ASSESSMENT OF RESPIRATORY SYSTEM MECHANICS IN ADULTS: EFFECT OF WEIGHT LOSS, POSTURE, BRONCHODILATION AND ARTEFACTS ON RESPIRATORY IMPEDANCE AND ITS REPEATABILITY

by

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To my family for their constant support

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Abstract

Obesity is associated with respiratory symptoms that often improve with weight loss. However, traditional methods of testing pulmonary function such as spirometry and plethysmography show little or no changes with weight loss. Oscillometry which measures respiratory system mechanics could potentially fill this knowledge gap, but it has never been used to measure weight-induced changes in respiratory system mechanics in the supine position, even though such assessment would be more relevant to the mechanics of breathing during sleep.

We evaluated 19 severely obese female subjects using spirometry, plethysmography, Pittsburgh Sleep Quality Index, and hand-held oscillometry to obtain respiratory system resistance (Rrs) and reactance from 6-19Hz before, and at five weeks and six months after weight loss surgery. These assessments were performed in both upright and supine positions, and pre- and post-bronchodilation with 200µg of salbutamol. An average weight loss of 11.9±2.7kg at 5 weeks was not associated with changes in upright respiratory mechanics, but Rrs at 19Hz (Rrs,19) was reduced by 13.1±3.8% in the supine position and this correlated with improvements in sleep quality. Weight loss also increased bronchodilator responsiveness, perhaps indicating improvements in airway-parenchymal tethering. At six months, greater mean weight-loss of 21.4±7.1kg caused significant changes in respiratory mechanics in both upright and supine positions. However, weight-loss induced a greater reduction in supine Rrs,19. Together with the early changes at 5 weeks, these results demonstrate the importance of breathing mechanics to sleep quality. By comparison, no significant changes were detected with spirometry.

We also characterized the biasing effects of inappropriate positioning of head-and-neck during oscillometry and compared these to the established effects of the upper airway shunt artefact. Impedance values were not significantly affected by 20° neck flexion, and although significant, changes in Rrs during 10° neck extension were small. Oscillometry outcomes were highly repeatable since within-test coefficient of variation (COV) was < 8%, and day-to-day COV was < 6% in all subjects. Furthermore, the test-retest reliability of oscillometry was also very high with Pearson's correlation coefficient of 0.99. Taken together, hand-held oscillometry was very repeatable, reliable and more sensitive than spirometry at detecting changes in lung mechanics with weight-loss.

List of Abbreviations and Symbols Used

AHR	airway hyperresponsiveness
ANOVA	analysis of variance
ASM	airway smooth muscle
ATS	American Thoracic Society
BAL	bronchoalveolar lavage
BD	bronchodilator
BMI	body mass index
C_{CW}	chest wall compliance
CI	confidence interval
C_L	lung compliance
cm H_2O	centimeter of water
COV	coefficient of variation
COPD	chronic obstructive pulmonary disease
Crs	respiratory system compliance
C_{RS}	respiratory system compliance
DEXA	dual-energy X-ray absorptiometry
E	elastance
Ec	central elastance
Ep	peripheral elastance
Ers	respiratory system elastance
ERS	European Respiratory Society
ERV	expiratory reserve volume
FEF_{25-75}	forced expiratory flow between 25-75% of FVC
FEF_{75}	forced expiratory flow at 75% of FVC
FEF_{50}	forced expiratory flow at 50% of FVC
FEF_{25}	forced expiratory flow at 25% of FVC
FEV_1	forced expiratory volume in one second
FFT	fast Fourier transform
FOT	forced oscillation technique

FRC	functional residual capacity
FVC	forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
hs-CRP	high-sensitivity C-reactive protein
Ic	central inertance
IC	inspiratory capacity
ICS	inhaled corticosteroid
IFN-γ	interferon-gamma
IgE	immunoglobulin E
IL	interleukin
IRV	inspiratory reserve volume
ISAAC	International Study of Asthma and Allergies in Childhood
IVC	inspiratory vital capacity
kg	kilogram
L	liter
LABA	long-acting beta-agonist
LTRA	leukotriene receptor antagonist
MCh	methacholine
MCP-1	monocyte chemoattractant protein-1
P	pressure
PC_{20}	provocative concentration of MCh that induces a 20% fall in FEV_1
PD_{20}	provocative dose of MCh that induces a 20% fall in FEV_1
PEF	peak expiratory flow
PFT	pulmonary function test
PSQI	Pittsburgh sleep quality index
R	resistance
R_{aw}	airway resistance
Rc	central resistance
RCT	randomized controlled trial
Rrs	respiratory system resistance

RV	residual volume
s	second or seconds
SABA	short-acting β_2-adreneric agonist
SD	standard deviation
SDXrs	standard deviation of respiratory system reactance
SE	standard error of the mean
sR_{aw}	specific airway resistance
T_H	T-helper
TLC	total lung capacity
TNF-α	tumor necrosis factor – alpha
VC	vital capacity
V	volume
VT	tidal volume
WC	waist circumference
Xrs	respiratory system reactance
Zrs	respiratory system impedance
Z_1	upper compartment impedance
Z_2	lower compartment impedance
Z_3	lower compartment impedance, representing the elastance of central airways
$\dot{V}$	flow

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Chapter 1: Introduction

In this chapter, I provide an overview of the thesis describing the motivation for the studies I conducted. The organization of the thesis chapters is provided in Section 1.2 and a list of original manuscripts and conference abstracts arising from this thesis is provided in Section 1.3.

1.1 Motivation for the Thesis

Obesity is associated with asthma [1], obstructive sleep apnea [2], and obesity hypoventilation syndrome [3]. It is also associated with respiratory symptoms such as wheeze and dyspnea. These symptoms can be difficult to manage in individuals with obesity, and bronchodilators are reported to exhibit limited effectiveness [4-8]. However, symptoms often improve with weight loss but traditional methods of testing lung function such as spirometry and plethysmography often show little or no changes with weight loss [9-11]. This lack of information from traditional lung function tests has hampered our ability to unravel the mechanisms that link obesity and weight loss to changes in respiratory health. Furthermore, lung function is typically assessed in the upright position but the respiratory symptoms associated with obesity are reported to be worse when subjects lie down and this affects their sleep quality [12, 13]. Unfortunately, there are no studies on the respiratory health consequences of weight loss in the supine position.

A novel technique for measuring lung mechanics and function known as oscillometry could potentially fill this knowledge gap by providing clinically useful information on the mechanisms that facilitate the relationship between obesity and respiratory symptoms. This thesis investigates the utility of this technique in detecting weight-induced changes in lung mechanics as compared to traditional methods of testing lung function.

We hypothesized that oscillometry outcomes would show larger changes in lung mechanics than spirometry and plethysmography outcomes after weight-loss surgery, and that these changes would be associated with improvements in sleep quality reported by the patients. We measured lung mechanics with oscillometry in the upright and supine

position and recorded the changes that occurred with weight loss. We then compared the changes in oscillometry outcomes to changes measured with spirometry and plethysmography. Furthermore, we hypothesized that changes in lung mechanics measured in the supine position following weight loss would be associated with changes in sleep quality. Finally, to investigate the contribution of obesity to the reported ineffectiveness of bronchodilators, we measured breathing mechanics with oscillometry before and after inhalation of salbutamol, and compared baseline bronchodilator responsiveness to the responsiveness measured after weight loss.

The clinical adoption of oscillometry is possibly hindered by the low repeatability of the technique, relative to spirometry. Repeatability is defined as the variation in an outcome measurement administered repeatedly by a specific individual or device on the same test subject, under the same test condition and within a short period of time. Repeatability is usually assessed from the standard deviation or percent coefficient of variation of the repeated measures. The percent coefficient of variation for oscillometry outcomes has been reported to be higher than 8 % in some oscillometry devices and this indicates low repeatability [14, 15]. By comparison, the percent coefficient of variation for spirometry is typically less than 3% and this indicates high repeatability [14]. In this thesis, we used a portable, handheld oscillometry device which allowed us to collect measurements in both upright and supine positions. However, the repeatability of this device has never been characterized before. Furthermore, it has been speculated that incorrect positioning of the head and neck during oscillometry could potentially affect the repeatability of oscillometry outcomes [14]. Thus, we hypothesized that a portable, handheld oscillometry device demonstrates high repeatability, and low and acceptable variability, even in the presence of artefacts such as variations in positioning of the head and neck.

The following section describes the structure of this thesis.

1.2 Thesis Organization

This thesis is divided into five chapters. Chapter 2 provides a broad review of published literature relevant to this thesis. It describes the state of the art in the field of lung

mechanics and function with a special focus on obesity and weight loss. Other topics reviewed include the clinical application of the oscillometry technique in the assessment of respiratory mechanics as well as a brief introduction into conventional methods of assessing pulmonary function. This section ends with a description of the research hypotheses, aims and approaches.

In Chapter 3, we investigated whether changes in lung mechanics relatively soon after weight loss surgery are detectable with oscillometry. Results obtained were compared to outcomes from spirometry and plethysmography. We also investigated whether the changes in lung mechanics measured in the supine position following weight loss are associated with changes in sleep quality. This work led to an original manuscript titled "Early detection of changes in lung mechanics with oscillometry following bariatric surgery in severe obesity" authored by Ubong Peters, and coauthored by Drs. Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. The manuscript is published in *Applied Physiology*, *Nutrition and Metabolism* and a modified version is presented in Chapter 3.

In Chapter 4, we extended the follow-up period of our previous weight loss study from 5 weeks to 6 months and re-evaluated lung function and sleep quality in our study participants using oscillometry, spirometry, plethysmography, and Pittsburgh Sleep Quality Index (PSQI). Results obtained were compared to those recorded earlier at baseline and 5 weeks after surgery. A manuscript for this study is being prepared for submission.

Chapter 5 investigates the effect of incorrect positioning of head and neck during oscillometry on the repeatability and variability of oscillometry outcomes. The effect of the upper airway shunt artefact on the repeatability and variability of oscillometry outcomes was also assessed, and the within-session repeatability and day-to-day reproducibility of a portable, handheld oscillometry device was characterized. A manuscript for this work is also currently under preparation.

A summary of the findings presented in this thesis is provided in Chapter 6. A discussion of the significance and implications of these findings is also presented along with a

summary of the original contributions of this thesis. This chapter ends with suggestions for future work.

1.3 Manuscripts, Conference Abstracts and Proceedings arising from the Thesis.

Journal Articles and Manuscripts

1. **Ubong Peters**, Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. Early Detection of Changes in Lung Mechanics with Oscillometry Following Bariatric Surgery in Severe Obesity. *Applied Physiology*, *Nutrition and Metabolism*. 2016 May; **41**(5) 538–47. doi: 10.1139/apnm-2015-0473.
2. **Ubong Peters**, Gail Dechman, Paul Hernandez, Thomas Schuessler, and Geoffrey Maksym. Repeatability, Variability and Reliability of Respiratory Impedance: Effect of Upper Airway Shunt Artefact, and Head and Neck Positioning (to be submitted)
3. **Ubong Peters**, Swati Bhatawadekar, Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. Improvements in Upright and Supine Lung Mechanics with Weight Loss Surgery: Sleep Quality and Bronchodilator Responsiveness (to be submitted)

Conference Abstracts and Proceedings

1. **Ubong Peters**, Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. Improvements in Lung Mechanics with Weight Loss in Severely Obese Subjects Following Bariatric Surgery: Sleep Quality and Bronchodilator Responsiveness. Poster discussion, Canadian Respiratory Conference, Halifax, Nova Scotia. April 2016.
2. **Ubong Peters**, Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. The Bronchodilatory Effects of Beta-Agonist and Weight Loss are Similar in Severely Obese Patients after Bariatric Surgery. Podium

presentation, Obesity and Metabolism Conference, University of Vermont, Burlington, Vermont. October 2015.

3. Hamed Hanafi, Kamal El-Sankary, **Ubong Peters**, Marwa Al-Amer, Dietrich Henzler, Andrew Milne, Jeremy Brown, and Geoffrey Maksym. Assessing the accuracy of oscillometry in tracking the mean values and temporal changes in impedance of children. Poster presentation, Annual Congress of the European Respiratory Society, Amsterdam, Netherlands. September 2015.
4. **Ubong Peters**, Swati Bhatawadekar, Gail Dechman, James Ellsmere, Paul Hernandez, and Geoffrey Maksym. Bariatric Surgery Improves Lung Mechanics of Severely Obese Patients in the Supine Position. Poster discussion, American Thoracic Society International Conference, Denver, Colorado. *Am J Respir Crit Care Med* 191; 2015: A1222, May 2015.
5. **Ubong Peters**, Gail Dechman, James Ellsmere, Thomas Schuessler, Paul Hernandez, and Geoffrey Maksym. Repeatability and Reliability of Respiratory Impedance in Obese and Nonobese Adults. Poster presentation, American Thoracic Society International Conference, Denver, Colorado. *Am J Respir Crit Care Med* 191; 2015: A2101, May 2015.
6. Marwa Al-Amer, **Ubong Peters**, Swati Bhatawadekar, Wade Watson, and Geoffrey Maksym. Repeatability of Respiratory Impedance and Bronchodilatory Response in Asthmatic Children. Poster presentation, American Thoracic Society International Conference, Denver, Colorado. *Am J Respir Crit Care Med* 191; 2015: A2105, May 2015.
7. **Ubong Peters**, Paul Hernandez, Gail Dechman, James Ellsmere, and Geoffrey Maksym. Lung Function Following Weight Loss. Podium presentation, Atlantic Respirology and Critical Care Medicine Conference, Halifax, Nova Scotia. November 2014.
8. **Ubong Peters**, James Ellsmere, Paul Hernandez, Thomas Schuessler, and Geoffrey Maksym. The Effect of Artefacts on Variability and Repeatability of Respiratory Impedance in Healthy Adults. Poster presentation, American Thoracic Society International Conference, San Diego, California. *Am J Respir Crit Care Med* 189; 2014: A3546, May 2014.

9. **Ubong Peters**, Gail Dechman, James Ellsmere, Paul Hernandez, and Geoffrey Maksym. The Role of Parenchymal Tethering on Lung Mechanics in Obesity before and after Bariatric Surgery. Poster presentation, American Thoracic Society International Conference, San Diego, California. *Am J Respir Crit Care Med* 189; 2014: A3517, May 2014.
10. **Ubong Peters**, Swati Bhatawadekar, Gail Dechman, James Ellsmere, Paul Hernandez, Dietrich Henzler, and Geoffrey Maksym. Lung mechanics in obesity and changes with bariatric surgery: role of airway smooth muscle tone and airway unloading. Poster presentation, Atlantic Respirology and Critical Care Conference, Halifax, Nova Scotia. November 2013.
11. **Ubong Peters**, Swati Bhatawadekar, Gail Dechman, James Ellsmere, Paul Hernandez, Dietrich Henzler, and Geoffrey Maksym. Lung mechanics in obesity and changes with bariatric surgery: role of airway smooth muscle tone and airway unloading. Poster discussion, Annual Congress of the European Respiratory Society, Barcelona, Spain. September 2013.
12. Yuanyuan Chen, Timothy Brown, Swati Bhatawadekar, Del Leary, **Ubong Peters**, Linhong Deng, and Geoffrey Maksym. A method for assessing glottis aperture variation on airway resistance by forced oscillation. Poster presentation, Annual Scientific Meeting of the Biomedical Engineering Society, Austin, Texas. October 2010.
13. **Ubong Peters**, Guy Drapeau, and Geoffrey Maksym. Testing and Validation of a Handheld Oscillation Spirometer Prototype. Poster presentation, Annual Scientific Meeting of the Biomedical Engineering Society, Pittsburgh, Pennsylvania. October 2009.

Chapter 2: Review of Relevant Literature

This chapter provides a broad review of published literature relevant to this thesis. It principally covers the role of obesity in altered lung function. Obesity often leads to asthma-like symptoms and asthma is a common comorbidity in these subjects. Therefore, this review also examines the state of the art in the field of lung mechanics with a specific focus on pulmonary mechanics in asthma. A general review of asthma is first provided followed by a detailed discussion of how asthma-like symptoms could possibly arise in individuals with obesity. Other topics reviewed include the clinical application of the oscillometry technique in the assessment of pulmonary mechanics. An introduction to conventional methods of assessing pulmonary function is also provided. This chapter ends with a description of the research hypotheses, aims and approaches.

2.1 The Respiratory System and the Mechanics of Breathing

The respiratory system can be described as a parallel combination of mechanical and physiological subsystems acting together in a synchronous fashion to facilitate one of the most vital processes of life – gas exchange. The physiological subsystem comprises of two functional zones namely, the conducting zone (pharynx, larynx, trachea, bronchi) and the respiratory zone (respiratory bronchioles, alveolar ducts, alveoli) while the mechanical subsystem is made up of the rib cage, pleural sac and muscles that assist in moving air into and out of the lungs during breathing. These muscles include the diaphragm and the nondiaphragmatic (intercostal) muscles. Other accessory muscles that also play a role in respiration include the abdominal muscles, the sternocleidomastoid, alae nasi and scalenus muscle.

During inspiration, the diaphragm contracts and moves downwards, the external intercostal muscles contract, the rib cage moves upwards and out, while the abdominal muscles relaxes [16]. The increase in chest cavity volume and the increase in the negative pressure within this region with respect to atmosphere results in the inflation of the lungs [17, 18]. Following inspiration, a relaxed exhalation of air from the lungs occurs as a

result of the passive elastic recoil of the lung and chest wall returning to their equilibrium state of balance, which is between inward recoil of the lung and outward recoil of the chest wall. This balance occurs at a defined lung volume known as the functional residual capacity (FRC). This is described in more detail in section 2.3. Unlike the act of inspiration which is an active event involving the respiratory muscles, expiration is usually passive, except during exercise or forced exhalation maneuvers when the abdominal muscles and internal intercostal muscles are recruited to assist.

The Structure of the Lungs

The lung is a highly complex structure made up of thousands of tree-like branching airways. Air comes into the lung through the trachea but the trachea divides into right and left main bronchus, which then divides into lobar bronchi, and then segmental bronchi. Penetrating further into the lungs, the airways become more numerous, they branch repeatedly, and they also become narrower and smaller. These structural changes continue up to the alveoli. The human airway tree is made up of about 23 generations of dichotomously-branching airways [19]. This process of dichotomous bifurcation at each airway generation continues even at the level of the terminal bronchioles, which make up the last set of conducting airways. The trachea and bronchi make up the conducting airways. These conducting airways are often referred to as the anatomic dead space because they have no alveoli, and as such they do not take part in gas exchange. The terminal bronchi then branch repeatedly to form the respiratory bronchioles, which are the site where alveolization begins as some alveoli can be found occasionally budding off the walls of the respiratory bronchioles. The bifurcation ends at the alveolar ducts, and the alveoli. The alveoli make up most of the lung parenchyma.

The alveolus is the main site in the respiratory system where physiological gas exchange actually occurs. In total there are about 300-500 million alveoli in a healthy adult lung and this covers an area of approximately 70-80 m^2 which is about the size of a tennis court [19, 20]. It is made up of a squamous epithelial cell layer lining the inner surface, supported by an extracellular matrix containing the blood capillaries. Deoxygenated blood containing carbon dioxide from all body tissues is exchanged for fresh oxygen

through the blood capillaries of the alveoli. The alveolated regions of the lung described here make up the respiratory zone.

The alveolar walls contain the proteins – collagen and elastin which are largely responsible for the structure and architecture of the lung parenchyma and also contribute to its compliance (i.e volume change per unit change in pressure). However, since the alveoli are lined with fluid, the compliance (inverse of elastance or stiffness) of the respiratory system arises from a combination of the elastic forces generated when the collagen and elastin fibers stretch during breathing, and from the surface tension of the alveolar lining fluid. The compliance of the respiratory system is also influenced by components of the chest wall which include the rib cage and diaphragm.

The following sections describe some common respiratory disorders and some of the tests that are used to understand them.

2.2 Lung Diseases

There are many types of respiratory diseases but the most common ones include asthma, chronic obstructive pulmonary disease (COPD), lung cancer, tuberculosis, cystic fibrosis, obstructive sleep apnea (OSA), respiratory distress syndrome (RDS), pneumonia and influenza. The main focus of this section is on asthma because it is a common comorbidity in obesity [21], and many individuals with obesity often present with asthma-like symptoms [22, 23]. A brief review of COPD is also provided here since patients with COPD were also studied in this thesis.

It is estimated that more than 3 million Canadians suffer every year from the five most prevalent lung diseases presented in Table 2.1 [24]. In the United Kingdom, respiratory diseases make up the second largest cause of death with more than 117,000 deaths recorded in 2004 [25] and in the United States, respiratory disease is rated as the third biggest killer with approximately 35 million Americans living with some form of chronic lung disease such as asthma or COPD [26].

Most respiratory diseases fall into two main categories, viz; obstructive respiratory diseases and restrictive respiratory diseases. Obstructive diseases are characterized by airflow limitation due to excessive mucus production or narrowing of the lung airways. Common examples of obstructive lung disease include: cystic fibrosis, asthma and COPD.

Table 2.1: Prevalence of some common respiratory diseases in Canada *

Respiratory Disease	**Affected Canadians**
Asthma – physician diagnosed (prevalence 2014) *	2,448,817
COPD – physician diagnosed (prevalence 2014) *	804,043
Lung cancer (new cases – 2014) *	26,100
Cystic fibrosis (prevalence from registry – 2013) †	4,077
Tuberculosis (new or reactivated cases – 2014) *	1,600

* Information sourced from [27].

† Information sourced from [28].

Restrictive respiratory diseases, on the other hand, are characterized by stiffer lungs, reduced lung volumes accompanied by a significant decline in lung compliance. Examples of restrictive lung diseases include: interstitial pulmonary fibrosis and interstitial lung disease (including sarcoidosis), pneumonia, and extra-pulmonary restrictive lung diseases (scoliosis). These pathologies manifest changes to the mechanics of the respiratory system which can, if measured, be used diagnostically.

A brief description of the two most prevalent respiratory diseases is provided below, followed by an introduction to various tests used in assessing changes in lung function caused by these diseases.

2.2.1 Asthma

Definition of Asthma

Asthma is a complex and heterogeneous disease. Historically, the term "asthma" was loosely applied to refer to any respiratory condition that resembled dyspnea. Despite tremendous advances in our understanding of the disease, the definition of asthma is still the subject of many scientific debates. According to the Global Initiative for Asthma (GINA) [29], asthma is defined as "a chronic inflammatory disease of the airways in which many cell types play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals, the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or early morning. These symptoms are usually associated with widespread but variable airflow obstruction that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli."

This definition of asthma recognized the central role chronic inflammation plays in the disease process. It also suggests that the best way to control the progression of the disease is to treat the underlying inflammatory process that is responsible for the changes in structure and function of the pulmonary airways. Indeed, structural remodeling of the airways is a well-documented pathological feature of asthma that is believed to be present in the airways of individuals with asthma [30, 31], including those newly diagnosed with the disease [32]. Such remodelling is characterized by increased airway smooth muscle (ASM) mass, angiogenesis, thickening of the reticular basement membrane and damage to bronchial epithelium [31, 33, 34].

Epidemiology of Asthma and its Economic Cost

Asthma is one of the most chronic diseases in North America with almost 2.5 million Canadians suffering from the disease (Table 2.1) [24, 35]. Epidemiological data have revealed that asthma is more prevalent among children with prevalence rates reported to

be about 10-15% of children and about 5% of the adult population [36]. In the US, it is estimated that 22.2 million Americans, representing 7.7% of the total population, have asthma [37]. The prevalence rate of the disease is reported to decline with age with a breakdown of the rate indicating that 8.9% of children and 7.2% of adults have the disease. Surprisingly, approximately 32% of asthmatic adults in the US are also obese [38], suggesting a relationship between obesity and asthma. This relationship is reviewed in more detail in Section 2.4.1.

According to the European Respiratory Society (ERS), the cost of asthma care in Europe alone is estimated to be over €17 billion and the loss of productivity due to the disease is over €9 billion annually [39]. The annual direct cost of asthma care in Canada is estimated to range from $504 to $684 million CAD [40] while the estimated direct and indirect cost of asthma care per year in the US is about $12.6 billion USD [41].

Pathophysiologic Mechanisms of Asthma

The symptoms of asthma develop through numerous pathophysiological mechanisms that are triggered by a wide variety of stimuli, ultimately resulting in bronchoconstriction and airflow obstruction [42]. The pathophysiology of classical asthma is reviewed in detail in Appendix A to enable us to draw parallels to the pathophysiology of asthma-like symptoms in obesity. This review is important because it is not yet known whether the mechanisms of classical asthma are distinct from the mechanisms of asthma or asthma-like symptoms in obese individuals. The pathophysiologic mechanisms of asthma-like symptoms in obesity will be reviewed in detail in Section 2.4.

Diagnosing Asthma

Since asthma is more frequently diagnosed in obese individuals than lean individuals, and the changes in pulmonary function can be similar in obesity and asthma, it is important to understand how asthma is diagnosed. The challenges in diagnosing asthma in obesity are described in the next section.

The criteria for diagnosing asthma in adults are presented in Figure 2.1. Asthma is usually diagnosed using a combination of different tools beginning with physical

examination and detailed documentation of the patient's medical history including history of wheeze, dyspnea, chest tightness and cough and how these symptoms vary in intensity. Other conditions such as COPD, upper airway obstruction, gastric esophageal reflux disease, pulmonary embolism and heart failure can cause wheezing and other symptoms that are similar to the symptoms of asthma; therefore, a differential diagnosis must be performed if the patient does not fit the criteria described here (i.e history and symptoms that vary in intensity) as shown in Figure 2.1. This is usually followed by lung function tests (see Section 2.3 for a detailed description) to document the presence of excessive variability in lung function, airflow limitation and reversible airflow obstruction, airway hyperresponsiveness, and to document the progression of the disease over time (Global Initiative for Asthma [43]).

Reversibility of airflow obstruction is usually assessed during a bronchial reversibility test with a short-acting beta$_2$-adrenergic agonist (SABA) such as salbutamol. When available, bronchial provocation test with a bronchoactive agent such as methacholine is also used to determine airway hyperresponsiveness (AHR). Tests for AHR and bronchial reversibility are described in detail in Section 2.3. Briefly, the airways are considered hyperresponsive if inhalation of 16 mg/mL of methacholine induces more than a 20 % drop in forced expired volume in one second (FEV_1) [44], while the clinical threshold for positive bronchial reversibility required to diagnose asthma is a 12 % increase in FEV_1 from baseline together with a minimum increase in volume of 200 ml [45]. According to guidelines used in diagnosing asthma, airflow obstruction occurs when the ratio of FEV_1 to forced vital capacity (FVC) is lower than 0.75 (Global Initiative for Asthma [43]). FEV_1 is defined as the volume of air that can be forcibly exhaled from the lung in the first second of a forced exhalation maneuver while FVC is defined as the total volume of air that is exhaled from the lungs during the forced exhalation maneuver. These tests are also described in detail in Section 2.3.

The Challenges in Diagnosing Asthma in Obese Individuals

As mentioned above, asthma is usually diagnosed using a combination of different tools that include: physical examination and past medical history (intermittent and variable wheeze, dyspnea, chest tightness and cough), lung function tests (to determine airflow

limitation), bronchial reversibility test (to determine reversibility of airflow obstruction) and bronchial provocation tests (to determine airway hyperresponsiveness) [46]. However, the most challenging problem in diagnosing asthma in obese patients is that many symptoms of asthma may not be present in these patients and, as will be discussed in Sections 2.4, obesity also modifies the clinical presentation of many of the distinguishing features of asthma (such as AHR [47]) making it even more difficult to ascertain whether the asthma-like symptoms of many obese individuals are indeed due to asthma.

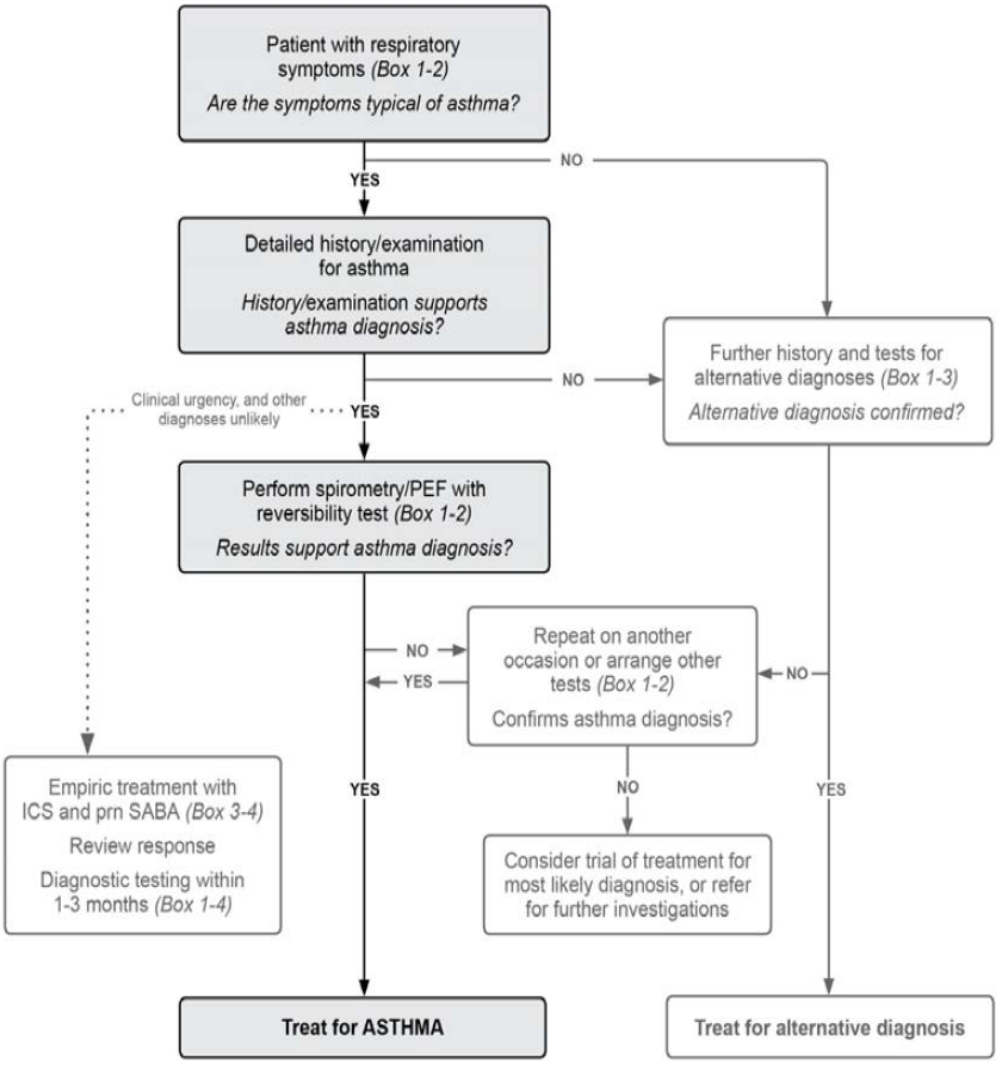

Figure 2.1. Diagnostic flowchart for asthma at initial presentation (GINA 2015 report).

PEF, peak expired flow; ICS, inhaled corticosteroid; SABA, short-acting bronchial agonist.

It is possible that obesity may confound the diagnosis of asthma by imitating some of the distinguishing characteristics of asthma. Clinicians currently have no way of discriminating obesity-induced respiratory symptoms such as dyspnea, wheeze, and AHR from asthma-induced symptoms which also include dyspnea, wheeze and AHR. Obesity is an important modifier of AHR in humans and mice where it leads to positive responses to bronchoactive agents [47-51]. Clinicians use negative methacholine response to rule out asthma; however, since most obese subjects respond positively to methacholine, the likelihood of making a false positive asthma diagnosis among this cohort is significantly increased [52].

In 2008, Aaron *et al* conducted a prospective study of 540 individuals with physician-diagnosed asthma and after rigorous assessment with bronchial reversibility and methacholine challenge, and withdrawal of asthma medication, they concluded that 31.8% of obese and 28.7% of nonobese patients diagnosed with asthma were actually misclassified as having asthma [53]. This finding highlights the challenges primary care physicians face in diagnosing and managing asthma in the obese population. Although Aaron *et al* reported that obese subjects were not any more likely than their nonobese counterparts to be misdiagnosed with asthma [53]; this is in contrast to Scott *et al* who reported evidence of increased likelihood of misdiagnosis among overweight and obese participants in their study [52].

These findings highlight the need for an effective tool that can readily detect changes in pulmonary mechanics induced by weight gain or loss such that its use can help in the phenotypic identification of new classes of asthma patients in whom certain treatment strategies might be more effective.

Classification systems for asthma

Asthma is usually classified based on the frequency of signs and symptoms (i.e intermittent or persistent), severity (i.e mild, moderate or severe) and control (i.e well-

controlled, not well-controlled, very poorly controlled) (Global Initiative for Asthma [43]); however, given the heterogeneous and complex nature of the disease, many experts have suggested other methods for classifying the disease. In 2006, a comprehensive and integrated method of classification for asthma was proposed [54]. This method classifies asthma into three distinct phenotypic categories based on its clinical features (i.e exacerbation-prone, chronic airflow obstruction, treatment resistant, age of onset), the stimulants that trigger an attack (i.e allergic, occupational, exercise-induced, aspirin-induced, menses-related, obesity-related) or based on its inflammatory phenotype (i.e eosinophilic, neutrophilic, paucigranulocytic) [54].

In the same vein, cluster analysis of demographic, clinical and/or pathophysiological characteristics of participants enrolled in the Severe Asthma Research Program have also led to the identification of five new asthma phenotypes [55]. The phenotypic characteristics of the five asthma clusters identified in this study were:

(i) early-onset atopic asthma with normal lung function treated with two or fewer controller medications and minimal healthcare utilization,
(ii) early-onset atopic asthma and well preserved lung function but increased medication requirements and healthcare utilization,
(iii) older obese women with late-onset non-atopic asthma and frequent use of oral corticosteroids.

Subjects in the remaining two clusters all had severe airflow obstruction and demonstrated bronchial reversibility but they differed in their age of asthma onset, atopic status and use of oral corticosteroids.

These findings suggest that each phenotype of asthma may be caused by a specific pathophysiologic mechanism implying that the treatment strategy must be designed to meet that specific phenotype, following a detailed characterization of the phenotype. Indeed, in the past 5 years, two new predominant phenotypes of asthma in obese subjects have emerged [56, 57]. These two phenotypes are: (i) atopic, early-onset, T_H2-high asthma phenotype characterized by high serum immunoglobulin-E (IgE) and the presence of eosinophilia, and (ii) non-atopic, late-onset, T_H2-low asthma phenotype characterized

by low serum IgE and the absence of eosinophilia. These phenotypes are described in more detail in section 2.4.

Given the complexity and phenotypic diversity of this disease, asthma should not be considered a single respiratory ailment; rather, it should be defined as a complex constellation of several respiratory conditions with many overlapping phenotypes. Perhaps in the future, a much deeper understanding of the pathophysiology of asthma will guide the development of novel treatment strategies that would target each asthma phenotype described in these classification systems. Indeed, there is a great need to carefully phenotype obese individuals with asthma to understand the mechanisms behind the increased risk of respiratory impairment in this patient group and their blunted response to asthma treatment.

2.2.2 Chronic obstructive pulmonary disease (COPD)

A brief review of chronic obstructive pulmonary disease (COPD) is provided here because, as described below, these patients present with impaired lung mechanics that lead to increased respiratory system impedance. COPD is a largely irreversible lung disorder that is characterized by damaged airway tissues and airways that narrow easily during exhalation, resulting in persistent and progressive airflow limitation and expiratory flow limitation [58]. It is strongly associated with inhaled particles such as cigarette smoke, occupational dust and/or chemicals, and polluted air. These particles irritate the lungs causing inflammatory changes in the walls of the small airways and alveoli, including the destruction of alveolar walls due to infiltration and accumulation of neutrophils and macrophages within the lung airways [59]. As a result of the destruction of alveolar walls, the parenchyma loses its elasticity, and the lung elastic recoil is consequently reduced. These pathological changes in lung parenchyma contribute to airflow limitation observed in patients with COPD. The destruction of the alveolar walls also attenuates airway-parenchymal tethering forces [60, 61]. Thus, the airways narrow easily because the elastic load that is typically applied around the airways through the parenchymal attachments is reduced. This results in an increased resistance to airflow.

There are several definitions for COPD. According to European Respiratory Society (ERS), COPD is defined as "reduced maximum expiratory flow and slow forced emptying of the lungs, which is slowly progressive and mostly irreversible to present medical treatment" [62]. The American Thoracic Society (ATS) defines COPD as "a diseased state characterized by the presence of airflow limitation due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, and may be partially reversible" [63]. However, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is defined as "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" [64].

The differences in these definitions show that there is no consensus yet on the precise definition and classification for COPD, therefore, its diagnostic criteria will also vary. Currently, the criteria for diagnosing COPD are based on the demonstration of COPD symptoms, particularly airflow obstruction which is defined as a post-bronchodilator FEV_1/FVC ratio less than 0.7. Spirometry is not only used to determine the presence of airflow obstruction in COPD, it is also used to classify the stages and severity of the disease as presented in Table 2.2. The clinical manifestations of COPD include: dyspnea, wheeze, and chronic cough that is sometimes accompanied by sputum production, poor exercise tolerance, and respiratory failure or cor pulmonale in very advanced stages of the disease. COPD patients sometimes demonstrate partial bronchial reversibility and airway hyperresponsiveness.

In 2001, COPD ranked as the 5th leading cause of death worldwide, and it is projected that it will become the 3rd leading cause of death by 2020 [65]. However, in the US, COPD is already the 3rd leading cause of death and the 12th leading cause of mortality [66]. COPD is the leading cause of hospitalization among adults in the US, with about 662,000 hospitalizations (1.9% of total hospitalizations) reported in 1998 alone [41]. In the same year, COPD was also identified as a comorbidity in an additional 2,530,000 hospitalizations (7% of hospitalizations). In 1996, the National Health Interview Survey (NHIS) estimated that about 10.2 million American adults (5.9 % of the adult population)

aged ≥ 25 suffer from COPD [41]. In Canada, the National Population Health Survey conducted by Statistics Canada between 2013-14 indicated that about 804,043 Canadians have physician-diagnosed COPD with the national prevalence reported to be 4.0% (Table 2.1) [67].

Table 2.2. Stages and Severity of Chronic Obstructive Pulmonary Disease

Severity of COPD	**Clinical signs and symptoms**	**Spirometry outcomes**
Stage I: Mild COPD	Mild airflow limitation, patient sometimes present with chronic cough with or without sputum production	$FEV_1/FVC < 70\%$ and $FEV_1 \geq 80\%$ predicted
Stage II: Moderate COPD	Worsening airflow limitation, dyspnea typically on exertion	$FEV_1/FVC < 70\%$, and $50\% \leq FEV_1 < 80\%$
Stage III: Severe COPD	Further worsening of airflow limitation, worsening dyspnea, reduced exercise tolerance, and frequent exacerbation of symptoms impacting significantly on patient's quality of life	$FEV_1/FVC < 70\%$, and $30\% \leq FEV_1 < 50\%$
Stage IV: Very severe COPD	Severe airflow limitation together with chronic respiratory failure. Patients may have Stage IV COPD whenever they present with this complication, even if FEV1 > 30% predicted	$FEV_1/FVC < 70\%$, and $FEV_1 < 30\ \%$

The economic burden of COPD is extremely heavy. It is estimated that the annual direct medical costs of COPD in the US is about $14.7 billion USD while the indirect cost of the disease was about $9.2 billion USD bringing the total to $23.9 billion USD [41]. The estimated direct and indirect cost of asthma care per year in the US is $12.6 billion USD

and when added to that of COPD care, the total burden of obstructive lung disease in the US alone rises to about $36.1 billion USD [41].

The following section describes the tests used in assessing changes in pulmonary function caused by various respiratory diseases.

2.3 Pulmonary Function Tests

Pulmonary function tests (PFTs) provide an objective index of how well the respiratory system is ventilating its lung by measuring the flows and volume that are associated with normal tidal breathing, and forced inspiration and exhalation. Common ways of assessing pulmonary function include body plethysmography, spirometry, and recently, the forced oscillation technique (FOT), also known as oscillometry. The main focus of this thesis is oscillometry, spirometry, and plethysmography thus these tests are described in the following sections.

2.3.1 Spirometry

Spirometry is currently the most frequently performed and most widely accepted measure of pulmonary function [68]. The technique was invented by John Hutchinson in 1846 after he recognized that the volume of air exhaled from total lung capacity is indicative of lung function [69, 70]. Hutchinson's invention has evolved over the decades to find usefulness today as a diagnostic tool for restrictive and obstructive lung diseases, and also as a tool for the physiological investigation of lung function.

During the spirometry test, the patient is trained to inhale to total lung capacity (TLC), and then exhale forcibly into the spirometer while measures such as the forced vital capacity (FVC) and the forced expired volume in 1 second (FEV_1) are recorded. FEV_1, or frequently, FEV_1 normalized to FVC is the "de facto" gold standard for measuring lung function in healthy subjects and patients with asthma and COPD [71, 72] and loss of FEV_1 indicates airway obstruction. Table 2.3 provides a brief description of some of the

common clinical indices that can be derived from spirometry. These outcomes are usually presented as percentiles of predicted values estimated from reference equations that take into account the subject's age, height, gender, and sometimes ethnicity [73-75].

Spirometry can be used to discriminate between obstructive respiratory diseases and restrictive respiratory diseases. Obstructive lung diseases are those that lead to some degree of obstruction to the passage of air into and out of the lung [76, 77]. Notable examples of obstructive diseases include emphysema, chronic bronchitis, asthma and cystic fibrosis, all of which lead to significant reductions in forced expiratory flows when assessed with spirometry. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria for classification of severity of airflow obstruction based on post-bronchodilator FEV_1 is shown in Table 2.4 [78]. According to the GOLD criteria, airflow limitation is defined by an FEV_1/FVC ratio of less than 70%, and in addition to this criteria, FEV_1 is used in classifying airflow obstruction as either mild ($FEV_1 \geq 80\%$ predicted), moderate ($50\% \leq FEV_1 < 80\%$ predicted), severe ($30\% \leq FEV_1 < 50\%$ predicted), or very severe ($FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure), but there are other criteria that can be used which do not rely on a fixed ratio . Restrictive respiratory disorders, on the other hand, are those that result in significant reductions in lung volumes [76, 79]. In particular, restriction is indicated by a low FVC in the presence of a normal FEV_1/FVC ratio [45, 77, 80, 81].

Obstruction in the large and mid-sized airways, collectively known as central airways, can be easily detected with spirometry since narrowed airways expel air more slowly [68, 82, 83]; however, this index is not sensitive to changes in the small or peripheral airways. The distinction between central and peripheral airways that used here was first described in 1967 by Peter Macklem and Jere Mead [84]. The upper airways includes the larynx, pharynx, and glottis while the central airways extends from the trachea to the segmental bronchi and includes all airways greater than 2 mm in diameter. The small peripheral airways includes all airways less than 2 mm in diameters. Indeed, the small airways are considered a silent zone to spirometry because changes in this region of the lungs are typically not reflected in spirometry, unless the airway disease is at an advanced stage [85-87]. Interestingly, mid-expiratory flow measurements including the forced expiratory

flow at 50% (FEF_{50}), 75% (FEF_{75}), and 25-75% (FEF_{25-75}) of FVC are often cited in the literature as sensitive indices of small airways obstruction [88, 89]; however, there is general agreement that these measures lack specificity [80, 90, 91], with one recent study reporting a poor correlation between mid-expiratory flow measurements and other well-established indices of air trapping such as FVC and the ratio of residual volume to total lung capacity [92]. The influence of obesity on spirometry outcomes is discussed below in Section 2.4.

Table 2.3. Common spirometric measures of pulmonary function

Name	**Abbreviation**	**Description**
Forced vital capacity	FVC	This is the volume of air that can be forcibly exhaled from the lungs after a deep inspiration.
Forced expiratory volume in 1 second	FEV_1	This is the volume of air that can be forcibly exhaled from the lungs in the first second of exhalation, measured in litres.
FEV_1/FVC	FEV_1/FVC	This is the ratio of FEV_1 to FVC. In healthy adults this should be approximately 0.75 – 0.80.
Peak expiratory flow	PEF	This is the speed of the air moving out of your lungs at the beginning of the expiration, measured in litres per second.
Forced expiratory flow 25–75% or 25–50%	$FEF_{25–75\% \text{ or } 25–50\%}$	This is the average flow (or speed) of air coming out of the lung during the middle half of expiration also sometimes referred to as the maximal mid-expiratory flow (MMEF).
Tidal volume	V_T	This is the specific volume of air that is drawn into and then expired out of the lungs during the normal respiratory cycle.

Spirometry is considered the “de facto” gold standard for measuring the severity of respiratory disease [71]. The technique is safe and noninvasive and spirometry devices are cheap, portable and simple to use. Another important advantage of spirometry is that it has been widely adopted and standardized [45, 68, 93], and normative values are readily available [73].

