relations and friends of staff and patients at our clinic in the case of older healthy controls. While our center at the HSC and clinics at Mount Sinai Hospital are large and see patients who
live in all parts of Toronto and surrounding areas, our subject sample is likely not representative of the general population.

There was a lack of healthy infants between the ages of 2 to 3 years in our healthy control cohort. Our healthy control recruitment was limited to the CHILD Study; the oldest infants involved in the CHILD Study were only at the 2 year age mark at the time of writing this thesis. We therefore were unable to recruit subjects between the ages of 2 and 3 years. Furthermore, testing children in this age group can be particularly difficult; many children of this age are too tall to comfortably fit inside the infant plethysmography box and therefore cannot perform iPFT and sedated MBW testing. At the same time, these young children are also often unable to sit quietly during preschool-age MBW testing. This age group therefore represents a particular testing challenge which was not addressed in this project.

There are also general limitations of the LCI as a measure which also apply to this project. The clinical significance of LCI, especially in young children, has yet to be determined. While recent imaging research has conclusively linked LCI to structural small airway disease in older children with CF [218], similar investigations have yet to be done in other disease cohorts and in younger age ranges. Furthermore, while LCI is believed to be illustrative of VI linked to small airway obstructive disease based on published histological work [254], we did not compare LCI results with any physical or immunological markers of disease processes in this project. It is therefore difficult to identify the specific disease process that may be causing elevated VI in our disease subjects. In addition, it was not possible to confirm whether elevated LCI values caused by increased VI are related to disease processes that will progress and be clinically relevant, or whether these changes are an epiphenomenon present in most young children with CF or wheezy/asthma. The LCI also does not discriminate the type or the location of VI (conductive and/or diffusive) within the lung that caused its elevation due to the global and simple nature of the LCI as a measure.

Another limitation in the analysis of this project was a lack of healthy reference data for FRC calculated by SF₆ MBW. This type of data is crucial in order to determine if disease subjects differ significantly in their FRC estimation, as this could have important implications on LCI values. We have attempted to demonstrate that our disease subjects did not seem to have any
different trends in measured FRC compared to healthy controls and therefore it is unlikely that FRC estimation was a confounding variable in LCI values. Reference equations for FRC corrected for age and other significant maturity factors are needed in order to confirm our results.

5.6 Future Directions

A logical next step in the characterization of LCI in disease would be to study LCI in exacerbating subjects with obstructive disease such as CF and asthma in comparison to clinical gold-standards of assessing respiratory distress. In doing so, the degree of elevation in LCI may be able to be linked with severity of symptoms. This is important in order to define the utility of LCI in asthma and CF on an individual level. We have demonstrated in this thesis that while the overall mean LCI of our asthma cohort was greater than that of healthy controls, many of individual asthmatic subjects had LCI values within normal limits. This may be due to the fact that they were clinically stable, well-controlled asthmatic patients; the LCI in an exacerbating asthmatic subject may be quite different. Furthermore, LCI was elevated in a large proportion of CF subjects by preschool-age. While this is beneficial in discriminating CF from health, LCI needs to be correlated to specific pathological processes in order to gain insight into disease progression in CF on an individual basis.

While some important work has so far been done studying the response in LCI before and after treatment in CF children [140], this kind of investigation is also needed in asthmatic cohorts where effectiveness of treatment methods is often judged only by the resolution of symptoms. We have shown in this study that VI can be present even in the absence of symptoms and acute illness. We have suggested that wheezy/asthmatic subjects with normal LCI values may represent cases of good control of airway inflammation and AHR. There may therefore be a role for LCI in testing the efficacy of drug delivery and function in young children.

As mentioned previously, large reference populations are needed to understand how LCI and FRC measured by MBW are related to growth and other demographic parameters in health in order to interpret LCI in disease, especially within the clinical setting. A limitation in this
study was the non-existence of reference populations for FRC measurements by MBW. Such information is needed in order to control for confounding demographic variables in this measures (age, height, weight, sex), to allow for comparison of FRC measurements between diagnosis cohorts and to plethysmographic FRC values.

Tested quality control measures for MBW testing are needed. Inherent in this need is the knowledge of the variability and repeatability of MBW parameters, and how they may be affected in different age ranges and in disease. This study investigated within-test variability of MBW parameters, yet further work should also look at the long-term variability of parameters in health and disease. Furthermore, potentially confounding factors such as tidal volume and respiratory rate need to be fully described in different age ranges. In our subjects, VT and RR had some relationship with LCI, particularly in preschool-years; we purposely aimed for stable VT and RR in all of our subjects, and it may be due to the low variability of breathing patterns both within and between subjects that stronger trends between LCI and breathing pattern were not observed. Studies investigating how LCI changes with variations in VT and RR in subjects with health and disease are needed to determine quality control standards in regards to these parameters.

In summary, there is a need to more strictly define LCI as a physiological and clinical measure of disease in children. The results presented in this thesis and published previously support the notion that LCI is a sensitive and highly feasible marker of VI related to obstructive disease in young children. It is necessary, however, to first more carefully characterize LCI in health and disease in order to use the LCI as a future clinical tool.
References


