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Pulmonary Structure and Function in Chronic Obstructive Pulmonary Disease Evaluated using Hyperpolarized Noble Gas Magnetic Resonance Imaging

Miranda Kirby
*The University of Western Ontario*

Supervisor
Dr. Grace Parraga
*The University of Western Ontario*

Graduate Program in Medical Biophysics

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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PULMONARY STRUCTURE AND FUNCTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE EVALUATED USING HYPERPOLARIZED NOBLE GAS MAGNETIC RESONANCE IMAGING

(Thesis Format: Integrated Article)

by

Miranda Kirby, BSc

Graduate Program in Medical Biophysics

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

Chronic obstructive pulmonary disease (COPD) is the 4th leading cause of death worldwide and accounts for the highest rate of hospital admissions in Canada. The need for sensitive regional and surrogate measurements of lung structure and function in COPD continues to motivate the development of non-radiation based and sensitive imaging approaches, such as hyperpolarized helium-3 ($^3$He) and xenon-129 ($^{129}$Xe) magnetic resonance imaging (MRI). The static ventilation images acquired using these approaches allows us to directly visualize lung regions accessed by the hyperpolarized gas during a breath-hold, as well as quantify the regions without signal referred to as the percentage of the thoracic cavity occupied by ventilation defects (VDP). The lung micro-structure can also be probed using diffusion-weighted imaging which takes advantage of the rapid diffusion of $^3$He and $^{129}$Xe atoms to generate surrogate measurements of alveolar size, referred to as the apparent diffusion coefficient (ADC). Here we evaluated COPD lung structure and function using hyperpolarized gas MRI measurements longitudinally, following treatment and in early disease. In COPD ex-smokers, we demonstrated $^3$He VDP and ADC worsened significantly in only 2 years although there was no change in age-matched healthy volunteers, suggestive of disease progression. We also evaluated COPD ex-smokers pre- and post-bronchodilator and showed regional improvements in gas distribution following bronchodilator therapy regardless of spirometry-based responder classification; the ADC measured in these same COPD ex-smokers also revealed significant reductions in regional gas trapping post-bronchodilator. Although $^3$He MRI has been more widely used, the limited global quantities necessitates the transition to hyperpolarized $^{129}$Xe, and therefore we directly compared $^3$He and $^{129}$Xe MRI in the same COPD ex-smokers and showed significantly greater gas distribution abnormalities for $^{129}$Xe compared to $^3$He MRI that were spatially and significantly related to lung regions with elevated ADC. Finally, we demonstrated that ex-smokers with normal spirometry but abnormal diffusion capacity of the lung for carbon monoxide ($DL_{CO}$) had significantly worse symptoms, exercise capacity and $^3$He ADC than ex-smokers with normal $DL_{CO}$. These important findings indicate that hyperpolarized gas MRI can be used to improve our understanding of lung structural and functional changes in COPD.
Keywords

Hyperpolarized $^3$He Magnetic Resonance Imaging, Hyperpolarized $^{129}$Xe Magnetic Resonance Imaging, Chronic Obstructive Pulmonary Disease, Emphysema, Apparent Diffusion Coefficient, Ventilation Defect
Co-Authorship Statement