Table 2.4. Spirometric classification of severity of airflow obstruction

Class	Condition	Description
Stage I	Mild	$FEV_1/FVC < 0.70$
		$FEV_1 \geq 80\%$ predicted
Stage II	Moderate	$FEV_1/FVC < 0.70$
		$50\% \leq FEV_1 < 80\%$ predicted
Stage III	Severe	$FEV_1/FVC < 0.70$
		$30\% \leq FEV_1 < 50\%$ predicted
Stage IV	Very Severe	$FEV_1/FVC < 0.70$
		$FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure

Assessment of Bronchial Reversibility with Spirometry

Bronchial reversibility or bronchodilator (BD) responsiveness is measured by assessing pulmonary function before and after inhalation of a short acting β_2-adrenergic agonist such as salbutamol. In order to prevent the deposition of the BD in the upper airways and to facilitate the deposition in the small airways, the BD is usually administered through an inhaler device such as a pressurized or breath-actuated metered-dose inhaler, dry powder inhaler, nebulizer, or soft mist inhaler [94]. The subject is instructed to exhale completely and then asked to inhale the drug to TLC. A breath-hold of about 5-10

seconds is recommended upon reaching TLC to ensure widespread distribution of the agonist within the central and small airways of the lung. According to ATS/ERS guidelines, the clinical threshold for positive bronchial reversibility required to diagnose asthma is at least a 12 % increase in FEV_1 from baseline together with a minimum increase in volume of 200 ml [45]. The 12 % increase in FEV_1 represents the 95^{th} percentile for percent change from baseline after bronchodilator administration in a large sample of the general population and patient population [45].

The bronchial reversibility test is highly specific; therefore, it is very useful in confirming (ruling in) an asthma diagnosis, if the result is positive. This test is sometimes used in clinical practice to distinguish subjects with asthma from those with COPD, if the FEV_1/FVC ratio normalizes following BD or if FEV_1 increases by more than 10 % [45]; however, some investigators have argued that this test does not possess such discriminatory powers [95, 96]. Moreover, because of its poor sensitivity, a negative bronchodilator response does not conclusively rule out asthma [97], a position that has also been adopted by the British Thoracic Society Scottish Intercollegiate Guidelines Network [98].

The poor sensitivity and high specificity of bronchial reversibility tests could prevent many asthma patients from taking part in randomized controlled trials (RCTs) and this could limit the generalizability of RCTs especially considering the fact that the outcome of these trials form the basis of many clinical guidelines and recommendations developed for the management of asthma. This is because the inclusion criteria for most asthma RCTs is the demonstration of BD responsiveness, defined as a 12 % increase in FEV_1 from baseline [99, 100]. In fact, according to one estimate, up to 76 % of adult subjects with asthma drawn from a random sample (n = 179) would not have met the entry criteria for many RCTs because they did not demonstrate positive BD reversibility [101].

There is a paucity of data on the effect of obesity on BD responsiveness and this effect has been investigated by only three studies to date. These studies which relied on spirometry to investigate BD responsiveness in obesity showed conflicting results. The first of these studies conducted by Castro-Rodriguez *et al* showed that girls who became overweight or obese between the ages of 6 and 11 were more likely to demonstrate

bronchial reversibility than girls who did not become overweight or obese over the same interval [102]. Children whose BMI are above the 85th percentile of their age- and gender-matched counterparts are considered overweight while children whose BMI are above the 95th percentile are considered obese [103]. However, Tantisira *et al* later reported that BD responsiveness decreased with increasing body mass index (BMI) in obese children [104]. A third study failed to establish a relationship between obesity and reversible airway obstruction, but this was in adults. In that study, Dixon *et al* showed that BD responsiveness was not associated with BMI in obese and non-obese patients with asthma [105]. To date, there are no published studies that have investigated bronchial reversibility in obesity using oscillometry. Furthermore, there are no published data in the literature on the effect of weight reduction on BD responsiveness in obese adults without asthma.

Assessment of Airway Hyperresponsiveness with Spirometry

Airway hyperresponsiveness (AHR) is a distinguishing characteristic of asthma. In simple terms, AHR refers to a heightened response of the airway to a variety of contractile stimuli in at risk individuals, as compared with healthy individuals who show no clinically significant response. Bronchial provocation testing is used to measure AHR. A heightened response to methacholine and histamine was first reported in healthy subjects and subjects with asthma, as far back as the 1940s [106, 107]. The degree of AHR was later discovered to correlate with asthma severity [108]. From then onwards, this test has become standardized [44, 109] and employed in many studies to deepen our understanding of the pathogenesis and pathophysiology of asthma and its use as a tool to support the diagnosis and management of asthma is now recommended (Global Initiative for Asthma [110]).

Bronchial challenge test involves the assessment of FEV_1 every 30 to 90 seconds after inhalation of increasing doses of methacholine administered through a nebulizer [44] or dosimeter [111], until a cut off dose of 16 mg/ml is reached, or a 20% fall in FEV_1 from baseline is achieved. The test begins with the administration of saline as control, and then doubling of methacholine dose starting from 0.03 mg/ml to 16 mg/ml. The result is usually expressed as the provocative dose (PD) or concentration (PC) of methacholine

that induced a 20% fall in FEV_1, i.e PD20 or PC20. The airways are considered hyperresponsive if inhalation of 16 mg/ml of methacholine induces more than a 20 % drop in FEV_1 [44] but as a precautionary measure, challenge testing is not recommended if the baseline FEV_1 is below 60 % of the predicted FEV_1. Another reason why challenge testing is not recommended when baseline FEV_1 is below 60 % of the predicted FEV_1 is because the volume changes would be so small that they may be undetectable by spirometry. The response to the stimuli is estimated from the percent drop in FEV_1 in response to histamine or methacholine as follows:

$$100 * \left(\frac{baselineFEV_1 - lowestFEV_1 postchallenge}{baselineFEV_1} \right)$$

Direct bronchial provocation test is a moderately sensitive test (GINA 2015 report), therefore it is very useful in ruling out asthma (if the result is negative) but its weakness is that it lacks specificity, implying that healthy subjects could sometimes be misclassified as asthmatics, particularly at higher methacholine concentrations (if the result is positive) [112]. However, there is a clinically significant difference in AHR between healthy subjects and patients with asthma. In fact, as shown in Figure 2.2, most healthy subjects do not present with significant AHR since their FEV_1 does not drop by up to 20%.

Healthy subjects exhibit a characteristic plateau in their dose-response curve whereas subjects with asthma are unable to reach a similar plateau, even when FEV_1 drops by 60% from baseline. As demonstrated in the seminal work of Woolcock *et al*, healthy subjects can be distinguished from current asthmatics and those with mild asthma based on the shape of their dose-response curve to histamine [113]. Subjects with severe asthma demonstrate a steep fall in FEV_1 at relatively small concentrations of methacholine while subjects with mild asthma demonstrate a characteristic pattern whereby larger concentration of methacholine is required to induce a clinically significant fall in FEV_1 (Figure 2.2). As described earlier, the risk for AHR increases significantly with increasing severity of obesity in both humans [47-50] and mice [51] but this will be described in detail in Section 2.4.

In summary, bronchial provocation test is a very important pulmonary function test with high diagnostic value. However, the main limitation of this test is that it has high technical requirements and healthy individuals and patients suffering from respiratory conditions other than asthma such as obesity could also present with positive AHR [47-50]. Therefore, the test is more useful in ruling out asthma than in confirming asthma.

Limitations of Spirometry

Although spirometry is sensitive to changes in pulmonary function [114], its downside is that it is highly effort dependent. In order to obtain reliable results, the test maneuver must be learned, and active subject cooperation must be achieved. Results are considered reliable if the patient produces at least two repeatable FEV_1s, within 150 ml of each other [68]. Reliable results also depend on the subject's psychological state, and the pulmonary technologist's ability to properly coach the subject during the maneuver. However, the cost of training the technician and the time needed to obtain satisfactory and reliable results significantly increases the cost of health care. In fact, about 10 % of patients are unable to perform spirometry to acceptable standards, even when coached by an experienced respiratory technician [115]. This rate is presumably higher among preschool children and seniors over 80 years of age. Due to these issues, spirometry is not recommended in those with cognitive limitations and those with a limited psychological reserve who lack the motivation to perform spirometric maneuvers. Spirometry is also not recommended in children below the age of 6 [116], even though the International Study of Asthma and Allergies in Childhood (ISAAC) reports that children in this age bracket self-report symptoms such as wheezing and shortness of breath which is consistent with asthma [35].

Furthermore, FEV_1 is typically normal in those with obesity [22, 23], while FEV_1/FVC ratio is typically well preserved or slightly elevated [22, 23, 117-119], even though these obese individuals exhibit respiratory symptoms like wheezing and shortness of breath [22, 23]. Thus, more effective methods of measuring lung function are badly needed for the elderly, obese populations, children and those unable to perform spirometry to acceptable standards.

Recent evidence has also revealed that spirometry is not routinely performed during the diagnosis of respiratory disorders. Indeed, according to some estimates, approximately 44–48 % of patients with physician-diagnosed asthma have never performed spirometry [120, 121]. These patients are diagnosed solely on the basis of positive symptom history and physical examination findings because there are many barriers that could potentially prevent primary care physicians from administering these pulmonary function tests (PFTs). These barriers include lack of necessary equipment at the doctor's office and lack of the training or support staff to conduct and interpret the PFTs. PFT equipment are expensive and require regular calibration, training and time to acquire reliable data and also to interpret the data.

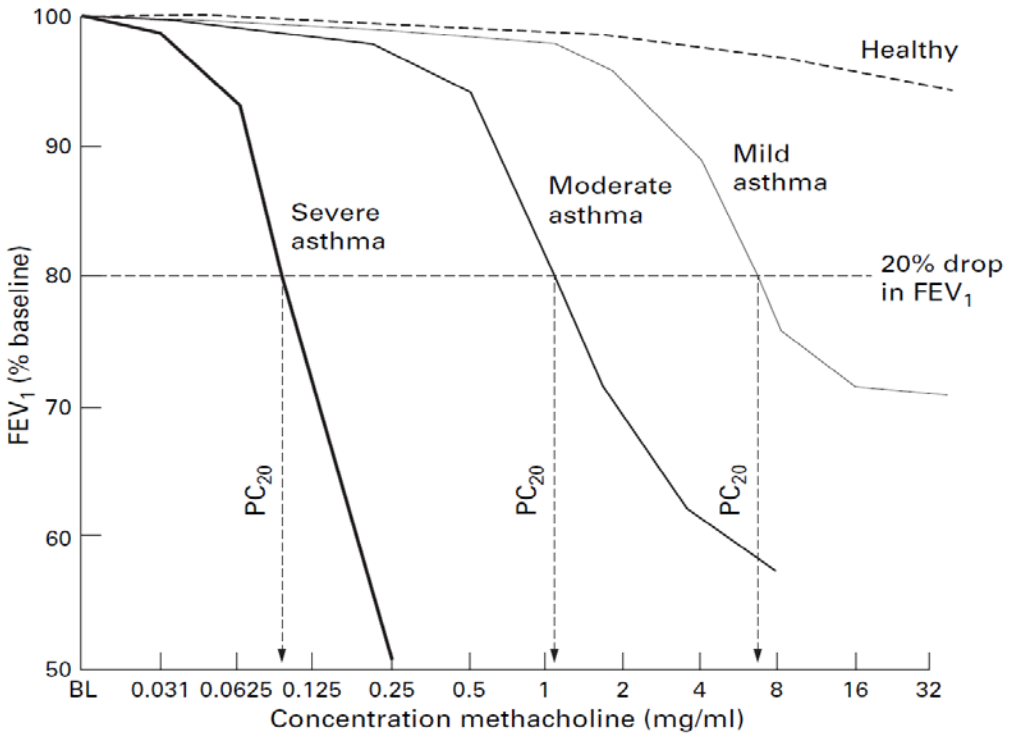

Figure 2.2. Change in FEV_1 from baseline versus increasing concentrations of methacholine in mild, moderate and severe asthma compared to healthy subjects [122].

Apart from these barriers, there is also no concrete evidence from RCTs suggesting that the use of spirometry in primary care could positively influence asthma outcomes and

improve its diagnosis and management. In fact, one study reported that office spirometry did not add any significant benefit to conventional evaluation with symptom history and physical examination when confirming an asthma diagnosis [123], but another study found the opposite to be true [124]. Available evidence also suggest that bronchial reversibility testing with spirometry only confirmed asthma diagnosis in 16% of asthma cases while methacholine challenge confirmed asthma in only 72% of patients studied [53]. Many primary care physicians therefore rely on the patient's response to trial of therapy for asthma to confirm their diagnosis [125]. Although trial of therapy is only recommended when spirometry is not readily available [98], this practice has likely contributed to the high rate of overdiagnosis of asthma in the developed countries [53, 121].

Taken together, these studies suggest that the use of spirometry as the only tool in clinical decision making could potentially mislead clinicians to withhold treatment from patients with respiratory disorders who absolutely need it. This problem is also compounded by the fact that many patients with respiratory disease have normal spirometry [123], and according to one Canadian spirometry interpretation algorithm, reversibility testing is only recommended in patients with demonstrable airflow obstruction, i.e patients who present with FEV_1/FVC ratio of less than 70% [126]. For example, in the study by Lusuardi *et al*, only 21% of asthmatic patients met this criteria [123]; therefore, if the Canadian spirometry interpretation algorithm was to be strictly adhered to, a very large proportion of asthma patients would either be left untreated or these patients would have to be diagnosed through alternate means such as methacholine challenge.

Given the limitations of spirometry outlined here, there are many reasons why other methods of assessing pulmonary function should be included in the armamentarium of respirologists, primary care physicians and physiologists. These methods include oscillometry for measurement of respiratory mechanics, and plethysmography which provide clinically useful indices of lung volumes. The plethysmography technique is described below in Section 2.3.2 because obesity affects lung volumes and the oscillometry technique is described in Section 2.3.3 because this is the primary

measurement technique used in this thesis to assess weight-induced changes in lung mechanics.

2.3.2 Plethysmography

The constant-volume (or constant-pressure) whole body plethysmograph, also known as the body box, is an air-tight, rigid-walled chamber used in measuring lung volumes. During measurement, the subject performs a combination of inspirations and expirations while seated within the chamber which is similar in size (about 700–1000 L) and shape to a telephone booth, and a number of important lung volumes and capacities are measured including TLC, functional residual capacity (FRC), residual volume (RV) as well as one non-volume measure known as airway resistance (R_{aw}) [127, 128].

The application of body plethysmography in modern clinical practice began more than 60 years ago following many technical contributions from Dubois and others [129]. Body plethysmography reflects many structural, physiological and functional aspects of the lung and the test is now frequently performed along with spirometry to detect the presence of obstructive or restrictive respiratory diseases [45]. For example, an abnormally high specific airway resistance (sR_{aw}) from plethysmography can help confirm the clinical diagnosis of airway obstruction indicated from an abnormally low FEV_1. Body box tests are also performed prior to surgery or to monitor the lung function of individuals at risks of developing lung ailments, as well as to assess disease progression or the effectiveness of therapy. The test is also highly valuable in the differential diagnosis of chronic dyspnea when used along with spirometry and chest X-ray.

Table 2.5 provides a brief description of a variety of plethysmography-derived indices. Some of these measures can also be derived using the Helium dilution technique as well as the Nitrogen washout technique, but these methods will not be reviewed here because they fall outside the scope of this thesis. The interested reader is encouraged to consult the following sources for comprehensive reviews of these techniques [127, 130-132].

Boyle's law is the basic principle applied in the measurement of lung volumes during plethysmography. Boyle's law states that, under constant temperature, when a constant mass of gas is compressed or decompressed, its volume (V) decreases or increases and its pressure (P) changes such that the product of volume and pressure at any given moment is constant [133, 134]. However, when it is not isothermal, the appropriate mathematical expression is: $P \cdot V^{\gamma}$ = constant, where γ varies between 1.4 for adiabatic gas compression and 1.0 for isothermal gas compression [135].

The body plethysmograph measures respiratory flow using either a pneumotachograph, anemometer, or ultrasound transducer. The body box also has two pressure transducers; one transducer measures the pressure within the box relative to atmospheric pressure while the other is placed close to the mouth and is used in measuring alveolar pressure when there is no flow and the subject is asked to pant against a closed shutter. During this maneuver, a shutter mechanism allows the respiratory technologist administering the test to transiently occlude air from flowing through the mouthpiece into the patient's lung while sensors record the pressure and volume changes within the body box and the lungs.

For example, during the measurement of FRC, Boyle's law is simultaneously applied to two separate but related situations: i.e, the gas in the body box, and the gas in the lungs. As shown in Table 2.6, the initial pressure and volume within the body box is indicated as P_1 and V_1, respectively, while the initial pressure and volume in the lungs is denoted by P_3 and FRC, respectively. During the panting maneuver, the test subject attempts to inspire against a closed breathing circuit. The subject cannot get much gas to flow through the mouthpiece into his/her lungs since the breathing circuit has been transiently occluded. However, the panting maneuver rarefies the air column in the lungs, and this results in a small increase in lung volume (i.e FRC + the change in volume, ΔV), and a proportional decrease in the volume of the body box (i.e $V_1 - \Delta V$). The accompanying changes in pressure within the body box (P_2) and the lungs (P_4) are also recorded. Once ΔV is calculated from the body box equation shown in Table 2.6, the FRC can then be estimated, since it is the only unknown term [136].

Table 2.5. Typical lung volume measurements obtained from plethysmography

Name	Abbreviation	Description
Total lung capacity	TLC	This is the volume of air the lungs can hold at maximal inspiration. It is estimated from: TLC = FRC + IC or less preferably from TLC = RV + inspiratory vital capacity (IVC).
Functional residual capacity	FRC	This is the volume of air left in the lungs at the end of a normal tidal exhalation. It can be estimated from: FRC = ERV + RV
Vital Capacity	VC	This is the total volume of air at the end of a full inspiration measured from a complete expiration.
Residual volume	RV	This is the volume of air left in the lungs at the end of a maximal exhalation. It can only be measured indirectly as follows: RV = FRC – ERV or RV = TLC – VC.
Specific Airway resistance	sR_{aw}	sRaw sensitive to airway caliber and is increased in the presence of airway obstruction. sRaw is computed by normalizing airway resistance by the FRC.
Airway resistance	R_{aw}	Derived resistance of the airways to airflow, i.e ratio of alveolar pressure minus mouth pressure to flow rate
Tidal Volume	V_T	This is the volume of air from the beginning of inspiration to the end of expiration during a normal respiratory breath cycle.
Inspiratory capacity	IC	Inspiratory capacity is the maximum volume of air that can be inspired from FRC.
Expiratory reserve volume	ERV	This is the largest volume of gas that can be expired starting from FRC.
Inspiratory reserve volume	IRV	This is the largest volume of gas that can be inspired from resting end-inspiration.

Note: The information presented in this table was sourced from [136, 137]

As with spirometry, results from plethysmography are typically presented as percentiles of predicted values estimated from reference equations that take into account the subject's age, height, gender, and ethnicity [137, 138]. The FRC is the volume of air left in the lungs at the end of a normal tidal exhalation and it is physiologically determined by a counteracting balance of inward retractile forces of the lung and the outward recoil forces of the chest wall. FRC is dramatically reduced as a result of restrictive respiratory disorders such as idiopathic pulmonary fibrosis. FRC is also reduced in obesity [139-144], due to obesity-induced reductions in the compliance of the chest wall and total respiratory system [145, 146].

The residual volume (RV) is another important lung volume index that can be estimated from whole body plethysmography. This parameter reflects the volume of air left in the lungs at the end of a full exhalation but it is usually well preserved in obesity [117, 147-149]. A reduced RV is sometimes the only physiological abnormality in patients with chest wall problems (skeletal deformity, fibrothorax, scoliosis) or diseases of the lung parenchyma such as sarcoidosis [150]. However, due to the nature of the complex interaction between the lungs and chest wall [151], it is important that such diagnosis also take into account results from other lung volume indices such as the total lung capacity (TLC), and the expiratory reserve volume (ERV) (Table 2.3) [45].

Table 2.6. Plethysmographic estimation of FRC using Boyle's law

	Initial Condition		**Final Condition**
Body box:	P_1V_1	=	$P_2(V_1 - \Delta V)$
Lung:	P_3FRC	=	$P_4(FRC + \Delta V)$

Note: P_1, initial pressure within body box; V_1, initial volume of body box; P_2, new pressure within the body box due to inspiration against closed breathing circuit; ΔV, change in volume caused by the attempted inspiration against a closed breathing circuit;

P_3, initial pressure within the lung; FRC, functional residual capacity; P4, new pressure within the lung caused by the attempted inspiration against a closed breathing circuit.

TLC is absolutely essential in confirming the diagnosis of restrictive disorders [127]. The threshold for confirming restrictive disorders is defined as TLC below the 5th percentile of normal values [45]. However, only 50% of patients who demonstrate a restrictive pattern on spirometry (i.e reduced FVC and normal or elevated FEV_1/FVC ratio) also demonstrate low TLC on plethysmography. The remaining 50% of patients are classified as presenting a "mixed ventilatory" or "non-specific" pattern since they exhibit both obstructive and restrictive ventilatory patterns on spirometry and plethysmography [81, 137]. Interestingly, patients presenting with such non-specific pattern suffer from a wide variety of conditions, in particular obesity [81] but the clinical course of these conditions are generally benign [152]. Marked reductions in ERV are also observed in obesity [139-144]. The changes in lung mechanics and function induced by obesity are discussed in greater detail in Section 2.4.

In conclusion, the determination of lung volumes using whole-body plethysmography is a highly technical procedure that is actually very easy for subjects to perform. This unique technique provides reliable information on the structural, physiological and functional aspects of the lungs in health and disease and measurements can be obtained in a relatively short amount of time. However, the main weaknesses of this technique include its bulky size, its cost and the strong technical requirements of the method. Active subject cooperation is also required for reliable results to be achieved.

2.3.3 Oscillometry

History, development, and principles of measurement

The forced oscillation technique (FOT) was introduced in the late 1950s as a tool for assessment of respiratory mechanics [153]. Dubois and his colleagues developed the technique to apply flow oscillations of varying frequencies at the airway opening during

voluntary apnea [153] but it was not widely accepted at the time because it required specialized equipment, was too difficult to perform, and it was also time consuming. However, the advent of microprocessor techniques in the 1970s that allow for complex analysis of signals by the Fourier transform have made it possible to further develop the FOT as a tool for routine assessment of pulmonary function [154, 155].

The principle behind the technique is that forced oscillatory pressure waves of about 1–2 cm H_2O are generated by an excitation source (typically a loudspeaker), and superimposed on a subject's spontaneous breathing at predetermined frequencies, unlike in the past when Dubois collected measurements during voluntary apnea [153]. The mechanical properties of the respiratory system are then estimated from the impedance of the respiratory system (Zrs) to the resulting flow oscillations [156]. Zrs is the spectral ratio of the fast Fourier transform (FFT) of the pressure and flow measured at the subject's airway opening (Equation 2.1).

$$Zrs(f) = \frac{P(f)}{\dot{V}(f)} \tag{2.1}$$

Where f is the oscillatory frequency. The details of the actual mathematical implementation can include repeated calculation of Zrs (Equation 2.1) from multiple, often overlapping windows or periods extracted from continuous recordings of $P(f)$ and $\dot{V}(f)$. This computation can also include filtering and other signal analysis methods to improve quality of the computed Zrs.

In the mathematical sense, and by convention in the frequency domain, Zrs is represented as a complex frequency dependent quantity and is made up of a real and an imaginary part (Equation 2.2). The real part depicts the in-phase component of the pressure and flow, which describes the resistance of the respiratory system (Rrs), while the imaginary part describes the 90º out-of-phase component of the pressure and flow, which describes the reactance of the respiratory system (Xrs). Rrs is largely governed by airway caliber and lung tissue properties while Xrs is largely governed by both the balance between the elasticity of the lung tissues and the inertia of the oscillating air, depending on the

oscillating frequency. Although with airway heterogeneity, the elastic element of the respiratory system can affect Rrs and the resistive dissipative element of the respiratory system can also affect Xrs [157].

$$Zrs(\omega) = Rrs(\omega) + jXrs(\omega) \tag{2.2}$$

where, ω is the angular frequency and is mathematically expressed as: $\omega = 2\pi f$.

The respiratory system is sometimes described using a mathematical model. A simple model that is often developed to describe pulmonary mechanics is known as the single compartment model. It consists of a single resistance (R) representing airflow resistance (i.e Rrs = R), and Xrs modeled as an inertance (I) and elastance (E) as shown below in Equation 2.3.

$$Xrs = \omega I - \frac{E}{\omega} \tag{2.3}$$

If R is to be estimated from $Rrs(\omega)$, then often the average Rrs is employed and if I and E are to be estimated from Xrs, then a least squares fit is often employed. A reciprocal relationship exists between elastance and forced oscillation respiratory system compliance (Crs); therefore, Crs can be computed from Equation 2.3 as follows:

$$Crs = \frac{-1}{\omega(Xrs - \omega I)} \tag{2.4}$$

At very low frequencies, inertance is negligible thus Equation 2.4 can be rewritten as follows (Equation 2.5).

$$Crs \approx \frac{-1}{\omega Xrs} \tag{2.5}$$

Rrs is the most commonly measured FOT index and patients with airway obstruction often exhibit a negative frequency-dependent Rrs [158-160] while healthy subjects are characterized by a lower and largely frequency-independent Rrs [161], within the normal FOT range from 5 to 40 Hz. Xrs has received less attention but it is thought to be sensitive and specific to changes in peripheral lung mechanics [162, 163]. This index has

been used as a surrogate measure for lung tissue stiffness, and to estimate the amount of recruited airspaces in the lung, since stiffness increases when there are fewer alveolar airspaces available for the forced oscillations from the oscillometry device [162, 163]. Xrs can be used to differentiate patients with stiffer lungs from healthy subjects through a characteristic rightward shift in the Xrs versus frequency curve resulting in a significantly higher resonant frequency which normally occurs between 8-10 Hz in healthy adults [154, 158, 164-166]. The resonant frequency is the frequency where the inertive and elastic components of Xrs are equal in magnitude (Equation 2.3). Xrs is dominated by elastic components of Xrs at low oscillation frequencies but inertive forces dominate at high oscillation frequencies and the resonant frequency is the oscillation frequency where that transition from elastic to inertive dominance occurs. Since the elastic and inertive forces are equal in magnitude at the resonant frequency, the elastance and inertance terms both cancel out (Equation 2.4), and Xrs becomes zero, implying that Zrs can be modelled by a pure resistance at the resonance frequency (Equation 2.2).

Clinical Application of Oscillometry

As described above, oscillometry measures how much pressure the respiratory system must generate in order to move air into and out of the lung. Thus, obstructed patients such as individuals with asthma, present with increased Rrs and decreased Xrs at low oscillation frequencies [167], and as a consequence, the pressure the respiratory system must generate in order to move the same quantity of air to and from the lungs is also increased. Oscillometry provides a direct assessment of Rrs and Xrs during quiet tidal breathing.

Furthermore, the oscillometric evaluation of Xrs and the frequency dependence of Rrs are known to be sensitive to small airway dysfunction [158, 168]. This means that oscillometry is thought to be useful for early detection of changes in the small peripheral airways which is where many respiratory diseases such as asthma and COPD, originate [169]. It is also thought that transpulmonary resistance can be estimated from measurements of inspiratory reactance [170] and this measure has been shown to discriminate patients with asthma from those with COPD [171]. This is potentially important especially given the overlap between both disorders [172].

Moreover, the assessment of Rrs at multiple oscillation frequencies can reveal ventilation heterogeneity within the lung, indicated by an inverse dependence of Rrs on frequency and this is thought to be a marker of heterogeneous disease [173]. Oscillometry is capable of identifying subjects with asthma or COPD even when they present with normal FEV_1 and mid-expiratory flows [174, 175]. In fact, in a case-controlled study of symptomatic and asymptomatic subjects exposed to dust and fumes from the 2011 World Trade Center disaster, oscillometric Rrs and frequency dependence of resistance detected small airway dysfunction in symptomatic subjects while spirometry remained within the normal range [176]. Also, assessment of Rrs and Xrs over a range of frequencies enables application of the data to models of respiratory mechanics, providing greater insight than can be obtained from spirometry [177, 178].

The oscillometry technique has been used in a large number of studies and in various clinical settings to monitor the progression of respiratory disease and the efficacy of treatment. Recent evidence from a randomized crossover study showed that improvements in small airway function measured with oscillometry correlated with improvements in asthma symptoms, airway inflammation and AHR [179]. As described above in Section 2.3.1, an important limitation of spirometry which oscillometry overcomes is the fact that spirometry is highly effort dependent and requires active subject cooperation for reliable assessment of pulmonary function. This implies that oscillometry can be used to assess lung mechanics in subjects who are unable to perform spirometry and this cohort includes elderly and geriatric subjects [180, 181], children below the age of 6 in whom asthma is first diagnosed [182], and sick and paralyzed subjects [183] as well as subjects with obesity.

Oscillometry has also been shown to be suitable for assessing the severity of airway obstruction in asthma [167]. It has been successfully used in studies involving infants [184, 185], in anaesthetized subjects [186], and in patients on mechanical ventilation [186, 187]. Studies investigating the response of asthmatic children to bronchial provocation challenges [188] as well as studies investigating the effects of bronchodilator (BD) on the respiratory system [189-192] have all relied on the oscillometry technique.

In almost all the studies, reproducible and repeatable estimates of Rrs and Xrs have been reported.

Reference values for oscillometry outcomes as a function of sex, age, height and ethnicity are now available for both children and adults [161, 182, 190, 191, 193, 194]. Moreover, in 2003, the ATS and ERS taskforce on FOT published guidelines and recommendations on the standardization and use of the FOT in clinical practice [177] indicating that the technique is indeed gaining wider acceptance in the respiratory community.

Limitations of Oscillometry

There are many challenges that have hindered the clinical adoption of this novel assessment tool. Unlike spirometry used with plethysmography, oscillometry cannot discriminate between obstructive and restrictive lung diseases [177]. Also, despite the fact that oscillometry directly measures the mechanical factors affecting breathing, the assessment of respiratory system impedance with oscillometry is a fairly technical concept. Conversely, while spirometry is simple in its technology, it does require substantial training in its interpretation to relate to pathophysiology, but this training is currently well in place for clinical practitioners. Currently, clinicians are unfamiliar with the terminology for measurement of respiratory mechanics. For example, respiratory reactance is usually negative at low frequencies and so clinicians find it unappealing and difficult to make sense of, despite a more negative reactance being easily relatable to a stiffer lung [162, 167]. Furthermore, respiratory impedance data is usually presented as an average value computed over several respiratory cycles. However, it is now established that in subjects with airflow obstruction such as COPD, expiratory Rrs is greater than inspiratory Rrs and expiratory Xrs is significantly more negative than inspiratory Xrs due to the presence of expiratory flow limitation [195].

The clinical adoption of oscillometry is also hindered by what is thought to be its low repeatability and reproducibility compared to spirometry, and the fact that noise from labored breathing can affect the results, implying it can have a low signal-to-noise ratio.

Until very recently, another significant drawback of oscillometry had been the lack of universal age- and height-related predicted values for comparison of results. Unlike

spirometry, the range of normal predicted values appeared to have some specificity to the device from which the measurements were obtained, although it is not clear whether this was also due to the differences in subjects measured. Recently, data from Oostveen *et al* have attempted to address this issue by measuring lung mechanics in a large population using multiple oscillometry devices [196]. Data were also collected from different centers and thus from different populations. The data obtained were used in developing age- and height-related predicted equations for oscillometry which can be applied across different oscillometry systems; however, since individual devices demonstrate differences in frequency dependence, it is not yet known whether these differences will impact the predicted values [197]. These differences are likely due to either inherent differences within the electronics of each device, or differences in the perturbation waveforms of the devices. One device uses impulses while the other oscillometry devices use combinations of sinusoidally varying oscillations.

Lung mechanics are typically obtained in less than three minutes with oscillometry from three repeated measures and the average of the three repeated measures is reported as the outcome variable. Throughout the maneuver, the subject breathes ambient air through a resistance mesh or high impedance bias tube. This feature, together with the widely used speaker-based forced-excitation generator, is responsible for the bulky size and the expensive cost of most conventional forced oscillatory devices. Commercially available FOT devices such as the MasterScreen-IOS device (E. Jaeger, Wuerzburg, Germany), and the Quark PFT from Cosmed (Chicago, IL, USA) can cost as much as $18,900 CAD and $21,995 CAD, respectively.

Despite these limitations, oscillometry is a convenient technique for noninvasive assessment of respiratory mechanics. Unlike spirometry, it does not require subject cooperation and useful measurements can be acquired within a very short duration. Since oscillometry provides a direct assessment of pulmonary mechanics and it is sensitive to respiratory diseases, this tool has a significant role to play in the monitoring of pulmonary function as well as in the diagnosis and management of asthma and other respiratory conditions such as COPD where small airway dysfunction may be involved, as the small airways is considered a silent zone to spirometry.

2.4 Lung Mechanics and Function in Obesity

Obesity is a medical condition characterized by an excessive accumulation of fat in the body. The Body Mass Index (BMI) is the standard index for classification of obesity [198]. It is calculated as the ratio of weight in kilograms to the square of height in meters and is expressed in units of kg/m^2. In adults, overweight is defined by a BMI greater than 25 kg/m^2 and obese by a BMI greater than 30 kg/m^2, but in children, the classification system is based on age- and gender-matched normative data of BMI [199, 200]. As described in Section 2.3.1, children with BMI above the 85^{th} percentile of their age- and gender-matched counterparts are considered overweight while those above the 95^{th} percentile are considered obese [103].

The BMI is a very simple and convenient method of classifying the severity of obesity. Its main limitation is that it is highly nonspecific since it does not distinguish between fat mass and lean (muscle) mass. More importantly, it does not account for the pattern of regional fat distribution in the body. There are two typical regional fat distribution patterns, namely central obesity and peripheral obesity. Central (abdominal or android) obesity is characterized by increased deposition of fat in the thorax, abdomen and visceral organs (apple-like body shape) while peripheral (gynoid) obesity is characterized by deposition of fat in the hips, thighs, limbs or in subcutaneous tissue (pear like body shape). This distinction is important because compared to gynoid obesity, android obesity is likely to have a more direct effect on pulmonary mechanics but the inflammatory state is likely to be similar in both types of obesity.

Obesity is a common comorbidity in asthma; it is associated with an increased risk for developing asthma. According to the National Health and Nutrition Examination Survey, approximately 32% of asthmatic adults are obese in the United States [38] whereas, as described in Section 2.2.1, the overall prevalence of asthma in the US is 7.7 % of the adult population [37]. In 2014, The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) demonstrated that 58% of patients with severe uncontrolled asthma were obese [201]. Other studies have also shown that

severely obese subjects have worse asthma outcomes and require higher doses of controller medication [4, 55, 202]. Interestingly, surgical and nutritional weight loss interventions effectively improve clinical asthma outcomes and overall respiratory health of obese patients [11, 203-205]. However, as will be described in the following sections, there are still many critical gaps in our current understanding of the relationship between asthma (including asthma-like symptoms such as dyspnea and wheeze) and obesity and the mechanism underpinning this relationship is yet to be clearly elucidated.

2.4.1 Asthma and the respiratory symptoms of obesity

Over the last two decades, a large body of epidemiological evidence suggesting a relationship between obesity and asthma has emerged. A meta-analysis of several prospective studies involving over 300,000 adults revealed a dose-response gradient between these two conditions whereby asthma risk increases with increasing BMI [206]. In that study, the odds ratio of incident asthma in the overweight group was 1.5 (95% CI; 1.2-1.6) compared to normal weight subjects, whereas the odds ratio for the obese group was 1.9 (95% CI; 1.4-2.6). The odds ratio of 1.9 measured in the meta-analysis of the obese group implies that the presence of obesity increases the odds of developing asthma by a factor of 1.9.

The relationship between asthma and obesity has also been established in studies of weight gain or loss. Castro-Rodriguez *et al* showed that girls who became overweight or obese between the ages of 6 and 13 were 7 times more likely to demonstrate new asthma symptoms than girls who did not become overweight or obese during the same time interval [102]. These new asthma symptoms included wheezing, bronchial reversibility, peak expired flow variability and airway hyperresponsiveness. They also showed that 54.2% of girls who became overweight or obese were atopic as indicated by a positive skin-prick test at Year 6. Interestingly, only 35.9% of girls who did not become overweight or obese during the same time interval were atopic at Year 6. However, in a large cross section of obese children, Tantisira *et al* found that increased BMI does not significantly contribute to other measures of asthma severity such as school absence and

emergency room visits [104]. The reasons for the differences are unclear. However, in adults, Hakala *et al* demonstrated that weight loss was associated with a reduction in airways obstruction (reduced airway resistance) and peak flow variability, and an increase in FRC, mid-expiratory flows and ERV [207], clearly linking lung function indices typically associated with asthma to obesity.

The conflicting results described above highlights the complexities in characterizing the obesity-asthma association. Factors such as heterogeneous study design, diagnostic bias and the effects of gender, race and age group are among some of the issues that could possibly account for these conflicting results and differences in interpretation. Despite these differences, there is a general agreement that weight gain and obesity significantly impacts respiratory health of millions around the world regardless of whether or not they have asthma [22, 23, 47, 208].

Two important biological mechanisms could potentially lead to asthma or asthma-like symptoms in obesity. These mechanisms are: (i) chronic systemic inflammation due to excess adiposity, and (ii) obesity-induced mechanical compression of the respiratory system. In other words, the asthma or asthma-like respiratory symptoms of obesity may be due to the mechanical effects of weight-induced loading, narrowing the airways and resulting in symptoms, or the symptoms could be due to systemic asthma or asthma-like effects that arise from a cascade of inflammatory mediators and hormones linked to adipose tissue secretions in the obese. These mechanisms are explored in detail in Sections 2.4.2 and 2.4.3 of this thesis.

2.4.2 Pathogenesis of asthma in obesity: role of inflammation

This section examines the role of obesity-induced inflammation in the pathogenesis of asthma or asthma-like respiratory symptoms. In section 2.4.2.1, evidence of the contribution of adipose tissue inflammation to the pathogenesis of obese asthma will be reviewed followed by a review of the contribution of systemic inflammation to the pathogenesis of obese asthma in section 2.4.2.2, and lastly, in section 2.4.2.3, a similar review will be conducted for the airways.

2.4.2.1 Evidence of elevated adipose tissue inflammation

Adipose tissue was once considered a storage depot for excess lipids but today is considered to be an important endocrine organ that secretes adipocyte-derived factors known as adipokines in addition to various signaling molecules and inflammatory mediators for regulation of numerous physiologic and immune functions. Adipokines are energy-regulating proteins synthesized and secreted by the adipose tissue. They include leptin, adiponectin, resistin, visfatin, and adipsin. Of these adipokines, leptin is the most studied and best characterized as will be described in later sections of this thesis.

Obesity significantly affects the types of immune and inflammatory cells that infiltrate the adipose tissue. On one hand, expression of adiponectin–the most abundant anti-inflammatory adipokine in the adipose tissue is markedly reduced in obese patients with asthma, while on the other hand, expression of leptin–a pro-inflammatory adipokine, is increased, compared to obese patients without asthma [1]. In clinical studies, visceral fat leptin expression was strongly correlated to AHR [1], a distinguishing feature of asthma. Leptin is an anorexigenic hormone; it induces satiety, increases metabolism and also plays an important role in the regulation of immune function. The fact that leptin level is upregulated in the adipose tissue while adiponectin is downregulated suggests that the adipose tissue of obese patients is an important source of chronic inflammation.

In addition to the changes in adipose-derived adipokines, the number of adipose tissue macrophages is significantly increased in the adipose tissue of obese humans and obese mice. Weisberg *et al* isolated subcutaneous adipose tissue of obese individuals and determined that adipose tissue macrophages accounted for up to 50% of the cells in the tissue when stained for the macrophage antigen–CD68 [209]. Sideleva *et al* also reported increased infiltration of subcutaneous adipose tissue and visceral adipose tissue by macrophages in obese individuals with asthma [1]. One might ask why the number of adipose tissue macrophages is amplified in obesity. While the exact reason is not completely understood, it is thought that enlargement of adipocytes in obesity affects the ability of the capillaries to supply blood to these cells with the result being hypoxia,

apoptosis and buildup of necrotic tissues which the macrophages are recruited to phagocytose [210].

In addition to macrophages that are recruited to the site of necrotic adipocytes, there are also large numbers of CD8+ and CD4+ T lymphocytes in the adipose tissue of obese humans [211] and obese mice [212, 213]. In obesity, the CD4+ T lymphocytes tend to differentiate into T-helper 1 (T_H1) cell lineage rather than T-helper 2 (T_H2) cell lineage since expression of T_H2 cells is known to decline with increasing severity of obesity [213]. Consistent with this notion, eosinophils that normally play a role in the differentiation of CD4+ T lymphocytes towards a T_H2 phenotype in lean individuals [214], are reduced in mice with diet-induced obesity [215]. Thus, it has been suggested that neutrophilic inflammation may be the predominant phenotypic characteristic of asthma in obesity whereas asthma is usually characterized by an eosinophilic inflammatory phenotype in lean individuals [216]. Unlike eosinophilic inflammation, the pathways involved in the recruitment and activation of neutrophils in asthma and many other aspects of neutrophilic inflammation have yet to be fully established [217].

Interestingly, increased adipose tissue mass is also synergistically associated with increased mast cell propagation. Mast cells are key mediators of allergic reaction and recent evidence from animal models of mast cell activation and dysfunction have demonstrated that the adipose tissue is an important source of mast cell progenitor cells [218]. Indeed, compared to lean individuals, the burden of mast cells is reportedly increased in obese humans and mice [219] suggesting that obesity-induced mast cell proliferation may be an important driver of asthma in these subjects.

In summary, although the development of inflammation in the adipose tissue of obese individuals is likely multifactorial and involves complicated interactions between various signaling proteins (leptin, adiponectin), inflammatory mediators and immune cells (macrophages, T lymphocytes, and mast cells), there is evidence to suggests that the adipose tissue is the site and source of chronic inflammation that could likely lead to the development of respiratory symptoms of asthma in obese individuals.

2.4.2.2 Evidence of elevated systemic inflammation

Pro-inflammatory adipokines such as leptin secreted by the adipose tissue (described above in section 2.4.2.1) continuously leak into the systemic circulation and this leads to an increased concentration of leptin in the serum [220]. Leptin is not only produced by adipose tissue; bronchial epithelial cells, type II alveolar cells and macrophages in peripheral lung tissue also produce this protein [221], and this further amplifies the circulating levels of this adipokine. As described in the preceding section, leptin is an anorexigenic hormone and this means that its presence induces satiety and increases metabolism. However, the increased levels of leptin measured in the serum of obese humans [220] and obese mice [222] suggests that leptin resistance occurs in obesity in a similar manner to insulin resistance in patients with type II diabetes [223].

Given its pro-inflammatory nature, the increased levels of leptin in the serum of obese patients invariably lead to recruitment of pro-inflammatory cytokines, chemokines and acute phase proteins in obesity. Specific inflammatory moieties that have been shown to increase in obesity include: tumor necrosis factor alpha (TNF-α) [224, 225], interleukin (IL-) 8 and IL-6 [226], high-sensitivity C-reactive protein (hs-CRP) and monocyte chemoattractant protein-1 (MCP-1) [227]. In addition, the level of circulating leukocytes in the blood of obese subjects is increased compared to lean individuals [228].

Leptin receptors have been observed on the surface of hematopoietic stem cells [229] and binding of these receptors to leptin stimulate monocytes and macrophages to produce cytokines through CD4+ T-lymphocytes [230]. T_H1 response is enhanced by leptin whereas T_H2 response is suppressed by leptin [213]. Given that allergic inflammation such as atopic asthma is usually mediated by T_H2 cells, the involvement of leptin in asthma is not through typical pathways of allergic inflammation, rather non-atopic mechanisms appear to be involved. This notion is supported by the recent identification of two predominant phenotypes of asthma in obese subjects [56, 57]. These two phenotypes are: (i) atopic, early onset, T_H2-high asthma characterized by high serum IgE and the presence of eosinophilia and (ii) non-atopic, late onset, T_H2-low asthma characterized by low serum IgE, presence of neutrophilic inflammation and the absence

of eosinophilia [231, 232]. In other words, asthma most likely predates the development of obesity in atopic subjects with T_H2-high asthma while obesity may play a causative role for asthma in non-atopic subjects with T_H2-low asthma.

The role of leptin in the respiratory system has been extensively studied in animal models of obesity, and to a lesser extent in clinical studies. The *ob/ob* mouse is a commonly used animal model of systemic inflammation in obesity because these mice lack functional leptin due to mutation of the leptin gene; therefore, they are extremely obese and demonstrate hyperphagia and low metabolism. Compared to wild type, *ob/ob* mice have significantly higher pulmonary resistance at baseline and elevated responsiveness to intravenous methacholine and ozone–both of which are prominent features of asthma (Figure 2.3) [233]. Similar results were observed in genetically diabetic (*db/db*) mice, the carboxypeptidase E-deficient (Cpe^{fat}) mice [234, 235] and mice with diet induced obesity (Figure 2.3), suggesting that the increased responsiveness in obese mice has more to do with increased adiposity than modality of obesity (*db/db* mice lack the receptor for leptin due to a genetic mutation and Cpe^{fat} mice lack the enzyme that regulates satiety neuropeptides). These results suggest that agents other than leptin may contribute to the development of airway hyperresponsiveness (AHR) and increased pulmonary resistance since similar results were obtained in the different strains of obese mice regardless of the levels of leptin in the circulatory system.

Evidence from clinical human studies on the role of leptin in obese asthma has been less insightful. There is some evidence that leptin is associated with asthma in obesity. In 2006, Sood *et al* showed that high BMI and high serum leptin concentration are both strongly associated with asthma in adults [236], Sood and coworkers also found that adjusting for leptin did not affect the association between BMI and asthma suggesting that the relationship between obesity and asthma is not mediated by the leptin pathway alone. There is similar evidence from children. Overweight children with asthma are reported to have twice the serum concentration of leptin compared to overweight nonasthmatics children [237]. However, Dixon and coworkers found no significant difference in the serum concentrations of leptin in obese asthmatics and obese nonasthmatics adults [1], in contrast with the above findings [236, 237]. While there is

general consensus that serum leptin levels are strongly correlated with excess adiposity [220], but the result from Dixon *et al* raises some doubt if this influences asthma [1].

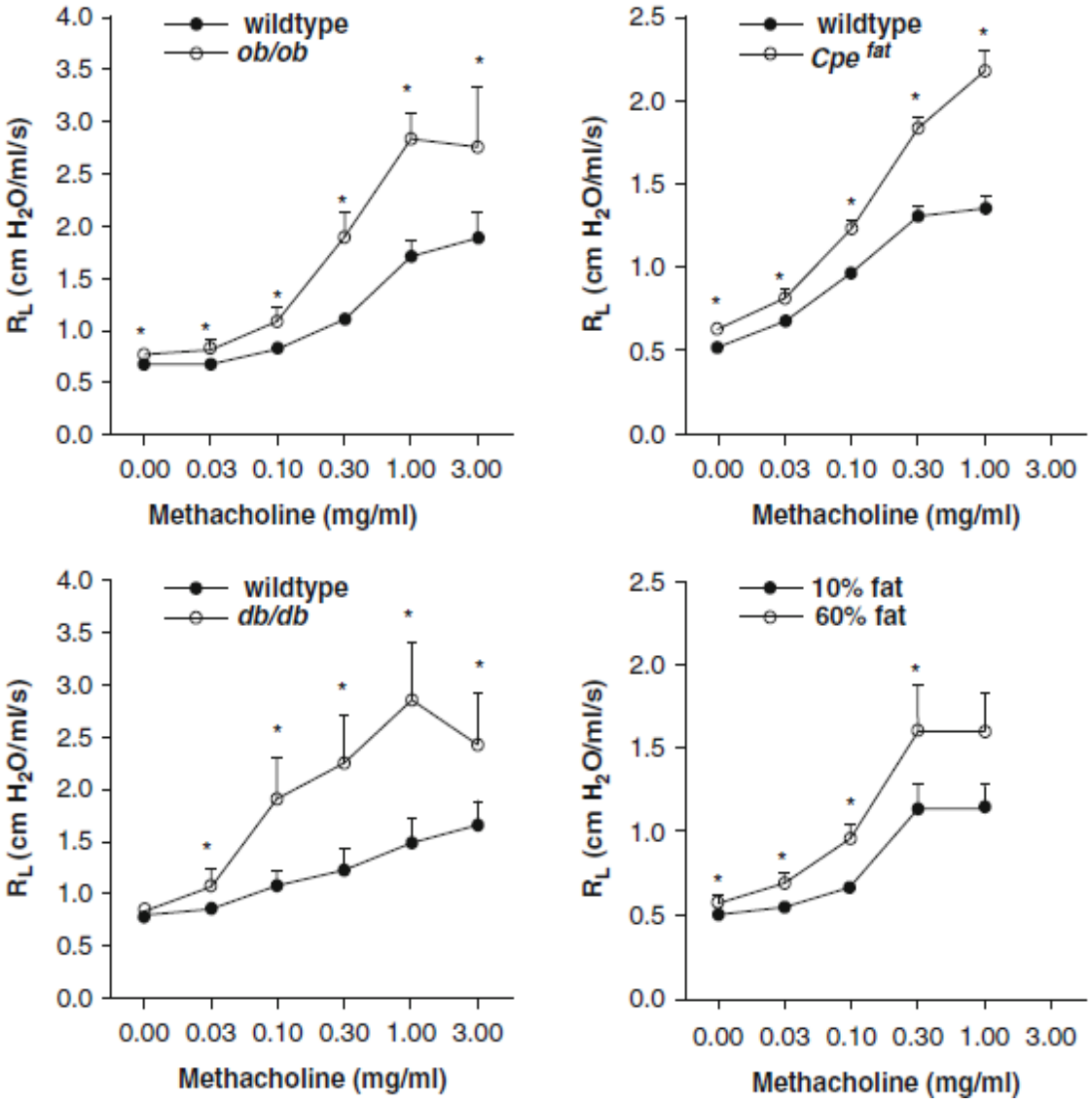

Figure 2.3**.** Methacholine-induced changes in pulmonary resistance in four types of obese mice compared to lean controls.
Notice that *ob/ob* and *cpe* ^{fat} mice have significantly higher pulmonary resistance compared to wildtype at baseline. Data expressed as mean ±SE. * $p < 0.05$. Compiled from previous studies conducted at Shore's lab [233, 234, 238] and published in a review article by Shore [51].

In summary, the role of increased systemic inflammation on the development of asthma in obesity was examined in this section. Although there is good evidence that leptin is significantly increased in obesity, current evidence also appears to suggest that the association between obesity and asthma is not mediated by the leptin pathway alone. Rather, it appears that leptin is involved in the asthma of obesity through non-atopic mechanisms since T_H2 response is suppressed by leptin and typical pathways of allergic inflammation is usually mediated by T_H2 cells.

2.4.2.3 Evidence of elevated airway inflammation

As described earlier, obesity is commonly associated with two phenotypes of asthma [56, 57]. One phenotype is described as a non-atopic (non-allergic), late onset, T_H2-low asthma with low serum IgE and absence of eosinophilia while the second phenotype is described as an atopic (allergic), early onset, T_H2-high asthma that is marked by high serum IgE and presence of eosinophilia [231]. In most obese patients, sputum eosinophils are decreased with increasing BMI, consistent with the reduction in exhaled nitric oxide associated with reduced airway eosinophilia and reduced underlying airway inflammation [232]. Interestingly, sputum neutrophils were shown to increase with increasing BMI in a study of obese women with asthma [231]. The drop in sputum eosinophils and the rise in sputum neutrophils with increasing BMI suggests that neutrophilic inflammation, rather than eosinophilic inflammation may play a more dominant role in the pathogenesis of asthma in the non-atopic, late onset, T_H2-low obese group.

The effect of obesity on airway inflammation has also been assessed from bronchoalveolar lavage (BAL) fluid of obese humans and mice. Elevated levels of pro-inflammatory mediators such as IL-6, MCP-1 and neutrophils were observed in the BAL fluid of Cpe^{fat} mice compared to lean controls following ozone exposure but not air exposure [235]. In fact, exogenous administration of the pro-inflammatory adipokine–leptin to lean control mice prior to ozone exposure reportedly augments ozone-induced increases in IL-6 in BAL fluid [233].

However, in human subjects, there is no concrete evidence of elevated airway inflammation in BAL fluid of obese patients. Similar cell counts of macrophages, neutrophils, lymphocytes and eosinophils were found in BAL fluid of obese asthmatics and nonasthmatics [56]. Furthermore, alveolar macrophages in obese asthmatics and nonasthmatics have been shown to produce similar amounts and types of cytokines [1]. Although increasing severity of obesity is associated with increased concentration of leptin in BAL fluid, no association was found between obesity and markers of airway inflammation such as exhaled nitric oxide [239]. As shown in the previous sections, inflammatory cells are present in higher quantities in the adipose tissue and serum of obese individuals but it is possible that these cells do not migrate into the airways, hence the lack of such evidence.

Taken together, there is no concrete evidence to support the notion that asthma in obesity is an inflammatory disease of the airways, besides the evidence of increased neutrophilic inflammation in women. However, there is some evidence suggesting that the disease is mediated by adipokines and other inflammatory mediators released from the adipose tissue which lead to a state of low grade chronic systemic inflammation. These adipose-derived adipokines appear to have indirect effects on airway hyperreactivity and lung function, despite the lack of evidence of airway inflammation. Thus, while not characteristic of the inflammation in asthma, this indirect route to airway reactivity may contribute ultimately to asthma or asthma-like symptoms although the mechanisms are unclear. However, as described in the next section (Section 2.4.3), the mechanical effects of obesity may also play an important role in this process.

2.4.3 Mechanical compression of the respiratory system in obesity

Obesity alters the mechanical properties of the lungs and chest wall mainly through the accumulation of fat in the mediastinum, and in the abdominal and thoracic cavities [240]. Increased fat deposition in obesity limits the downward excursion of the diaphragm and lowers the operating volume of the lungs. Consequently, pleural pressure is increased [241] and the functional residual capacity (FRC), which is physiologically determined by

a counteracting balance of inward retractile forces of the lung and the outward recoil forces of the chest wall, is reduced [242, 243]. In fact, FRC is reduced by 10%, 22% and 33% in overweight, mildly obese and severely obese subjects without asthma, respectively [243].

The pressure-volume curves of the chest wall (C_{CW}), lung (C_L) and entire respiratory system (C_{RS}) in lean and obese subjects is shown in Figure 2.4 (Dixon *et al*. [244]). As demonstrated here, obesity is thought to have profound effects on the compliance of the lungs [245-247], chest wall and entire respiratory system [145, 247]. For example, compared to C_L of a normal weight subject, the obese C_L shows an inflection implying that increased pressure must be generated to open airways that closed when lung volume fell below FRC. Also, the obese C_{CW} is shifted to the right towards higher transpulmonary pressures, even though the overall shape of the curve is preserved. Together, the reduced compliance of the lung and chest wall result in a reduced respiratory system compliance where FRC is significantly reduced.

The reduction in respiratory system compliance (increased stiffness) alters the breathing pattern. Obese individuals therefore breathe in a rapid shallow pattern with reduced tidal volumes [148] perhaps minimizing the peak pressures required during breathing. Furthermore, obese subjects breathe at very low FRC (Figure 2.4) and their expiratory reserve volume (ERV) is drastically diminished. Therefore, their tidal flow-volume loop approaches the region of maximal flow and this increases the risk of expiratory flow limitation especially in the supine position [13].

The mechanical effects of obesity are associated with respiratory signs and symptoms of asthma such as dyspnea [23], wheeze [22], increased respiratory system resistance [117], airway narrowing and closure [248, 249], ventilation heterogeneity [250], and airway hyperresponsiveness [47]. However, an important question that must be answered is: How do the mechanical effects of obesity bring about these asthma-like symptoms? The following sections attempt to answer this important question by examining the mechanical consequences of obesity on some distinguishing characteristics of asthma such as AHR, airway narrowing and closure.

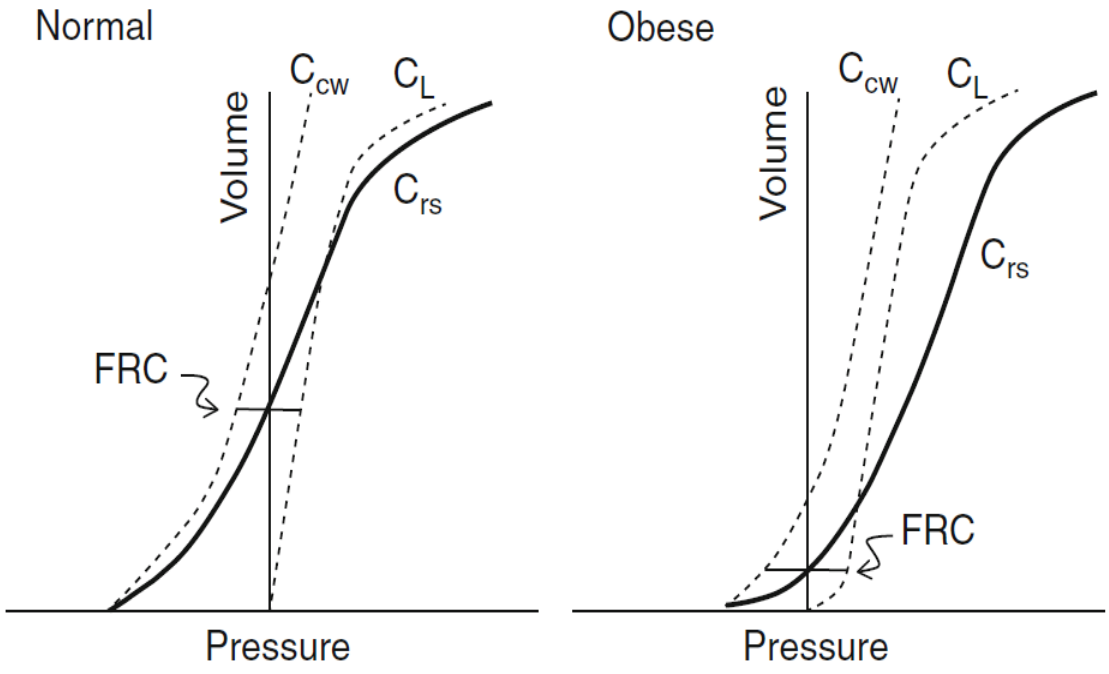

Figure 2.4. Campbell diagram for the chest wall (C_{CW}), lung (C_L) and respiratory system (C_{RS}) in normal weight and obese subjects.
The mechanical effects of obesity results in a reduction in the functional residual capacity (FRC) and higher transpulmonary pressures in the obese individual [244].

Effect of Obesity on Airway Hyperresponsiveness

Many large studies report positive associations between BMI and AHR, but this is not a uniform finding because smaller studies do not show this association [51]. The only prospective longitudinal cohort study that investigated the relationship between obesity and AHR in more than 7,000 adults found that the risk for AHR increases as BMI increases [47]. They also found that weight gain was a risk factor for developing AHR. These findings were established after controlling for the effects of age, smoking, serum IgE and baseline FEV_1.

Similar results were obtained in three other large cross-sectional epidemiologic studies in China (7,109 participants [48]), Europe (11,277 participants [49]) and the United States

(1,725 participants [50]) where AHR or symptomatic AHR was more prevalent in obese adults compared to lean subjects. In contrast, several smaller studies failed to find a consistent relationship between AHR and obesity [22, 251]. This inconsistency may be explained by several factors such as the small sample sizes, or possibly differences between the outcome indicator used (Rrs, Xrs, FEV_1, and maximum expiratory flow at 60% of TLC), atopic status, severity of obesity in the participants and other study inclusion and exclusion criteria such as enrolling only nonasthmatic obese subjects. It would be expected that these findings would be more consistent if the various studies used the same outcome measure, and since the association is more consistent with larger studies, this tends to favor the presence of a positive association between AHR and obesity.

Effect of Weight Loss on Airway Hyperresponsiveness

If obesity-induced alterations in respiratory mechanics represent an important factor in the development of AHR, then it is reasonable to assume that weight loss could reverse these changes. This issue has been addressed in three important studies by Aaron *et al* [252], Dixon *et al* [56], and Chapman *et al* [248]. The data from Aaron *et al* indicates a trend towards reduced AHR with weight loss following an intense diet-induced weight reduction program; however, this effect was not statistically significant ($p = 0.23$) [252]. In contrast to these findings, Dixon *et al* reported that a 27% weight loss in asthmatic women who underwent bariatric surgery was associated with a significant reduction in AHR ($p = 0.03$) [56]. Interestingly, they also found that the effect of weight loss on AHR could be predicted from the atopic status of their subjects. In other words, subjects who were classified as atopic based on their serum IgE concentration did not demonstrate any changes in their AHR with weight loss, whereas weight loss resulted in significant reductions in AHR in nonatopic subjects. Subjects in the atopic group reported that they had asthma since childhood and their serum IgE concentrations were unchanged following weight loss suggesting that they had the allergic form of the disease while subjects in the nonatopic group had asthma with an onset that was later in life (nonallergic asthma phenotype). The nonatopic group had a diagnosis of asthma, but without allergy suggesting that another mechanism is responsible for their asthma

symptoms, and the leading mechanism at play here is thought to be the direct effect of mechanical compression of the lung due to obesity [11].

As described earlier, these results gave rise to the notion that there are two distinct clinical phenotypes of asthma in obesity – those with early onset asthma and high serum IgE (T_H2-high) and those with late onset asthma and low serum IgE (T_H2-low). Chapman *et al* further investigated these findings by assessing the effect of weight loss on sensitivity to small airway closure during methacholine challenge in the two obese asthmatic groups (allergic and nonallergic) compared to an obese control arm [248]. They found significantly higher airway responsiveness to methacholine in the two obese asthmatic groups compared to the control group. Furthermore, sensitivity to airway closure and AHR was not significantly improved following weight loss in T_H2-high asthmatics whereas weight loss was associated with a reduction in small airway responsiveness in T_H2-low asthmatics.

Taken together, the results of these weight loss studies on AHR suggest that obesity exerts a strong mechanical effect on the respiratory system and depending on the asthma phenotype (atopic versus nonatopic), obesity could potentially increase the risk of AHR. The mechanical effects of obesity on the respiratory system together with the inflammatory effects of excess adiposity could indirectly contribute to AHR likely through mechanisms such as airway narrowing and airway remodeling that is commonly associated with asthma. The next section will examine these mechanisms.

Effect of Obesity on Airway Narrowing and Airway Closure

Compared to healthy weight controls, airway narrowing in obesity has been shown to correlate with airway closure and AHR [249]. Airway narrowing and closure leads to gas trapping and ventilation inhomogeneity [250]. While gas trapping and thus the effect of airway closure can be assessed from elevated RV/TLC ratio by body plethysmography [253], a recently developed method to assess airway closure is from the oscillometric evaluation of Xrs. For example, in a comparative study of lung mechanics in obese versus nonobese nonasthmatics, Salome *et al* used the oscillometry technique to demonstrate that obesity does not alter airway responsiveness to methacholine; rather, it significantly

reduces Xrs and increases Rrs [254]. They reported that methacholine-induced reduction in Xrs was greater among obese nonasthmatics compared to their nonobese counterparts suggesting that excess adiposity could promote peripheral airway closure. Similar findings have been observed in other studies [11, 248].

The mechanisms behind the increase in Rrs and reduction in Xrs observed in obesity [117, 254] are not completely understood but it is thought that the reduction in FRC attenuates the forces of interdependence between the airway and parenchyma and this reduces airway caliber [245]. Indeed, airway caliber is sensitive to airway-parenchymal tethering forces, and breathing at a lowered lung volume due to reduced FRC plays a dominant role in increasing Rrs in obese subjects [117, 119]. In addition, breathing at low lung volumes also increases AHR [255]. Van Noord *et al*, Torchio *et al*, and Navajas *et al* conducted studies involving rib cage strapping [256, 257] and supine lying [258] to reduce lung volume in a similar manner to obesity-induced mechanical compression of the respiratory system. These experiments resulted in increased Rrs and in one of the studies [257], AHR was also increased. These results show that the reduction in the operating volume of the lung in obesity, measured as a reduction in FRC, could potentially amplify the manifestation of symptoms related to airway narrowing or airway closure with notable examples being the increased risk for AHR [47], dyspnea [23], and wheeze [22].

Another hypothesis that has been postulated to explain the increased AHR in obesity is that breathing at low FRC, with a rapid breathing pattern and a reduced tidal volume could affect the contractility of airway smooth muscle (ASM). Smooth muscle contractility is usually modulated by stretching during regular tidal breathing and deep inspirations [259]. The outcome of breathing at reduced tidal volume in obesity is potentially two-fold. The first outcome is that the load applied on the ASM is reduced and this leads both to increased shortening velocity of the ASM, and the ASM consequently operates at a shorter length possibly due to ASM length adaptation (mechanical plasticity) i.e, the ASM adapts to a shorter length at reduced lung volume and rearranges its contractile apparatus in the best possible way to maintain its force generating capacity [257, 260-266]. Thus, it can now generate more force at a shorter

length than it would have without the length adaptation. A second mechanism potentially leading to increased force and AHR with obesity is that the reduced tidal volume breathing does not disrupt actin-myosin cross-bridges, thus the ASM becomes stiffer than it would have been at larger amplitude stretches. This was demonstrated in tissue strip experiments that showed that with large tidal oscillations and large stretches, ASM force reduces, and ASM dramatically lengthened and the muscle became softened (fluidized) [259, 267-269]. This mechanism could explain the maintenance of airway dilation in healthy subjects despite inhaled agonists, and how smaller tidal stretching in obesity might impair this effect. Currently, there is some debate arising from data using intact airways which appear to indicate that this effect can only be produced by very large stretches, and the effect is less substantial than that recorded in the tissue strip experiments [270]. Nevertheless, it is possible that reduced length with reduced tidal stretching in obesity could lead to reduced disruption of actin-myosin cross-bridges thus leading to AHR.

2.4.4 Summary

Obesity is associated with asthma and asthma-like respiratory symptoms such as wheeze and dyspnea. The diagnosis and management of asthma in obese individuals is complicated as a result of excess adiposity, and bronchodilators are reported to exhibit limited effectiveness [4-8]. However, symptoms often improve with weight loss but traditional methods of testing lung function such as spirometry and plethysmography often show little or no changes with weight loss [9-11]. Lung function is typically assessed in the upright position but the respiratory symptoms of obesity worsens when subjects lie down [12, 13] and this affects their sleep quality [271]. Unfortunately, there is a paucity of data on the respiratory health consequences of weight loss, particularly in the supine position. There is also no data on the effect of weight loss on responsiveness to short-acting β_2–adrenergic bronchial agonists, despite reports of limited effectiveness of long-acting bronchodilators in this patient group. This information gap has hampered our ability to unravel the mechanisms that link obesity and weight loss to changes in respiratory health.

It seems likely that there is an additive or potentially synergistic relationship between obesity-induced mechanical compression of the respiratory system and obesity-related hormonal, metabolic and inflammatory effects. Evidence from several studies reviewed here appear to suggest that these two factors could synergistically act to worsen lung mechanics and function in obese individuals and also modify their response to asthma controller therapies and bronchodilators. As reviewed in this chapter, there is ample evidence that the pro-inflammatory environment in obesity could contribute to the development of asthma and asthma-like respiratory symptoms in individuals with obesity but evidence of the effect of obesity and weight loss on lung mechanics is rather scant. It is quite possible that the inflammatory environment in obesity and the mechanical effects of excess adiposity could act together to produce an overall effect that is greater than the sum of the individual effects of these two factors.

2.5 Research Hypothesis and Aims

The general hypotheses for this thesis is that improvements in lung function after weight loss can be reliably measured with oscillometry and would be related to improvements in sleep quality and these changes in lung mechanics would reflect lung-volume-induced changes in airway-parenchymal tethering affecting airway diameters.

2.5.1 Early changes in lung mechanics following weight loss surgery

Specific Hypothesis 1: Oscillometry can be used effectively to show early changes in pulmonary mechanics with weight loss in obese subjects, and is more sensitive than spirometry and plethysmography.

Specific Hypothesis 2: Changes in lung mechanics measured in the supine position are associated with improvements in sleep quality reported by the patients.

Specific Hypothesis 3: Obesity and weight loss alters bronchodilator responsiveness due to changes in lung volumes.

Brief approach: I evaluated lung mechanics and function in 19 severely obese female subjects using spirometry, oscillometry, whole-body plethysmography and the Pittsburgh Sleep Quality Index (Appendix F) before and 5 weeks after bariatric surgery. Spirometry and oscillometry were conducted in both the upright and the supine position, and pre- and post-bronchodilation with 200 μg of the short-acting β_2–adrenergic agonists, salbutamol.

The aims of this part of my thesis were to a) assess pulmonary mechanics in both the upright and supine position in obese individuals, and assess the early changes in upright and supine pulmonary mechanics that occur within the first 5 weeks of weight loss surgery by measuring Rrs and Xrs with oscillometry and comparing these assessments with results from spirometry and plethysmography, b) correlate the early changes in lung mechanics induced by weight loss with changes in sleep quality assessed during the 5-week study period, and c) study the effect of obesity and weight loss on bronchodilator responsiveness.

2.5.2 Improvement in upright and supine lung mechanics with bariatric surgery

Specific Hypothesis 1: Compared to the modest weight loss recorded at 5 weeks after bariatric surgery, further weight loss would induce greater improvements in upright pulmonary mechanics, and changes in supine pulmonary mechanics will be greater, and this would in turn lead to greater improvements in sleep quality.

Specific Hypothesis 2: Weight loss will lead to decreased airway heterogeneity via recruitment of small airways due to improved tethering between the airway and parenchyma, and this would be detectable by improvements in bronchodilator responsiveness, and reduced frequency dependence of Rrs.

Brief approach: I extended the follow-up period of our weight-loss study (section 2.5.1) to 6 months and re-evaluated our study participants using oscillometry, spirometry, plethysmography, and Pittsburgh Sleep Quality Index (PSQI). Results obtained were compared to those recorded earlier at baseline and 5 weeks after surgery.

The aims of the second part of this thesis were to a) evaluate the changes in lung mechanics measured in the upright and supine positions at 6 months after weight loss surgery, b) assess the changes in sleep quality with further weight loss achieved at 6 months after bariatric surgery, and c) investigate the changes in frequency dependence of Rrs at 6 months and assess the changes in lung mechanics induced by the short-acting β_2–adrenergic agonists following weight loss.

2.5.3 Repeatability, variability and reliability of respiratory impedance

The third and final objective of this thesis was to a) assess the effect of incorrect positioning of the head and neck during oscillometry, as well as the effect of cheek support for comparison, b) assess within-day repeatability and variability on the respiratory impedance values, and c) assess day-to-day repeatability and variability of oscillometric indices.

Brief approach: I evaluated 6 healthy adults and 6 COPD patients with oscillometry on two separate study visits to obtain Rrs and Xrs. At baseline, subjects were instructed to follow recommended guidelines for use of oscillometry in clinical practice. Oscillometry was then repeated without cheek-support, and artefacts were introduced through flexion and extension of the head and neck. Three consecutive sets of measurements were obtained under each condition, and standard deviation and percent coefficient of variation were computed as indices of variability and repeatability. Also, Pearson's product moment correlation coefficient [Pearson's (r)] was computed to determine the test-retest reliability of oscillometry from day to day.

Chapter 3: Early Detection of Changes in Lung Mechanics with Oscillometry Following Bariatric Surgery in Severe Obesity

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3.1 Abstract

Obesity is associated with respiratory symptoms that are reported to improve with weight loss; but this is poorly reflected in spirometry and few studies have measured respiratory mechanics with oscillometry. We investigated whether early changes in lung mechanics following weight loss is detectable with oscillometry. Furthermore, we investigated whether the changes in lung mechanics measured in the supine position following weight loss is associated with changes in sleep quality. Nineteen severely obese female subjects (mean body mass index: 47.2±6.6kg/m^2) were evaluated using spirometry, oscillometry, plethysmography and the Pittsburgh Sleep Quality Index before and 5 weeks after bariatric surgery. These tests were conducted in both upright and supine positions, and pre- and post-bronchodilation with 200mcg of salbutamol. Five weeks after surgery, mean weight loss of 11.5±2.5kg was not associated with changes in spirometry and plethysmography, except for functional residual capacity. There was also no change in upright respiratory system resistance (Rrs) or reactance following weight loss. Importantly, however, in the supine position, weight loss substantially reduced Rrs. In addition, sleep quality significantly improved and was highly correlated with the reduction in supine Rrs. Prior to weight loss, subjects did not respond to bronchodilator when assessed in the upright position with either spirometry or oscillometry, but with modest weight loss, bronchodilator response was regained to the normal range. Improvements in lung mechanics occur very early following weight loss but mostly in the supine position, resulting in improved sleep quality. These improvements are detectable with oscillometry but not with spirometry.

Keywords: Oscillometry (Forced oscillation technique); Obesity; Body mass index; Lung function; Lung mechanics; Bronchial reversibility.

3.2 Background

The worldwide prevalence of obesity has nearly doubled over the past three decades, now exceeding 10 % of the adult population [272]. Obesity is associated with impaired lung function and respiratory conditions including asthma [21] and obstructive sleep apnea [2], and increased risk of respiratory symptoms such as wheeze [22], dyspnea [23], and orthopnea [13].

Increasingly, bariatric surgery is used successfully to achieve and maintain weight loss, and this is accompanied by significant improvements in morbidity [273, 274], sleep quality [271], and lung function [144, 275]. While several studies have examined the effects of surgically induced weight loss on lung function in obese subjects with asthma, there is a paucity of data on the effects of bariatric surgery on obese individuals without asthma or other concomitant lung disease. The most significant lung function changes with weight loss in individuals without asthma are improvements in expiratory reserve volume (ERV) and functional residual capacity (FRC), with modest changes in total lung capacity (TLC) and residual volume (RV), accompanied by minor improvements in forced expiratory volume in one second (FEV_1) [276]. Expiratory reserve volume and FRC were shown to increase by as much as 54 % and 37 % respectively, following a 34.2 kg weight loss [144].

Dyspnea and wheeze in obesity are thought to arise largely from compression of the lungs by visceral fat around the mediastinum and in the abdominal and thoracic cavities which leads to decreased lung volumes [146, 240, 277]. While the reduced lung volumes alter airway-parenchymal interdependence [278], there is little apparent effect on airway obstruction, at least as reflected in FEV_1, despite the reductions in airway diameters [117]. This has led to the notion that obesity predominantly affects small airways, and is therefore not likely to be fully reflected in spirometry [276]. However, evaluation of lung mechanics by oscillometry, also known as the forced oscillation technique, in obesity has revealed an increase in respiratory system resistance (Rrs) [117, 254, 279] and a decrease in respiratory system reactance (Xrs), primarily at low frequencies [254, 279]. Rrs is sensitive to central airway caliber, while low frequency Xrs can be used to determine respiratory system elastance (Ers), which is the inverse of respiratory system compliance

[280]. In obesity, Xrs is thought to be significantly decreased due to closure of small airways in the lung periphery [254]. Indeed, low frequency Xrs is sensitive to peripheral small airway closure [162, 281, 282] making it a suitable tool for assessment of small airways [132], which is considered a silent zone to spirometry [283].

There have been very few studies of changes in lung mechanics with weight loss [9-11, 248]. Moreover, there are no studies of how weight loss affects lung mechanics in the supine position, despite reports of increased severity of dyspnea and poor sleep quality in obesity [13, 271]. While the reduced lung volumes in obesity alters airway-parenchymal interdependence by decreasing tethering forces and shifting the pressure-volume relationship of the respiratory system [117, 278], it is not well understood how the reduced tethering forces in obesity might affect the responsiveness to bronchodilator (BD). Indeed, responsiveness to long acting bronchodilators and inhaled corticosteroids is impaired in obese subjects with asthma [5-8], but the effect of obesity and weight loss on responsiveness to short-acting $beta_2$-adrenergic agonists has not been studied.

We hypothesized that oscillometry is more sensitive than spirometry and plethysmography to early changes in lung mechanics measured at 5 weeks following weight loss surgery and this may be associated with improvements in sleep quality reported by the patients. We assessed the mechanics of moving air into and out of the lungs in both upright and supine positions and recorded the changes that occurred with weight loss by measuring Rrs and Xrs with oscillometry and comparing these assessments with results from spirometry and plethysmography. Furthermore, we hypothesized that changes in lung mechanics measured in the supine position following weight loss is associated with the changes in sleep quality. Finally, to probe the role of obesity and weight loss on bronchodilator response, we measured lung mechanics before and after salbutamol inhalation and compared the changes in lung mechanics to changes in spirometry values.

3.3 Methods

3.3.1 Selection of study participants and consent

Nineteen severely obese individuals without a diagnosis of asthma or other lung diseases were recruited from the Bariatric Surgery Clinic at the Queen Elizabeth II Health Sciences Center. This study was approved by the Nova Scotia Health Authority Research Ethics Board (reference number: CDHA-RS/2012-109). All participants provided written informed consent to participate in the study and also consented to have their data published.

3.3.2 Inclusion and exclusion criteria

All participants in the study had a body mass index (BMI) greater than 40 kg/m^2 and were scheduled for bariatric surgery within a few days of the baseline assessments described here. Subjects were excluded from the study if their BMI was less than 40 kg/m^2, or if they presented with a physician-diagnosis of chronic lung disease (including asthma and chronic obstructive pulmonary disease). Other exclusion criteria included: a greater than 10 pack year smoking history, cognitive impairments that prevented accurate completion of study questionnaires or unacceptable performance of pulmonary function tests. Subjects were also excluded if they were unable to lie on their back for up to 10 minutes.

3.3.3 Study design

We assessed lung mechanics pre- and post-bronchodilator (BD) in the upright and supine postures, prior to and 5 weeks after bariatric surgery. We define all measurements before surgery to be baseline. At each assessment, oscillometry, whole body plethysmography and spirometry were performed as described below. The testing sequence is outlined in Figure 3.1.

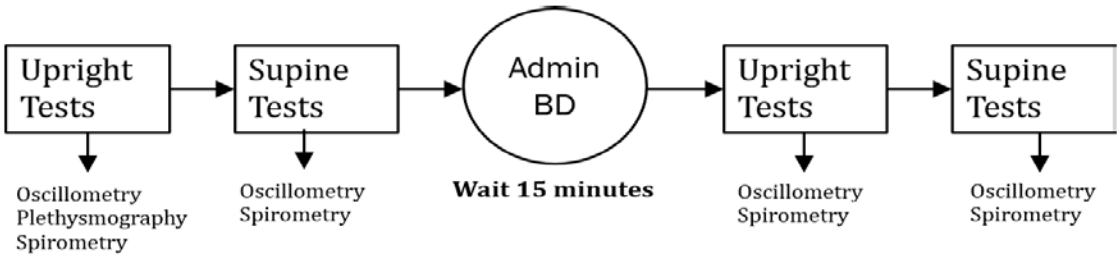

Figure 3.1. Testing sequence for assessment of the effect of weight loss on lung mechanics.

3.3.4 Sleep quality questionnaire

Participants were asked to complete the Pittsburgh Sleep Quality Index (PSQI) before lung function assessments at baseline and 5 weeks after bariatric surgery (Appendix F). The PSQI was analyzed using previously described methodology with permission from Buysse and coworkers [284]. Briefly, the PSQI is a 19-item questionnaire that provides validated measurements of sleep disturbance and usual sleep habits in the previous month. The questions are grouped into seven components that analyze various aspects of sleep quality such as: subjective sleep quality, sleep latency, sleep duration, habitual sleep quality, sleep disturbances, use of sleep medication and daytime dysfunction due to sleepiness. Respondents rated their sleep quality as "very good," "fairly good," "fairly bad" or "very bad" and this represented a score of 0, 1, 2, or 3, respectively. The PSQI also assessed various factors that disrupt sleep including breathing discomfort, waking up to use the bathroom, coughing or snoring loudly, feeling too hot or cold, having bad dreams and experiencing pain. The global sleep score was calculated as the sum of the seven components that make up the PSQI. A score of ≤ 5 is associated with good sleep quality while a score > 5 is associated with poor sleep quality.

The effect of weight loss on dyspnea in obesity was specifically assessed with the PSQI by analyzing the response of participants to perceptions of breathing discomfort. A score of zero was assigned when no breathing discomfort was reported in the past month; a score of 1 was assigned when breathing discomfort occurred less than once a week, corresponding to mild discomfort; a score of 2 was assigned when breathing discomfort occurred once or twice a week, corresponding to moderate discomfort, while a score of 3

was assigned when breathing discomfort occurred three or more times a week, corresponding to severe discomfort.

3.3.5 Weight, height and waist circumference measurements

Participant weight and height were measured without wearing shoes or heavy clothing; these parameters were used to calculate BMI. Waist circumference (WC) was measured as the circumferential distance around the midpoint between the lowest rib and the iliac crest.

3.3.6 Pulmonary function tests

Spirometry and whole body plethysmography were performed according to recommended international guidelines using a spirometer-equipped body box (SensorMedics Corporation, Yorba Linda, CA, USA) [45, 127]. Forced expiratory flows, including forced expiratory volume in one second (FEV_1) and expiratory flow at 50 % (FEF_{50}), 75 % (FEF_{75}), and 25 – 75 % ($FEF_{25–75}$) of forced vital capacity (FVC) were obtained. Lung volumes and capacities, including expiratory reserve volume (ERV), inspiratory capacity (IC), functional residual capacity (FRC) and vital capacity (VC) were also measured. The average ERV, FRC and IC; and the best VC and forced expiratory flows were selected as the final result from three technically acceptable measurements [285]. TLC was calculated as the sum of IC and FRC while RV was calculated as the difference between TLC and VC. Results obtained from these lung function tests were expressed as percentages of predicted values from Crapo *et al* [73].

3.3.7 Oscillometry

The mechanical properties of the respiratory system were measured according to recommended guidelines [286] using the tremoFlo^{TM} Airwave Oscillometry System (Appendix G, Thorasys Thoracic Medical Systems Inc., Montreal, QC, Canada). During each measurement, subjects wore a nose-clip and firmly held their cheeks and mouth

floor so as to minimize the upper airway shunt artefact [286]. The Airwave Oscillometry System delivered a multi-frequency composite oscillatory pressure waveform of about 1-2 cm H_2O superimposed on a subject's spontaneous breathing at 6, 11 and 19 Hz for 16 seconds. Mechanical parameters of the respiratory system were then computed from the impedance of the respiratory system (Zrs) to the resulting flow oscillations. The frequency range allowed us to examine the frequency-dependent mechanics of the respiratory system and how it changed with weight-loss. Respiratory impedance measured at the 6 Hz oscillation frequency probed the respiratory mechanics of the entire respiratory system while the impedance measured at the 19 Hz oscillation frequency probed the respiratory mechanics of the upper and central airways of the lung.

Respiratory system impedance is the spectral ratio of the fast Fourier transform (FFT) of the pressure and flow measured at the subject's mouth, and was computed by averaging 1 second windows of the pressure and flow recordings multiplied by Hamming windows with 50% overlap. Zrs is a complex quantity where the real part depicts the portion of the pressure that is in phase with flow and thus describes Rrs, while the imaginary part depicts the portion of the pressure that is in phase with volume and acceleration of volume and thus describes Xrs. Rrs is largely governed by airway caliber and some lung tissue properties, while Xrs is governed by the elasticity of the chest wall and lung tissues, and the inertia of the oscillating column of gas in the central airways. Furthermore, while the resistance of the two components of the chest wall – the rib cage and diaphragm-abdomen accounts for a small part of the Rrs, the contribution of these two components to the total Rrs may be increased in the supine position particularly in obesity.

Within each 16 second recording, the effects of artefacts were minimized by automatic rejection of any 1 second window containing negative Rrs or Rrs values greater than three standard deviations and using the mean Rrs and Xrs to minimize the effect of any outliers. Additionally, measurements were repeated four times with periodic breaks of about 30 seconds between measurements. The average from these measurements was computed as the final result.

At low frequencies Xrs is negative and is dominated by the elastance of the respiratory system (Ers) while at high frequencies when Xrs is positive, it is dominated by the inertia of the oscillating air column. For all subjects, Xrs at 6 Hz was less than -1 $cmH_2O/(L \cdot s^{-1})$ indicating that Xrs was dominated by elastance and an estimate of Ers was obtained [287] as: $Ers = -\omega Xrs_6$ where, ω is the angular frequency and is mathematically expressed as: $\omega = 2\pi f$.

The coefficient of variation (COV) for Rrs was computed from the standard deviation of the four consecutive Rrs measurements normalized to the mean to determine the repeatability and variability of Rrs. Coherence ≥ 0.90 was used as an acceptance criteria for each 16 second measurement of Zrs at each frequency.

3.3.8 Bronchial reversibility test

Lung mechanics and function was assessed before, and 15 minutes after inhaling 200 µg of the BD – salbutamol, administered with a metered-dose inhaler and valved-holding chamber spacer device (CareStream Medical Ltd., Pickering, ON, Canada). This assessment was performed before and 5 weeks after bariatric surgery.

3.3.9 Bariatric surgery

All subjects underwent a laparoscopic sleeve gastrectomy – a minimally invasive procedure that results in the complete removal of about 85% of the stomach. The surgery was performed under general anesthesia and patients were discharged home 48-72 hours post-surgery. The postoperative diet regimen consisted of 4-6 weeks of a high protein, semi-liquid diet followed by a return to normal diet.

3.3.10 Statistical analysis

Results are expressed as means ± standard deviations (SD). Weight, BMI, waist circumference, PSQI and plethysmography results obtained before and after bariatric

surgery were analyzed using a one-way repeated measures analysis of variance (ANOVA) while the effects of position and bronchodilation on spirometry and oscillometry results obtained before and after bariatric surgery were analyzed using two-way repeated-measures ANOVA with surgery and condition (position plus bronchodilation) as factors. Surgery was divided into two levels (i.e. before and after surgery) while condition was divided into four levels (i.e. upright pre-BD, supine pre-BD, upright post-BD and supine post-BD). Where significance was found, separate post hoc pairwise testing was then performed with Student's t-test to identify differences due to surgery, position, or BD and the Benjamini-Hochberg procedure was applied to control the effect of multiple comparisons [288]. Correlation analyses were performed using linear regression methods in MATLAB R2013b (Natick, MA, USA) while all other analyses were performed in IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY, USA). *P*-values < 0.05 were considered statistically significant.

3.4 Results

3.4.1 Demographics and obesity parameters

Table 3.1 shows the age distribution and anthropometric characteristics of the subjects before and 5 weeks after bariatric surgery. Following bariatric surgery, there was a significant reduction in weight, BMI and WC ($p < 0.001$). Study participants lost about 9.4 ± 2.1 % of their initial weight (Table 3.1). The ANOVA summary table of within-subjects effects for oscillometry is presented in Table 3.2. With the exception of Rrs,19, weight loss surgery did not have a significant effect on any of the oscillometric (Table 3.2) or spirometric measures (not shown). Furthermore, position and bronchodilation significantly changed all oscillometric measures (Table 3.2) while the only spirometric indices that significantly changed were FEV_1, FEV_1/FVC, FEF_{25-75} and FEF_{75} (not shown). Also, there was a significant cross interaction between surgery and conditions (position plus bronchodilation) in Xrs,6 and Ers (Table 3.2).

3.4.2 Changes in lung mechanics with weight loss: posture and bronchodilation

Although weight loss had no effect on upright pre-BD Rrs measured at all the frequencies, weight loss significantly reduced pre-BD Rrs,19 measured in the supine position by 13.1 ± 3.8%, but not Rrs,6 and Rrs,11 (Table 3.2, Figures 3.2 and 3.3A). In addition, moving from an upright to supine position elicited changes in lung mechanics that were further influenced by weight loss and by bronchodilation. For instance, prior to weight loss, moving to the supine position pre-BD resulted in a 23.7 ± 11 % increase in pre-BD Rrs,6, but there was no change post-BD; however, after weight loss, the increase in Rrs,6 was 24.4 ± 8 % post-BD (Figure 3.3A). Ers was consistently sensitive to changes in posture increasing by similar amounts on moving from upright to supine position, both pre- and post-BD, and before and after weight loss (Figure 3.3B).

Table 3.1. Age distribution and anthropometric characteristics of study participants.

	Before Surgery	**After Surgery**	***p*-value**
Number of participants	19	19	
Sex (Females, Males)	19, 0		
Age (years)	48.3 ± 7.6		
Mean weight (kg)	123.4 ± 19.0	111.9 ± 18.1	< 0.001
Mean BMI (kg/m^2)	47.2 ± 6.6	42.8 ± 6.6	< 0.001
Mean Waist Circumference	1.30 ± 0.04 m (134 ± 4 cm)	1.20 ± 0.03 m (121 ± 3 cm)	< 0.001
Average weight loss (kg)	N/A	11.5 ± 2.5	
Percent of initial weight lost (%)	N/A	9.4 ± 2.1	

Note: Weight, BMI and waist circumference significantly reduced after bariatric surgery. Data are expressed here as mean ± standard deviation.

3.4.3 Weight loss and bronchodilator responsiveness

Figure 3.4 shows the effects of BD at each frequency. Prior to weight loss, BD only had a modest effect on Rrs,11 measured in the upright position (Figure 3.4A); however, following weight loss, BD had a substantial effect by decreasing Rrs at all frequencies (Figure 3.4C). In contrast to measurements in the upright position, in the supine position, BD significantly decreased Rrs at all frequencies before weight loss but following weight loss, BD response was only observed in Rrs,6 (Figure 3.3A) and not in Rrs,11 and Rrs,19 (not shown). There was also no BD response measured with Ers before surgery in both the upright and supine positions; however, significant response to BD was measured following weight loss in both the upright and supine positions (Figure 3.3B). Unlike Ers, Xrs measured at 11 and 19 Hz was consistently increased in response to BD in the upright (Figure 3.4B and 3.4D) and supine (not shown) positions both before and after weight loss.

Table 3.2. Analysis of Variance for Oscillometry

	Surgery		Conditions (Position + Bronchodilation)		Interactions (Surgery*Conditions)	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Rrs6	.562	.463	7.873	< .001	2.355	.082
Rrs11	3.251	.088	8.783	.001	2.053	.117
Rrs19	5.627	.029	4.421	.017	2.353	.082
Xrs6	.214	.650	26.415	< .001	1.207	.029
Xrs11	1.331	.264	25.551	< .001	.717	.546
Xrs19	.011	.919	24.687	< .001	.266	.850
Ers	.214	.650	26.415	< .001	1.207	.029

Examining the responses due to BD in Rrs again, and evaluating the percent changes in Rrs to compare to the percent changes in Ers in Figure 3.5, we saw that at baseline, the percent change in upright Rrs at all frequencies in response to BD was negligible;

however after surgery, the response was significant and significantly greater than before surgery at all frequencies, increasing on average by 17.6 ± 4.0 % (Figure 3.5A). By comparison, prior to surgery, BD induced only a modest 7.1 ± 10 % decrease in Ers measured in the upright position, but after surgery a larger reduction in Ers of 33 ± 8 % was seen (Figure 3.5A). Similarly in the supine position, the BD-induced reduction in Ers after weight loss was much larger than before weight loss and larger than the change in Rrs, which was only significant at 11 Hz (Figure 3.5B).

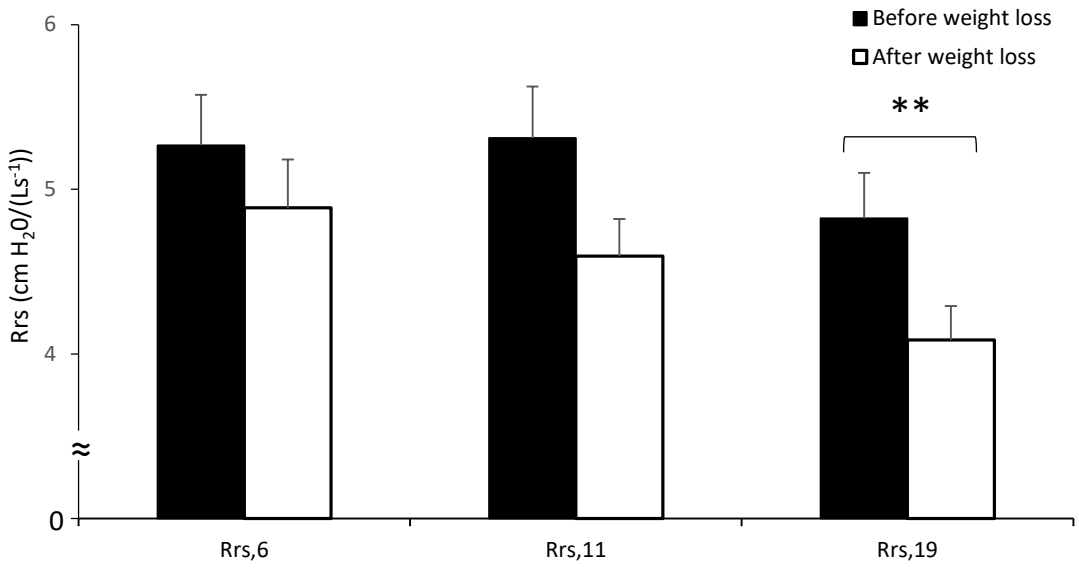

Figure 3.2. Effect of weight loss on pre-BD resistance measured in the supine position. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Error bars indicate standard error of the mean.

Table 3.3 demonstrates results from spirometry measured in the upright position. Prior to bariatric surgery, only FEF_{25-75}, changed significantly in response to BD. After surgery, FEV_1, FEV_1/FVC, FEF_{25-75} and FEF_{75} all increased significantly in response to BD. FEF_{75} and FEF_{25-75} increased by 28.2 ± 7.0 % and 18.8 ± 5.4 % respectively, while FEV_1 increased by only 5 ± 2 % post-BD. In the supine position, BD induced a 4.0 ± 2 % increase in FEV1 before surgery and a 5.1 ± 1.7 % increase after surgery (not shown).

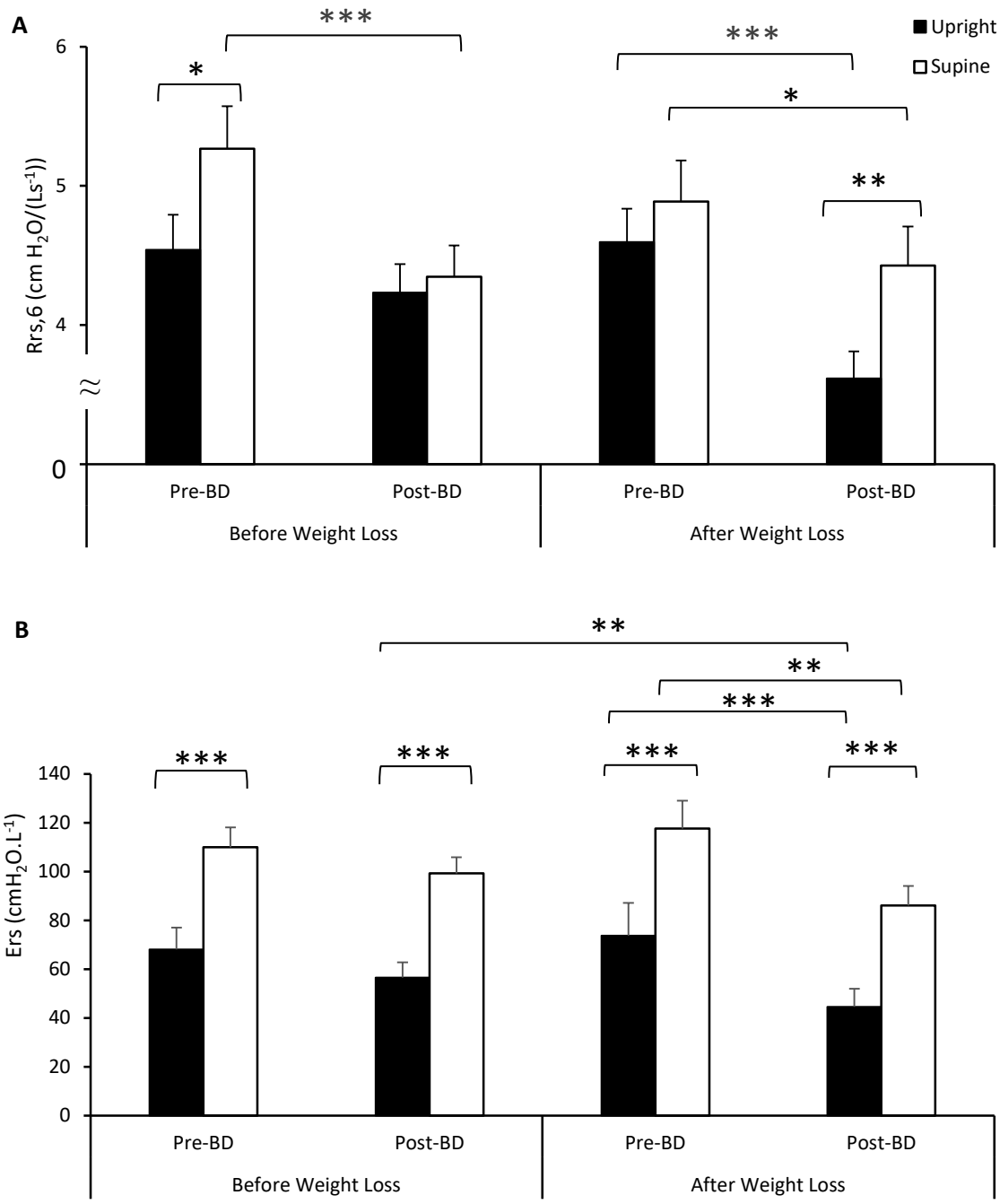

Figure 3.3. Effect of moving from an upright to supine position on Pre- and Post-BD Rrs,6 (**A**) and Ers (**B**) measured before and after bariatric surgery.
In Panel B, moving to the supine position significantly increased Ers for all conditions ($p < 0.001$). (* $p < 0.05$; ** $p < 0.01$; ** $p < 0.001$). Error bars indicate standard error of the mean.

3.4.4 Weight loss and lung volumes

There was a 9.5 ± 2.5 % increase in FRC with weight loss following bariatric surgery but no change in other lung volume measurements (Table 3.4). There was a significant correlation between the change in FRC, expressed as a percent of predicted, and the percent weight loss following surgery ($r = 0.56$, $p = 0.019$).

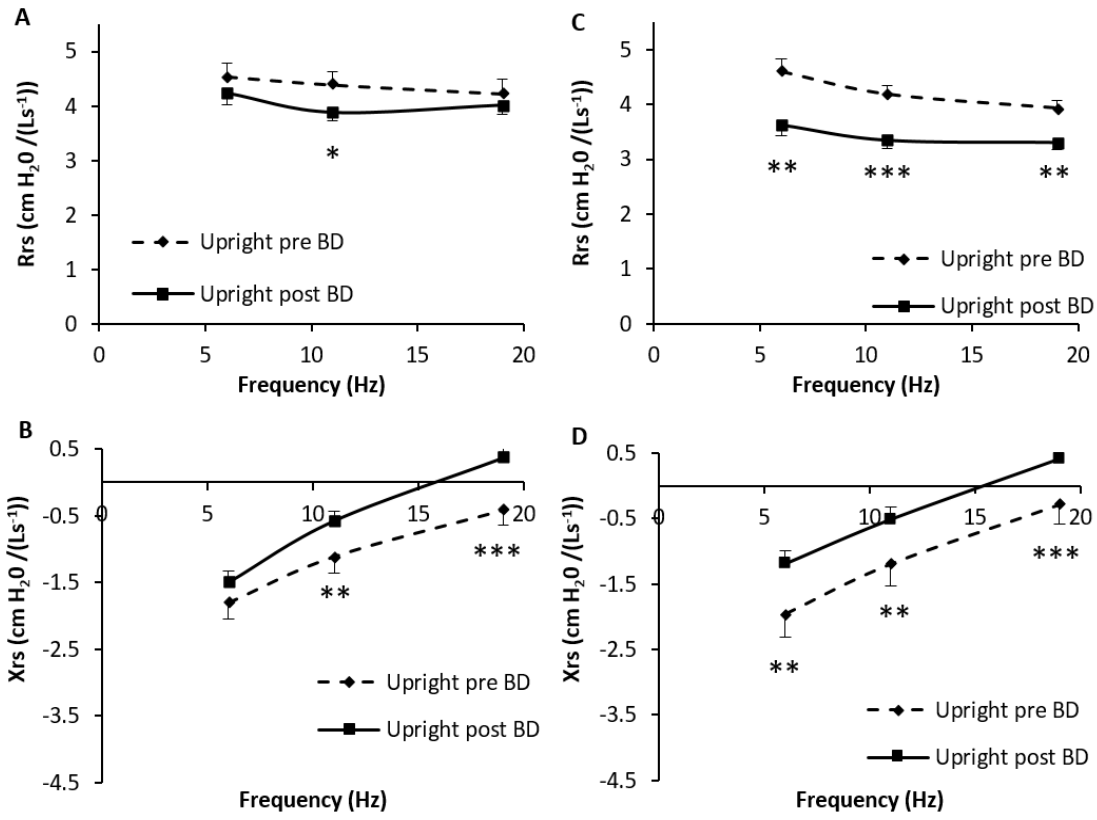

Figure 3.4**.** The effect of BD on Rrs and Xrs measured in the upright posture before (**A** & **B**) and five weeks after bariatric surgery (**C** & **D**).
(* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Error bars indicate standard error of the mean.

3.4.5 Variability and repeatability of respiratory resistance

The variability and repeatability of respiratory system resistance was determined from the percent COV (Table 3.5) obtained from multiple measurements at each time and in each test condition (e.g. before and after bariatric surgery, in the upright and supine position,

and pre- and post-bronchodilation). The variability in Rrs measurements obtained from oscillometry was less than 10 % in all tests indicating that the measurement was highly repeatable. Measures of variation in Xrs were estimated from the standard deviation of Xrs (SDXrs) and was found to be less than 0.5 $cmH_2O/L/s$ in all tests. For example, at baseline, SDXrs was 0.46 $cmH_2O/L/s$; however, 5 weeks after surgery, the variation in Pre-BD Xrs dropped to 0.31 $cmH_2O/L/s$.

Table 3.3. Pre- and Post-BD spirometric data obtained before and 5 weeks after bariatric surgery.

	Before weight loss			After weight loss		
	Pre-BD	Post-BD	*p*-value	Pre-BD	Post-BD	*p*-value
FVC	102 ± 3	101 ± 3	0.397	102 ± 3	102 ± 4	0.926
FEV_1	95 ± 3	96 ± 4	0.490	94 ± 4	98 ± 4	0.031
FEV_1/FVC	94 ± 2	96 ± 3	0.056	93 ± 3	97 ± 3	0.002
PEF	104 ± 6	103 ± 6	0.649	103 ± 6	103 ± 6	0.850
FEF_{25-75}	85 ± 7	92 ± 8	0.005	84 ± 9	97 ± 9	<0.001
FEF_{50}	105 ± 8	112 ± 10	0.068	102 ± 11	110 ± 10	0.124
FEF_{75}	72 ± 11	82 ± 12	0.108	69 ± 9	86 ± 12	<0.001

Note: Values are expressed as % predicted mean ± standard deviation. (n = 17) FVC: forced vital capacity, FEV_1: forced expiratory volume in 1 sec, FEV_1/FVC: ratio of forced expiratory volume in 1 sec to forced vital capacity, PEF: peak expired flow, FEF_{25-75}: forced expiratory flow between 25% and 75% of FVC, FEF_{50}: forced expiratory flow at 50% of FVC, FEF_{75}: forced expiratory flow at 75% of FVC.

3.4.6 Sleep quality

Weight loss was associated with significant improvements in sleep quality as measured by the PSQI. Table 6 shows the component scores from the PSQI collected before and 5 weeks following bariatric surgery. Improvements in subjective sleep quality, sleep disturbances and daytime dysfunction due to sleepiness led to an improvement in the global sleep score. The overall improvement in sleep quality with weight loss was highly correlated with the reduction in Pre-BD Rrs,19 measured in the supine position (r = 0.71,

$p = 0.009$) but not with any reductions in Pre-BD Rrs,6 ($r = 0.53$, $p = 0.075$) or Rrs,11 ($r = 0.43$, $p = 0.163$). We did not find any correlation between the reduction in waist circumference and improvement in sleep quality.

Prior to bariatric surgery, 8 of the 19 participants reported mild to severe breathing discomfort on the PSQI in the month prior to examination. Four respondents reported mild breathing discomfort (score of 1); 1 respondent reported moderate breathing discomfort (score of 2), while 3 respondents reported severe breathing discomfort (score of 3). Significant improvements were noted after bariatric surgery as follows: 16 respondents reported no breathing discomfort at all; 2 respondents reported mild breathing discomfort, while 1 respondent reported moderate breathing discomfort in the month prior to re-examination.

Table 3.4**.** Lung Volumes obtained before and five weeks after bariatric surgery.

LUNG VOLUMES	**PRE-SURGERY**	**POST-SURGERY**	***p*-value**
TLC	90 ± 9	91 ± 10	0.223
FRC	69 ± 8	75 ± 12	0.003
RV	61 ± 17	65 ± 18	0.356
VC	104 ± 12	104 ± 14	0.950
IC	115 ± 17	112 ± 16	0.326
ERV	72 ± 16	83 ± 25	0.143

Note: Values are expressed as % predicted mean ± standard deviation. TLC: total lung capacity, FRC: functional residual capacity, RV: residual volume, VC: vital capacity, IC: inspiratory capacity, ERV: expiratory reserve volume. (n = 17)

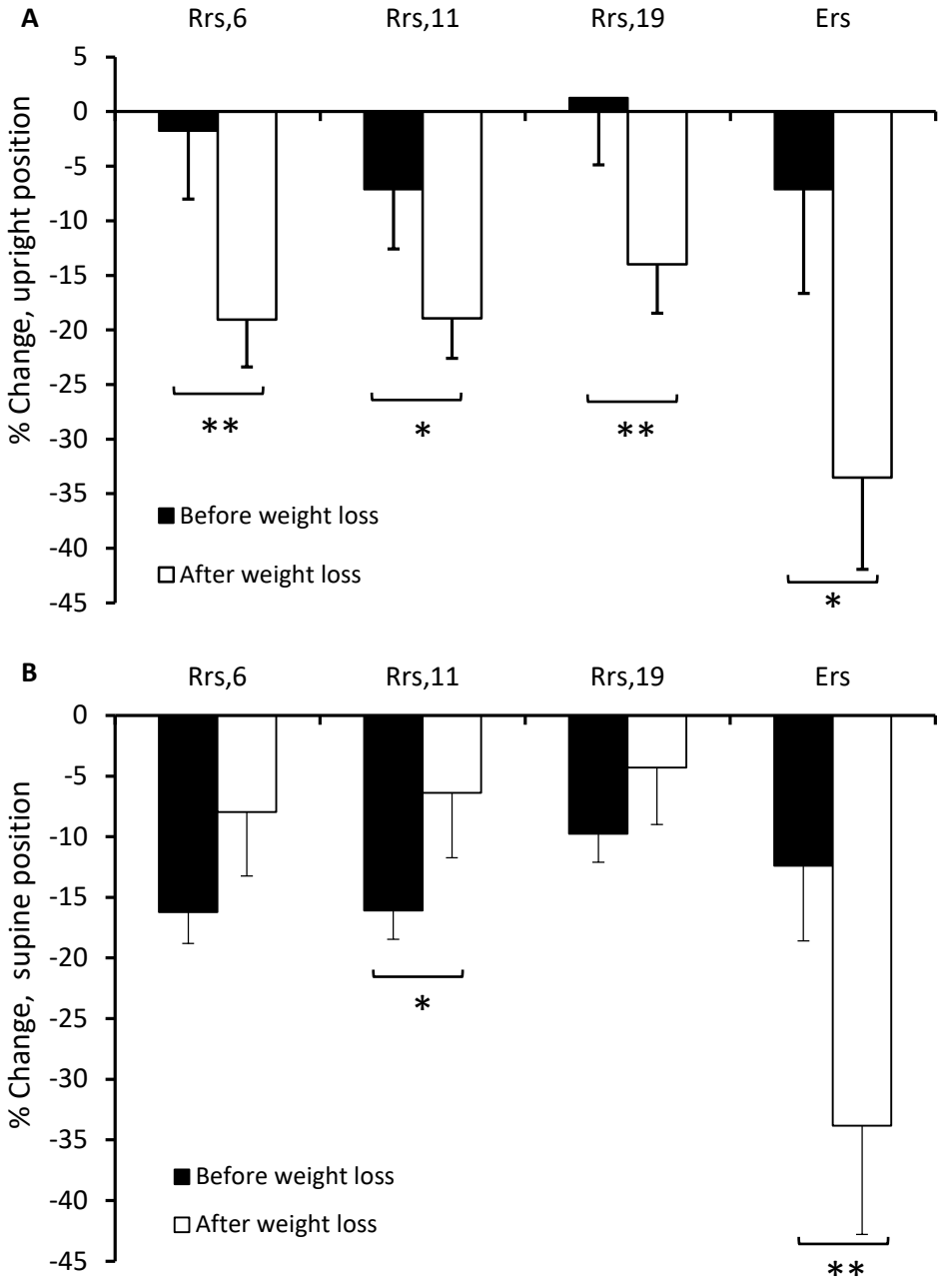

Figure 3.5. Percent drop in Rrs and Ers in the upright (**A**) and supine position (**B**) measured in response to BD before and after surgery.
The effect of BD on upright Rrs measured before and after surgery is shown in Figure 4A and 4C. Prior to surgery, BD significantly lowered supine Rrs at all frequencies, however after surgery, only Rrs,6 reduced following inhalation of BD. Error bars indicate standard error of the mean. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Table 3.5. Percent Coefficient of Variation (COV) for oscillometric Rrs obtained under various test conditions.

	Pre-Surgery	**Post-Surgery**	***p*-value**
Upright Pre-BD	7.3 ± 2.9	5.2 ± 2.2	0.010
Supine Pre-BD	7.0 ± 1.8	6.5 ± 1.7	0.181
Upright Post-BD	5.5 ± 1.7	5.4 ± 2.2	0.362
Supine Post-BD	6.4 ± 2.3	7.0 ± 2.5	0.230

Note: Values are expressed as % mean ± standard deviation.

Table 3.6. Component scores of the PSQI collected before and 5 weeks after bariatric surgery.

Components of the PSQI	**Before surgery**	**After surgery**	***p*-value**
Subjective Sleep Quality	1.2 ± 0.8	0.7 ± 0.8	0.025
Sleep Latency	1.6 ± 1.1	1.4 ± 1.1	0.297
Sleep Duration	1.0 ± 1.2	0.7 ± 1.0	0.297
Habitual Sleep Efficiency	1.2 ± 1.0	1.2 ± 1.2	1.00
Sleep Disturbances	1.8 ± 0.5	1.4 ± 0.5	0.007
Use of Sleeping Medication	0.3 ± 0.8	0.2 ± 0.5	0.506
Daytime Dysfunction	1.2 ± 0.9	0.8 ± 0.6	0.042
Global score	**8.4 ± 3.5**	**6.5 ± 3.7**	**0.049**

Note: Values are expressed as mean ± standard deviation

3.5 Discussion

We investigated the early changes in lung mechanics and lung function associated with weight loss in severely obese subjects at 5 weeks following bariatric surgery. Our principle finding was that despite normal spirometry and no changes in lung mechanics in the upright position, lung mechanics assessed by oscillometry significantly improved in the supine position with weight loss. Secondly, these changes were accompanied by a

significant increase in BD responsiveness in the upright position; thirdly, these changes were accompanied by improvements in sleep quality.

This is the first study to examine changes in lung mechanics induced by weight loss in both the upright and supine positions. Although weight loss had no effect on Rrs measured in the upright position, it significantly reduced Rrs measured supine (Figures 3.2 and 3.3A). Previous studies have consistently reported significant reductions in Rrs measured in the upright position following weight-loss surgery, but those assessments were conducted 12-24 months after surgery [9-11]. Thus, the amount of weight loss achieved by the participants only a few weeks after bariatric surgery in our study was likely insufficient to induce a decrease in Rrs measured in the upright position. However, the modest weight loss was sufficient to reduce the Rrs measured in the supine position likely because moving to the supine position augments lung compression associated with obesity [258, 279] and that could amplify the changes in mechanics with weight loss. Compression reduces lung volume and lung elastic recoil [250], resulting in weaker airway-parenchymal tethering and the collapse of dependent airways [289]. Thus, the modest weight loss present at 5 weeks after surgery, which was accompanied by a decrease in waist circumference (Table 3.1), was sufficient to reduce lung compression in the supine position. Indeed, prior to weight loss, moving from an upright to supine position increased Rrs,6 by 23.7 ± 11 %, while after surgery, the increase in Rrs was not significant (Figure 3.3A).

The reduction in supine Rrs with weight loss measured at 19 Hz (Figure 3.2) may be due to a greater increase in the caliber of the central airways. This is because Rrs at higher oscillometry frequencies (Rrs,19) is usually dominated by the upper and central airways while the effect of small peripheral airways on Rrs is normally undetectable unless there is sufficient heterogeneous peripheral airway obstruction present [173]. It is possible that with further weight loss larger changes in peripheral airway caliber might be detectable, leading to improvements in Rrs at lower frequencies as well. In some severely obese patients improvements in Rrs with weight loss might occur with resolution of upper airway obstruction associated with obstructive sleep apnea [290]. Since the prevalence of obstructive sleep apnea in severely obese patients presenting for weight loss surgery is

about 74 % [291], it is quite likely that some of our study participants may have had undiagnosed sleep apnea that could possibly have improved with weight loss resulting in improved sleep quality (Table 3.6).

The fact that we did not find any changes in spirometry but did with oscillometry may be because oscillometry measures lung mechanics near FRC, and particularly in the supine position, the effects may be greater than might occur during the forced exhalation manoeuvres performed during spirometry. Surprisingly, even at 18-24 months, spirometry appeared to be insensitive to weight-loss-induced improvements in upright lung function as reported previously [9, 10], although Al-alwan *et al* reported slight improvements in FEV_1 and FVC at 12 months [11].

Weight loss affected the response to BD in our study participants. Prior to bariatric surgery, inhalation of salbutamol did not result in significant reduction in Rrs measured in the upright position or in spirometry values. This is consistent with the results of other studies in obese individuals with asthma who demonstrated little or no change in spirometry values following BD [105, 292]. In a study of obese subjects without respiratory disease, less than 15 % of subjects demonstrated a positive BD response and examining mean values, post BD FEV_1% was only 0.25 % higher than pre BD FEV_1% [293]. We are the first group to publish data on the effect of weight reduction on BD responsiveness in obese adults without asthma. Our results demonstrate that weight loss is associated with an increased BD response in both oscillometric and spirometric indices (Figure 3.5A and Table 3.3). Although the BD response in Rrs was increased to 17 %, it was still within the normal range which is reported as an 11 % drop in Rrs [196]. This response was also below traditional thresholds for reporting acute reversibility in both spirometry (<12%) [45] and oscillometry (<33%) [196].

The lack of BD responsiveness before weight loss could be due to decreased airway-parenchymal tethering forces with obesity [278]. Reduced tethering uncouples the airway smooth muscle from the parenchyma and as a consequence, the airways fail to dilate with BD. This same mechanism has been suggested in nocturnal asthma where the airway is uncoupled from the parenchyma during sleep resulting in increased resistance that persisted throughout sleep [294, 295]. Weight loss reduces lung compression and

accounts for the increase in FRC that we measured 5 weeks following bariatric surgery (Table 3.4). This effect could partially restore airway-parenchymal coupling, resulting in greater airway wall tension that would be responsive to bronchodilation. This may explain the increased BD response recorded in the upright position as an increased percent drop in post-BD Rrs following weight loss (Figure 3.5A), suggesting an increase in central airway caliber.

Another explanation for the reduced BD responsiveness measured in the upright position prior to weight loss surgery is that some of the bronchodilator may have been deposited within the oropharynx despite the use of a metered dose inhaler and valved-holding chamber in the administration of the drug. With weight loss, it is possible that there was a reduction in the amount of redundant supraglottic oropharyngeal tissue which increased BD deposition within the lung. However, this effect was likely modest since we found that BD reduced Rrs and FEV1 in the supine position indicating successful BD delivery to the lung. Regardless of the cause, reduced BD responsiveness is an important problem for severely obese subjects with asthma since they are usually prescribed short acting bronchial agonists for sudden symptoms. It is also possible that changes in systemic inflammation with weight loss may have led to physiological changes in the airways. Inflammation is associated with obesity and, in particular, increased secretion of pro-inflammatory adipokines by the visceral adipose tissue is suggested to lead to airway remodeling and bronchial hyperreactivity [1]. However, changes in inflammatory status with weight loss is particularly associated with obese subjects who also have asthma [1], whereas our study focused on subjects without asthma. Furthermore, progesterone has been shown to potentiate BD-stimulated ASM relaxation [296], and it is down-regulated in obesity [297], suggesting a possible mechanism for the reduced BD responsiveness at baseline, since our study was limited to only female participants. Thus perhaps, even with modest weight-loss, these hormonal and inflammatory mediators could have a functional effect on lung mechanics. Nevertheless, our subjects were severely obese before bariatric surgery and remained severely obese after surgery, despite significant weight loss.

In contrast to the upright position, supine Rrs was significantly reduced post-BD at all frequencies before weight loss; however, only Rrs,6 was reduced post-BD after weight

loss (Figures 3.5B and 3.3A). Before weight loss, Rrs was highest in the supine position, indicating narrowed airways that may be attributed largely to the central airways, as we did not observe any frequency dependence indicative of small airways heterogeneity [281]. Due to the inverse power relationship between radius and resistance, Rrs is exquisitely sensitive to airway diameter. Thus, small changes in airway caliber can cause large effects on Rrs. It is possible that despite diminished airway-parenchymal tethering in obesity, the BD was able to induce central airway dilation in the supine position before weight loss because of the reduced airway caliber. However, following weight loss, the beta-agonist had little effect in dilating the airways in the supine position (Figure 3.5B), since weight loss had already induced significant airway dilation in this position as shown in Rrs,19 (Figure 3.2). Moving from upright to supine position can cause significant changes in upper, and central airway geometries as well as changes to the lung periphery [258, 279, 298]. Xrs is thought to be an indicator of airway closure [162, 254, 282] and is decreased in the supine position [258, 279, 298] consistent with our findings. Xrs may also reflect changes in chest wall elastance that may occur with changes in posture [299]. Interestingly, we found that the BD was very effective at reducing upright and supine Ers after weight loss likely due to recruitment of small airways (Figures 3.3B and 3.5B). The larger relative change in Ers compared to Rrs following weight loss may mean a greater improvement in lung function due to recruitment of small airways with weight loss.

The biggest effect of weight loss at baseline was the improvement in supine lung mechanics which may help explain the improvements observed in sleep quality (Table 3.6). Eight (42%) out of the 19 subjects interviewed in this study reported mild to severe breathing discomfort at baseline. However, after weight loss, only three (16%) of the respondents reported any breathing difficulty in the month prior to examination. This is in agreement with the findings of Oppenheimer *et al* where they reported that the prevalence of respiratory symptoms such as dyspnea, wheeze and cough was greatly reduced after weight loss [9]. A PSQI of 8.4±3.5 was measured at baseline in our cohort and this is quite similar to the findings of Toor *et al* who reported a PSQI of 8.8 in their cohort [271]. A global sleep score of ≤ 5 is associated with good sleep quality while a global sleep score > 5 is associated with poor sleep quality [284]. The PSQI reduced to

6.5±3.7 following weight loss surgery (Table 3.6), unlike the findings of Toor *et al* [271] where global sleep score reduced to ≤ 5, although their follow-up assessment was conducted after much greater weight loss was achieved at 3-6 months after bariatric surgery. Nevertheless, even with modest weight loss at 5 weeks, there were significant improvements in PSQI that was highly correlated with the reduction in $Rrs_{,19}$ measured in the supine position. In fact, about 51% of the variance in the improvement in sleep quality was accounted for by the reduction in supine $Rrs_{,19}$ suggesting that breathing discomfort due to narrowing of upper and central airways in the supine position may contribute more to poor sleep quality than previously thought.

We have shown that the assessment of lung mechanics with oscillometry in the supine position or with BD was sensitive to early changes in lung function associated with modest weight loss following bariatric surgery while spirometry and plethysmography, except for FRC, was not. Weight loss likely improved lung mechanics by restoring the airway-parenchymal tethering forces in the supine position and likely helps explain improvements in sleep quality. In addition, the tethering forces were likely sufficiently restored to allow the BD to dilate the airways even after weight loss. These findings demonstrate the sensitivity of oscillometry and that improvements in lung mechanics occur early after weight loss surgery improving sleep quality.

Chapter 4: Improvement in upright and supine lung mechanics with bariatric surgery: sleep quality and bronchodilator responsiveness

4.1 Abstract

Introduction: Obesity is associated with respiratory symptoms such as wheezing and dyspnea, but its effect and the effect of weight loss on lung function particularly in the supine position is insufficiently documented.

Methods: We evaluated fifteen severely obese female participants (mean±SD BMI 48.2±6.0 kg/m^2) using spirometry, plethysmography, Pittsburgh Sleep Quality Index (PSQI), and oscillometry to obtain various indices of lung mechanics including respiratory system resistance (Rrs,6, Rrs,11 and Rrs,19) and elastance (Ers). These tests were conducted in the upright and supine position, and pre- and post-bronchodilation with 200 μg of salbutamol before, and 5 weeks and 6 months after bariatric surgery.

Results: An average weight loss of 11.9±2.7kg was recorded at the 5 week-postoperative assessment but this had no significant effect on respiratory mechanics. Six months after surgery, a 21.4±7.1kg weight loss was associated with a 14.1±6.1% reduction in Rrs,6 and a 25.7±9.4% reduction in Ers measured in the upright position, with no significant changes in spirometry. Similar changes in Rrs and Ers were recorded in the supine position and the sleep quality of study participants improved from a PSQI of 8.4±3.5 to 4.1±2.9. The reduction in Rrs and Ers induced by weight loss was comparable to that produced by salbutamol. Furthermore, the frequency dependence of Rrs was modestly reduced with weight loss indicating reduced heterogeneity. Fits of respiratory impedance spectra to a mathematical model suggests reduced resistance in the central and peripheral airways as well as reduced stiffness in the peripheral airways.

Conclusion: Respiratory mechanics during tidal breathing were more greatly affected by weight loss than the mechanics of forced expiration, likely due to both airway recruitment and reduced chest wall stiffness. Our findings provide new evidence that severely obese subjects may have poor sleep quality due to poor respiratory mechanics but weight loss reverses these changes.

Keywords: Oscillometry (Forced oscillation technique); Obesity; Body mass index; Lung function; Lung mechanics; Bronchial reversibility; Sleep quality.

4.2 Background

Obesity is associated with respiratory symptoms like wheeze [22], dyspnea [23], orthopnea [13], and sleep disturbances [271]. These symptoms can be difficult to treat with bronchodilators but improvements have been widely reported with weight loss [6, 300]. However, spirometry often shows little or no change in lung function with weight loss [9-11, 300]. This lack of insight from spirometry has made it challenging to explore the mechanisms that link obesity and weight loss to changes in lung function. Furthermore, lung function is typically assessed in the upright position but the respiratory symptoms of obesity worsen in the supine position [13], and this is thought to contribute to sleep disturbances [271].

Mechanically, the lung is compressed in obesity and this causes a number of changes in lung mechanics and function, including a reduction in lung volumes, particularly, the functional residual capacity (FRC) [147, 242, 279]. The changes in lung mechanics include: an increase in respiratory system stiffness and a reduction in the diameter of the airways [245]. These changes can be indirectly assessed from respiratory system resistance (Rrs) and reactance (Xrs) using oscillometry. We recently used oscillometry to study the effects of modest weight loss induced by bariatric surgery [300]. We found significant changes in supine Rrs as early as 5 weeks after bariatric surgery and these changes correlated strongly with improvements in sleep quality. Interestingly, however, we found no other significant changes in respiratory system mechanics measured in the upright position, and similarly no changes in spirometric outcomes [300]. While the effects of weight loss on supine respiratory mechanics have never been probed, improvements in oscillometry outcomes (Rrs and Xrs) obtained in the upright position have been consistently reported after 12-24 month of bariatric surgery-induced weight loss [9-11].

Here, because of the likely importance of supine respiratory mechanics to sleep quality, and because it had not been previously investigated, we were interested in tracking the progress of the changes in respiratory system mechanics with weight loss in the supine position, and to assess these changes in comparison to the changes in upright respiratory mechanics. Lastly, we wanted to assess the changes in airway heterogeneity using the

frequency dependence of Rrs, and also the role of weight-loss-induced restoration of airway-parenchymal tethering on bronchodilator responsiveness. Thus, we evaluated the effect of weight loss on sleep quality and respiratory system mechanics at the 6 month-postoperative assessment using the PSQI, oscillometry, spirometry, and plethysmography, and we compared the results obtained to weight-changes recorded at the 5 week-postoperative assessment.

4.3 Methods

4.3.1 Selection of study participants and consent

Twenty four severely obese individuals without a diagnosis of asthma or other lung diseases were recruited into the study from the bariatric surgery clinic at the Queen Elizabeth II Health Sciences Centre. Of this, 5 subjects were lost to follow-up at 5-weeks while 4 were lost to follow-up at 6 months. This study was approved by the Nova Scotia Health Authority Research Ethics Board (reference number: CDHA-RS/2012-109). All participants provided written informed consent to participate in the study and consented to have their data published.

4.3.2 Inclusion and exclusion criteria

Inclusion criteria were the presence of severe obesity defined as body mass index (BMI) greater than 40 kg/m^2 and scheduled bariatric surgery. Exclusion criteria were: BMI less than 40 kg/m^2, physician diagnosis of chronic lung disease (including asthma and chronic obstructive pulmonary disease), smoking history of more than 10 pack years, inability to lie on the back for up to 10 minutes, inability to perform PFTs acceptably, and cognitive limitations that prevented completion of sleep questionnaire. Subjects who did not show up for their follow-up appointments were also excluded from the study.

4.3.3 Study design

This prospective observational study includes assessments performed prior to bariatric surgery and post-operative assessments at 5 weeks and 6 months. Some of the data obtained at baseline and 5 weeks after bariatric surgery have been previously published

[300]. During follow-up, sleep quality was reassessed and plethysmography, spirometry and oscillometry were repeated. Spirometry and oscillometry was performed in the upright and supine position, and pre- and post-bronchodilation (BD). The testing sequence for the measurements conducted has been described previously in Chapter 3 (Figure 3.1).

4.3.4 Sleep quality questionnaire

Sleep quality was reassessed using the Pittsburgh Sleep Quality Index (PSQI) with permission from Buysse and coworkers [284]. Briefly, the PSQI is a 19-item questionnaire that provides validated measurements of sleep disturbance and usual sleep habits in the previous month. The questions are grouped into seven components that analyze various aspects of sleep quality such as: subjective sleep quality, sleep latency, sleep duration, habitual sleep quality, sleep disturbances, use of sleep medication and daytime dysfunction due to sleepiness. Respondents rated their sleep quality as "very good," "fairly good," "fairly bad" or "very bad" and this represented a score of 0, 1, 2, or 3, respectively. The PSQI also assessed various factors that disrupt sleep including breathing discomfort, waking up to use the bathroom, coughing or snoring loudly, feeling too hot or cold, having bad dreams and experiencing pain. The global sleep score is calculated as the sum of the seven components that make up the PSQI. A score of ≤ 5 was associated with good sleep quality while a score > 5 was associated with poor sleep quality. Responder analysis of the PSQI was also performed by estimating the number of subjects that reported good sleep quality at baseline, 5 weeks after surgery, and at 6 months after surgery.

4.3.5 Anthropometric measurements

Weight and height were measured using a calibrated scale and stadiometer and used in calculating BMI.

4.3.6 Pulmonary function tests

Spirometry and whole-body plethysmography were performed according to recommended international guidelines [127, 285], using a calibrated spirometer-equipped body plethysmograph (SensorMedics Corporation, Yorba Linda, CA, USA). Forced

expiratory flows, including forced expiratory volume in 1 s (FEV_1) and expiratory flow at 50 % (FEF_{50}), 75 % (FEF_{75}), and 25% – 75 % ($FEF_{25–75}$) of vital capacity (VC) were obtained. Lung volumes and capacities, including residual volume (RV), expiratory reserve volume (ERV), FRC (estimated as the sum of RV and ERV), inspiratory capacity (IC) and TLC (estimated as the sum of FRC and IC) were also measured. The lowest residual volume (RV); the average ERV, FRC and TLC; and the best VC and forced expiratory flows were selected as the final result from three technically acceptable measurements and expressed as percentages of predicted values from Crapo and colleagues [73].

4.3.7 Oscillometry

Respiratory system impedance was measured according to recommended guidelines [286] using the tremoFlo™ airwave oscillometry system (Appendix G, Thorasys Thoracic Medical Systems Inc., Montreal, QC, Canada). This technique has been described in detail previously [300]. Briefly, a multi-frequency composite oscillatory pressure waveform of about 1-2 cm H_2O was generated by the oscillometry device and superimposed on a subject's spontaneous breathing at 6, 11 and 19 Hz for 16 s. The impedance of the respiratory system (Zrs) to the resulting flow oscillations was then estimated from the spectral ratio of the pressure and flow signals measured at the mouth. Respiratory system resistance (Rrs) and reactance (Xrs) were computed at each frequency by separating Zrs into its in-phase component of the pressure and flow and 90 degrees out-of-phase component of the pressure and flow, respectively. The frequency dependence of Rrs, which is an indicator of airway heterogeneity, was computed by subtracting Rrs,19 from Rrs,6, that is: Rrs,6 minus Rrs,19. Predicted Rrs and Xrs was also computed using each subject's age, height, sex and ideal body weight using reference equations published by Oostveen and colleagues [196]. Xrs measured at 6 Hz was used in estimating respiratory system elastance (Ers) using the relationship: $Ers = -\omega Xrs, 6$, where ω is the angular frequency (f), mathematically expressed as: $\omega = 2\pi f$.

4.3.8 Mathematical modeling of respiratory system impedance

To investigate changes in central versus peripheral lung mechanics, we employed the two-compartment series model originally developed by Mead *et al* [301], and recently

applied by Al-Alwan *et al* to study lung mechanics in obese subjects [11]. The model was fit to respiratory system impedance spectra measured before and after weight loss surgery in order to obtain the parameters of the respiratory system using MATLAB R2016a (Mathworks Inc., Natick, Mass., USA). The model consists of a series arrangement of an upper airway resistance, representing central resistance (Rc), and an upper airway compliance, representing central compliance (1/Ec) connected through a single peripheral airway, representing the peripheral resistance (Rp), to peripheral compliance (1/Ep) to account for the alveolar compartments (Figure 4.1) [301]. The upper compartment pathway impedance (Z_1) included a central inertance (Ic) and is described by Equation 4.1, while the lower compartment impedance (Z_2) is described by Equation 4.2 as shown below.

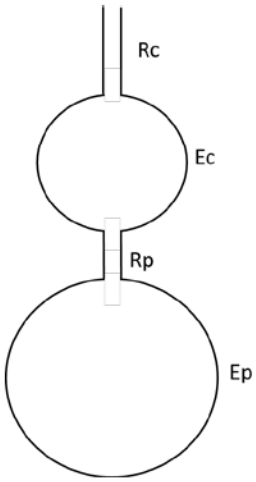

Figure 4.1. Schematic illustration of the series two-compartment model of the lung.

$$Z_1 = Rc + i2\pi fIc \tag{4.1}$$

$$Z_2 = Rp - i\frac{Ep}{2\pi f} \tag{4.2}$$

Also included in the upper compartment was an impedance Z_3, representing the elastance of the central airways (Ec) as shown in Equation 4.3 below.

$$Z_3 = -i\frac{Ec}{2\pi f} \tag{4.3}$$

The total static elastance (Etotal) was computed as the parallel sum of Ec and Ep as shown in Equation 4.4, assuming zero-flow conditions.

$$Etotal = \frac{E_c E_p}{E_c + E_p} \tag{4.4}$$

Z_1 was added to the parallel sum of Z_2 and Z_3 to obtain the total impedance (Ztotal) of the model as shown in Equation 4.5.

$$Ztotal = Z_1 + \frac{Z_2 Z_3}{Z_2 + Z_3} \tag{4.5}$$

We also calculated the coefficient of determination (CD) in order to determine the goodness of fit of the model impedance to the measured impedance spectra and CD greater than 0.95 was used as an acceptance criterion.

4.3.9 Bronchial reversibility test

Lung mechanics were assessed before and 15 minutes after relaxation of airway wall tension with inhalation of 200 μg of the short-acting β_2-adrenergic agonist–salbutamol delivered through a pressurized metered-dose inhaler and valved-holding chamber (CareStream Medical Ltd, Pickering, ON, Canada).

4.3.10 Bariatric surgery

All subjects underwent a laparoscopic sleeve gastrectomy, a minimally invasive procedure that results in the complete removal of about 85% of the stomach. The postoperative diet regimen consisted of 4–6 weeks of a high-protein, semiliquid diet, followed by a return to a normal diet.

4.3.11 Statistical analyses

Results are expressed as means ± standard deviation (SD) unless specified otherwise. Normality of data was determined with Shapiro-Wilk test and normality plots. Weight, height, BMI, PSQI, and plethysmography data obtained before and at 5 weeks and 6

months after bariatric surgery were analyzed using a 1-way repeated-measures analysis of variance (ANOVA) with 3 levels (i.e before surgery, 5 weeks after surgery, and 6 months after surgery). The effects of position and bronchodilation on spirometry results were analyzed using 2-way repeated-measures ANOVA with time and intervention (position plus bronchodilation) as factors. Time was divided into 3 levels (i.e before surgery, 5 weeks after surgery, and 6 months after surgery), and intervention was divided into 4 levels (i.e upright pre-BD, supine pre-BD, upright post-BD, and supine post-BD). The same procedure was repeated for oscillometry results at each frequency. When significance was found, separate post hoc pair-wise testing was performed with a Student's *t* test to identify differences caused by weight loss surgery, position, or BD and the Benjamini-Hochberg procedure was applied to control the effect of multiple comparisons [288]. Statistical analyses were performed in IBM Statistics for Windows, version 22.0 (IBM Corporation, Armonk, NY, USA) and p-values < 0.05 were considered statistically significant.

4.4 Results

4.4.1 Demographics and obesity parameters

Twenty four severely obese individuals were recruited into the study through the informed consent process. Demographics and anthropometric data from 15 study participants that completed the study protocol are presented in Table 4.1. Bariatric surgery significantly reduced the weight and BMI of the participants with an average weight loss of 11.9 ± 2.7 kg and 21.4 ± 7.1 kg recorded at the 5 week- and 6 month-postoperative assessments, respectively.

4.4.2 Lung function before and after bariatric surgery

At baseline, FEV_1, FVC, PEF and other spirometric parameters, expressed as percentages of predicted, were within normal limits (Figure 4.2). Weight loss did not induce any significant changes in any of the spirometric parameters at the 5 week- or 6 month-postoperative assessment.

4.4.3 Lung volumes before and after bariatric surgery

Similar to the spirometry outcomes reported above, TLC, VC and IC, were also within normal limits at baseline but RV, ERV and FRC were below the range of expected values (Figure 4.3). With the exception of FRC, weight loss was not associated with any significant changes in these lung volumes at the 5 week- or 6 month-postoperative assessment (Figure 4.3). Weight loss was associated with an increase in FRC of 8.1 ± 3.8 % and 13.4 ± 3.9 % from baseline at 5 weeks and 6 months, respectively.

Table 4.1**.** Demographics and anthropometric characteristics of study participants.

	Baseline	**5 weeks After Surgery**	**6 months After Surgery**	***p*-value**
Sex (Females, Males)	15, 0			
Age (years)	45.5 ± 7.0			
Mean weight (kg)	125.7 ± 18.0	113.7 ± 17.3	104.3± 15.4	< 0.001
Mean BMI (kg/m^2)	48.2 ± 6.0	43.7 ± 6.1	40.1 ± 5.7	< 0.001
Avg weight loss (kg)	na	11.9 ± 2.7	21.4 ± 7.1	
% of initial weight lost	na	9.6 ± 2.3	16.9 ± 5.2	

BMI, body mass index; Avg, average; na, not applicable. Data are expressed as means ± SD. $p < 0.05$ was considered statistically significant.

4.4.4 Effect of weight loss and bronchodilation on oscillometry outcomes

The effect of weight loss on Rrs measured in the upright position is shown in Figure 4.4A. Upright Rrs measured at 5 weeks after bariatric surgery was not significantly different from baseline at any oscillation frequency. However, with further weight loss, there was a significant drop across all oscillation frequencies at 6 months, approaching the mean predicted Rrs estimated from the participants' ideal body weights, particularly at Rrs,19 (Figure 4.4A).

Similar results were observed in Xrs obtained in the upright position. At baseline, upright Xrs was quite negative and well below the predicted Xrs (Figure 4.4B). Weight loss had

no significant effect on upright Xrs at the 5 week-postoperative assessment. However, at 6 months, a significant increase in Xrs,6 and Xrs,11 was observed and the Xrs versus oscillation frequency curve moved towards the predicted Xrs.

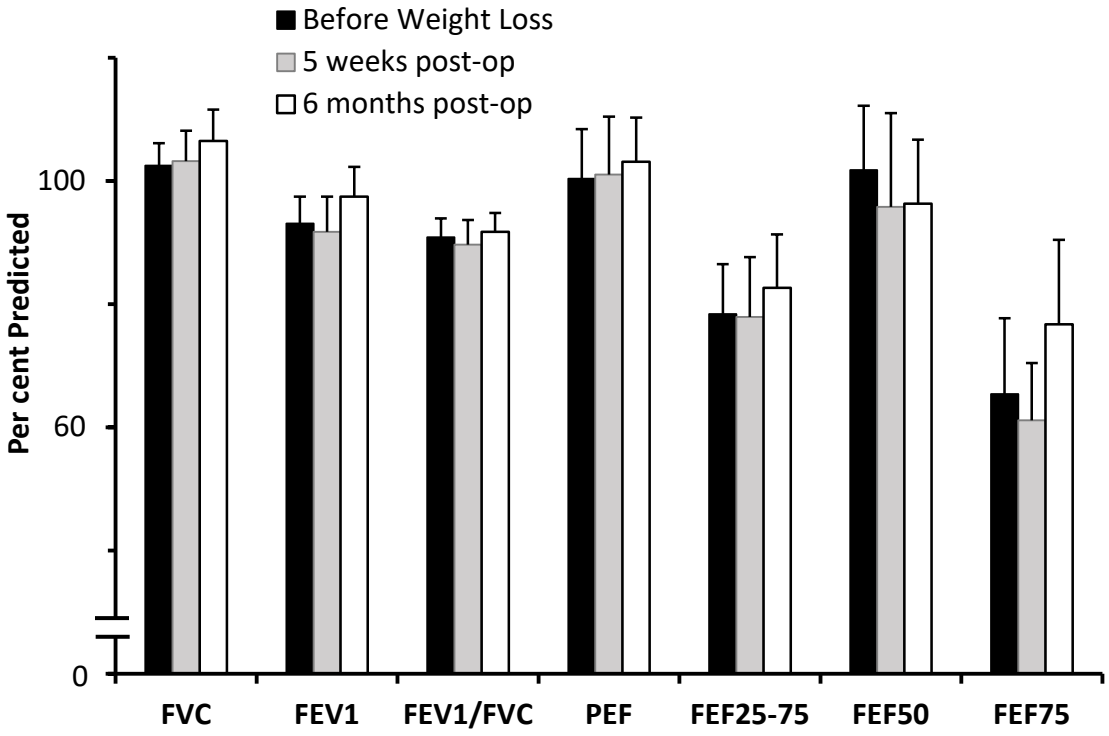

Figure 4.2. Spirometric measures before and after weight loss presented as percent predicted.
Error bars indicate SE. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; FEV1/FVC; ratio of forced expiratory volume in 1 s to forced vital capacity; PEF, peak expired flow; FEF_{25-75}, forced expiratory flow between 25% and 75% of FVC; FEF_{50}, forced expiratory flow at 50% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC.

As previously reported, supine Rrs significantly reduced at the 5 week-postoperative assessment but only at the 19 Hz oscillation frequency [300]. Here, we found that further weight loss at 6 months led to greater reductions in supine Rrs across all oscillation frequencies (Figure 4.4C). Also, moving to the supine position shifted the Rrs versus oscillation frequency curve upwards (Figures 4.4A and 4.4C). Compared to Xrs measured in the upright position, supine Xrs was much more negative at baseline and weight loss

had no significant effect at the 5 week-postoperative assessment (Figure 4.4D). Interestingly, weight loss induced significant increases in supine Xrs,6 and Xrs,11 at 6 months but similar to the upright position (Figure 4.4B), supine Xrs,19 was not significantly affected. These improvements in supine Rrs and Xrs with weight loss were found to be comparable to the improvements recorded in the upright position, with the only exception being Rrs,19 which improved more in the supine position

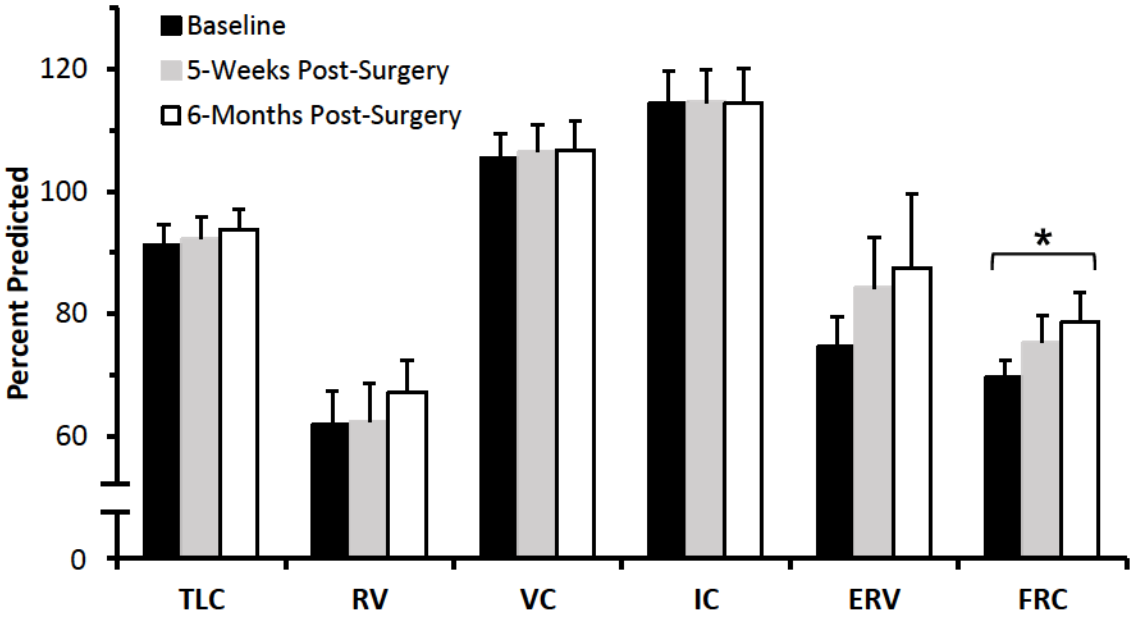

Figure 4.3. Plethysmography outcomes before and after weight loss presented as percent predicted.
Error bars indicate SE. TLC, total lung capacity; RV, residual volume; VC, vital capacity; IC, inspiratory capacity; ERV, expiratory reserve volume; FRC, functional residual capacity. *, $p < 0.05$.

Figure 4.5 shows the percent change in key oscillometry outcomes and FEV1 produced by weight loss in the upright and supine positions. Weight loss induced a 14.1 ± 6.1 % reduction in upright Rrs,6 and a 25.7 ± 9.4 % reduction in upright Ers at the 6 month-postoperative assessment but FEV1 only demonstrated a 4.6 ± 2.8 % increase in the upright position (Figure 4.5) and this increase was not statistically significant. Compared to the upright position, weight loss was also associated with a greater reduction in supine

Rrs,19 of 23.4 ± 6.7 % (p = 0.040) (Figure 4.5). Supine Rrs,6 and Ers also reduced by 17.8 ± 5.4 % and 20.2 ± 7.2 % but these reductions were not statistically different from the percent reductions reported above for the upright position (Figure 4.5).

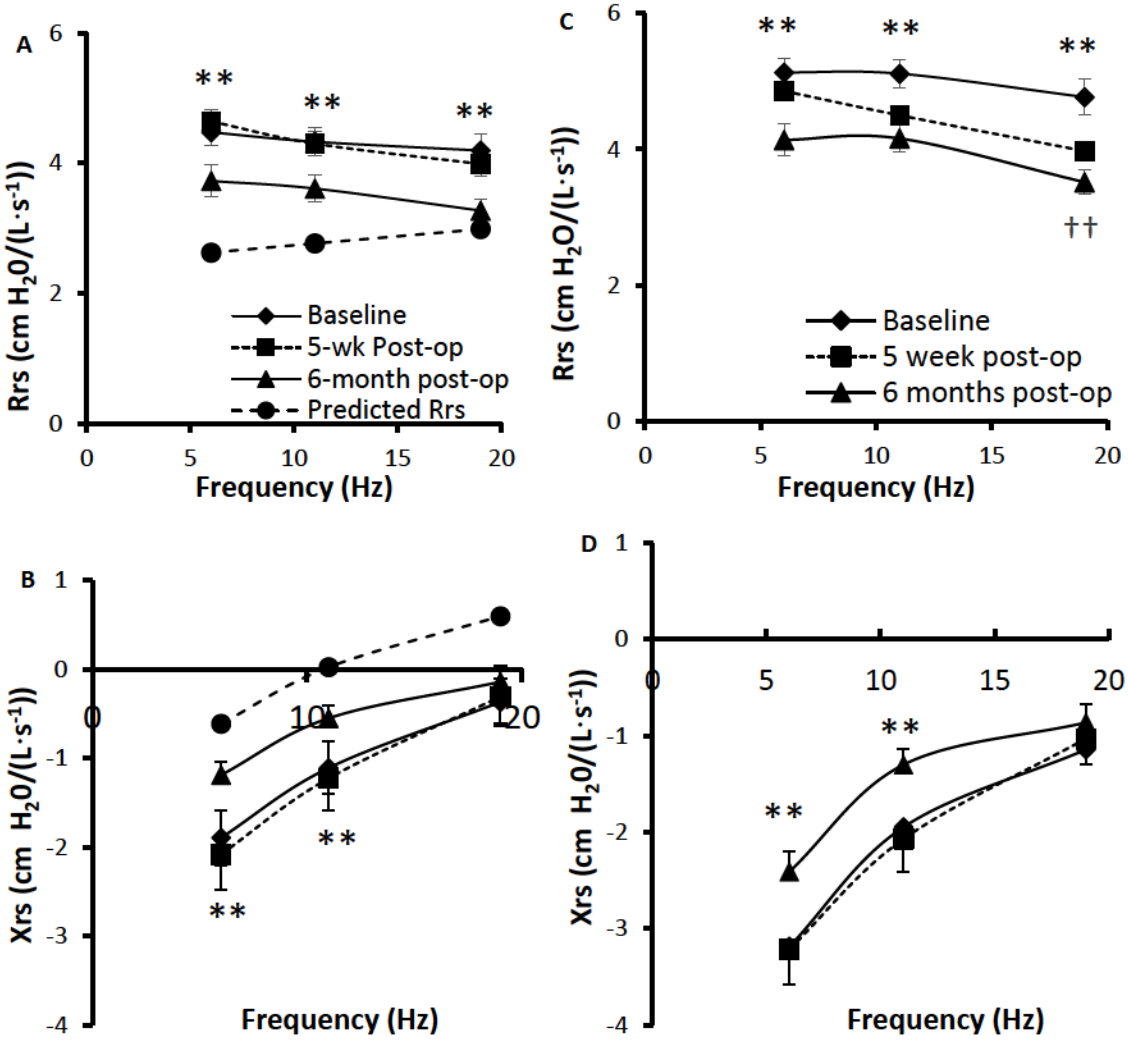

Figure 4.4. Changes in Rrs and Xrs measured in the upright (A & B) and supine positions (C & D) before and after bariatric surgery.
Note that improvements in upright Rrs and Xrs only occur at the 6 month post-op assessment, while improvements in supine Rrs occurred as early as 5 weeks after bariatric surgery. Error bars indicate SE. Rrs, respiratory system resistance; Xrs, respiratory system reactance. ††, $p < 0.01$ at 5 week post-op assessment and **, $p < 0.01$ at 6 month post-op assessment compared to baseline.

When we administered the short-acting β-agonist, we measured very little changes in lung function at baseline in both oscillometry and spirometry outcomes obtained in the upright position (Figure 4.6A). However, BD responsiveness increased significantly 6 months after surgery and this response was comparable to the bronchodilatory effect produced by weight loss (Figure 4.5). In contrast, significant BD responsiveness was measured in the supine position at baseline and at the 5 week- (Figure 3.5) and 6 month-postoperative assessment (Figure 4.6B), unlike the upright position (Figure 4.6A).

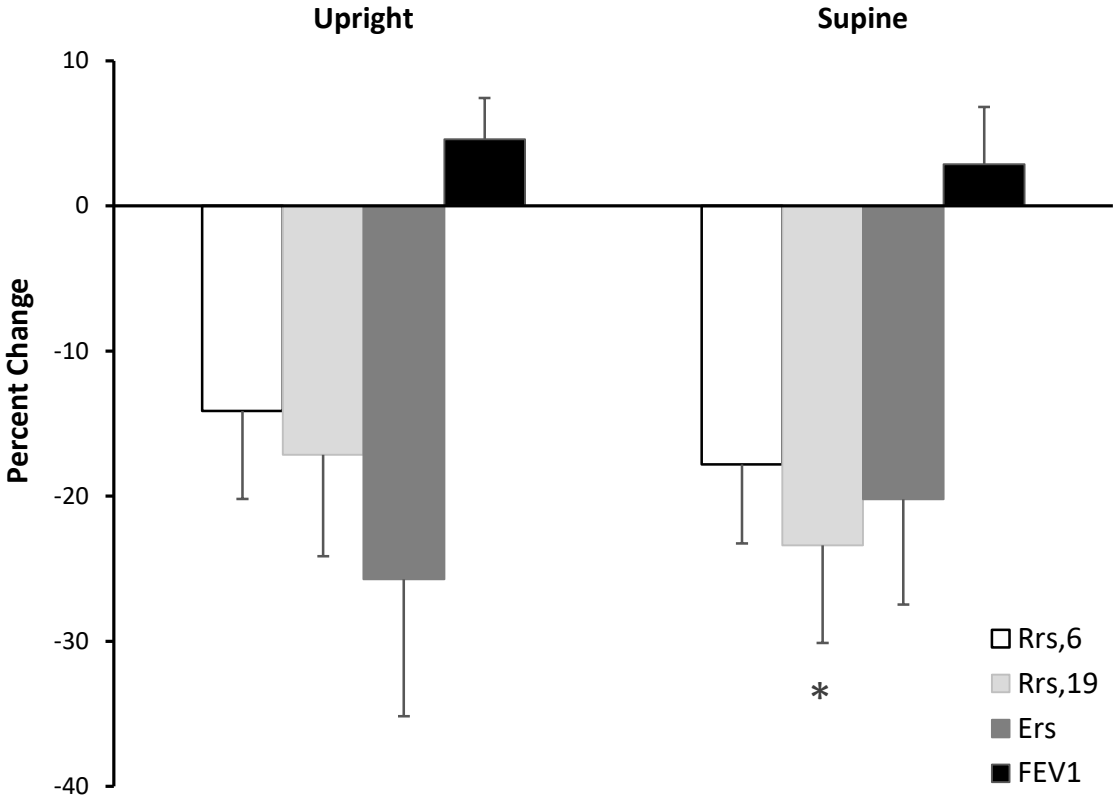

Figure 4.5. Percent change in key oscillometry outcomes and FEV_1 produced by weight loss in the upright and supine positions.
Improvements in upright lung mechanics measured here at the 6 month-postop assessment were comparable to improvements in supine lung mechanics, except for Rrs,19 where the reduction due to weight loss was slightly greater (*, $p < 0.05$). Error bars indicate SE. Rrs,6 and Rrs,19 respiratory system resistance at 6 and 19 Hz; Ers, respiratory system elastance.

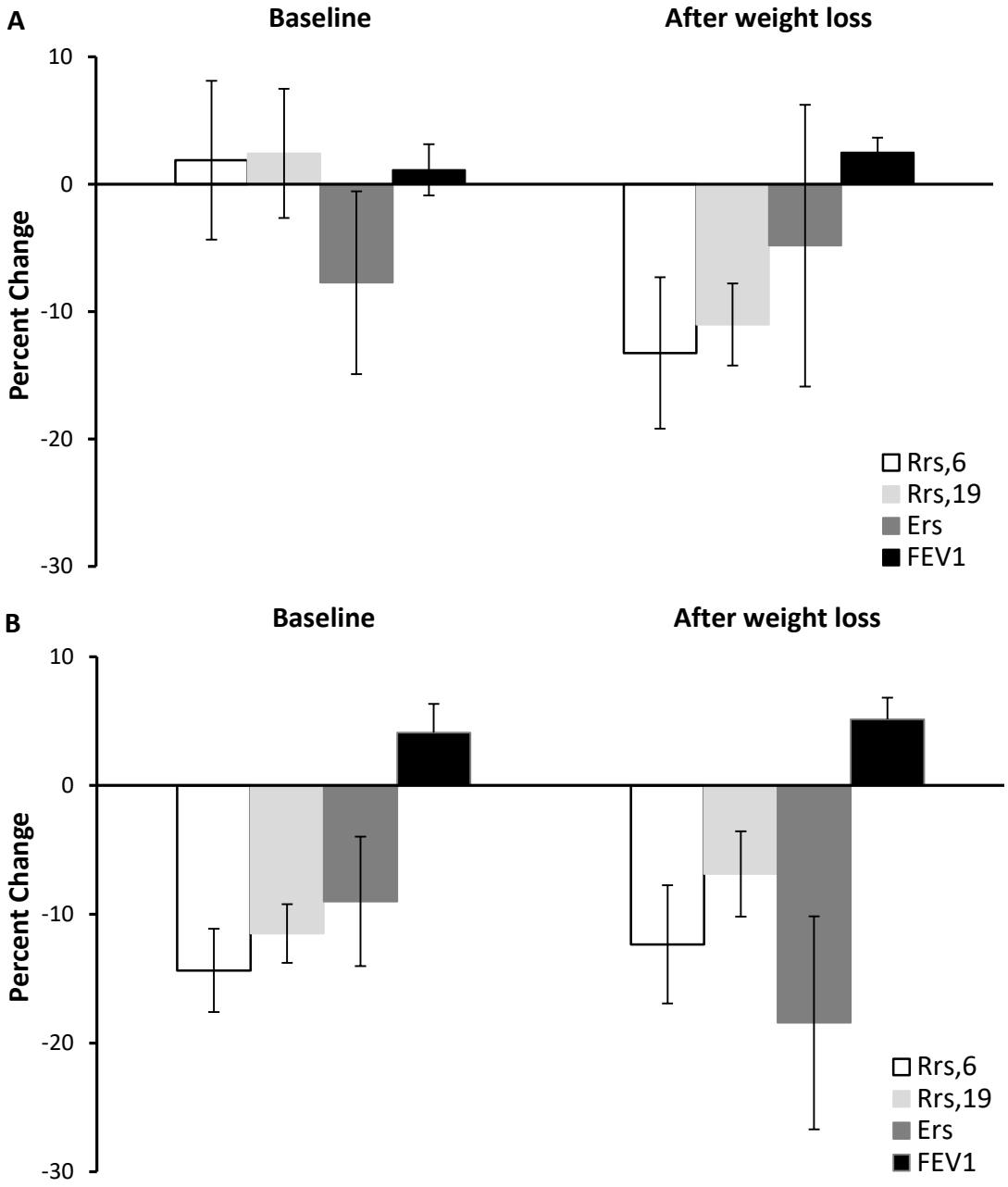

Figure 4.6. Percent change in key oscillometry outcomes and FEV1 produced by bronchodilator in the upright (A) and supine positions (B) before and 6 months after weight loss surgery.
Error bars indicate SE. Rrs,6 and Rrs,19 respiratory system resistance at 6 and 19 Hz; Ers, respiratory system elastance.

4.4.5 Improvements in sleep quality with weight loss

Previously, we reported that weight loss was associated with significant improvements in sleep quality at the 5 week-postoperative assessment [300]. However, our participants' sleep quality was still rated as poor because their global sleep score was above the threshold for good sleep quality. According to the PSQI, a global sleep score > 5 is associated with poor sleep quality, whereas a global sleep score ≤ 5 is associated with good sleep quality [284]. At the 6 month-follow-up assessment, however, we measured greater improvements in sleep quality and the global sleep score of our participants exceeded the threshold for good sleep quality (Table 4.2). Subjects reported significant improvements in their subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, and daytime dysfunction, but they reported no significant changes in their use of sleeping medication.

Responder analysis of the PSQI revealed that only 10.5% of subjects reported good sleep quality at baseline but this increased to 47.4% at the 5 week-postoperative assessment. This proportion continued to increase with further weight loss reaching 80% at the 6 month-postoperative assessment.

4.4.6 Mathematical model of changes in respiratory impedance

Figure 4.7A-D shows weight-induced changes in the mechanical parameters of the respiratory system obtained from fitting the series two-compartment model (Figure 4.1) to respiratory impedance spectra measured before and after weight loss. Weight loss had no significant effect on Ec but Ep reduced significantly and this led to a significant reduction in Etotal (Figure 4.7A). Similarly, weight loss caused Rc and Rp to decrease significantly (Figure 4.7C and 4.7D) but Ic was unaffected (Figure 4.7B).

4.4.7 Changes in frequency dependence of Rrs with weight loss

We were interested in knowing whether the frequency dependence of Rrs improved in subjects who exhibited definitive frequency dependence at baseline. Thus, we also quantified the changes in frequency dependence of Rrs induced by weight loss in subjects whose Rrs,6 minus Rrs,19 reached a critical threshold of 0.2 cm $H_2O/(L \cdot s^{-1})$, which

would be large enough to be unaffected by variability or noise. Seven study participants met this threshold and their frequency dependence of Rrs before and after weight loss is presented in Figure 4.7E. In those subjects with baseline frequency dependence of Rrs, weight loss was associated with a significant reduction in the frequency dependence.

Table 4.2. Component scores of the Pittsburgh sleep quality index collected before and 6 months after bariatric surgery.

	Before surgery	**6 months after surgery**	***p*-value**
Subjective sleep quality	1.3±0.7	0.6±0.5	0.003
Sleep latency	1.7±1.1	1.1±1.0	0.022
Sleep duration	1.1±1.1	0.2±0.5	0.003
Habitual sleep efficiency	1.2±1.0	0.5±1.0	0.033
Sleep disturbances	1.9±0.5	1.1±0.3	< 0.001
Use of sleep medication	0.4±0.9	0.3±0.6	0.327
Daytime dysfunction	1.1±0.9	0.3±0.5	0.003
Global score	8.6±3.5	4.1±2.9	< 0.001

Data are presented as means ± SD.

4.6 Discussion

In this study, we sought to study the changes in lung mechanics and function at 6 months after weight loss surgery. The main results of this study are that (i) surgically-induced weight loss caused large changes in Rrs, Xrs, Ers and FRC at the 6 month-postoperative assessment but no significant changes were detected with spirometry, and (ii) compared to the 5 week-postoperative assessment described in Chapter 3 [300], a greater improvement in sleep quality was recorded at the 6 month-postoperative assessment and study participants reported good sleep quality. These findings provide new evidence that severely obese subjects may have poor sleep quality due to poor pulmonary mechanics but weight loss reverses these changes.

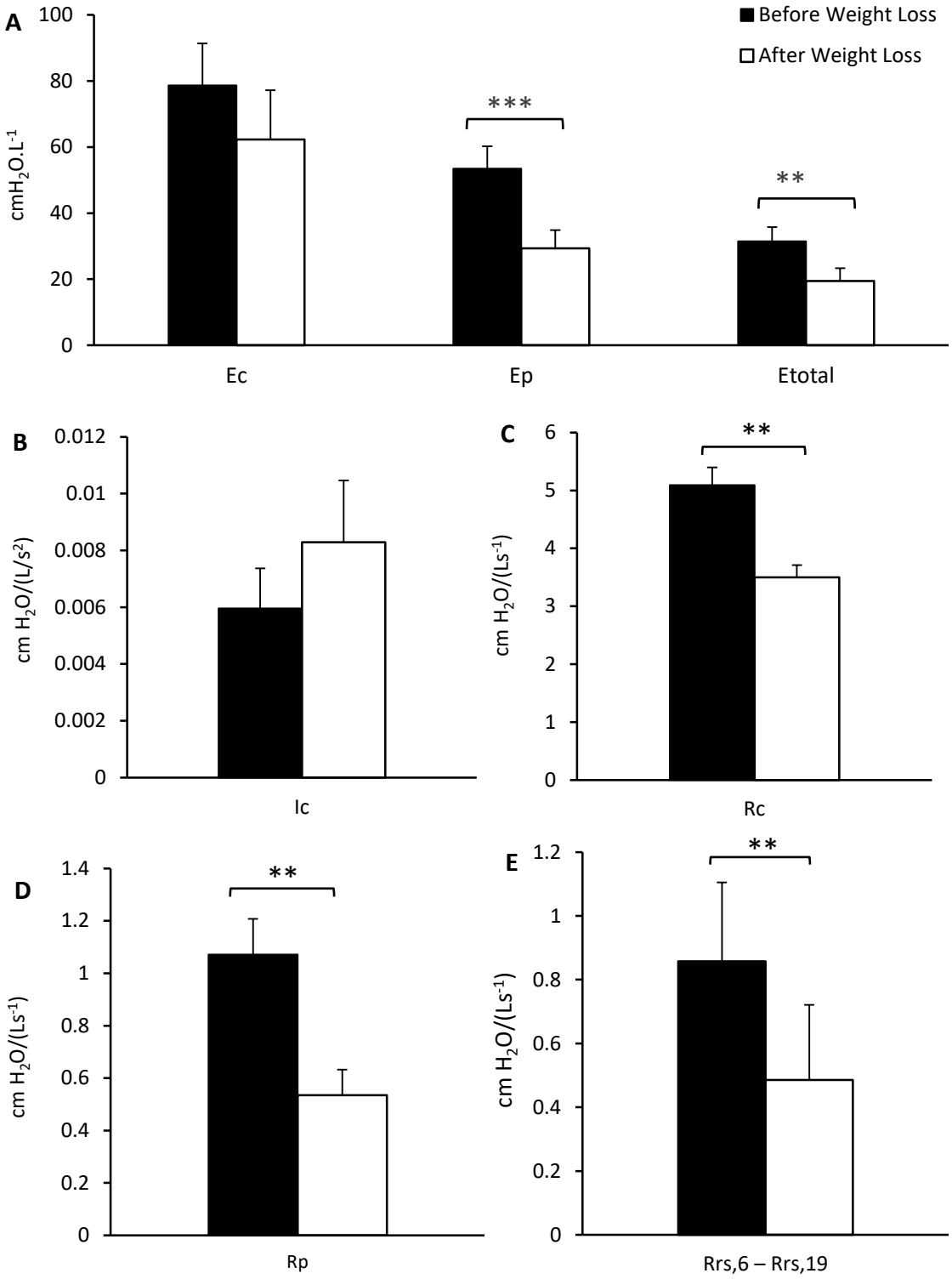

Figure 4.7. Parameters of the two-compartment series model of the respiratory system before and weight loss at 6 months (Panels A – D).

Panel E shows the frequency dependence of Rrs computed by subtracting Rrs,19 from Rrs,6 in seven subjects that demonstrated substantial frequency dependence at baseline. Ec, central elastance; Ep, peripheral elastance; Etotal, total elastance; Ic, central inertance of air column; Rc, central resistance; Rp, peripheral resistance; Rrs,6, respiratory system resistance at 6 Hz; Rrs,19, respiratory system resistance at 19 Hz. Error bars indicate SE. **, $p < 0.01$.

The association between weight loss and improvements in lung mechanics in the upright position has been consistently reported in previous studies at 12-24 months after weight-loss surgery [9-11]. To the best of our knowledge, we are the first group to study the effect of weight loss on lung mechanics in both upright and supine positions and at earlier time points [300]. We specifically investigated the effect of weight loss at earlier time points because most of the surgically-induced weight loss typically occurs within the first 5 weeks to 6 months following bariatric surgery. Thus, although the weight loss achieved by our study participants at the end of the study period was slightly smaller than those reported in previous studies [9, 11], we can show that the weight loss led to dramatic improvements in both upright and supine lung mechanics.

At baseline, respiratory system resistance was significantly higher than the predicted Rrs in the upright position (Figure 4.4A) in agreement with earlier studies which concluded that the increase was due to lung compression and narrower airways in obesity [11]. Six months after bariatric surgery, the resultant 21.4 ± 7.1 kg weight loss was associated with a significant reduction in both upright and supine Rrs measured at all the oscillation frequencies (Figure 4.4A and 4.4C), suggesting reversal of airway narrowing and an overall improvement in breathing mechanics. Further analysis revealed that the bronchodilation induced by weight loss (Figure 4.5) was substantial, and was comparable to the bronchodilatory response that was induced by salbutamol at the end of the study period (Figure 4.6). Rrs,19 was particularly close to the predicted Rrs at the 6 month-postoperative assessment suggesting normalization of upper and central airway caliber. This implies greater improvement in upper and central airways because Rrs at high oscillometry frequencies (Rrs,19) largely reflects these sources, whereas the effect of the small peripheral airways on Rrs is normally undetectable unless significant heterogeneous peripheral airway obstruction is present [173].

While Rrs,6 was significantly greater than Rrs,19 in our obese subjects, which could be indicative of peripheral airway heterogeneity, this was not as large as that reported in other studies [9, 11]. However, we did find a significant reduction in the frequency dependence of Rrs with weight loss (Figure 4.7E) in individuals with observable frequency dependence at baseline. Since frequency dependence is an index of peripheral airway heterogeneity, this indicates the presence of heterogeneous obstruction of the small airways, but this reduced after weight loss. Notably, using the series two compartment model, Rp was significantly reduced after weight loss, potentially indicating airway recruitment as described by Al-Alwan *et al* [11]. Frequency dependence of Rrs is caused by heterogeneous obstruction of the small airways in the lung periphery, and is a hallmark feature of asthma [158, 173, 302-304]. However, in this study, the degree of heterogeneity was likely modest in all subjects. Furthermore, our subjects did not have asthma and none reached the clinical threshold for positive bronchial reversibility in response to BD. Similar studies on obese subjects without asthma do show larger frequency dependence of Rrs [9, 11, 305] than our study. This could be due to our small sample size, or differences in the sensitivity to frequency dependence in oscillometry devices. Oostveen *et al*. (2013) noted that one of the more common oscillometry devices demonstrated a larger frequency dependence than three other devices used in their study. Although the differences between devices were small, they might arise due to differences in the perturbation waveforms employed [196, 197] and could have contributed to frequency dependence of Rrs in other studies [9, 11, 305].

Weight loss was also associated with a significant increase in upright and supine Xrs but only at the 6 and 11 Hz frequencies (Figure 4.4B and 4.4D). Similar to the frequency dependence of Rrs, Xrs is thought to be a sensitive indicator of peripheral airway closure and respiratory system stiffness [162, 254, 282]. At baseline, Xrs was highly negative and well below the predicted Xrs in both upright and supine positions (Figure 4.4B and 4.4D) and this could be due to collapse and closure of small airways in the lung periphery. In fact, Xrs measured in the supine position at low oscillation frequencies was comparable to results obtained from individuals with moderately severe asthma [167]. With weight loss, Xrs,6 and Xrs,11 in both upright and supine positions increased indicating a reduction in the stiffness of the respiratory system.

While the highly negative Xrs (Figure 4.4B and 4.4D) and large Ep (Figure 4.7A) and their significant improvement could be attributed to peripheral airway recruitment, this change in elastic properties could also be due in part to changes in chest wall stiffness which is thought to be increased in obesity [145, 247]. Indeed, for peripheral airway heterogeneity and recruitment to play a large role in our obese subjects, we would expect larger and comparable changes in the magnitude of Rrs,6–Rrs,19 to changes in the magnitude of Xrs. However, the baseline frequency dependence was modest, as was its decrease with weight loss and this may mean that changes in chest wall stiffness with weight loss may have played a more substantial part. If true, then the role of the changes in chest wall stiffness with weight loss may have been less appreciated previously [11]. Although the series model differentiates between central and peripheral lung compartments, these compartments are individually homogeneous, and both must account for the chest wall elastance which is part of the total respiratory system impedance. Thus, changes in chest wall cannot be easily differentiated from changes in Ep.

Nevertheless, the notion that there might be peripheral airway recruitment with weight loss is further supported by evidence of a 13.4 ± 3.9 % increase in FRC with weight loss (Figure 4.3), consistent with other findings in the literature [162, 282]. However, this might also be due to changes in the mechanical balance of components of the respiratory system as well as changes in the chest wall. Thus, since Rrs,6 minus Rrs,19 decreased with weight loss in some subjects, it is likely that weight loss induced some airway recruitment; however, as this was modest relative to the changes in Xrs, we believe the contribution of chest wall mechanics to the improved pulmonary mechanics may be more important.

Obesity affects the forces of mechanical interdependence between the airway and parenchyma [245, 278]. Using oscillometry, we explored how weight loss changed the balance of forces between the airway and parenchyma using two approaches, viz: (i) increased lung compression caused by the supine position, and (ii) by reducing airway smooth muscle tone to induce bronchodilation with inhaled salbutamol. Figures 4.4A and 4.4C show that moving to the supine position increased Rrs as a result of a significant

reduction in the caliber of the airways due to mechanical compression of the lungs. The shift to more negative Xrs recorded when subjects moved to the supine position (Figures 4.4B and 4.4D) further supports the notion of mechanical compression of the lung in obesity, since both involve shifts in abdominal mass in the thorax. With both obesity and the supine position, the Xrs at baseline is at its most negative, and obesity likely amplifies increases in chest wall stiffness, especially in the supine position [299] which results in a worsening of breathing mechanics in these subjects. The reduction in lung volume leads to attenuation of tethering forces between the parenchyma and the airways [245], and this consequently leads to potential derecruitment of small airways in the parenchyma as previously reported [258, 279, 298]. Interestingly, weight loss appears to restore airway-parenchymal tethering forces, illustrated by the increased responsiveness to BD, at least in the upright position (Figure 4.6) [300], as well as improvements in multiple measures of lung mechanics including Rrs,6, and Ers (Figure 4.4 and Figure 4.5), echoed by improvements in model parameters Rc, Rp and Ep (Figure 4.7E).

The worsening of lung mechanics in the supine position likely contributes to the severity of sleep-disordered breathing and poor sleep quality in obesity. We previously found a strong correlation between improvements in supine Rrs,19 and improvements in sleep quality following a modest 11.5 ± 2.5 kg weight loss at the 5 week-postoperative assessment [300]. However, despite the improvements in sleep quality, the global sleep score of our subjects was 6.5 ± 3.7 at the end of the 5-week study period. According to the PSQI, a global sleep score > 5 is associated with poor sleep quality, whereas a global sleep score ≤ 5 is associated with good sleep quality [284]. Here, with greater weight loss, the global sleep score of our subjects improved to 4.1 ± 2.9. Thus, our study participants achieved good sleep quality at the 6 month-postoperative assessment only after much greater weight loss. This result is consistent with the findings of Toor *et al* who also found improvements in sleep quality after much greater weight loss was achieved at 3-6 months following bariatric surgery [271] but they did not measure respiratory mechanics or lung function.

One of the main limitations of this study is that our study protocol required participants to perform both spirometry and oscillometry and it is known that the deep inspirations

associated with the forced breathing maneuvers of spirometry can transiently modify bronchomotor tone and airway caliber [306, 307]. It is possible that oscillometry performed in the supine position may have been affected by the deep inspirations of the preceding upright spirometry. To control for this effect, we ensured that oscillometry was performed at least 5 minutes after upright spirometry and participants were breathing normally. We also maintained the same testing sequence at all 3 time points of the study to help control for any effect this might have on the study outcomes.

In summary, lung compression occurs in obesity and in agreement with other studies [11]; this reduces airway-parenchymal tethering forces, resulting in narrower airways and stiffer lungs. Weight loss likely restored the airway-parenchymal tethering forces and this resulted in improvements in oscillometric Rrs and Xrs, sleep quality and BD responsiveness, but not spirometry. Impairments in respiratory mechanics were amplified in the supine position, but substantial improvements were noted following weight loss. The improvements in supine respiratory mechanics appeared to have greater impact on the subject's sleep quality and overall breathing mechanics.

Chapter 5: Repeatability and Variability of Respiratory Impedance and its Reliability: Effect of Cheek Support and Variations in Head and Neck Positioning

5.1 Abstract

Background: Oscillometry is increasingly used in clinical practice and in research as an objective tool for assessment of respiratory system mechanics. Certain recommendations have been outlined for avoiding the biasing effects from the upper airway such as those caused by inappropriate head and neck angles, and oscillometry requires cheek-support to minimize upper airway shunting. However, the effects of variations in positioning of the head and neck during oscillometry have never been carefully characterized, so it is difficult to assess the importance of these criteria. Here we examined the effects of small and large neck angles on oscillometry outcomes, and compared these to the established effects of the upper airway shunt artefact. We also compared within-session repeatability and variability of oscillometric indices to day-to-day reproducibility and variability. **Methods:** Six healthy adults and 6 COPD patients were evaluated using handheld airwave oscillometry (tremoFlo) on two separate study visits to obtain respiratory system resistance (Rrs) and reactance (Xrs). At baseline, subjects were instructed to follow recommended guidelines for use of oscillometry in clinical practice. Oscillometry was then repeated without cheek-support, and also during flexion and extension of the head and neck. Three consecutive sets of measurements were obtained under each condition, and standard deviation and percent coefficient of variation (COV) were computed as indices of variability, repeatability and reproducibility. Also, Pearson's product moment correlation coefficient [Pearson's (*r*)] was computed to determine the test-retest reliability of oscillometry. **Results:** Oscillometry outcomes were not affected by flexion of the neck but significant changes were recorded during extension. Despite the changes induced by these artefacts, oscillometry demonstrated high within-day repeatability with a COV < 15 % for Rrs,5. The test-retest reliability and day-to-day reproducibility of oscillometry was also very high. Furthermore, Rrs and Xrs were not significantly affected by cheek support in healthy subjects, but significant effects from the upper airway shunt artefact were observed in COPD patients. **Conclusions:** Handheld oscillometry demonstrated high repeatability, and low and acceptable variability. It was also not susceptible to artefacts caused by incorrect head and neck angles.

Keywords: Oscillometry; Repeatability; Reproducibility; Variability; COPD; Cheek support; Respiratory system resistance; Respiratory system reactance; Upper airway shunt

5.2 Introduction

Respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) affect millions of people around the world and are responsible for hundreds of thousands of deaths every year [40, 41, 67]. These diseases represent a huge global health burden impacting significantly on global economics [29, 72]. Currently, spirometry is the gold standard applied in the diagnosis, staging, and monitoring of respiratory disease progression. However, the main limitations of spirometry are that it is a highly effort dependent test that is also insensitive to changes in the small airways – the predominant locus for lung diseases. In the mid-1950s, Dubois *et al*. developed a new tool for assessment of respiratory system mechanics known as the forced oscillation technique or more recently as oscillometry [153].

Oscillometry is increasingly being used in clinical practice and in research to derive many useful indices of respiratory system mechanics, such as respiratory system resistance (Rrs) and reactance (Xrs). These indices have been reported in normal subjects [196] and in patients with various conditions such as asthma [167], COPD [163], sleep apnea/hypopnea [308, 309] and obesity [117, 247, 279], as well as to assess changes induced by weight loss [9, 11, 300]. One key advantage oscillometry has over spirometry is that it allows the assessment of pulmonary function during regular tidal breathing, and it can be used when spirometry is difficult or unfeasible as is the case in preschool children [182], and in patients with cognitive impairments and/or limited psychological reserve [180, 181]. Oscillometry is also a valuable alternative in cases where symptomatic patients present with normal or nearly normal spirometric values [174, 175].

In an effort to standardize the use of oscillometry in clinical practice, the American Thoracic Society (ATS) and the European Respiratory Society (ERS) published a joint guideline in 2003 with recommendations for its proper use [177]. According to the

ATS/ERS guideline, oscillometry should be performed while sitting upright with the neck slightly extended forward or in the neutral position and with hands firmly supporting the cheeks and the floor of the mouth. This procedure minimizes biasing effects from the upper airway such as those caused by inappropriate head and neck angles, and upper airway shunting. It has been speculated that incorrect positioning of the head and neck during oscillometry could potentially affect the accuracy as well as the repeatability, and contribute to the variability of test outcomes [14]; however, the effects of different head and neck angles have never been carefully characterized, so it is difficult to assess the importance of these criteria. Furthermore, there is need for more data on the acceptable variability for an oscillometry test from repeated measures, referred to here as the within-test variability. More data is also needed on the day-to-day reproducibility, variability and reliability of oscillometric parameters in healthy subjects and patients with respiratory disease, particularly patients with airflow obstruction such as COPD and asthma patients. Therefore, the objectives of this study were to: (i) assess the magnitude of the effect of incorrect angles in the positioning of head and neck during oscillometry, and compare these with the effect of cheek support to reduce upper airway shunt artefact, and (ii) assess within-test (also referred to as within-day) repeatability and day-to-day reproducibility, variability and reliability of oscillometric indices in healthy subjects and patients with airway obstruction.

5.3 Methods

5.3.1 Selection of study participants and consent

Six healthy adult subjects without a history of chronic respiratory disease were recruited from the local community (Halifax Regional Municipality) and 6 patients with a confirmed diagnosis of chronic obstructive pulmonary disease (COPD) were recruited from the Pulmonary Rehabilitation Program at the Mumford Professional Centre in Halifax. This study was approved by the Nova Scotia Health Authority Research Ethics Board (reference number: CDHA-RS/2013-219). All participants provided written informed consent to participate in the study and consented to have their data published.

5.3.2 Inclusion and exclusion criteria

Male or female subjects between the ages of 16 and 70 with no history of asthma or other chronic respiratory condition and no recent respiratory tract infection (within 6 weeks), and less than 10 pack-years were enrolled in this study as healthy subjects. Inclusion criteria for COPD patients was a confirmed diagnosis of COPD (GOLD stage II [moderate COPD] to stage IV [very severe COPD]) based on the Canadian Thoracic Society COPD guidelines [310]. Male and female COPD patients between the ages of 16 and 70 with a baseline post-bronchodilator forced expiratory volume in 1 second (FEV_1) < 80% of predicted and FEV_1/forced vital capacity ratio < 70% predicted were included in the study. COPD patients who were current or ex-smokers with a smoking history of more than 10 pack-years were also included in the study.

Exclusion criteria for COPD patients were: (i) known respiratory disease other than COPD (e.g. lung cancer, sarcoidosis, tuberculosis, lung fibrosis, cystic fibrosis, upper and/or lower respiratory tract infection, (ii) having undergone lung surgery (e.g. lung reduction or lung transplant), and (iii) any other uncontrolled disease likely to interfere with the study or impact on subject safety.

5.3.3 Study design

Respiratory system impedance was assessed in 6 healthy adult subjects and 6 patients with COPD on two study visits. During the first study visit, oscillometry was recorded according to the ATS/ERS criteria [177] which recommend that during oscillometry, subjects seat upright in a straight-backed chair, with their head in the neutral position and with hands supporting their cheeks and mouth floor to minimize the upper airway shunt artefact. Data obtained was used as a baseline reference, and to determine within-day repeatability of the device. Oscillometry was then performed without hand support of cheeks to assess the effect of upper airway shunt, and also during flexion and extension of the neck to assess the effect of incorrect head-and-neck angles on oscillometry outcomes. Each test was repeated 3 times with periodic breaks of about 30 s between the measurements and within-test repeatability was assessed from these 3 measurements.

In healthy subjects, we sought to assess the potential magnitude of large head-and-neck angles on oscillometry outcomes, so oscillometry was conducted with the head and neck comfortably near maximum forward flexion, and with the head and neck extended backwards in comfortable maximum extension. The maximum flexion and maximum extension described here established the effect of extreme deviations from the ATS/ERS criteria, but these would not be expected to occur clinically.

We also investigated the potential magnitude of smaller deviations in head and neck angles on oscillometry outcomes in a clinically relevant patient population. To achieve this objective, COPD patients were asked to flex and extend their neck submaximally by only 20° and 10°, respectively. These angles were chosen to approximate extreme situations that would likely occur during assessment of respiratory system mechanics in clinical practice and a goniometer was used in verifying the angle between the head and neck.

Respiratory system mechanics was re-evaluated 2 days later with the head in the neutral position according to the ATS/ERS criteria to determine day-to-day reproducibility, and the test-retest reliability of oscillometry.

5.3.4 Weight and height measurements

The weight and height of each participant was measured and used in computing the BMI of each subject.

5.3.5 Oscillometry

Respiratory system mechanics were assessed by using the tremoFlo C-100 Airwave Oscillometry System (Appendix G, Thorasys Thoracic Medical Systems Inc, Montreal, QC, Canada) to superimpose a multifrequency composite oscillatory pressure waveform of about 1-2 cm H_2O on a subject's spontaneous breathing for 16 s. The handheld oscillometry device delivered a multifrequency waveform at 5, 11, 13, 17, 19, 23, 29, 31, and 37 Hz and mechanical parameters of the respiratory system (Rrs and Xrs) were then estimated from the impedance of the respiratory system (Zrs) to the resulting flow oscillations. Other experiments conducted as part of the calibration and validation of this handheld oscillometry device are described in Appendix H.

Zrs was computed as the spectral ratio of the fast Fourier transform of the pressure and flow signals by averaging 1 second windows of the pressure and flow signals. Each 1 second window from the 16 second recording was multiplied by a Hamming windows with 50% overlap in order to reduce the error caused by spectral leakage, and the Rrs and Xrs from each 1 second window was calculated at each frequency. In each 16 s recording, the effects of artefacts were minimized by automatic rejection of any 1 second window that contains negative Rrs or Rrs (and Xrs) values greater than 3 standard deviations (SDs) prior to computing the mean Rrs and Xrs. Coherence ≥ 0.90 was used as an acceptance criterion. Subjects wore a nose-clip during all measurements as recommended in the ATS/ERS criteria [177].

5.3.6 Statistical analyses

Statistical analyses were performed in IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Within-day repeatability of Rrs was estimated from the coefficient of variation (COV) calculated by dividing the SD of the 3 consecutive measurements obtained on the first study visit by the mean of those 3 consecutive measurements. Since Xrs can have values that approach zero making COV less representative of its variation, the variability in Xrs was estimated from the SD of Xrs. Day-to-day reproducibility of Rrs was estimated from the COV computed by dividing the SD of study visits 1 and 2 by the mean of study visits 1 and 2. Pearson's correlation coefficient (Pearson's *r*) was computed from the Rrs measured on both study visits to determine the test-retest reliability of oscillometry. All results were analyzed using a 1-way analysis of variance (ANOVA) with repeated measures. When significance was found, separate post hoc pairwise testing was performed with a Student's *t* test to identify differences due to the upper airway shunt artefact, incorrect head-and-neck angles, and day-to-day variability. The Benjamini-Hochberg procedure was then applied to *p*-values obtained to control the effect of multiple comparisons and to minimize the false discovery rate [288]. The Benjamini-Hochberg procedure ranked the *p*-values from the multiple comparisons and computed a critical value using the relationship: $(i/m)q$, where i is the rank, m is the total number of comparisons performed, and q is the false discovery

rate which was set to 5%. A *p*-value less than the Benjamini-Hochberg critical value was considered statistically significant.

5.4 Results

5.4.1 Anthropometric characteristics of study participants

Anthropometric and oscillometric variables of study participants are presented as mean ± SD in Table 5.1. The COPD patients were significantly older than the healthy subjects and their weight and BMI were also significantly higher. Importantly, compared to the healthy group, baseline respiratory mechanics was poorer among COPD patients as demonstrated by the significantly higher Rrs,5 and significantly lower Xrs,5 ($p < 0.05$).

Table 5.1. Anthropometric characteristics of study participants

	COPD Patients	**Healthy Subjects**	***p*-value**
Sex, (male/female)	4/2	2/4	na
Age (years)	64.0 ± 4.1	29 ± 7.5	< 0.001
Height (m)	1.72 ± 0.1	1.67 ± 0.1	0.151
Average weight (kg)	95.2 ± 19.1	67.0 ± 3.4	0.008
Average BMI ($kg \cdot m^{-2}$)	32.0 ± 4.0	24.3 ± 1.7	0.001
Rrs,5	4.6 ± 1.0	2.4 ± 0.2	0.003
Xrs,5	-4.4 ± 3.3	-1.1 ± 0.1	0.042

Note: Data are expressed as mean ± SD. COPD, chronic obstructive pulmonary disease; BMI, body mass index; na, not applicable.

5.4.2 Effects of flexion, extension and cheek support on mean Zrs

Oscillometry outcomes were not significantly affected by flexion of the neck but significant changes were recorded during neck extension (Figures 5.1 and 5.2). In COPD patients, the small 20º flexion of the neck during oscillometry did not significantly affect Rrs and Xrs; however, the 10º extension reduced Rrs significantly while Xrs was

unaffected (Figure 5.1). For example, an 8.4 ± 3.8 % reduction in Rrs was recorded at 5 Hz as a result of the 10º extension. In healthy subjects, maximum flexion and extension of the neck during oscillometry did not also affect oscillometric Rrs and Xrs (Figure 5.2) significantly, but there was a significant reduction in Xrs at 11 – 37 Hz during maximal extension (Figure 5.2B).

In healthy subjects, cheek support had no effect on Rrs and Xrs (Figure 5.3). However, in COPD patients, Rrs was significantly lower without cheek support, while Xrs was significantly higher at all the oscillation frequencies compared to when the cheeks were supported (Figure 5.3).

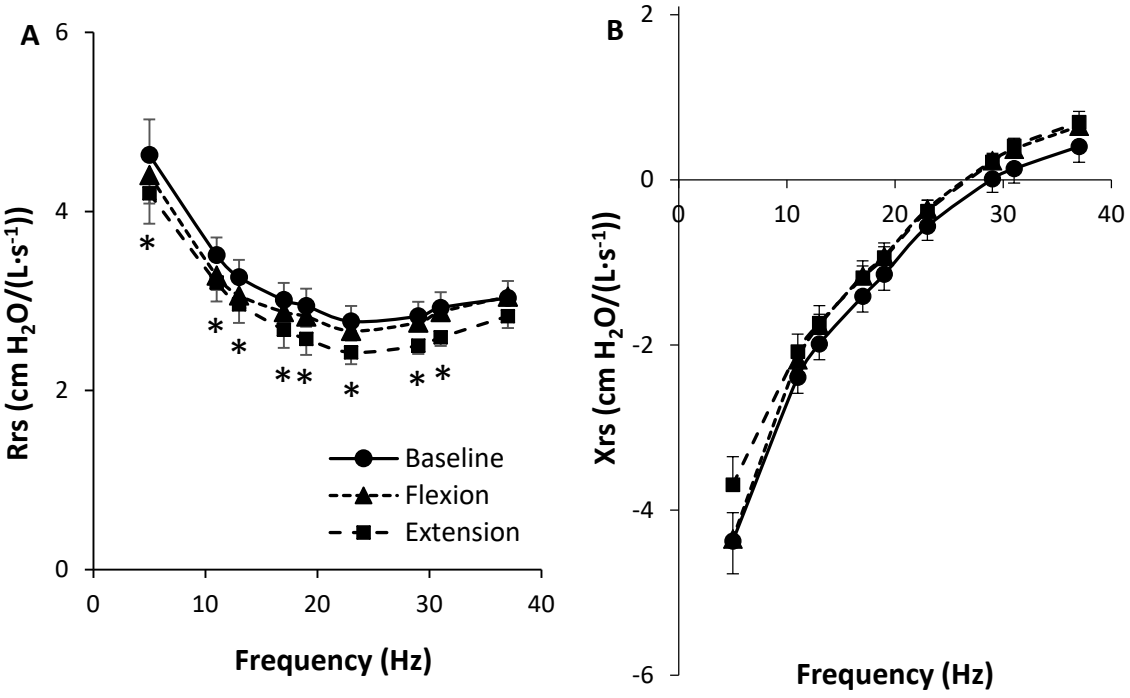

Figure 5.1. Effect of 20° flexion and 10° extension of neck on Rrs (A) and Xrs (B) in COPD patients.
Error bars indicate SE. Rrs, respiratory system resistance; Xrs, respiratory system reactance. *, $p < 0.05$.

5.4.3 Effects of flexion, extension and cheek support on COV of Rrs

In both healthy and COPD subjects, the percent COV in Rrs was generally larger during flexion and extension compared to their baselines (Table 5.2 and 5.3) but COV was less than 15 % in all test scenarios including measurements with no cheek support. Despite the increased variability in the measurements, COV < 15 % demonstrates that Rrs was quite repeatable even in the presence of artefacts caused by incorrect head and neck angles as well as the upper airway shunt.

Furthermore, in COPD patients, the upper airway shunt artefact and small deviations in head-and-neck angle did not significantly affect the percent COV for Rrs,5 (Table 5.3) but in healthy subjects, maximum flexion significantly increased the percent COV for Rrs at 5, 19 and 37 Hz while maximum extension deceased the percent COV for Rrs at 37 Hz (Table 5.2). In healthy subjects, the lack of cheek support did not have any significant effect on the percent COV, except for a large reduction in the percent COV for Rrs,37 (Table 5.2). However, in COPD patients, a substantial reduction in the percent COV for Rrs,19 was recorded when there was no cheek support (Table 5.3). The percent COV for Rrs,37 was also substantially reduced when these patients extended their neck during oscillometry (Table 5.3).

Table 5.2. Percent COV for Rrs at selected frequencies obtained when healthy subjects performed oscillometry without cheek support, and during maximum flexion and extension of the neck.

	5 Hz	**19 Hz**	**37 Hz**
Baseline	4.7±1.7	3.1±0.4	4.4±0.9
No cheek support	3.6±1.2	3.0±0.9	1.9±0.3
Flexion	12.1±3.4	12.1±3.1	13.3±3.1
Extension	10.1±4.0	5.3±1.3	2.8±0.8

Note: Data are presented as % means ± SE. COV, coefficient of variation.

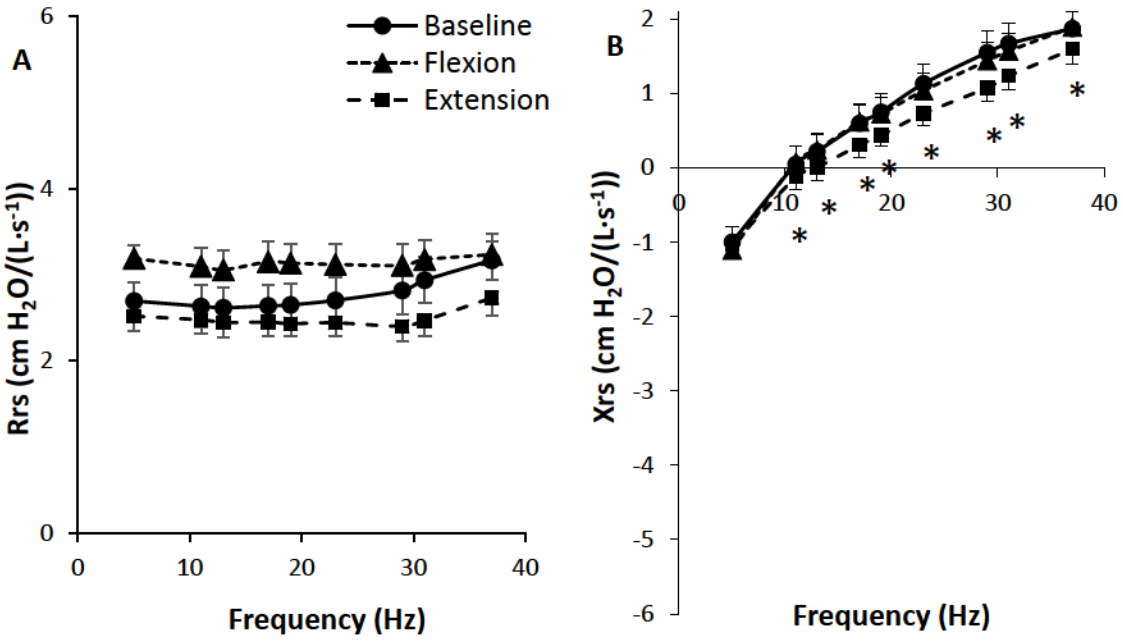

Figure 5.2. Effect of maximal forward flexion and maximal extension on Rrs (A) and Xrs (B) in healthy subjects.
Error bars indicate SE. Rrs, respiratory system resistance; Xrs, respiratory system reactance. Xrs for all frequencies other than 6 Hz was significantly different from baseline during extension of the head and neck ($p < 0.05$).

Table 5.3. Percent COV for Rrs at selected frequencies obtained when COPD patients performed oscillometry without cheek support, and during 20° flexion and 10° extension of the neck.

	5 Hz	**19 Hz**	**37 Hz**
Baseline	4.9±1.1	6.7±1.3	6.4±1.2
No cheek support	5.2±1.6	2.1±0.3	2.5±0.8
Flexion	5.7±0.5	4.4±1.1	3.1±0.6
Extension	8.1±2.3	2.7±0.6	2.3±0.5

Note: Data are presented as % means ± SE. COV, coefficient of variation; COPD, chronic obstructive pulmonary disease.

5.4.4 Effects of flexion, extension and cheek support on SD of Xrs

The mean variability in Xrs assessed through the standard deviation was less than 0.6 $cm \cdot H_2O/(L \cdot s^{-1})$ at all frequencies and in all test scenarios in both healthy subjects and COPD patients (Table 5.4 and 5.5). At baseline, the variability in Xrs was less than 0.1

cm·H_2O/(L·s^{-1}) and 0.5 cm·H_2O/(L·s^{-1}) in healthy subjects and COPD patients, respectively, and was not significantly different with other test conditions.

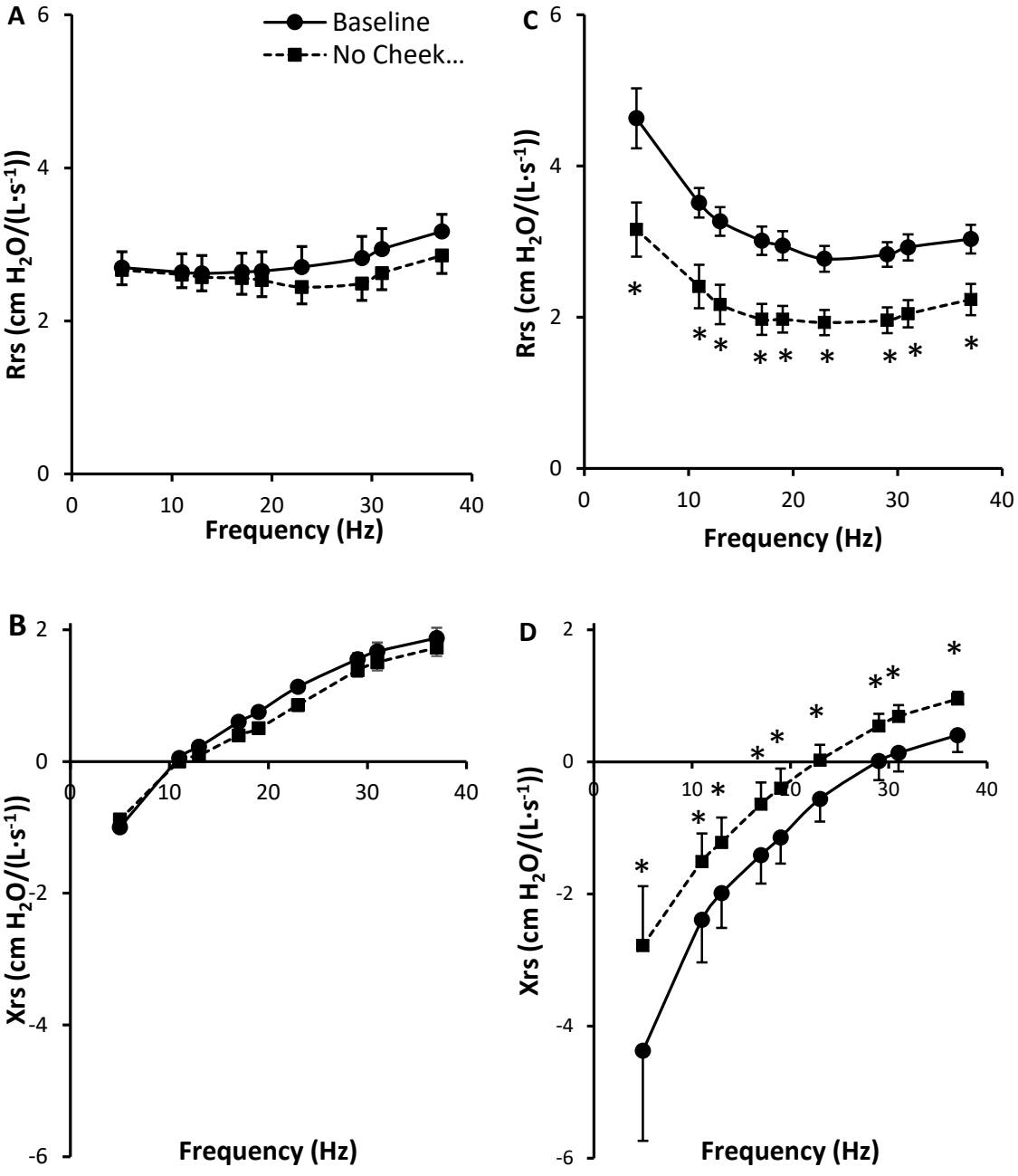

Figure 5.3. Effect of upper airway shunt artefact on Rrs and Xrs measured in healthy subjects (A & B) and COPD patients (C & D).
Error bars indicate SE. Rrs, respiratory system resistance; Xrs, respiratory system reactance. *, $p < 0.05$.

Table 5.4. Standard deviation of Xrs (in $cm \cdot H_2O/(L \cdot s^{-1})$) at selected frequencies obtained when healthy subjects performed oscillometry with and without cheek support, and during maximum flexion and extension of the neck.

	5 Hz	**19 Hz**	**37 Hz**
Baseline	0.08	0.10	0.07
No cheek support	0.06	0.06	0.10
Flexion	0.12	0.14	0.17
Extension	0.12	0.12	0.11

Note: Xrs, respiratory system reactance.

5.4.5 Reliability and reproducibility of oscillometry outcomes

Respiratory impedance values were not significantly different from day to day in all the study participants (Figure 5.4) and the test-retest reliability of oscillometry from day-to-day was very high as demonstrated by a Pearson's *r* of 0.99. The day-to-day reproducibility of Rrs computed from the two study visits was surprisingly less than 1 % in healthy adults, and less than 6 % in COPD patients. In healthy adults, day-to-day COV for Rrs,5, Rrs, 19 and Rrs,37 was 0.3 %, 0.5 %, and 0.5 %, respectively, while COPD patients demonstrated a COV of 5.2 %, 4.1 %, and 3.5 % at similar frequencies. Furthermore, in healthy adults and COPD patients, the within-test COV for Rrs across all oscillation frequencies obtained from the 3 consecutive measures was below 8 % on both study visits (Figure 5.5). The within-day variability of Xrs was not significantly different from day to day (not shown). While the within-day repeatability of Rrs was generally not significantly different from day to day (Figure 5.5), a slight improvement was noted at the high-frequency range (29 – 37 Hz) on the second day in patients with COPD (Figure 5.5B).

5.5 Discussion

In the present study, we assessed the effect of incorrect head and neck angles and the repeatability and variability of respiratory impedance in a portable, handheld oscillometry

device. Day-to-day reproducibility and variability were also assessed. The main finding of this study was that respiratory impedance was not significantly affected by flexion of the neck. Indeed, while significant changes were detected during extension of the neck, the changes were still modest. In addition, handheld oscillometry demonstrated high within-day repeatability and low variability in both healthy adults and patients with COPD. Furthermore, day-to-day reproducibility of Rrs, and the test-retest reliability of oscillometry was also very high. Thus, the assessment of respiratory mechanics using handheld oscillometry is accurate, repeatable and reliable.

Table 5.5. Standard deviation of Xrs (in $cm \cdot H_2O/(L \cdot s^{-1})$) at selected frequencies obtained when COPD patients performed oscillometry with and without cheek support, and during 20° flexion and 10°extension of the neck.

	5 Hz	**19 Hz**	**37 Hz**
Baseline	0.41	0.15	0.19
No cheek support	0.29	0.08	0.09
Flexion	0.54	0.14	0.11
Extension	0.47	0.16	0.13

Note: Xrs, respiratory system reactance; COPD, chronic obstructive pulmonary disease.

To the best of our knowledge, this is the first study to carefully characterize the effects of incorrect head and neck angles on oscillometry outcomes. It is also the first study to assess the repeatability and variability of respiratory impedance in a portable, handheld oscillometry device. Compared to previous reports [14, 311], we found slightly better repeatability of Rrs. Indeed, the mean within-day COV for Rrs was less than 8 % in both healthy adults and COPD patients, and this indicates high repeatability. Similarly, the day-to-day COV for Rrs was lower than 1 % in healthy subjects, and less than 6 % in COPD patients. By comparison, within-day COV for Rrs in healthy and COPD patients in other studies is between 8–16 % [14, 15, 311], while day-to-day COV for Rrs from 3 or more consecutive days is about 11 % in healthy normals [312, 313], and about 16 % in COPD patients [313]. Furthermore, baseline variability in Xrs in this study was less than

0.1 cm·H_2O/(L·s^{-1}) and 0.5 cm·H_2O/(L·s^{-1}) in healthy subjects and COPD patients, respectively and these results are comparable to those reported by Timmins *et al* [14].

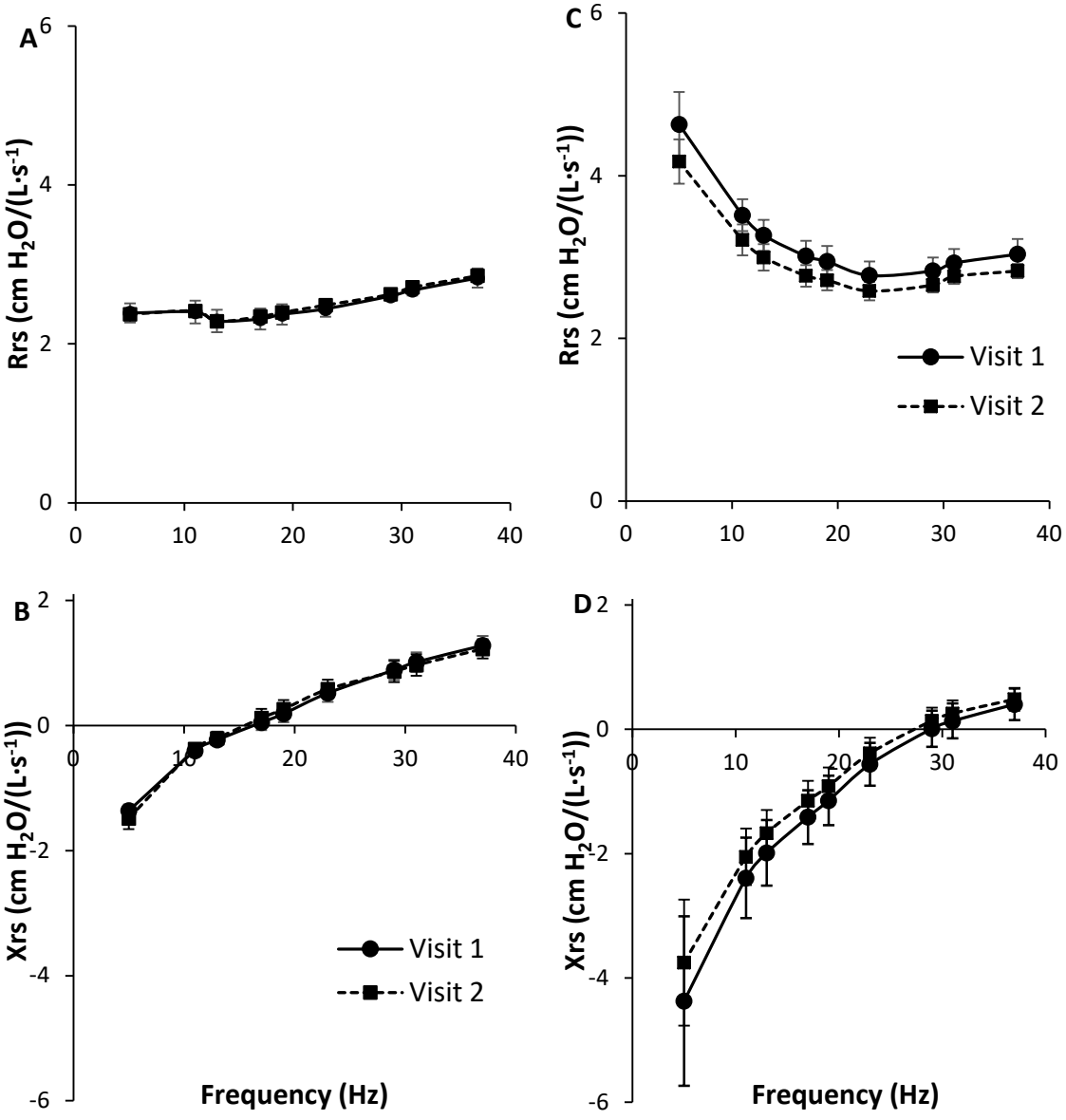

Figure 5.4. Rrs and Xrs measured during two separate study visits in healthy adults (A & B) and COPD patients (C & D).
Error bars indicate SE. Rrs, respiratory system resistance; Xrs, respiratory system reactance.

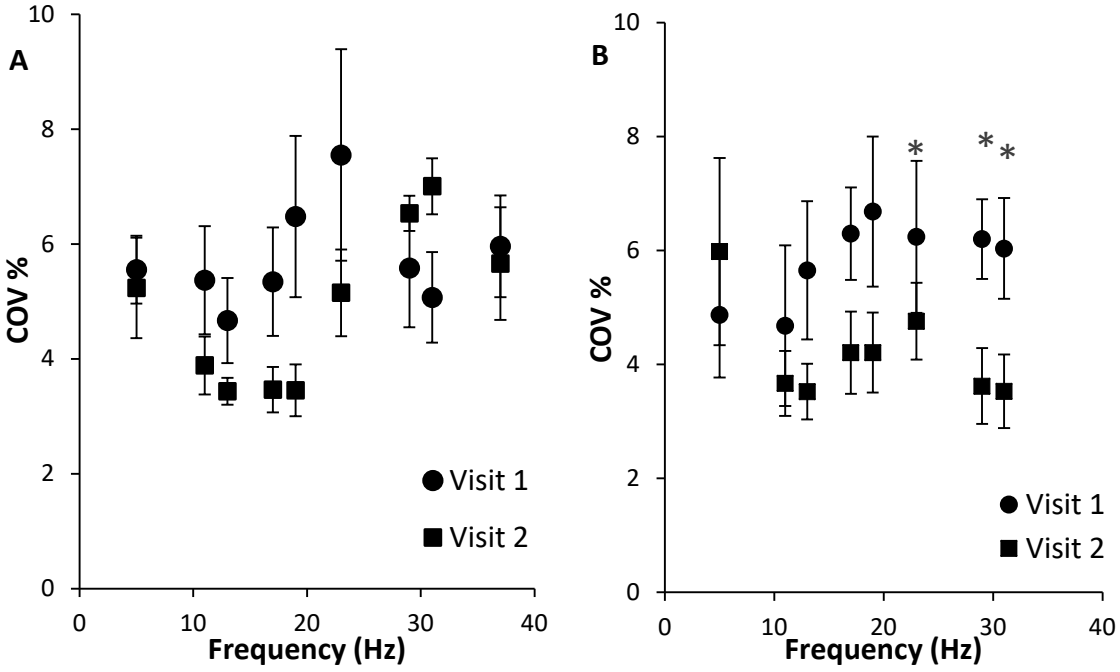

Figure 5.5. Within-day COV for Rrs versus frequency from three consecutive measures obtained during study visits 1 and 2 in healthy adults (A) and COPD patients (B). Error bars indicate SE. COV, coefficient of variation; *, $p < 0.05$.

We specifically explored the effects of 20° flexion and 10°extension of the neck on respiratory impedance in COPD patients because these angles are perhaps at the borderline of head-and-neck position that would not be rejected during clinical oscillometry. To compare, we also measured the effect of maximal flexion and extension of the neck on respiratory impedance in healthy subjects to get an idea of the possible magnitude of this effect at its extremes. We reasoned that the effect would be larger in healthy subjects because the change in upper airway impedance would be a larger fraction of the total respiratory impedance [314-317]. However, the overall magnitude of this effect was modest since maximal extension only affected Xrs at mid- and high-frequencies (Figure 5.2). We also recorded significant reductions in Rrs during 10°extension of the neck in COPD patients, but this reduction was also modest – with only 8.4 ± 3.8 % reduction in Rrs recorded at 5 Hz. Although the ATS/ERS guidelines recommend that flexion of the head should be avoided during oscillometry [177], our results indicate that this artefact had no significant effect on respiratory impedance in

both healthy subjects and patients with COPD. However, maximal flexion in healthy individuals increased the COV for Rrs to 13.3±3.1 % at high oscillation frequencies (Table 5.2), thereby reducing measurement repeatability.

The upper airway shunt is an established artefact that is known to bias oscillometric outcomes, especially in patients with high respiratory system impedance [315]. This artefact occurs when a significant portion of the forced oscillatory energy directed at the central and peripheral airways is diverted to compliant structures of the upper airways with only a small portion reaching the lower respiratory system [314, 318]. This is commonly observed in patients with high respiratory impedance, such as asthma and COPD patients [171, 175], as well as young children who have low Crs and high Rrs [189, 319]. To the best of our knowledge, this is the first study to investigate the biasing effect of upper airway shunt artefact on the repeatability and variability of oscillometry outcomes. Indeed, the upper airway shunt significantly affected respiratory impedance in patients with COPD but no significant effect was observed among the healthy subjects. This finding is not entirely consistent with previous reports in the literature which established that cheek-support can significantly affect respiratory impedance in both healthy subjects and patients with COPD [314, 318, 320]. The lack of significant difference we found is likely due to the small number of subjects in our study. Also, the mechanical properties of the chest wall are a significant contributor to total respiratory system mechanics [155], and although it is likely smaller than the contribution arising from the shunt provided by the soft tissues of the upper airway, it is possible that the contribution from the chest wall might be significantly altered when study participants raise their arms to support the cheeks, as suggested by Goldman *et al* [321].

We did not measure the effect of small angle variations on oscillometry outcomes in healthy subjects; however, the magnitude of this effect would have been smaller than the modest changes we reported at extreme angles. While we anticipate that small angle flexion and extension of head and neck would have similar effects to that reported in COPD (Figure 5.1), it is also possible that such deviations in recommended head-and-neck position may produce larger changes than those measured in the patients with COPD. This is because the changes in the upper airway in a healthy subject would

normally be a greater fraction of the total respiratory impedance, since lung impedance is lower [314]. However, given that baseline Rrs at higher frequencies which largely represent central airway [304] (Figure 5.3) was comparable between healthy subjects and COPD patients, it is quite likely that changes in respiratory impedance caused by small deviations in head-and-neck angles would be similar in both subject groups.

In summary, we have shown that oscillometry is not easily susceptible to upper airway artefacts caused by flexion of the head and neck, but small angle extension of the neck slightly reduced Rrs in COPD patients while maximal neck extension reduced Xrs in healthy subjects. We also demonstrated that cheek support only affected oscillometry outcomes in high impedance patients. Lastly, this study demonstrated that oscillometry is highly repeatable with low variability and high test-retest reliability. These results provide important information on the clinical validity of handheld oscillometry as a novel tool for assessment of respiratory system mechanics.

Chapter 6: Discussion

This chapter provides a brief description of the novel findings and contributions from this thesis. This is followed by a description of the significance and implications of the findings presented in this thesis. A summary of original contributions from this thesis is also provided and the chapter ends with suggestions for future work.

6.1 Overview of findings

As reviewed in Chapters 1 and 2, obesity is a condition associated with respiratory diseases such as asthma, obstructive sleep apnea, and obesity hypoventilation syndrome. The most common respiratory symptoms in obesity are wheeze and dyspnea. These symptoms can be difficult to manage in individuals with obesity, and bronchodilators are reported to exhibit limited effectiveness. However, symptoms often improve with weight loss but traditional methods of testing lung function such as spirometry and plethysmography often show little or no changes with weight loss. This lack of information from traditional lung function tests has hampered our ability to unravel the mechanisms that link obesity and weight loss to changes in respiratory health.

Furthermore, lung function is typically assessed in the upright position either with spirometry or oscillometry but the respiratory symptoms of obesity are reported to be worse when subjects lie down and this affects their sleep quality. Because this information was important to asses, as was the role weight loss or weight gain could play in altering lung function, we directly assessed the respiratory consequences of obesity and weight loss in the supine position. We used a portable oscillometry system which facilitated comparison of upright pulmonary mechanics to supine mechanics, and used this to characterize weight-induced changes in upright and supine pulmonary mechanics. Results were also compared to traditional methods of testing lung function. Finally, to investigate the contribution of obesity to the reported ineffectiveness of bronchodilators, we measured breathing mechanics with oscillometry before and after inhalation of

salbutamol, and compared baseline bronchodilator responsiveness to the responsiveness measured after weight loss.

In Chapter 3, we reported that prior to weight loss, subjects did not respond to the bronchodilator when assessed in the upright position with either spirometry or oscillometry; however, a remarkable response to the bronchodilator was observed after bariatric surgery at the 5-week postoperative assessment. We also found that oscillometry was sensitive to early changes in pulmonary mechanics induced by weight loss at the 5-week postoperative assessment, while spirometry was not. While oscillometry and spirometry had been previously reported to change with obesity and weight loss [9-11, 117, 204, 305], the difference in sensitivity was not well characterized. The higher sensitivity of oscillometry to the changes induced by weight loss indicate that oscillometry is a more appropriate technique to probe and monitor changes in pulmonary function in these subjects.

As described above, we found that modest weight loss at the 5 week time point induced a substantial reduction in supine Rrs,19 and this change correlated strongly with improvements in sleep quality (Chapter 3). Thus, in Chapter 4, we were interested to see if greater magnitudes of weight loss would lead to larger changes in supine respiratory mechanics so we extended the follow-up period of our study to 6 months. We hypothesized that additional weight loss would induce greater improvements in pulmonary mechanics, which would in turn lead to greater improvements in sleep quality. We also hypothesized that the magnitude of the changes in pulmonary mechanics would be larger in the supine position than in the upright position. To test these hypotheses, we re-evaluated sleep quality and pulmonary mechanics and function in our study subjects using the PSQI, oscillometry, spirometry, and plethysmography. The main findings of this study were that (i) surgically-induced weight loss of 21.4 ± 7.1 kg induced large changes in Rrs, Xrs, Ers and FRC at the postoperative assessment conducted at 6 months but no significant changes were detected with spirometry, and (ii) compared to the postoperative assessment conducted at 5 weeks (Chapter 3)[300], a greater improvement in sleep quality was recorded at the postoperative assessment conducted at 6 months and, for the first time, the global sleep score of our study participants exceeded the threshold

for good sleep quality. While there was a small but significant role for airway recruitment with weight loss, it is likely that changes in chest wall stiffness played an important role in reducing overall respiratory system stiffness.

Lastly, in Chapter 5, this thesis investigated the biasing effects of upper airway shunt artefact and inappropriate positioning of head and neck during oscillometry on the repeatability of oscillometry outcomes. The within-session, and day-to-day reproducibility of a portable, handheld oscillometry device was also characterized. Oscillometry outcomes were not significantly affected by flexion of the neck but significant changes were recorded during extension. In general, outcomes from oscillometry were highly repeatable, even in the presence of artefacts caused by the upper airway shunt artefact and incorrect head and neck angles. Furthermore, the test-retest reliability of the device was also found to be very high.

6.2 Significance and Implications of Findings

We demonstrated for the first time that improvements in respiratory mechanics and BD responsiveness occur very early after weight loss, mostly in the supine position, and this resulted in improved sleep quality. These findings are significant because it further confirms the notion that obese individuals have poor lung mechanics and this leads to respiratory symptoms in susceptible individuals. In particular, this may be the first study to indicate the likely importance of the association between improvements in respiratory mechanics and sleep quality. This study also demonstrates that oscillometry is an effective tool to objectively and sensitively measure the improvements in breathing mechanics that occur with weight loss or with changes induced by pharmacological intervention.

The oscillometry technique objectively measures the mechanics of moving air into and out of the lungs and it revealed that obese individuals have some difficulty moving air into and out of their lungs, by quantifying respiratory impedance. Our study revealed that obese individuals have worse respiratory mechanics and likely experience greater

difficulty moving air into and out of the lungs, particularly in the supine position. Obesity has long been associated with respiratory symptoms such as wheeze [22] and dyspnea [23] but traditional methods of testing pulmonary function such as spirometry and plethysmography are usually insensitive to these symptoms [22, 23, 117, 143, 149, 243]. For example, FEV_1, which is the gold standard for assessing airway obstruction, is typically normal in those with obesity [22, 23, 322] possibly because spirometry predominantly measures different mechanical factors of the lung, particularly the dynamic flow limitation that occur during forced exhalation at lung volumes well above FRC. This behavior may be normal in obese individuals. Indeed, we have shown that obesity has greater effects on lung mechanics near FRC (since oscillometry is conducted at FRC). These effects may be greater at FRC than might occur during the forced exhalation maneuvers performed during spirometry. The early improvements in lung mechanics measured with oscillometry after weight loss indicate that oscillometry is sensitive to breathing difficulty (assessed from the PSQI) and small changes in lung mechanics.

When we used oscillometry to probe the contribution of obesity and weight loss to the efficacy of the short-acting bronchodilator – salbutamol, we found that prior to weight loss, subjects did not respond to bronchodilator when evaluated in the upright position with spirometry and oscillometry; however, with modest weight loss, bronchodilator responsiveness returned to the normal range. This finding may be important because it may indicate that obesity plays an important role in impairing response to bronchodilators. Our subjects were severely obese, and this result implies that obesity could impair therapeutic effectiveness of the BD. An important result is that weight loss potentiates responsiveness to BD, likely through mechanisms such as restoration of airway-parenchymal tethering (Figure 6.1).

This study has also contributed to the discussion on the role of obesity and weight loss on changes in respiratory system mechanics. Obesity reduces chest wall compliance and this results in lung compression and narrower airways (Figure 6.1). The resultant reduction in airway-parenchymal tethering forces can also lead to collapse of small airways in the lung periphery (Figure 6.2). These changes could contribute to respiratory symptoms and

poor sleep quality which many obese individuals exhibit. Here, we have shown that weight loss increases the compliance of the chest wall and this results in lung decompression and increased airway caliber (Figures 6.1 and 6.2). These changes restore the tethering forces that alveolar walls exert around the small airways likely leading to increased recruitment of small airways and perhaps explaining the increased responsiveness to BD. That is, at baseline, due to weak tethering forces, the changes in airway caliber induced by BD might be relatively small, despite the attendant relaxation of airway smooth muscle. This may explain the restoration of BD responsiveness to the normal range with weight loss.

The improvements in multiple measures of respiratory system mechanics, sleep quality and BD responsiveness reported here underscore the importance of weight loss in improving pulmonary function, response to inhaled BD therapy, and overall quality of life. To reiterate, these findings have provided new evidence that severely obese subjects may have poor sleep quality due to poor pulmonary mechanics but weight loss can reverse these changes.

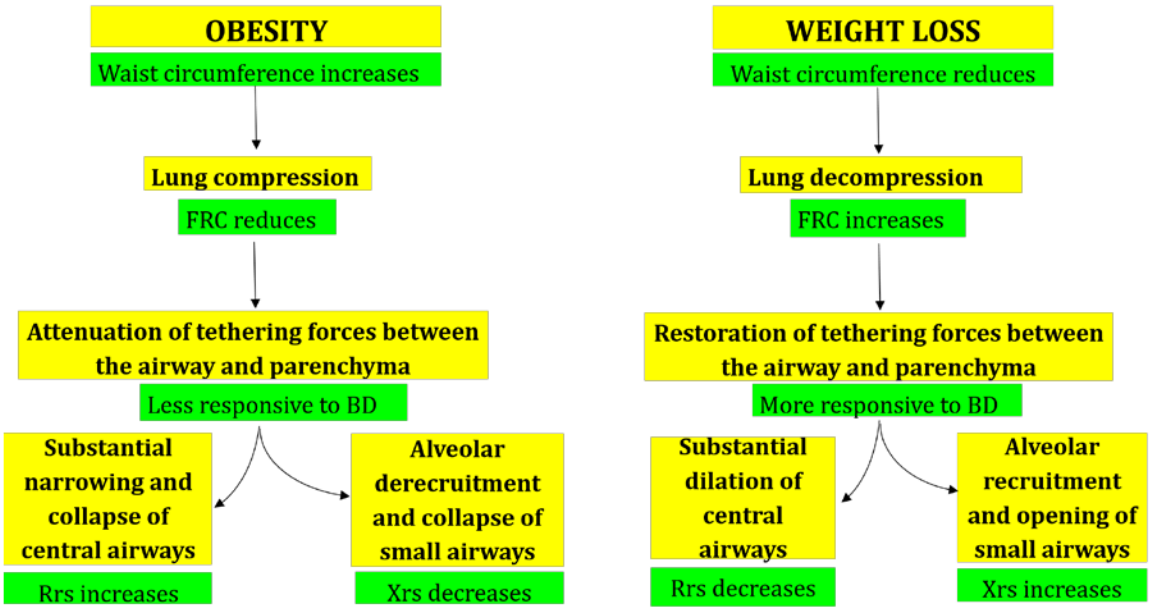

Figure 6.1. Summary of findings at baseline and after weight loss.
The likely mechanisms for these findings are also briefly provided.

The changes in respiratory system mechanics and function reported here were measured with a novel handheld oscillometry device. We measured the reliability and repeatability of this device and also characterized its response to the biasing effects of inappropriate neck angles with respect to the well-established effects of the upper airway shunt artefact. Our results demonstrate that the oscillometry technique is highly repeatable and reliable, and is not easily susceptible to changes due small deviations in the recommended positioning of the head and neck.

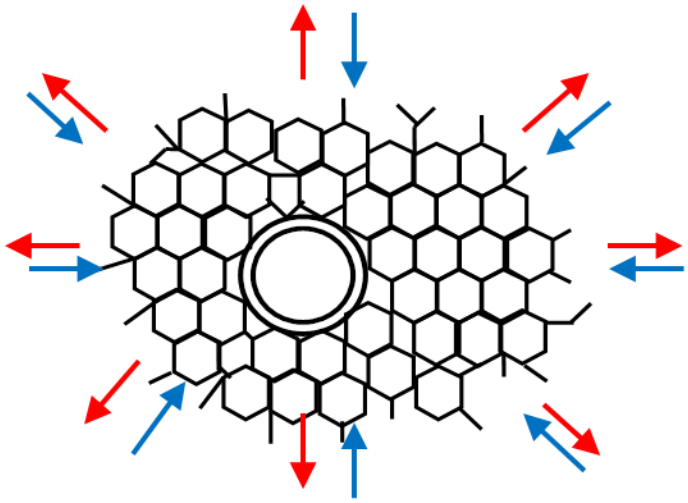

Figure 6.2. Schematic showing how stress distribution within the lung can alter airway caliber.
The circle in the center represents a single airway that is tethered by parenchyma (alveoli) represented by hexagons. The blue arrows pointing inward show the forces of compression that could potentially act on the parenchyma in obesity while the red arrows pointing outwards show how weight loss can decompress the lung resulting in a significant increase the tethering forces between the airway and parenchyma

6.3 Limitations

The findings presented in this thesis must be interpreted in light of the limitations of this work. Oscillometry by itself cannot be used to separate the contributions of the chest wall from lung mechanics, in particular from peripheral lung elastance. This is because respiratory impedance measurements represents contribution from all the components of

the respiratory system, including the upper airway, lungs and chest wall. This means that when we assessed the changes in pulmonary mechanics due to obesity and weight loss, we cannot directly distinguish changes due to chest wall elastance from changes in lung elastance that occur with recruitment following improved tethering of the peripheral airways to the parenchyma. However, changes in peripheral airway recruitment can be assessed from its association with changes in ventilation heterogeneity caused by obstruction of the small airways in the lung periphery. This heterogeneity can be indirectly assessed from the negative frequency dependence of Rrs, which is typically a hallmark feature of asthma [173, 302-304].

Interestingly, we did not find substantial evidence of peripheral airway heterogeneity from assessing frequency dependence of Rrs in our study participants. Indeed, we only recorded slight negative frequency dependence of Rrs in our obese cohort, probably because these subjects do not have asthma. Other studies of respiratory mechanics in obesity have reported significant negative frequency dependence of Rrs which also appears to have been larger than we report [9, 11, 305]. The exact reason for this potential difference in frequency dependence of Rrs is not yet known, but it may be due to differences in the devices used [9-11, 117, 305]. Depending on the manufacturer, some oscillometry devices may exhibit inherently larger frequency dependence of Rrs [196] likely be due to differences arising from the hardware of each device, and also differences in the perturbation waveforms generated, since the device with the highest frequency dependence of Rrs used impulses rather than a mixed sine wave oscillatory signal [196, 197].

Another important factor indicating that ventilation heterogeneity was mild in our subjects was that the coefficient of determination obtained from fitting our Zrs data to a 2-compartment model of the respiratory system was higher than 0.95, and this suggests that the behavior of our Zrs data can be explained by the series 2-compartment model, which is a homogenous model. Indeed, we found that the frequency dependence of Rrs measured by subtracting Rrs,19 from Rrs,6 decreased significantly with weight loss, indicating some reduction in ventilation heterogeneity. This means that while peripheral airway recruitment likely played some role in the changes measured in Xrs,6 and model

elastance with weight loss, it is likely that the reduction in the stiffness of the chest wall may have contributed more to these changes. It is well-established that obesity changes the compliance of the chest wall [245] and it is possible that the weight loss reported by our subjects contributed to changes in the stiffness of the chest wall; thus contributing to the reduction in Ers.

Our study only focused on obese women, and this could limit the generalizability of our findings to obese men and children. Indeed, the effects of weight gain on pulmonary function is different in men and women. While weight gain is associated with small reductions in both FEV_1 and FVC, the effect has been found to be greater in men than women [323, 324], presumably because men comparatively gain more abdominal fat than women. This may imply that compared to women, larger changes in Rrs and Xrs would occur with weight loss in men.

Another potential limitation of this study is that our study protocol required participants to perform both spirometry and oscillometry. However, the deep inspirations associated with the forced breathing maneuvers of spirometry are known to transiently modify bronchomotor tone and airway caliber, particularly in studies comparing asthma to health [306, 307]. This could potentially have some effect on our oscillometry results obtained in the supine position since these measurements followed spirometry in the upright position. However, our subjects did not have asthma, so we anticipated that they had low bronchomotor tone; thus this effect was likely small. In any case, to help control for this effect, we ensured that participants followed the same testing sequence at all 3 time points of the study.

We cannot reliably infer from our data that respiratory impedance is not affected by cheek support in healthy subjects because of our small sample size. Cheek support significantly affected respiratory impedance in patients with COPD but no significant effect was observed among the healthy subjects. This finding is not entirely consistent with previous reports in the literature which established that cheek-support can significantly affect respiratory impedance in both healthy subjects and patients with COPD [314, 318, 320]. The lack of significant difference which we report here is likely due to the small number of subjects in our study. Also, the mechanical properties of the

chest wall are a significant contributor to total respiratory system mechanics [155], and although it is likely smaller than the contribution arising from the shunt provided by the soft tissues of the upper airway, it is possible that the contribution from the chest wall might be significantly altered when study participants raise their arms to support the cheeks, as suggested by Goldman *et al* [321].

6.4 Statement of Original Contributions

The following is a brief summary of the original contributions from this thesis.

1) I measured changes in respiratory mechanics in both the upright and supine positions. My study was the first to measure pulmonary mechanics in obese subjects in the supine position with oscillometry. This allowed us to compare the changes measured in the supine position with weight loss to those recorded in the upright position.

2) I showed that improvements in respiratory mechanics occur very early after weight loss in the supine position. This was the first study to report changes in lung mechanics as early as 5 weeks after weight loss surgery, and also the first to report changes in respiratory mechanics from baseline at both 5 weeks and 6 months.

3) I measured the effect of weight reduction on BD responsiveness in obese female adults without asthma. This is the first study to examine the effect of weight loss on responsiveness to short-acting β_2-adrenergic agonist. My study showed that BD responsiveness was impaired in severe obesity, but weight loss restored this response to the normal range.

4) I demonstrated a significant correlation between reduction in supine Rrs with weight loss and improvement in sleep quality. This is the first study to examine how weight loss affects the relationship between supine respiratory mechanics

and sleep quality. Here, I demonstrated that the breathing discomfort caused by narrowing of the upper and central airways in the supine position contributes significantly to poor sleep quality in obesity.

5) I showed that oscillometry was more sensitive than spirometry to changes in respiratory system mechanics and function induced by weight loss and short-acting bronchial agonist. Indeed, spirometry was not sensitive enough to detect changes in pulmonary function even after 6 months of substantial weight loss.

6) I showed that a portable handheld oscillometry device demonstrated high within-day repeatability and low and acceptable variability in the assessment of respiratory mechanics in health and in COPD. I also showed that handheld oscillometry demonstrated high day-to-day reproducibility and high test-retest reliability.

7) I showed that portable handheld oscillometry device was not easily susceptible to artefacts caused by small deviations in the recommended positioning of the head and neck during oscillometry.

6.5 Suggestions for Future Research

There are several possible research questions that may be pursued as a result of the studies in my thesis.

6.5.1 Upright and supine respiratory mechanics in obese subjects with asthma and changes following bariatric surgery

The weight loss study described in this thesis focused only on obese subjects without asthma because we wanted to explore the respiratory symptoms of obesity by itself. A future direction for research would be to replicate this study in obese subjects with

asthma and compare the results to those without asthma. Compared to healthy weight individuals, asthma is more severe in obese individuals [55] and several studies have shown that obese asthmatics are less likely to respond favorably to controller therapies [4]. There are several types of controller therapies available for treating asthma. These include: inhaled short-acting $beta_2$ adrenergic agonist, inhaled corticosteroids (ICS), inhaled long acting $beta_2$ adrenergic agonist (LABA), leukotriene receptor antagonist (LTRA) and monoclonal antibodies such as omalizumab. We have shown that response to short-acting $beta_2$ adrenergic agonist is attenuated in obese subjects without asthma but the responsiveness to this bronchodilator is restored to the normal range following weight loss. Given the clinical implications of this findings, it is important to conduct a similar study in obese subjects with asthma since they are usually prescribed short-acting bronchodilators for relief of sudden symptoms. This study would help determine whether weight loss is effective in reversing asthma symptoms in obese individuals with asthma. It would also help determine whether short acting bronchodilators become more effective in these subjects after weight loss.

6.5.2 The contribution of chest wall and airway-parenchymal tethering to overall mechanics of the respiratory system in obesity and the changes following weight loss

My thesis has shown that obesity significantly affected the impedance of the respiratory system and significant changes in respiratory mechanics were measured with weight loss. However, we were unable to quantitatively determine the relative contribution of the chest wall and lungs to the overall changes in respiratory mechanics caused by obesity and weight loss. A future study could quantify the contribution of the chest wall or lung airways to the changes in pulmonary mechanics. Such a study would involve the use of an esophageal balloon to differentiate lung from chest wall mechanics, and thus help identify the degree of airway derecruitment occurring in obesity relative to changes in chest wall.

6.5.3 Changes in upper airway geometry with weight loss assessed with dual-energy X-ray absorptiometry

In Chapter 3, we speculated that two possible mechanisms could account for the lack of BD responsiveness in our obese subjects prior to weight loss. One mechanism was that prior to weight loss, most of the bronchodilator administered to the subjects was most likely deposited within the oropharynx. However, with weight loss, there was a reduction in the amount of redundant supraglottic oropharyngeal tissue and this resulted in the exposure of these subjects to effectively higher doses of the drug. The increased responsiveness to BD measured after weight loss in these subjects is therefore a direct consequence of increased exposure to the drug. The second possible mechanism behind the lack of BD responsiveness prior to weight loss has to do with the attenuation of airway-parenchymal tethering forces with obesity. The increased responsiveness to BD is therefore due to the restoration of the tethering forces with weight loss. A future study could use imaging modalities such as dual-energy X-ray absorptiometry (DEXA) to determine whether the reduction in the amount of redundant supraglottic oropharyngeal tissue with weight loss correlates with the increase in BD responsiveness. The outcome of the study on the contribution of chest wall mechanics and airway-parenchymal tethering to changes in lung mechanics with weight loss suggested in 6.5.1 would also be pivotal here because it would provide crucial information on whether effective airway-parenchymal tethering is absolutely required for inhaled bronchodilators to take effect.

6.6 Conclusion

In conclusion, the work outlined in this thesis demonstrates that the oscillometry technique is more sensitive than spirometry at measuring breathing difficulty. Changes in respiratory mechanics induced by weight loss were detected with the oscillometry technique as early as 5 weeks following bariatric surgery, but no significant changes in spirometry outcomes were recorded even after 6 months of massive weight loss. This thesis also demonstrates that portable handheld oscillometry is not easily susceptible to upper airway artefacts caused by small deviations in recommended head-and-neck

positioning during oscillometry. Handheld oscillometry was shown to be highly repeatable and highly reliable with low and acceptable variability. Taken together, these findings provide important information on the clinical utility of handheld oscillometry as a novel tool for assessment of respiratory mechanics.

References

[1] Sideleva O, Suratt B T, Black K E, Tharp W G, Pratley R E, Forgione P, Dienz O, Irvin C G and Dixon A E 2012 Obesity and asthma: an inflammatory disease of adipose tissue not the airway *Am J Respir Crit Care Med* **186** 598-605

[2] Romero-Corral A, Caples S M, Lopez-Jimenez F and Somers V K 2010 Interactions between obesity and obstructive sleep apnea: implications for treatment *Chest* **137** 711-9

[3] Piper A J and Grunstein R R 2011 Obesity hypoventilation syndrome: mechanisms and management *Am J Respir Crit Care Med* **183** 292-8

[4] Sutherland E R, Goleva E, Strand M, Beuther D A and Leung D Y 2008 Body mass and glucocorticoid response in asthma *Am J Respir Crit Care Med* **178** 682-7

[5] Camargo C A, Boulet L P, Sutherland E R, Busse W W, Yancey S W, Emmett A H, Ortega H G and Ferro T J 2010 Body mass index and response to asthma therapy: fluticasone propionate/salmeterol versus montelukast *J Asthma* **47** 76-82

[6] Peters-Golden M, Swern A, Bird S S, Hustad C M, Grant E and Edelman J M 2006 Influence of body mass index on the response to asthma controller agents *Eur Respir J* **27** 495-503

[7] Boulet L P and Franssen E 2007 Influence of obesity on response to fluticasone with or without salmeterol in moderate asthma *Respir Med* **101** 2240-7

[8] Rodrigo G J and Plaza V 2007 Body mass index and response to emergency department treatment in adults with severe asthma exacerbations: a prospective cohort study *Chest* **132** 1513-9

[9] Oppenheimer B W, Macht R, Goldring R M, Stabile A, Berger K I and Parikh M 2012 Distal airway dysfunction in obese subjects corrects after bariatric surgery *Surg Obes Relat Dis* **8** 582-9

[10] Zerah-Lancner F, Boyer L, Rezaiguia-Delclaux S, D'Ortho M P, Drouot X, Guilloteau-Schoennagel I, Ribeil S, Delclaux C, Adnot S and Tayar C 2011 Airway responsiveness measured by forced oscillation technique in severely obese patients, before and after bariatric surgery *J Asthma* **48** 818-23

[11] Al-Alwan A, Bates J H, Chapman D G, Kaminsky D A, DeSarno M J, Irvin C G and Dixon A E 2014 The nonallergic asthma of obesity. A matter of distal lung compliance *Am J Respir Crit Care Med* **189** 1494-502

[12] Peppard P E, Ward N R and Morrell M J 2009 The impact of obesity on oxygen desaturation during sleep-disordered breathing *Am J Respir Crit Care Med* **180** 788-93

[13] Ferretti A, Giampiccolo P, Cavalli A, Milic-Emili J and Tantucci C 2001 Expiratory flow limitation and orthopnea in massively obese subjects *Chest* **119** 1401-8

[14] Timmins S C, Coatsworth N, Palnitkar G, Thamrin C, Farrow C E, Schoeffel R E, Berend N, Diba C, Salome C M and King G G 2012 Day-to-day variability of oscillatory impedance and spirometry in asthma and COPD *Respir Physiol Neurobiol*

[15] van den Elshout F J, van de Woestijne K P and Folgering H T 1990 Variations of respiratory impedance with lung volume in bronchial hyperreactivity *Chest* **98** 358-64

[16] Macklem P T 1998 The mechanics of breathing *Am J Respir Crit Care Med* **157** S88-94

[17] Ward S A 2004 Physiology of breathing II *Surgery (Oxford)* **22** 230-4

[18] Ward J 2005 Physiology of breathing I *Surgery (Oxford)* **23** 419-24

[19] Weibel E 1963 *Morphometry of the Human Lung* (New York: Academic Press)

[20] Daniels C B and Orgeig S 2003 Pulmonary surfactant: the key to the evolution of air breathing *News Physiol Sci* **18** 151-7

[21] Sideleva O and Dixon A E 2014 The many faces of asthma in obesity *J Cell Biochem* **115** 421-6

[22] Schachter L M, Salome C M, Peat J K and Woolcock A J 2001 Obesity is a risk for asthma and wheeze but not airway hyperresponsiveness *Thorax* **56** 4-8

[23] Sin D D, Jones R L and Man S F 2002 Obesity is a risk factor for dyspnea but not for airflow obstruction *Arch Intern Med* **162** 1477-81

[24] Editorial Board Respiratory Diseases in Canada. Respiratory Diseases in Canada. Ottawa, ON: Canadian Lung Association, Health Canada, Statistics Canada, the Canadian Institute for Health Information; 2001. Available from: http://www.statcan.ca/

[25] BTS 2006 The Burden of Lung Disease. A Statistics Report from the British Thoracic Society - 2nd Edition.

[26] American Lung Association: Chronic Obstructive Pulmonary Disease (COPD) Fact Sheet. 2009. Available: http://www.lungusa.org [2009, 28 August]

[27] Canada S *Table 105-0501* - Health indicator profile, annual estimates, by age group and sex, Canada, provinces, territories, health regions and peer groups.

[28] 2013 Annual Report. The Canadian Cystic Fibrosis Registry.

[29] Bateman E D, Hurd S S, Barnes P J, Bousquet J, Drazen J M, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen S E, Pizzichini E, Sullivan S D, Wenzel S E and Zar H J 2008 Global strategy for asthma management and prevention: GINA executive summary *Eur Respir J* **31** 143-78

[30] Hamid Q 2003 Gross pathology and histopathology of asthma *J Allergy Clin Immunol* **111** 431-2

[31] Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, Zanin M E, Zuin R, Maestrelli P, Fabbri L M and Saetta M 2006 Epithelial damage and angiogenesis in the airways of children with asthma *Am J Respir Crit Care Med* **174** 975-81

[32] Laitinen L A, Laitinen A and Haahtela T 1993 Airway mucosal inflammation even in patients with newly diagnosed asthma *Am Rev Respir Dis* **147** 697-704

[33] Bousquet J, Jeffery P K, Busse W W, Johnson M and Vignola A M 2000 Asthma. From bronchoconstriction to airways inflammation and remodeling *Am J Respir Crit Care Med* **161** 1720-45

[34] Xiao C, Puddicombe S M, Field S, Haywood J, Broughton-Head V, Puxeddu I, Haitchi H M, Vernon-Wilson E, Sammut D, Bedke N, Cremin C, Sones J, Djukanović R, Howarth P H, Collins J E, Holgate S T, Monk P and Davies D E 2011 Defective epithelial barrier function in asthma *J Allergy Clin Immunol* **128** 549-56.e1-12

[35] 1998 Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee *Lancet* **351** 1225-32

[36] 2000 A Report from the National Asthma Control Task Force. The Prevention and Management of Asthma in Canada: A Major Challenge Now and in the Future.

[37] Akinbami, L. Asthma prevalence, Health Care Use and Mortality: United States, 2003-05. National Center for Health Statistics, Centers for Disease Control and Prevention, Available from: http://www.cdc.gov/nchs/data/hestat/asthma03-05/asthma03-05.htm. [2010, October 20th].

[38] Ford E S and Mannino D M 2005 Time trends in obesity among adults with asthma in the United States: findings from three national surveys *J Asthma* **42** 91-5

[39] Asthma Burden in Europe.

[40] Krahn M D, Berka, C., Langlois, P., Detsky, A. S. 1996 Direct and indirect costs of asthma in Canada, 1990 *CMAJ* **154** 821-31

[41] Mannino D M 2002 COPD* *Chest* **121** 121S-6S

[42] James A L, Pare P D and Hogg J C 1989 The mechanics of airway narrowing in asthma *Am Rev Respir Dis* **139** 242-6

[43] 2007 National Heart Lung and Blood Institute National Asthma Education and Prevention Program. Expert Panel Report 3: guidelines for the diagnosis and management of asthma. (Bethesda, MD

[44] Crapo R O, Casaburi R, Coates A L, Enright P L, Hankinson J L, Irvin C G, MacIntyre N R, McKay R T, Wanger J S, Anderson S D, Cockcroft D W, Fish J E and Sterk P J 2000 Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999 *Am J Respir Crit Care Med* **161** 309-29

[45] Pellegrino R, Viegi G, Brusasco V, Crapo R O, Burgos F, Casaburi R, Coates A, van der Grinten C P, Gustafsson P, Hankinson J, Jensen R, Johnson D C, MacIntyre N, McKay R, Miller M R, Navajas D, Pedersen O F and Wanger J 2005 Interpretative strategies for lung function tests *Eur Respir J* **26** 948-68

[46] Lougheed M D, Lemiere C, Ducharme F M, Licskai C, Dell S D, Rowe B H, Fitzgerald M, Leigh R, Watson W, Boulet L P and Assembly C T S A C 2012 Canadian Thoracic Society 2012 guideline update: diagnosis and management of asthma in preschoolers, children and adults *Can Respir J* **19** 127-64

[47] Litonjua A A, Sparrow D, Celedon J C, DeMolles D and Weiss S T 2002 Association of body mass index with the development of methacholine airway hyperresponsiveness in men: the Normative Aging Study *Thorax* **57** 581-5

[48] Celedón J C, Palmer L J, Litonjua A A, Weiss S T, Wang B, Fang Z and Xu X 2001 Body mass index and asthma in adults in families of subjects with asthma in Anqing, China *Am J Respir Crit Care Med* **164** 1835-40

[49] Chinn S, Jarvis D, Burney P and Survey E C R H 2002 Relation of bronchial responsiveness to body mass index in the ECRHS. European Community Respiratory Health Survey *Thorax* **57** 1028-33

[50] Sood A, Verhulst S J, Varma A, Eagleton L E, Henkle J Q and Hopkins-Price P 2006 Association of excess weight and degree of airway responsiveness in asthmatics and non-asthmatics *J Asthma* **43** 447-52

[51] Shore S A 2010 Obesity, airway hyperresponsiveness, and inflammation *J Appl Physiol (1985)* **108** 735-43

[52] Scott S, Currie J, Albert P, Calverley P and Wilding J P 2012 Risk of misdiagnosis, health-related quality of life, and BMI in patients who are overweight with doctor-diagnosed asthma *Chest* **141** 616-24

[53] Aaron S D, Vandemheen K L, Boulet L P, McIvor R A, Fitzgerald J M, Hernandez P, Lemiere C, Sharma S, Field S K, Alvarez G G, Dales R E, Doucette S, Fergusson D and Consortium C R C R 2008 Overdiagnosis of asthma in obese and nonobese adults *CMAJ* **179** 1121-31

[54] Wenzel S E 2006 Asthma: defining of the persistent adult phenotypes *Lancet* **368** 804-13

[55] Moore W C, Meyers D A, Wenzel S E, Teague W G, Li H, Li X, D'Agostino R, Castro M, Curran-Everett D, Fitzpatrick A M, Gaston B, Jarjour N N, Sorkness R, Calhoun W J, Chung K F, Comhair S A, Dweik R A, Israel E, Peters S P, Busse W W, Erzurum S C, Bleecker E R and National Heart L n, and Blood Institute's Severe Asthma Research Program 2010 Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program *Am J Respir Crit Care Med* **181** 315-23

[56] Dixon A E, Pratley R E, Forgione P M, Kaminsky D A, Whittaker-Leclair L A, Griffes L A, Garudathri J, Raymond D, Poynter M E, Bunn J Y and Irvin C G 2011 Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation *J Allergy Clin Immunol* **128** 508-15.e1-2

[57] Holguin F, Bleecker E R, Busse W W, Calhoun W J, Castro M, Erzurum S C, Fitzpatrick A M, Gaston B, Israel E, Jarjour N N, Moore W C, Peters S P, Yonas M, Teague W G and Wenzel S E 2011 Obesity and asthma: an association modified by age of asthma onset *J Allergy Clin Immunol* **127** 1486-93.e2

[58] Rabe K F, Hurd S, Anzueto A, Barnes P J, Buist S A, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C and Zielinski J 2007 Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary *Am J Respir Crit Care Med* **176** 532-55

[59] Baraldo S, Turato G and Saetta M 2012 Pathophysiology of the small airways in chronic obstructive pulmonary disease *Respiration* **84** 89-97

[60] Wiggs B R, Bosken C, Paré P D, James A and Hogg J C 1992 A model of airway narrowing in asthma and in chronic obstructive pulmonary disease *Am Rev Respir Dis* **145** 1251-8

[61] Paré P D and Mitzner W 2012 Airway-parenchymal interdependence *Compr Physiol* **2** 1921-35

[62] Siafakas N M, Vermeire P, Pride N B, Paoletti P, Gibson J, Howard P, Yernault J C, Decramer M, Higenbottam T and Postma D S 1995 Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force *Eur Respir J* **8** 1398-420

[63] 1995 Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society *Am J Respir Crit Care Med* **152** S77-121

[64] Global Initiative for Chronic Obstructive Lung Disease.

[65] Murray C J and Lopez A D 1997 Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study *Lancet* **349** 1498-504

[66] Miniño A M, Murphy S L, Xu J and Kochanek K D 2011 Deaths: final data for 2008 *Natl Vital Stat Rep* **59** 1-126

[67] Lacasse Y, Brooks D and Goldstein R S 1999 Trends in the Epidemiology of COPD in Canada, 1980 to 1995* *Chest* **116** 306-13

[68] Miller M R, Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten C P, Gustafsson P, Jensen R, Johnson D C, MacIntyre N, McKay R, Navajas D, Pedersen O F, Pellegrino R, Viegi G and Wanger J 2005 Standardisation of spirometry *Eur Respir J* **26** 319-38

[69] Hutchinson J 1846 On the capacity the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer. *Medico-Chirurgical Transactions (London)* **29** 137-61

[70] Petty T L 2002 John Hutchinson's mysterious machine revisited *Chest* **121** 219S-23S

[71] Enright P L, Lebowitz M D and Cockroft D W 1994 Physiologic measures: pulmonary function tests. Asthma outcome *Am J Respir Crit Care Med* **149** S9-18; discussion S9-20

[72] Vestbo J, Hurd S S, Agustí A G, Jones P W, Vogelmeier C, Anzueto A, Barnes P J, Fabbri L M, Martinez F J, Nishimura M, Stockley R A, Sin D D and Rodriguez-Roisin R 2013 Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary *Am J Respir Crit Care Med* **187** 347-65

[73] Crapo R O, Morris A H and Gardner R M 1981 Reference spirometric values using techniques and equipment that meet ATS recommendations *Am Rev Respir Dis* **123** 659-64

[74] Glindmeyer H W, Lefante J J, McColloster C, Jones R N and Weill H 1995 Blue-collar normative spirometric values for Caucasian and African-American men and women aged 18 to 65 *Am J Respir Crit Care Med* **151** 412-22

[75] Langhammer A, Johnsen R, Gulsvik A, Holmen T L and Bjermer L 2001 Forced spirometry reference values for Norwegian adults: the Bronchial Obstruction in Nord-Trøndelag Study *Eur Respir J* **18** 770-9

[76] Dykstra B J, Scanlon P D, Kester M M, Beck K C and Enright P L 1999 Lung volumes in 4,774 patients with obstructive lung disease *Chest* **115** 68-74

[77] Vandevoorde J, Verbanck S, Schuermans D, Kartounian J and Vincken W 2005 FEV1/FEV6 and FEV6 as an alternative for FEV1/FVC and FVC in the spirometric detection of airway obstruction and restriction *Chest* **127** 1560-4

[78] NHLBI/WHO workshop 1998. Global initiative for chronic obstructive lung disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Last updated: 2006. Available from: http://pharmacoclin.hug-ge.ch/formation/GOLD_WR_06.pdf [2009, August 28]

[79] Vandevoorde J, Verbanck S, Schuermans D, Broekaert L, Devroey D, Kartounian J and Vincken W 2008 Forced vital capacity and forced expiratory volume in six seconds as predictors of reduced total lung capacity *Eur Respir J* **31** 391-5

[80] Boros P W, Franczuk M and Wesolowski S 2004 Value of spirometry in detecting volume restriction in interstitial lung disease patients. Spirometry in interstitial lung diseases *Respiration* **71** 374-9

[81] Hyatt R E, Cowl C T, Bjoraker J A and Scanlon P D 2009 Conditions associated with an abnormal nonspecific pattern of pulmonary function tests *Chest* **135** 419-24

[82] 1995 American Thoracic Society. Standardization of Spirometry, 1994 Update. *Am J Respir Crit Care Med* **152** 1107-36

[83] Aggarwal A N and Agarwal R 2007 The new ATS/ERS guidelines for assessing the spirometric severity of restrictive lung disease differ from previous standards *Respirology* **12** 759-62

[84] Macklem P T and Mead J 1967 Resistance of central and peripheral airways measured by a retrograde catheter *J Appl Physiol* **22** 395-401

[85] Mead J 1970 The lung's "quiet zone" *N Engl J Med* **282** 1318-9

[86] Levine G, Housley E, MacLeod P and Macklem P T 1970 Gas exchange abnormalities in mild bronchitis and asymptomatic asthma *N Engl J Med* **282** 1277-82

[87] Hogg J C, Williams J, Richardson J B, Macklem P T and Thurlbeck W M 1970 Age as a factor in the distribution of lower-airway conductance and in the pathologic anatomy of obstructive lung disease *N Engl J Med* **282** 1283-7

[88] Cosio M, Ghezzo H, Hogg J C, Corbin R, Loveland M, Dosman J and Macklem P T 1978 The relations between structural changes in small airways and pulmonary-function tests *N Engl J Med* **298** 1277-81

[89] McFadden E R and Linden D A 1972 A reduction in maximum mid-expiratory flow rate. A spirographic manifestation of small airway disease *Am J Med* **52** 725-37

[90] Gelb A F, Williams A J and Zamel N 1983 Spirometry. FEV1 vs FEF25-75 percent *Chest* **84** 473-4

[91] Aaron S D, Dales R E and Cardinal P 1999 How accurate is spirometry at predicting restrictive pulmonary impairment? *Chest* **115** 869-73

[92] Sorkness R L, Bleecker E R, Busse W W, Calhoun W J, Castro M, Chung K F, Curran-Everett D, Erzurum S C, Gaston B M, Israel E, Jarjour N N, Moore W C, Peters S P, Teague W G, Wenzel S E and National Heart L n, and Blood Institute Severe Asthma Research Program 2008 Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation *J Appl Physiol (1985)* **104** 394-403

[93] Miller M R, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, van der Grinten C P, Gustafsson P, Jensen R, Johnson D C, MacIntyre N, McKay R, Navajas D, Pedersen O F, Pellegrino R, Viegi G and Wanger J 2005 General considerations for lung function testing *Eur Respir J* **26** 153-61

[94] Lavorini F 2013 The challenge of delivering therapeutic aerosols to asthma patients *ISRN Allergy* **2013** 102418

[95] Kesten S and Rebuck A S 1994 Is the short-term response to inhaled beta-adrenergic agonist sensitive or specific for distinguishing between asthma and COPD? *Chest* **105** 1042-5

[96] Richter D C, Joubert J R, Nell H, Schuurmans M M and Irusen E M 2008 Diagnostic value of post-bronchodilator pulmonary function testing to distinguish between stable, moderate to severe COPD and asthma *Int J Chron Obstruct Pulmon Dis* **3** 693-9

[97] Dundas I, Chan E Y, Bridge P D and McKenzie S A 2005 Diagnostic accuracy of bronchodilator responsiveness in wheezy children *Thorax* **60** 13-6

[98] Network B T S S I G 2008 British Guideline on the Management of Asthma *Thorax* **63 Suppl 4** iv1-121

[99] Herland K, Akselsen J P, Skjønsberg O H and Bjermer L 2005 How representative are clinical study patients with asthma or COPD for a larger "real life" population of patients with obstructive lung disease? *Respir Med* **99** 11-9

[100] Boushey H A, Sorkness C A, King T S, Sullivan S D, Fahy J V, Lazarus S C, Chinchilli V M, Craig T J, Dimango E A, Deykin A, Fagan J K, Fish J E, Ford J G, Kraft M, Lemanske R F, Leone F T, Martin R J, Mauger E A, Pesola G R, Peters S P, Rollings N J, Szefler S J, Wechsler M E, Israel E and National Heart L n, and Blood Institute's Asthma Clinical Research Network 2005 Daily versus as-needed corticosteroids for mild persistent asthma *N Engl J Med* **352** 1519-28

[101] Travers J, Marsh S, Williams M, Weatherall M, Caldwell B, Shirtcliffe P, Aldington S and Beasley R 2007 External validity of randomised controlled trials in asthma: to whom do the results of the trials apply? *Thorax* **62** 219-23

[102] Castro-Rodríguez J A, Holberg C J, Morgan W J, Wright A L and Martinez F D 2001 Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years *Am J Respir Crit Care Med* **163** 1344-9

[103] Barlow S E and Committee E 2007 Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report *Pediatrics* **120 Suppl 4** S164-92

[104] Tantisira K G, Litonjua A A, Weiss S T, Fuhlbrigge A L and Group C A M P R 2003 Association of body mass with pulmonary function in the Childhood Asthma Management Program (CAMP) *Thorax* **58** 1036-41

[105] Dixon A E, Shade D M, Cohen R I, Skloot G S, Holbrook J T, Smith L J, Lima J J, Allayee H, Irvin C G, Wise R A and Centers A L A-A C R 2006 Effect of obesity on clinical presentation and response to treatment in asthma *J Asthma* **43** 553-8

[106] Curry J J 1946 THE ACTION OF HISTAMINE ON THE RESPIRATORY TRACT IN NORMAL AND ASTHMATIC SUBJECTS *J Clin Invest* **25** 785-91

[107] Curry J J 1947 COMPARATIVE ACTION OF ACETYL-BETA-METHYL CHOLINE AND HISTAMINE ON THE RESPIRATORY TRACT IN NORMALS, PATIENTS WITH HAY FEVER, AND SUBJECTS WITH BRONCHIAL ASTHMA *J Clin Invest* **26** 430-8

[108] Murray A B, Ferguson A C and Morrison B 1981 Airway responsiveness to histamine as a test for overall severity of asthma in children *J Allergy Clin Immunol* **68** 119-24

[109] Sterk P J, Fabbri L M, Quanjer P H, Cockcroft D W, O'Byrne P M, Anderson S D, Juniper E F and Malo J L 1993 Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society *Eur Respir J Suppl* **16** 53-83

[110] Program N A E a P 2007 Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007 *J Allergy Clin Immunol* **120** S94-138

[111] Chai H, Farr R S, Froehlich L A, Mathison D A, McLean J A, Rosenthal R R, Sheffer A L, Spector S L and Townley R G 1975 Standardization of bronchial inhalation challenge procedures *J Allergy Clin Immunol* **56** 323-7

[112] Cockcroft D W, Murdock K Y, Berscheid B A and Gore B P 1992 Sensitivity and specificity of histamine PC20 determination in a random selection of young college students *J Allergy Clin Immunol* **89** 23-30

[113] Woolcock A J, Salome C M and Yan K 1984 The shape of the dose-response curve to histamine in asthmatic and normal subjects *Am Rev Respir Dis* **130** 71-5

[114] Schneider A, Gindner L, Tilemann L, Schermer T, Dinant G J, Meyer F J and Szecsenyi J 2009 Diagnostic accuracy of spirometry in primary care *BMC Pulm Med* **9** 31

[115] Enright P L 2015 COUNTERPOINT: Should Oscillometry Be Used to Screen for Airway Disease? No *Chest* **148** 1135-7

[116] Kanengiser S and Dozor A J 1994 Forced expiratory maneuvers in children aged 3 to 5 years *Pediatr Pulmonol* **18** 144-9

[117] Zerah F, Harf A, Perlemuter L, Lorino H, Lorino A M and Atlan G 1993 Effects of obesity on respiratory resistance *Chest* **103** 1470-6

[118] Lazarus R, Sparrow D and Weiss S T 1997 Effects of obesity and fat distribution on ventilatory function: the normative aging study *Chest* **111** 891-8

[119] Rubinstein I, Zamel N, DuBarry L and Hoffstein V 1990 Airflow limitation in morbidly obese, nonsmoking men *Ann Intern Med* **112** 828-32

[120] Chapman K R, Boulet L P, Rea R M and Franssen E 2008 Suboptimal asthma control: prevalence, detection and consequences in general practice *Eur Respir J* **31** 320-5

[121] LindenSmith J, Morrison D, Deveau C and Hernandez P 2004 Overdiagnosis of asthma in the community *Can Respir J* **11** 111-6

[122] Lötvall J, Inman M and O'Byrne P 1998 Measurement of airway hyperresponsiveness: new considerations *Thorax* **53** 419-24

[123] Lusuardi M, De Benedetto F, Paggiaro P, Sanguinetti C M, Brazzola G, Ferri P and Donner C F 2006 A randomized controlled trial on office spirometry in asthma and COPD in standard general practice: data from spirometry in Asthma and COPD: a comparative evaluation Italian study *Chest* **129** 844-52

[124] Yawn B P, Enright P L, Lemanske R F, Israel E, Pace W, Wollan P and Boushey H 2007 Spirometry can be done in family physicians' offices and alters clinical decisions in management of asthma and COPD *Chest* **132** 1162-8

[125] Stanbrook M B and Kaplan A 2008 The error of not measuring asthma *CMAJ* **179** 1099-102

[126] He X O, D'Urzo A, Jugovic P, Jhirad R, Sehgal P and Lilly E 2015 Differences in spirometry interpretation algorithms: influence on decision making among primary-care physicians *NPJ Prim Care Respir Med* **25** 15008

[127] Wanger J, Clausen J L, Coates A, Pedersen O F, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten C P, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller M R, Navajas D, Pellegrino R and Viegi G 2005 Standardisation of the measurement of lung volumes *Eur Respir J* **26** 511-22

[128] Stocks J, Godfrey S, Beardsmore C, Bar-Yishay E, Castile R and Society E A T F o S f I R F T E R S A T 2001 Plethysmographic measurements of lung volume and airway resistance. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/ American Thoracic Society *Eur Respir J* **17** 302-12

[129] Dubois A B, Botelho S Y and Comroe J H, Jr. 1956 A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease *J Clin Invest* **35** 327-35

[130] Brown R, Leith D E and Enright P L 1998 Multiple breath helium dilution measurement of lung volumes in adults *Eur Respir J* **11** 246-55

[131] Sivan Y, Hammer J and Newth C J 1994 Measurement of high lung volumes by nitrogen washout method *J Appl Physiol (1985)* **77** 1562-4

[132] McNulty W and Usmani O S 2014 Techniques of assessing small airways dysfunction *2014*

[133] Dubois A B, Botelho S Y, Bedell G N, Marshall R and Comroe J H, Jr. 1956 A rapid plethysmographic method for measuring thoracic gas volume: a comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects *J Clin Invest* **35** 322-6

[134] Coates A L, Peslin R, Rodenstein D and Stocks J 1997 Measurement of lung volumes by plethysmography *Eur Respir J* **10** 1415-27

[135] Bates J 1989 Correcting for the thermodynamic characteristics of a body plethysmograph *Annals of Biomedical Engineering* **17** 647

[136] Criée C P, Sorichter S, Smith H J, Kardos P, Merget R, Heise D, Berdel D, Köhler D, Magnussen H, Marek W, Mitfessel H, Rasche K, Rolke M, Worth H, Jörres R A and Care W G f B P o t G S f P a R 2011 Body plethysmography--its principles and clinical use *Respir Med* **105** 959-71

[137] Quanjer P H, Tammeling G J, Cotes J E, Pedersen O F, Peslin R and Yernault J C 1993 Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society *Eur Respir J Suppl* **16** 5-40

[138] Quanjer J S a P 1995 Reference values for residual volume, functional residual capacity and total lung capacity. ATS Workshop on Lung Volume Measurements. Official Statement of The European Respiratory Society *Eur Respir J* **8:** 492-506

[139] BEDELL G N, WILSON W R and SEEBOHM P M 1958 Pulmonary function in obese persons *J Clin Invest* **37** 1049-60

[140] Barrera F, Reidenberg M M and Winters W L 1967 Pulmonary function in the obese patient *Am J Med Sci* **254** 785-96

[141] GILBERT R, SIPPLE J H and AUCHINCLOSS J H 1961 Respiratory control and work of breathing in obese subjects *J Appl Physiol* **16** 21-6

[142] Emirgil C and Sobol B J 1973 The effects of weight reduction on pulmonary function and the sensitivity of the respiratory center in obesity *Am Rev Respir Dis* **108** 831-42

[143] Ray C S, Sue D Y, Bray G, Hansen J E and Wasserman K 1983 Effects of obesity on respiratory function *Am Rev Respir Dis* **128** 501-6

[144] Thomas P S, Cowen E R, Hulands G and Milledge J S 1989 Respiratory function in the morbidly obese before and after weight loss *Thorax* **44** 382-6

[145] NAIMARK A and CHERNIACK R M 1960 Compliance of the respiratory system and its components in health and obesity *J Appl Physiol* **15** 377-82

[146] Sharp J T, Henry J P, Sweany S K, Meadows W R and Pietras R J 1964 Effects of mass loading the respiratory system in man *J Appl Physiol* **19** 959-66

[147] Watson R A and Pride N B 2005 Postural changes in lung volumes and respiratory resistance in subjects with obesity *J Appl Physiol* **98** 512-7

[148] Sampson M G and Grassino A E 1983 Load compensation in obese patients during quiet tidal breathing *J Appl Physiol Respir Environ Exerc Physiol* **55** 1269-76

[149] Collins L C, Hoberty P D, Walker J F, Fletcher E C and Peiris A N 1995 The effect of body fat distribution on pulmonary function tests *Chest* **107** 1298-302

[150] Owens M W, Kinasewitz G T and Anderson W M 1987 Clinical significance of an isolated reduction in residual volume *Am Rev Respir Dis* **136** 1377-80

[151] Yernault J C and Gibson G J 1982 Interactions between lung and chest wall in restrictive ventilatory defects *Bull Eur Physiopathol Respir* **18** 395-401

[152] Iyer V N, Schroeder D R, Parker K O, Hyatt R E and Scanlon P D 2011 The nonspecific pulmonary function test: longitudinal follow-up and outcomes *Chest* **139** 878-86

[153] Dubois A B, Brody A W, Lewis D H and Burgess B F, Jr. 1956 Oscillation mechanics of lungs and chest in man *J Appl Physiol* **8** 587-94

[154] Landser F J, Nagles J, Demedts M, Billiet L and van de Woestijne K P 1976 A new method to determine frequency characteristics of the respiratory system *J Appl Physiol* **41** 101-6

[155] Nagels J, Landser F J, van der Linden L, Clement J and Van de Woestijne K P 1980 Mechanical properties of lungs and chest wall during spontaneous breathing *J Appl Physiol* **49** 408-16

[156] Smith H J, Reinhold, P., Goldman, M. D. 2005 Forced oscillation technique and impulse oscillometry *European Respiratory Monograph* **31** 72 - 105

[157] OTIS A B, MCKERROW C B, BARTLETT R A, MEAD J, MCILROY M B, SELVER-STONE N J and RADFORD E P 1956 Mechanical factors in distribution of pulmonary ventilation. *J Appl Physiol* **8** 427-43

[158] Grimby G, Takishima T, Graham W, Macklem P and Mead J 1968 Frequency dependence of flow resistance in patients with obstructive lung disease. *J Clin Invest* **47** 1455-65

[159] OTIS A, MCKERROW C, BARTLETT R, MEAD J, MCILROY M, SELVER-STONE N and RADFORD E J 1956 Mechanical factors in distribution of pulmonary ventilation. *J Appl Physiol* **8** 427-43

[160] Kaczka D W, Ingenito E P, Body S C, Duffy S E, Mentzer S J, DeCamp M M and Lutchen K R 2001 Inspiratory lung impedance in COPD: effects of PEEP and immediate impact of lung volume reduction surgery *J Appl Physiol* **90** 1833-41

[161] Pasker H G, Mertens I, Clement J, Van de Woestijne KPP 1994 Normal values of total respiratory input resistance and reactance for adult men and women *Eur Respir Rev* **4** 134-7

[162] Dellaca R L, Andersson Olerud M, Zannin E, Kostic P, Pompilio P P, Hedenstierna G, Pedotti A and Frykholm P 2009 Lung recruitment assessed by total respiratory system input reactance. *Intensive Care Med* **35** 2164-72

[163] Dellaca R L, Santus P, Aliverti A, Stevenson N, Centanni S, Macklem P T, Pedotti A and Calverley P M 2004 Detection of expiratory flow limitation in COPD using the forced oscillation technique *Eur Respir J* **23** 232-40

[164] Fisher A, DuBois A and Hyde R 1968 Evaluation of the forced oscillation technique for the determination of resistance to breathing. *J Clin Invest* **47** 2045-57

[165] Peslin R, Papon J, Duviver C and Richalet J 1975 Frequency response of the chest: modeling and parameter estimation. *J Appl Physiol* **39** 523-34

[166] Michaelson E D, Grassman E D and Peters W R 1975 Pulmonary mechanics by spectral analysis of forced random noise *J Clin Invest* **56** 1210-30

[167] Cavalcanti J V, Lopes A J, Jansen J M and Melo P L 2006 Detection of changes in respiratory mechanics due to increasing degrees of airway obstruction in asthma by the forced oscillation technique *Respir Med* **100** 2207-19

[168] Cavalcanti J V, Lopes A J, Jansen J M and de Melo P L 2006 Using the forced oscillation technique to evaluate bronchodilator response in healthy volunteers and in asthma patients presenting a verified positive response *J Bras Pneumol* **32** 91-8

[169] Oppenheimer B W, Goldring R M, Herberg M E, Hofer I S, Reyfman P A, Liautaud S, Rom W N, Reibman J and Berger K I 2007 Distal airway function in symptomatic subjects with normal spirometry following World Trade Center dust exposure *Chest* **132** 1275-82

[170] Johnson M K, Birch M, Carter R, Kinsella J and Stevenson R D 2005 Use of reactance to estimate transpulmonary resistance *Eur Respir J* **25** 1061-9

[171] Paredi P, Goldman M, Alamen A, Ausin P, Usmani O S, Pride N B and Barnes P J 2010 Comparison of inspiratory and expiratory resistance and reactance in patients with asthma and chronic obstructive pulmonary disease *Thorax* **65** 263-7

[172] Postma D S and Rabe K F 2015 The Asthma-COPD Overlap Syndrome *N Engl J Med* **373** 1241-9

[173] Bates J H, Irvin C G, Farré R and Hantos Z 2011 Oscillation mechanics of the respiratory system *Compr Physiol* **1** 1233-72

[174] Vink G R, Arets H G, van der Laag J and van der Ent C K 2003 Impulse oscillometry: a measure for airway obstruction *Pediatr Pulmonol* **35** 214-9

[175] Di Mango A M, Lopes A J, Jansen J M and Melo P L 2006 Changes in respiratory mechanics with increasing degrees of airway obstruction in COPD: detection by forced oscillation technique *Respir Med* **100** 399-410

[176] Friedman S M, Maslow C B, Reibman J, Pillai P S, Goldring R M, Farfel M R, Stellman S D and Berger K I 2011 Case-control study of lung function in World Trade Center Health Registry area residents and workers *Am J Respir Crit Care Med* **184** 582-9

[177] Oostveen E, MacLeod D, Lorino H, Farre R, Hantos Z, Desager K and Marchal F 2003 The forced oscillation technique in clinical practice: methodology, recommendations and future developments *Eur Respir J* **22** 1026-41

[178] Bates J H T 2009 *Lung mechanics : an inverse modeling approach* (Cambridge, UK ; New York: Cambridge University Press)

[179] Kirsten A M, Watz H, Brindicci C, Piccinno A and Magnussen H 2015 Effects of beclomethason/formoterol and budesonide/formoterol fixed combinations on lung function and airway inflammation in patients with mild to moderate asthma--an exploratory study *Pulm Pharmacol Ther* **31** 79-84

[180] Ducharme F and Davis G 1998 Respiratory resistance in the emergency department: a reproducible and responsive measure of asthma severity. *Chest* **113** 1566-72

[181] Carvalhaes-Neto N, Lorino H, Gallinari C, Escolano S, Mallet A, Zerah F, Harf A and Macquin-Mavier I 1995 Cognitive function and assessment of lung function in the elderly. *Am J Respir Crit Care Med* **152** 1611-5

[182] Ducharme F M, Davis G M and Ducharme G R 1998 Pediatric reference values for respiratory resistance measured by forced oscillation *Chest* **113** 1322-8

[183] Janssens J P, Nguyen M C, Herrmann F R and Michel J P 2001 Diagostic value of respiratory impedance measurements in elderly subjects *Respiratory Medicine* **95** 415-22

[184] Desager K N, Buhr W, Willemen M, van Bever H P, de Backer W, Vermeire P A and Landser F J 1991 Measurement of total respiratory impedance in infants by the forced oscillation technique *J Appl Physiol* **71** 770-6

[185] Srikasibhandha S 1983 [Measurement of respiratory resistance in newborn infants with the oscillation method] *Anaesthesist* **32** 214-8

[186] Peslin R, Felicio da Silva J, Duvivier C and Chabot F 1993 Respiratory mechanics studied by forced oscillations during artificial ventilation *Eur Respir J* **6** 772-84

[187] Van de Woestijne K P 1993 The forced oscillation technique in intubated, mechanically-ventilated patients *Eur Respir J* **6** 767-9

[188] van Noord J A, Clement J, van de Woestijne K P and Demedts M 1989 Total respiratory resistance and reactance as a measurement of response to bronchial challenge with histamine *Am Rev Respir Dis* **139** 921-6

[189] Delacourt C, Lorino H, Herve-Guillot M, Reinert P, Harf A and Housset B 2000 Use of the forced oscillation technique to assess airway obstruction and reversibility in children *Am J Respir Crit Care Med* **161** 730-6

[190] HAYDEN MARK J, PETAK F, HANTOS Z, HALL G and SLY PETER D 1998 Using Low-frequency Oscillation to Detect Bronchodilator Responsiveness in Infants *Am. J. Respir. Crit. Care Med.* **157** 574-9

[191] Hellinckx J, De Boeck K and Demedts M 1998 No paradoxical bronchodilator response with forced oscillation technique in children with cystic fibrosis *Chest* **113** 55-9

[192] Klug B and Bisgaard H 1996 Measurement of lung function in awake 2-4-year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique, and transcutaneous measurement of oxygen versus whole-body plethysmography *Pediatr Pulmonol* **21** 290-300

[193] Clement J, Landser F J and Van de Woestijne K P 1983 Total resistance and reactance in patients with respiratory complaints with and without airways obstruction *Chest* **83** 215-20

[194] Gimeno F, van der Weele L T, Koeter G H and van Altena R 1992 Forced oscillation technique. Reference values for total respiratory resistance obtained with the Siemens Siregnost FD5 *Ann Allergy* **68** 155-8

[195] Dellacà R L, Duffy N, Pompilio P P, Aliverti A, Koulouris N G, Pedotti A and Calverley P M 2007 Expiratory flow limitation detected by forced oscillation and negative expiratory pressure *Eur Respir J* **29** 363-74

[196] Oostveen E, Boda K, van der Grinten C P, James A L, Young S, Nieland H and Hantos Z 2013 Respiratory impedance in healthy subjects: baseline values and bronchodilator response *Eur Respir J* **42** 1513-23

[197] Calverley P M and Farre R 2013 Putting noninvasive lung mechanics into context *Eur Respir J* **42** 1435-7

[198] 1998 Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health *Obes Res* **6 Suppl 2** 51S-209S

[199] Krebs N F, Himes J H, Jacobson D, Nicklas T A, Guilday P and Styne D 2007 Assessment of child and adolescent overweight and obesity *Pediatrics* **120 Suppl 4** S193-228

[200] Cole T J, Bellizzi M C, Flegal K M and Dietz W H 2000 Establishing a standard definition for child overweight and obesity worldwide: international survey *BMJ* **320** 1240-3

[201] Schatz M, Hsu J W, Zeiger R S, Chen W, Dorenbaum A, Chipps B E and Haselkorn T 2014 Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma *J Allergy Clin Immunol* **133** 1549-56

[202] Mosen D M, Schatz M, Magid D J and Camargo C A 2008 The relationship between obesity and asthma severity and control in adults *J Allergy Clin Immunol* **122** 507-11.e6

[203] Simard B, Turcotte H, Marceau P, Biron S, Hould F S, Lebel S, Marceau S and Boulet L P 2004 Asthma and sleep apnea in patients with morbid obesity: outcome after bariatric surgery *Obes Surg* **14** 1381-8

[204] Boulet L P, Turcotte H, Martin J and Poirier P 2012 Effect of bariatric surgery on airway response and lung function in obese subjects with asthma *Respir Med* **106** 651-60

[205] Scott H A, Gibson P G, Garg M L, Pretto J J, Morgan P J, Callister R and Wood L G 2013 Dietary restriction and exercise improve airway inflammation and clinical outcomes in overweight and obese asthma: a randomized trial *Clin Exp Allergy* **43** 36-49

[206] Beuther D A and Sutherland E R 2007 Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies *Am J Respir Crit Care Med* **175** 661-6

[207] Hakala K, Stenius-Aarniala B and Sovijärvi A 2000 Effects of weight loss on peak flow variability, airways obstruction, and lung volumes in obese patients with asthma *Chest* **118** 1315-21

[208] Sutherland T J, McLachlan C R, Sears M R, Poulton R and Hancox R J 2016 The relationship between body fat and respiratory function in young adults *Eur Respir J*

[209] Weisberg S P, McCann D, Desai M, Rosenbaum M, Leibel R L and Ferrante A W 2003 Obesity is associated with macrophage accumulation in adipose tissue *J Clin Invest* **112** 1796-808

[210] Strissel K J, Stancheva Z, Miyoshi H, Perfield J W, DeFuria J, Jick Z, Greenberg A S and Obin M S 2007 Adipocyte death, adipose tissue remodeling, and obesity complications *Diabetes* **56** 2910-8

[211] Travers R L, Motta A C, Betts J A, Bouloumié A and Thompson D 2015 The impact of adiposity on adipose tissue-resident lymphocyte activation in humans *Int J Obes (Lond)* **39** 762-9

[212] Rausch M E, Weisberg S, Vardhana P and Tortoriello D V 2008 Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration *Int J Obes (Lond)* **32** 451-63

[213] Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker D J, Engleman E, Winer D and Dosch H M 2009 Normalization of obesity-associated insulin resistance through immunotherapy *Nat Med* **15** 921-9

[214] Spencer L A and Weller P F 2010 Eosinophils and Th2 immunity: contemporary insights *Immunol Cell Biol* **88** 250-6

[215] Wu D, Molofsky A B, Liang H E, Ricardo-Gonzalez R R, Jouihan H A, Bando J K, Chawla A and Locksley R M 2011 Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis *Science* **332** 243-7

[216] Nijhuis J, Rensen S S, Slaats Y, van Dielen F M, Buurman W A and Greve J W 2009 Neutrophil activation in morbid obesity, chronic activation of acute inflammation *Obesity (Silver Spring)* **17** 2014-8

[217] Ciepiela O, Ostafin M and Demkow U 2015 Neutrophils in asthma--a review *Respir Physiol Neurobiol* **209** 13-6

[218] Poglio S, De Toni-Costes F, Arnaud E, Laharrague P, Espinosa E, Casteilla L and Cousin B 2010 Adipose tissue as a dedicated reservoir of functional mast cell progenitors *Stem Cells* **28** 2065-72

[219] Liu J, Divoux A, Sun J, Zhang J, Clément K, Glickman J N, Sukhova G K, Wolters P J, Du J, Gorgun C Z, Doria A, Libby P, Blumberg R S, Kahn B B, Hotamisligil G S and Shi G P 2009 Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice *Nat Med* **15** 940-5

[220] Considine R V, Sinha M K, Heiman M L, Kriauciunas A, Stephens T W, Nyce M R, Ohannesian J P, Marco C C, McKee L J and Bauer T L 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans *N Engl J Med* **334** 292-5

[221] Bruno A, Pace E, Chanez P, Gras D, Vachier I, Chiappara G, La Guardia M, Gerbino S, Profita M and Gjomarkaj M 2009 Leptin and leptin receptor expression in asthma *J Allergy Clin Immunol* **124** 230-7, 7.e1-4

[222] Van Heek M, Compton D S, France C F, Tedesco R P, Fawzi A B, Graziano M P, Sybertz E J, Strader C D and Davis H R 1997 Diet-induced obese mice develop peripheral, but not central, resistance to leptin *J Clin Invest* **99** 385-90

[223] Reaven G M 1995 Pathophysiology of insulin resistance in human disease *Physiol Rev* **75** 473-86

[224] Bulló M, Garcia-Lorda P and Salas-Salvadó J 2002 Plasma soluble tumor necrosis factor alpha receptors and leptin levels in normal-weight and obese women: effect of adiposity and diabetes *Eur J Endocrinol* **146** 325-31

[225] Bulló M, García-Lorda P, Megias I and Salas-Salvadó J 2003 Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression *Obes Res* **11** 525-31

[226] Bastard J P, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H and Hainque B 2000 Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss *J Clin Endocrinol Metab* **85** 3338-42

[227] Roth C L, Kratz M, Ralston M M and Reinehr T 2011 Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children *Metabolism* **60** 445-52

[228] Zaldivar F, McMurray R G, Nemet D, Galassetti P, Mills P J and Cooper D M 2006 Body fat and circulating leukocytes in children *Int J Obes (Lond)* **30** 906-11

[229] Zhou B O, Yue R, Murphy M M, Peyer J G and Morrison S J 2014 Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow *Cell Stem Cell* **15** 154-68

[230] Lord G M, Matarese G, Howard J K, Baker R J, Bloom S R and Lechler R I 1998 Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression *Nature* **394** 897-901

[231] Scott H A, Gibson P G, Garg M L and Wood L G 2011 Airway inflammation is augmented by obesity and fatty acids in asthma *Eur Respir J* **38** 594-602

[232] van Veen I H, Ten Brinke A, Sterk P J, Rabe K F and Bel E H 2008 Airway inflammation in obese and nonobese patients with difficult-to-treat asthma *Allergy* **63** 570-4

[233] Shore S A, Rivera-Sanchez Y M, Schwartzman I N and Johnston R A 2003 Responses to ozone are increased in obese mice *J Appl Physiol (1985)* **95** 938-45

[234] Lu F L, Johnston R A, Flynt L, Theman T A, Terry R D, Schwartzman I N, Lee A and Shore S A 2006 Increased pulmonary responses to acute ozone exposure in obese db/db mice *Am J Physiol Lung Cell Mol Physiol* **290** L856-65

[235] Johnston R A, Theman T A and Shore S A 2006 Augmented responses to ozone in obese carboxypeptidase E-deficient mice *Am J Physiol Regul Integr Comp Physiol* **290** R126-33

[236] Sood A, Ford E S and Camargo C A 2006 Association between leptin and asthma in adults *Thorax* **61** 300-5

[237] Mai X M, Böttcher M F and Leijon I 2004 Leptin and asthma in overweight children at 12 years of age *Pediatr Allergy Immunol* **15** 523-30

[238] Johnston R A, Theman T A, Lu F L, Terry R D, Williams E S and Shore S A 2008 Diet-induced obesity causes innate airway hyperresponsiveness to methacholine and enhances ozone-induced pulmonary inflammation *J Appl Physiol (1985)* **104** 1727-35

[239] Holguin F, Rojas M, Brown L A and Fitzpatrick A M 2011 Airway and plasma leptin and adiponectin in lean and obese asthmatics and controls *J Asthma* **48** 217-23

[240] Watson R A, Pride N B, Thomas E L, Fitzpatrick J, Durighel G, McCarthy J, Morin S X, Ind P W and Bell J D 2010 Reduction of total lung capacity in obese men: comparison of total intrathoracic and gas volumes *J Appl Physiol (1985)* **108** 1605-12

[241] Behazin N, Jones S B, Cohen R I and Loring S H 2010 Respiratory restriction and elevated pleural and esophageal pressures in morbid obesity *J Appl Physiol (1985)* **108** 212-8

[242] Li A M, Chan D, Wong E, Yin J, Nelson E A and Fok T F 2003 The effects of obesity on pulmonary function *Arch Dis Child* **88** 361-3

[243] Jones R L and Nzekwu M M 2006 The effects of body mass index on lung volumes *Chest* **130** 827-33

[244] 2013 *Obesity and lung disease: A guide to management* (New York: Springer)

[245] Pelosi P, Croci M, Ravagnan I, Tredici S, Pedoto A, Lissoni A and Gattinoni L 1998 The effects of body mass on lung volumes, respiratory mechanics, and gas exchange during general anesthesia *Anesth Analg* **87** 654-60

[246] Hedenstierna G and Santesson J 1976 Breathing mechanics, dead space and gas exchange in the extremely obese, breathing spontaneously and during anaesthesia with intermittent positive pressure ventilation *Acta Anaesthesiol Scand* **20** 248-54

[247] SHARP J T, HENRY J P, SWEANY S K, MEADOWS W R and PIETRAS R J 1964 THE TOTAL WORK OF BREATHING IN NORMAL AND OBESE MEN *J Clin Invest* **43** 728-39

[248] Chapman D G, Irvin C G, Kaminsky D A, Forgione P M, Bates J H and Dixon A E 2014 Influence of distinct asthma phenotypes on lung function following weight loss in the obese *Respirology* **19** 1170-7

[249] Chapman D G, Berend N, King G G and Salome C M 2008 Increased airway closure is a determinant of airway hyperresponsiveness *Eur Respir J* **32** 1563-9

[250] Pellegrino R, Gobbi A, Antonelli A, Torchio R, Gulotta C, Pellegrino G M, Dellacà R, Hyatt R E and Brusasco V 2014 Ventilation heterogeneity in obesity *J Appl Physiol (1985)* **116** 1175-81

[251] Bustos P, Amigo H, Oyarzún M and Rona R J 2005 Is there a causal relation between obesity and asthma? Evidence from Chile *Int J Obes (Lond)* **29** 804-9

[252] Aaron S D, Fergusson D, Dent R, Chen Y, Vandemheen K L and Dales R E 2004 Effect of weight reduction on respiratory function and airway reactivity in obese women *Chest* **125** 2046-52

[253] Naoum C, Kritharides L, Ing A, Falk G L and Yiannikas J 2015 Changes in lung volumes and gas trapping in patients with large hiatal hernia *Clin Respir J*

[254] Salome C M, Munoz P A, Berend N, Thorpe C W, Schachter L M and King G G 2008 Effect of obesity on breathlessness and airway responsiveness to methacholine in non-asthmatic subjects *Int J Obes (Lond)* **32** 502-9

[255] Ding D J, Martin J G and Macklem P T 1987 Effects of lung volume on maximal methacholine-induced bronchoconstriction in normal humans *J Appl Physiol* **62** 1324-30

[256] van Noord J A, Demedts M, Clément J, Cauberghs M and Van de Woestijne K P 1986 Effect of rib cage and abdominal restriction on total respiratory resistance and reactance *J Appl Physiol* **61** 1736-40

[257] Torchio R, Gulotta C, Ciacco C, Perboni A, Guglielmo M, Crosa F, Zerbini M, Brusasco V, Hyatt R E and Pellegrino R 2006 Effects of chest wall strapping on mechanical response to methacholine in humans *J Appl Physiol (1985)* **101** 430-8

[258] Navajas D, Farre R, Rotger M M, Milic-Emili J and Sanchis J 1988 Effect of body posture on respiratory impedance *J Appl Physiol* **64** 194-9

[259] Fredberg J J, Inouye D S, Mijailovich S M and Butler J P 1999 Perturbed equilibrium of myosin binding in airway smooth muscle and its implications in bronchospasm *Am J Respir Crit Care Med* **159** 959-67

[260] Shen X, Wu M F, Tepper R S and Gunst S J 1997 Mechanisms for the mechanical response of airway smooth muscle to length oscillation *J Appl Physiol (1985)* **83** 731-8

[261] Wang L, Paré P D and Seow C Y 2001 Selected contribution: effect of chronic passive length change on airway smooth muscle length-tension relationship *J Appl Physiol (1985)* **90** 734-40

[262] Gunst S J, Meiss R A, Wu M F and Rowe M 1995 Mechanisms for the mechanical plasticity of tracheal smooth muscle *Am J Physiol* **268** C1267-76

[263] Gunst S J and Wu M F 2001 Selected contribution: plasticity of airway smooth muscle stiffness and extensibility: role of length-adaptive mechanisms *J Appl Physiol (1985)* **90** 741-9

[264] Gunst S J 1983 Contractile force of canine airway smooth muscle during cyclical length changes *J Appl Physiol Respir Environ Exerc Physiol* **55** 759-69

[265] Seow C Y 2005 Myosin filament assembly in an ever-changing myofilament lattice of smooth muscle *Am J Physiol Cell Physiol* **289** C1363-8

[266] Pratusevich V R, Seow C Y and Ford L E 1995 Plasticity in canine airway smooth muscle *J Gen Physiol* **105** 73-94

[267] Trepat X, Deng L, An S S, Navajas D, Tschumperlin D J, Gerthoffer W T, Butler J P and Fredberg J J 2007 Universal physical responses to stretch in the living cell *Nature* **447** 592-5

[268] Krishnan R, Trepat X, Nguyen T T, Lenormand G, Oliver M and Fredberg J J 2008 Airway smooth muscle and bronchospasm: fluctuating, fluidizing, freezing. *Respir Physiol Neurobiol* **163** 17-24

[269] Fredberg J J, Inouye D, Miller B, Nathan M, Jafari S, Raboudi S H, Butler J P and Shore S A 1997 Airway smooth muscle, tidal stretches, and dynamically determined contractile states *Am J Respir Crit Care Med* **156** 1752-9

[270] Golnabi A H, Harris R S, Venegas J G and Winkler T 2014 Deep inspiration and the emergence of ventilation defects during bronchoconstriction: a computational study *PLoS One* **9** e112443

[271] Toor P, Kim K and Buffington C K 2012 Sleep quality and duration before and after bariatric surgery *Obes Surg* **22** 890-5

[272] 2013 Obesity: Situation and Trends. In: *WHO*: World Health Organization)

[273] Shah S S, Todkar J S, Shah P S and Cummings D E 2010 Diabetes remission and reduced cardiovascular risk after gastric bypass in Asian Indians with body mass index <35 kg/m(2) *Surg Obes Relat Dis* **6** 332-8

[274] Spivak H, Hewitt M F, Onn A and Half E E 2005 Weight loss and improvement of obesity-related illness in 500 U.S. patients following laparoscopic adjustable gastric banding procedure *Am J Surg* **189** 27-32

[275] Hasegawa K, Tsugawa Y, Chang Y and Camargo C A 2015 Risk of an asthma exacerbation after bariatric surgery in adults *J Allergy Clin Immunol*

[276] Littleton S W 2012 Impact of obesity on respiratory function *Respirology* **17** 43-9

[277] Babb T G, Wyrick B L, DeLorey D S, Chase P J and Feng M Y 2008 Fat distribution and end-expiratory lung volume in lean and obese men and women *Chest* **134** 704-11

[278] Mead J, Takishima T and Leith D 1970 Stress distribution in lungs: a model of pulmonary elasticity *J Appl Physiol* **28** 596-608

[279] Yap J C, Watson R A, Gilbey S and Pride N B 1995 Effects of posture on respiratory mechanics in obesity *J Appl Physiol* **79** 1199-205

[280] MacLeod D and Birch M 2001 Respiratory input impedance measurement: forced oscillation methods *Med Biol Eng Comput* **39** 505-16

[281] King G G, Downie S R, Verbanck S, Thorpe C W, Berend N, Salome C M and Thompson B 2005 Effects of methacholine on small airway function measured by forced oscillation technique and multiple breath nitrogen washout in normal subjects *Respir Physiol Neurobiol* **148** 165-77

[282] Goldman M D, Saadeh C and Ross D 2005 Clinical applications of forced oscillation to assess peripheral airway function *Respir Physiol Neurobiol* **148** 179-94

[283] Konstantinos Katsoulis K, Kostikas K and Kontakiotis T 2013 Techniques for assessing small airways function: Possible applications in asthma and COPD *Respir Med*

[284] Buysse D J, Reynolds C F, Monk T H, Berman S R and Kupfer D J 1989 The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research *Psychiatry Res* **28** 193-213

[285] Miller M R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten C P, Gustafsson P, Jensen R, Johnson D C, MacIntyre N, McKay R, Navajas D, Pedersen O F, Pellegrino R, Viegi G, Wanger J and Force A E T 2005 Standardisation of spirometry *Eur Respir J* **26** 319-38

[286] Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, Marchal F and Measurements o b o t E T F o R I 2003 The forced oscillation technique in clinical practice: methodology, recommendations and future developments *European Respiratory Journal* **22** 1026-41

[287] Kaczka D W and Dellacá R L 2011 Oscillation mechanics of the respiratory system: applications to lung disease *Crit Rev Biomed Eng* **39** 337-59

[288] Benjamini Y and Hochberg Y 1995 Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing *Journal of the Royal Statistical Society* **57** 289-300

[289] Salome C M, King G G and Berend N 2010 Physiology of obesity and effects on lung function *J Appl Physiol (1985)* **108** 206-11

[290] Schwartz A R, Gold A R, Schubert N, Stryzak A, Wise R A, Permutt S and Smith P L 1991 Effect of weight loss on upper airway collapsibility in obstructive sleep apnea *Am Rev Respir Dis* **144** 494-8

[291] Lopez P P, Stefan B, Schulman C I and Byers P M 2008 Prevalence of sleep apnea in morbidly obese patients who presented for weight loss surgery evaluation: more evidence for routine screening for obstructive sleep apnea before weight loss surgery *Am Surg* **74** 834-8

[292] Sutherland E R, Lehman E B, Teodorescu M, Wechsler M E and National Heart L n, and Blood Institute's Asthma Clinical Research Network 2009 Body mass index and phenotype in subjects with mild-to-moderate persistent asthma *J Allergy Clin Immunol* **123** 1328-34.e1

[293] Machado D, Tavares B, Loureiro G, Conde B, Cemlyn-Jones J, Calado G, Sousa N, Chieira C and Pereira C 2008 Body mass index and airway hyper-responsiveness in individuals without respiratory disease *Eur Ann Allergy Clin Immunol* **40** 130-7

[294] Ballard R D, Irvin C G, Martin R J, Pak J, Pandey R and White D P 1990 Influence of sleep on lung volume in asthmatic patients and normal subjects *J Appl Physiol (1985)* **68** 2034-41

[295] Irvin C G, Pak J and Martin R J 2000 Airway-parenchyma uncoupling in nocturnal asthma *Am J Respir Crit Care Med* **161** 50-6

[296] Foster P S, Goldie R G and Paterson J W 1983 Effect of steroids on beta-adrenoceptor-mediated relaxation of pig bronchus *Br J Pharmacol* **78** 441-5

[297] Hernández García I A, Gutiérrez Gutiérrez A M and Gallardo Lozano E 1999 [Effect of weight reduction on the clinical and hormonal condition of obese anovulatory women] *Ginecol Obstet Mex* **67** 433-7

[298] Lorino A M, Atlan G, Lorino H, Zanditenas D and Harf A 1992 Influence of posture on mechanical parameters derived from respiratory impedance *Eur Respir J* **5** 1118-22

[299] Hantos Z, Daróczy B, Suki B, Galgóczy G and Csendes T 1986 Forced oscillatory impedance of the respiratory system at low frequencies *J Appl Physiol (1985)* **60** 123-32

[300] Peters U, Hernandez P, Dechman G, Ellsmere J and Maksym G 2016 Early detection of changes in lung mechanics with oscillometry following bariatric surgery in severe obesity *Appl Physiol Nutr Metab* **41** 538-47

[301] Mead J 1969 Contribution of compliance of airways to frequency-dependent behavior of lungs *J Appl Physiol* **26** 670-3

[302] Lutchen K R and Gillis H 1997 Relationship between heterogeneous changes in airway morphometry and lung resistance and elastance *J Appl Physiol (1985)* **83** 1192-201

[303] Lutchen K R, Jensen A, Atileh H, Kaczka D W, Israel E, Suki B and Ingenito E P 2001 Airway constriction pattern is a central component of asthma severity: the role of deep inspirations *Am J Respir Crit Care Med* **164** 207-15

[304] LaPrad A S and Lutchen K R 2008 Respiratory impedance measurements for assessment of lung mechanics: focus on asthma *Respir Physiol Neurobiol* **163** 64-73

[305] Oppenheimer B W, Berger K I, Segal L N, Stabile A, Coles K D, Parikh M and Goldring R M 2014 Airway dysfunction in obesity: response to voluntary restoration of end expiratory lung volume *PLoS One* **9** e88015

[306] Nadel J A and Tierney D F 1961 Effect of a previous deep inspiration on airway resistance in man *J Appl Physiol* **16** 717-9

[307] Gimeno F, Berg W C, Sluiter H J and Tammeling G J 1972 Spirometry-induced bronchial obstruction *Am Rev Respir Dis* **105** 68-74

[308] Badia J R, Farre R, Rigau J, Uribe M E, Navajas D and Montserrat J M 2001 Forced oscillation measurements do not affect upper airway muscle tone or sleep in clinical studies *Eur Respir J* **18** 335-9

[309] Reisch S, Daniuk J, Steltner H, Rühle K H, Timmer J and Guttmann J 2000 Detection of sleep apnea with the forced oscillation technique compared to three standard polysomnographic signals *Respiration* **67** 518-25

[310] O'Donnell D E, Aaron S, Bourbeau J, Hernandez P, Marciniuk D D, Balter M, Ford G, Gervais A, Goldstein R, Hodder R, Kaplan A, Keenan S, Lacasse Y, Maltais F, Road J, Rocker G, Sin D, Sinuff T and Voduc N 2007 Canadian Thoracic Society recommendations for management of chronic obstructive pulmonary disease - 2007 update *Can Respir J* **14 Suppl B** 5B-32B

[311] Borrill Z L, Houghton C M, Woodcock A A, Vestbo J and Singh D 2005 Measuring bronchodilation in COPD clinical trials *Br J Clin Pharmacol* **59** 379-84

[312] Neild J E, Twort C H, Chinn S, McCormack S, Jones T D, Burney P G and Cameron I R 1989 The repeatability and validity of respiratory resistance measured by the forced oscillation technique *Respir Med* **83** 111-8

[313] Gimeno F, van der Weele L T, Koeter G H, de Monchy J G and van Altena R 1993 Variability of forced oscillation (Siemens Siregnost FD 5) measurements of total respiratory resistance in patients and healthy subjects *Ann Allergy* **71** 56-60

[314] Peslin R, Duvivier C, Didelon J and Gallina C 1985 Respiratory impedance measured with head generator to minimize upper airway shunt *J Appl Physiol* **59** 1790-5

[315] Peslin R, Duvivier C, Gallina C and Cervantes P 1985 Upper airway artifact in respiratory impedance measurements *Am Rev Respir Dis* **132** 712-4

[316] Habib R H and Jackson A C 1993 Total respiratory input impedance with the upper airway wall shunt minimized *J Appl Physiol* **74** 1045-55

[317] Farre R, Peslin R, Oostveen E, Suki B, Duvivier C and Navajas D 1989 Human respiratory impedance from 8 to 256 Hz corrected for upper airway shunt *J Appl Physiol* **67** 1973-81

[318] Cauberghs M and Van de Woestijne K P 1989 Effect of upper airway shunt and series properties on respiratory impedance measurements *J Appl Physiol* **66** 2274-9

[319] Delacourt C, Lorino H, Fuhrman C, Herve-Guillot M, Reinert P, Harf A and Housset B 2001 Comparison of the forced oscillation technique and the interrupter technique for assessing airway obstruction and its reversibility in children *Am J Respir Crit Care Med* **164** 965-72

[320] Uchida A, Ito S, Suki B, Matsubara H and Hasegawa Y 2013 Influence of cheek support on respiratory impedance measured by forced oscillation technique *Springerplus* **2** 342

[321] Goldman M D, Carter R, Klein R, Fritz G, Carter B and Pachucki P 2002 Within- and between-day variability of respiratory impedance, using impulse oscillometry in adolescent asthmatics *Pediatr Pulmonol* **34** 312-9

[322] Schachter L M, Peat J K and Salome C M 2003 Asthma and atopy in overweight children *Thorax* **58** 1031-5

[323] Chen Y, Horne S L and Dosman J A 1993 Body weight and weight gain related to pulmonary function decline in adults: a six year follow up study *Thorax* **48** 375-80

[324] Wise R A, Enright P L, Connett J E, Anthonisen N R, Kanner R E, Lindgren P, O'Hara P, Owens G R, Rand C S and Tashkin D P 1998 Effect of weight gain on pulmonary function after smoking cessation in the Lung Health Study *Am J Respir Crit Care Med* **157** 866-72

[325] Corrigan C J and Kay A B 1990 CD4 T-lymphocyte activation in acute severe asthma. Relationship to disease severity and atopic status *Am Rev Respir Dis* **141** 970-7

[326] Berger A 2000 Th1 and Th2 responses: what are they? *BMJ* **321** 424

[327] Gaurav R and Agrawal D K 2013 Clinical view on the importance of dendritic cells in asthma *Expert Rev Clin Immunol* **9** 899-919

[328] Ziegler S F and Liu Y J 2006 Thymic stromal lymphopoietin in normal and pathogenic T cell development and function *Nat Immunol* **7** 709-14

[329] Zhou B, Comeau M R, De Smedt T, Liggitt H D, Dahl M E, Lewis D B, Gyarmati D, Aye T, Campbell D J and Ziegler S F 2005 Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice *Nat Immunol* **6** 1047-53

[330] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben T Y, Karp C L and Donaldson D D 1998 Interleukin-13: central mediator of allergic asthma *Science* **282** 2258-61

[331] Clutterbuck E J, Hirst E M and Sanderson C J 1989 Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF *Blood* **73** 1504-12

[332] Burrows B, Martinez F D, Halonen M, Barbee R A and Cline M G 1989 Association of asthma with serum IgE levels and skin-test reactivity to allergens *N Engl J Med* **320** 271-7

[333] Bousquet J, Chanez P, Lacoste J Y, Barnéon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J and Godard P 1990 Eosinophilic inflammation in asthma *N Engl J Med* **323** 1033-9

[334] Peslin R, Jardin P, Duvivier C and Begin P 1984 In-phase rejection requirements for measuring respiratory input impedance *J Appl Physiol* **56** 804-9

[335] Làndsér F, Clément J and Van de Woestijne K 1982 Normal values of total respiratory resistance and reactance determined by forced oscillations: influence of smoking. *Chest* **81** 586-91

Appendix A: Pathophysiologic Mechanisms of Asthma

The symptoms of asthma develops through numerous pathophysiological mechanisms that are triggered by a wide variety of stimuli, ultimately resulting in bronchoconstriction and airflow obstruction [42]. It is not yet known whether these mechanisms are quite distinct in obese individuals, who may develop asthma as a consequence of obesity. The pathophysiologic mechanisms of asthma-like symptoms in obesity is reviewed in detail in Section 2.4. Here, the pathophysiology of classical asthma is reviewed to enable us draw parallels to the pathophysiology of asthma-like symptoms in obesity. The stimuli that could provoke an asthma attack include pollen, house dust mite, cigarette smoke, animal fur, cold air, fog, certain drugs such as aspirin, exercise, anxiety and even stress. These triggers set off a cascade of immunological reactions that involve the CD4 T-lymphocytes known as T-helper (T_H) cells [325]. There are two types of T_H cells: T_H1 and T_H2 cells and the cytokines they produce are known as T_H1-type cytokines (eg interleukin (IL-) 2, interferon gamma (IFN-γ) and lyphotoxin) and T_H2-type cytokines (eg IL-4, -5, -6, -9, -10 and -13), respectively [326]. T_H1 cells are normally found in the lungs whereas T_H2 are not commonly found in the lungs. It is well established that T_H1 cells stimulate cell-mediated immunity (delayed-type hypersensitivity reaction or autoimmune response) while T_H2 cells mediate allergic inflammation by increasing humoral-mediated immunity (B-cell antibody production particularly IgE).

When inhaled allergens, microorganisms and other environmental pollutants disrupt the airway epithelial layer in an individual with asthma, dendritic cells in the submucosa intercept the offending antigen and migrate to regional lymph nodes where the antigen is presented to CD4 T-lymphocytes. Depending on the nature of the interaction between the processed antigen and the microenvironment, dendritic cells polarize naïve CD4+ T-lymphocytes to differentiate into T_H1, T_H2, or regulatory T lymphocytes which normally inhibit T_H2 cells [327]. At the same time, airway epithelial cells respond by releasing various bronchoactive agents such as nitric oxide and thymic stromal lymphopoietin–an important factor that conditions and activates the dendritic cells to upregulate production of T_H2 cell attracting chemokine – CCL17 [328, 329]. Activated T_H2 cells perform several functions. They stimulate plasma cells through IL-4 and IL-13 to increase production of IgE antibody [330]. In addition, T_H2 cells stimulate the production of mast

cells through IL-9 and also promotes eosinophil production in the bone marrow through IL-5 [331].

Immunoglobulin E released from the peripheral blood then binds to IgE receptors present on the surface of inflammatory cells such as mast cells, basophils, lymphocytes, eosinophils and macrophages to form a complex. When this complex encounters the offending antigen, it degranulates and this results in the release of pro-inflammatory mediators including histamine, cysteinyl-leukotrienes, tryptase, and prostaglandin D_2 as well as further amplification of the inflammatory response. These inflammatory mediators induce airway hyperresponsiveness (AHR), bronchoconstriction, mucus hypersecretion, airway edema, tissue damage, hypertrophy and hyperplasia of airway smooth muscle (ASM), as well as other typical symptoms of asthma. Some of these respiratory symptoms have also been reported in individuals with obesity [22, 23, 249].

Atopic or allergic asthma is thought to be due to a preference towards increased T_H2-lymphocyte production along with a predisposition towards an IgE-mediated response resulting in the eosinophilic inflammation that is known to characterize this asthma phenotype and is responsible for its chronicity [332, 333]. The pathophysiology of nonatopic or nonallergic asthma is very similar to that of atopic asthma. However, compared to atopic asthma, many aspects of the pathophysiology of nonatopic asthma have yet to be fully established, especially the role of IgE and neutrophils [217].

Appendix B: Permission letter for Figure 2.1

Ubong Peters

From: Rebecca Decker <goldandgina@gmail.com>
Sent: Tuesday, August 9, 2016 12:27 PM
To: Ubong Peters
Subject: RE: GINA COPYRIGHT REQUEST

Hi Ubong, permission is granted for this request.

Rebecca Decker

Program Director, GOLD and GINA

Global Initiative for Chronic Obstructive Lung Disease Global Initiative for Asthma
P.O. Box 558 Fontana, WI 53125 Mobile: 262-812-6741 email:
goldandgina@gmail.com

www.goldcopd.org @gold_copd

www.ginasthma.org @ginasthma

-----Original Message-----

From: Ubong Peters [mailto:wordpress@ginasthma.org]

Sent: Monday, August 8, 2016 2:11 PM

To: goldandgina@gmail.com

Subject: GINA COPYRIGHT REQUEST

1. Name: Ubong Peters

2. Address: 5981 University Avenue, Halifax, NS, Canada. B3H 4R2

3. Email Address: ubong.peters@dal.ca

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Ubong Peters

From: Buysse, Daniel <BuysseDJ@upmc.edu>
Sent: Friday, July 5, 2013 10:34 AM
To: Ubong Peters
Subject: RE: Permission to use the PSQI

Dear Ubong,

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Please note that Question 10 is not used in scoring the PSQI. This question is for informational purposes only, and may be omitted during data collection per requirements of the particular study.

Good luck with your research.

Sincerely,

Daniel J. Buysse, M.D.
Professor of Psychiatry and Clinical and Translational Science
University of Pittsburgh School of Medicine
E-1127 WPIC
3811 O'Hara St.
Pittsburgh, PA 15213
T: (412) 246-6413
F: (412) 246-5300
buyssedj@upmc.edu

From: Ubong Peters [mailto:Ubong.Peters@Dal.Ca]
Sent: Thursday, July 04, 2013 4:15 PM

To: Buysse, Daniel
Subject: Permission to use the PSQI

4th July, 2013

Hello Dr. Buysse,

My name is Ubong Peters – a PhD Student in Biomedical Engineering at Dalhousie University. I collaborate with a multidisciplinary team of researchers and clinicians from my University and Capital District Health Authority to investigate how rapid weight loss affects sleep quality in morbidly obese subjects. This project is funded by the Nova Scotia Lung Association.

I am writing to request your permission to use the Pittsburgh Sleep Quality Index in our research study. The PSQI will not be modified in any way. Please let us know if we have your approval to use this important tool in our research.

Best regards,
-Ubong

Ubong Peters, *MASc*
PhD Candidate
School of Biomedical Engineering
Dalhousie University
5981 University Avenue
Halifax, NS B3H 1W2

Tel: (902) 494-1261
Fax: (902) 494-6621
e-mail: Ubong.Peters@dal.ca

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-Benjamin Disraeli

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ID Code _____

DATE _____

AGE _____

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past months only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?
   Usual bedtime: _____

2. During the past month, how long has it usually taken you to fall asleep each night?
   Number of minutes: _____

3. During the past month, when have you usually gotten up in the morning?
   Usual getting up time: _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)
   Hours of sleep per night: _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you…

a) **Cannot get to sleep within 30 minutes**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

b) **Wake up in the middle of the night or early morning**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

c) **Have to get up to use the bathroom**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

d) **Cannot breathe comfortably**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

e) **Cough or snore loudly**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

f) **Feel too cold**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

g) **Feel too hot**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

h) **Had bad dreams**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

i) **Have pain**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

j) **Other reason(s), please describe:** _____

_____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

6. During the past month, how would you rate your sleep quality overall?

Very good ☐ Fairly good ☐ Fairly bad ☐ Very bad ☐

7. During the past month, how often have you taken medicine (prescribed, or "over the counter") to help you sleep?

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

8. During the past month, how often have you had trouble staying awake while driving, eating meals or engaging in social activity?

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all ☐ Only a very slight problem ☐

Somewhat of a problem ☐ A very big problem ☐

10. Do you have a bed partner or roommate?

No bed partner or roommate ☐ Partner/roommate in other room ☐

Partner in same room, but not same bed ☐ Partner in same bed ☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

a) **Loud snoring**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

b) **Long pauses between breaths while asleep**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

c) **Legs twitching or jerking while you sleep**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

d) **Episodes of disorientation or confusion during sleep**

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

e) **Other restlessness while you sleep, please describe:** _____

_____

_____

Not during the past month ☐ Less than once a week ☐

Once or twice a week ☐ Three or more times a week ☐

Appendix G: Pictorial and Schematic Representation of tremoFlo™

Figure G1. The tremoFlo C-100 Airwave Oscillometry System used in collecting respiratory impedance measurements in this thesis.

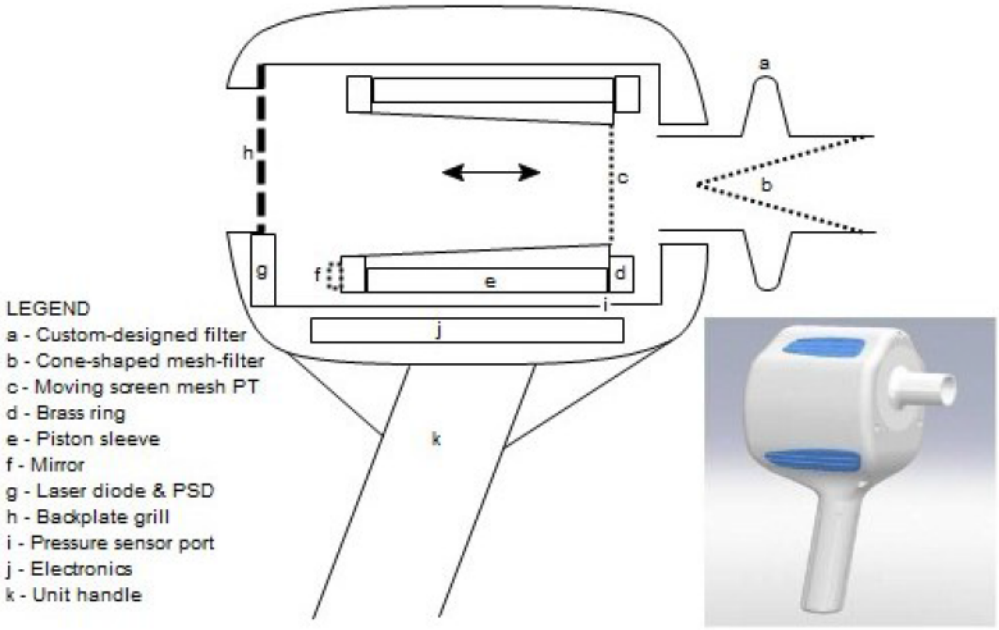


Figure G2. A schematic illustration of the inner components of one of the early prototypes of the handheld oscillometry device. The inset is a 3D model of the handheld oscillometry prototype.

Appendix H: Validation of Resistance and Reactance

Resistive test loads with nominal values of 1, 2, 3, 5, 10, 20 and 40 cm·H_2O/(L·s^{-1}) from Hans Rudolph Inc (Shawnee, KS, USA) were used as reference impedances to determine the accuracy of measurements obtained from the handheld oscillometry device. These test loads were measured using a 5-frequency composite signal that contained 4, 6, 10, 14 and 19 Hz oscillation frequencies. Resistance validation tests were also conducted with a 7-frequency composite signal that contained 22 and 26 Hz oscillation frequencies, in addition those frequencies outlined in the 5-frequency composite signal. The predicted resistance closely matched the measured resistance, indicating that the device can accurately measure resistance (Figure H1).

Reactance was validated using the 7-frequency composite signal with the aid of a mechanical analogue of the lung developed in a similar manner to the study by Peslin *et al*. [334]. The parameters of this mechanical analog of the lung were chosen to match the *E* and *I* of an average man reported by Landser *et al.* [335] to be 30 cm H_2O L^{-1} and 0.01 cm H_2O L^{-1} s^{-2}, respectively. This test-bed mainly consisted of a 0.23 m rigid pipe with an inner diameter of 0.021 m and an inertance (*I*) of 0.0106 cm H_2O L^{-1} s^{-2} connected in series to a 26.4 L glass chamber with an elastance (*E*) of 38.297 cm H_20 L^{-1} (Figure H2).

Basically, a defined volume of gas has a fixed *E* which can be predicted from the equation: $E = \frac{P}{V}$ and a rigid pipe with a defined dimension has a fixed inertance which can also be predicted from the equation: $I = \frac{\eta \rho l}{\pi r^2}$, where ρ is density of air, *l* is length of the pipe, *r* is the radius of the pipe and η is a constant that represents the velocity profile, with $\eta = 1$ when the velocity profile is blunt and $\eta = 1.3$ when the velocity profile is parabolic. The *E* and *I* were used in computing the predicted reactance of the mechanical analog of the lung model according to the equation: $X = \omega I - \frac{E}{\omega}$ and the result was compared to the actual reactance measured with the handheld oscillometry device.

The results obtained confirm that the predicted closely matched the measured reactance, indicating that the device can accurately measure reactance. The broken lines in Figure H3 represent the predicted inertance from the above equation, while the solid line represents the predicted elastance. The behavior of the combined model starts very close to the pure elastance and this implies that elastic forces dominate at low frequencies. However, at high frequencies, the reactance is very close to the pure inertance and this implies that inertive forces dominate reactance at high frequencies.

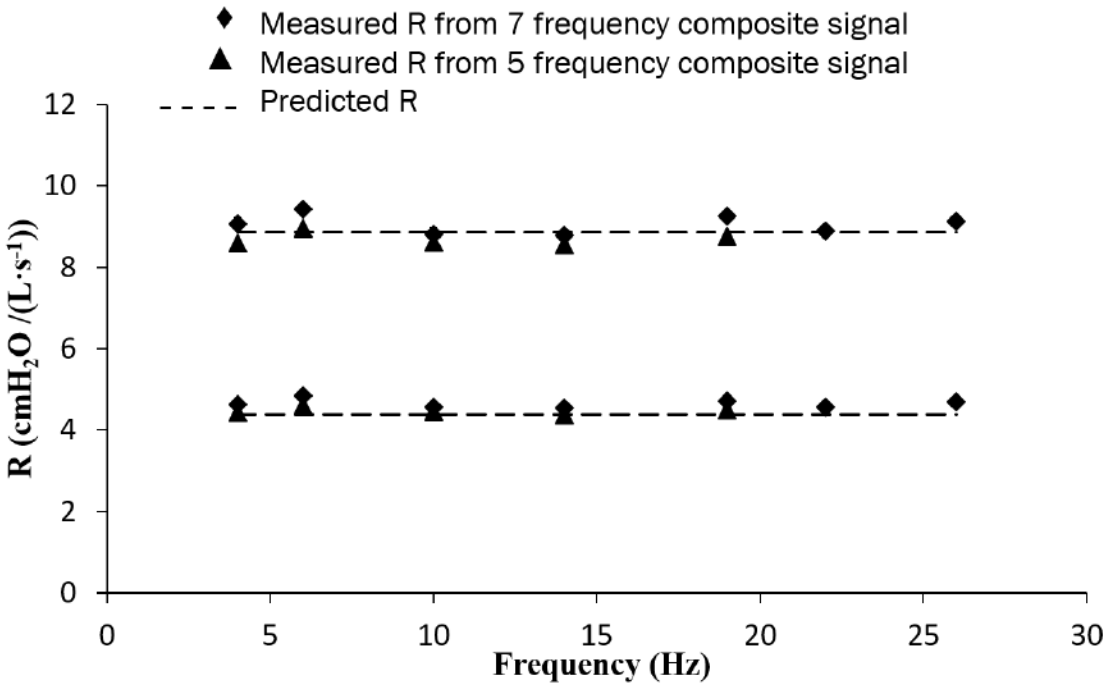
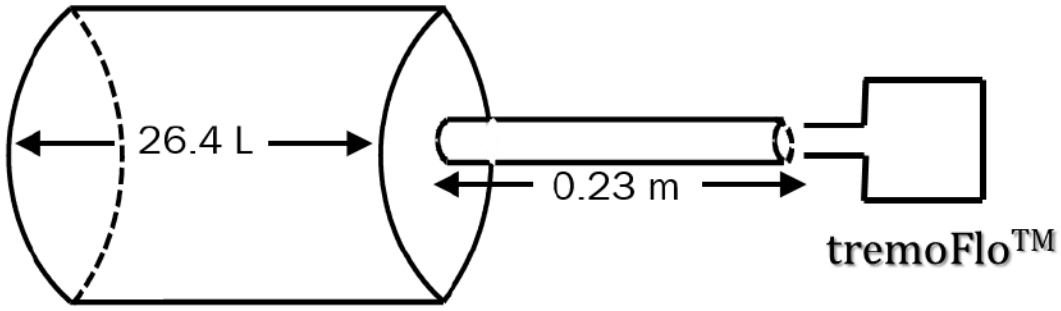


Figure H1. Predicted and measured resistance versus frequency in a handheld oscillometry device. The predicted resistance closely matched the measured resistance, indicating that the device can accurately measure resistance. Note: Error bars cannot be seen because the measurements were highly repeatable.



$E = 38.297\ cm\ H_20\ L^{-1}$

$I = 0.0106\ cm\ H_20\ L^{-1}s^{-2}$

Figure H2. Reactance validation testbed.

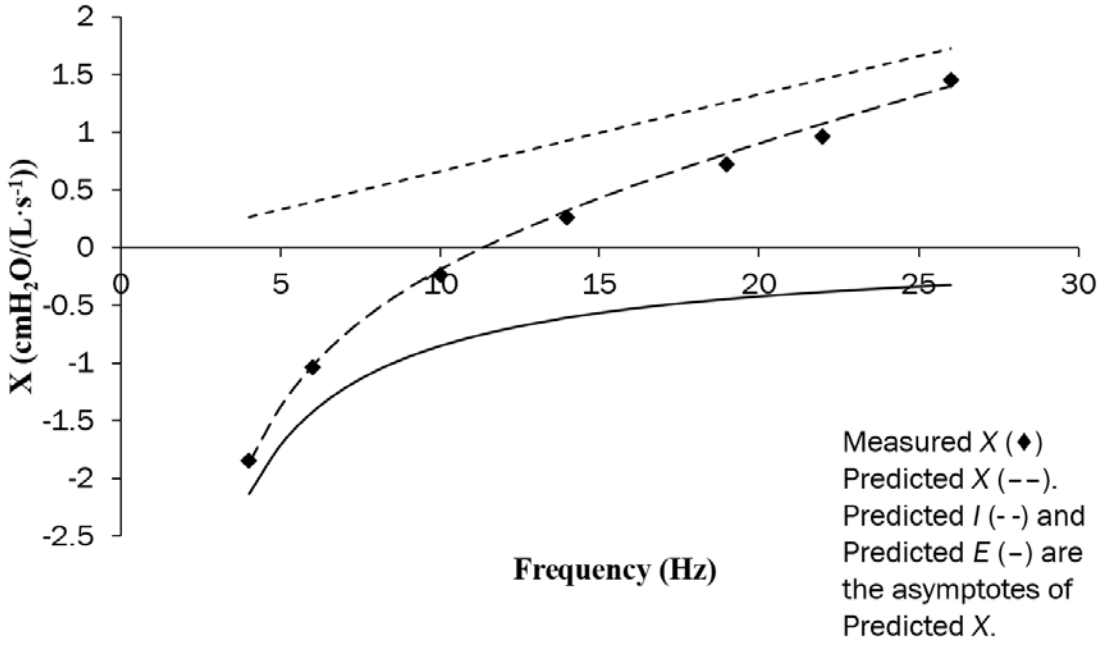


Figure H3. Predicted and measured reactance versus frequency in a handheld oscillometry device. The predicted reactance closely matched the measured reactance, indicating that the device can accurately measure reactance.