The following thesis contains seven manuscripts published in scientific journals. Chapter 2 is an original research article titled “Chronic Obstructive Pulmonary Disease: Longitudinal Hyperpolarized $^3$He MR Imaging” and was published in the journal *Radiology* in 2010. This manuscript was co-authored by Miranda Kirby, Lindsay Mathew, Andrew Wheatley, Giles E Santyr, David G McCormack and Grace Parraga. Chapter 3 is an original research article titled “Hyperpolarized $^3$He Magnetic Resonance Functional Imaging Semiautomated Segmentation” and was published in the journal *Academic Radiology* in 2012. This manuscript was co-authored by Miranda Kirby, Mohammadreza Heydarian, Sarah Svenningsen, Andrew Wheatley, David G McCormack, Roya Etemad-Rezai and Grace Parraga. Chapter 4 is an original research article titled “Chronic obstructive pulmonary disease: quantification of bronchodilator effects by using hyperpolarized $^3$He MR imaging” and was published in the journal *Radiology* in 2011. This manuscript was co-authored by Miranda Kirby, Mohammadreza Heydarian, Mohammadreza Heydarian, Ro Etemad-Rezai, David G McCormack and Grace Parraga. Chapter 5 is an original research article titled “Evaluating Bronchodilator Effects in Chronic Obstructive Pulmonary Disease using Diffusion-Weighted Hyperpolarized Helium-3 Magnetic Resonance Imaging” and was published in the *Journal of Applied Physiology* in 2012. This manuscript was co-authored by Miranda Kirby, Mohammadreza Heydarian, Andrew Wheatley, Ro Etemad-Rezai, David G McCormack and Grace Parraga. Chapter 6 is an original research article titled “Hyperpolarized Helium-3 and Xenon-129 Magnetic Resonance Imaging in Healthy Volunteers and Subjects with Chronic Obstructive Pulmonary Disease” and was published in the journal *Radiology* in 2012. This manuscript was co-authored by Miranda Kirby, Sarah Svenningsen, Amir Owrangi, Andrew Wheatley, Adam Farag, Alexei Ouriadov, Giles E Santyr, Ro Etemad-Rezai, Harvey O Coxson, David G McCormack and Grace Parraga. Chapter 7 is an original research article titled “Pulmonary Ventilation Visualized using Hyperpolarized Helium-3 and Xenon-129 Magnetic Resonance Imaging: Differences in COPD and Relationship to Emphysema” and was published in the *Journal of Applied Physiology* in 2012. This manuscript was co-authored by Miranda Kirby, Sarah Svenningsen, Nikhil Kanhere, Amir Owrangi, Andrew Wheatley, Harvey O Coxson, Giles E Santyr, Nigel AM Paterson, David G McCormack and Grace Parraga. Chapter 8 is an original research article titled “On the
role of abnormal DL_{CO} in ex-smokers without airflow limitation: Symptoms, exercise capacity and hyperpolarized helium-3 magnetic resonance imaging” and was accepted and is currently in press in *Thorax* (Accepted March 28, 2013). This manuscript was co-authored by Miranda Kirby, Amir Owrangi, Sarah Svenningsen, Andrew Wheatley, Harvey O Coxson, Nigel AM Paterson, David G McCormack and Grace Parraga. Appendix A is an original research article titled “Quantitative Evaluation of Hyperpolarized Helium-3 Magnetic Resonance Imaging of Lung Function Variability in Cystic Fibrosis” and was published in the journal *Academic Radiology* in 2011. This manuscript was co-authored by Miranda Kirby, Sarah Svenningsen, Hassan Ahmed, Nigel AM Paterson and Grace Parraga. Appendix B is a case report titled “Hyperpolarized Helium-3 Magnetic Resonance Imaging of Chronic Obstructive Pulmonary Disease Exacerbation: Case Report” and was published in the *Journal of Magnetic Resonance in Medicine* in 2012. This manuscript was co-authored by Miranda Kirby, Nikhil Kanhere, Roya Etemad-Rezai and David G McCormack and Grace Parraga.

As the 1st author of these published peer-reviewed manuscripts, I assisted with the acquisition of the data including subject scanning and coaching breath-hold maneuvers during the subject study visits and I also assisted with the acquisition of pulmonary function test measurements. I also contributed to the conception and design of the experiments, performed the statistical data analysis and assisted with interpretation of the results, as well as drafting, final revisions and final approval of the manuscript. Dr. Lindsay Mathew (Chapter 2,4), Dr. Amir Owrangi (Chapter 6-8), Sarah Svenningsen (Chapter 3,6-8, Appendix A), Hassan Ahmed (Appendix A) and Nikhil Kanhere (Chapter 7, Appendix B) assisted with the acquisition of data, provided assistance with statistical analysis and interpretation of results as well as contributed editorial assistance for the manuscripts they co-authored and provided final approval of the manuscript. Dr. Mohammadreza Heydarian (Chapter 3-5) and Andrew Wheatley (Chapter 2,3,5-8) provided assistance with data analysis and contributed to the development of image registration/segmentation software as well as contributed editorial assistance for the manuscripts they co-authored and provided final approval of the manuscript. Drs. Giles E Santyr (Chapter 2,6,7), Alexei Ouriadov (Chapter 6), Roya Etemad-Rezai (Chapter 3-6, Appendix B), Nigel AM Paterson (Chapter 7,8), Harvey O Coxson (Chapter 6-8) and Adam Farag (Chapter 6) provided additional clinical
and physics expertise and aided in interpretation of the results for the respective manuscripts they co-authored as well as contributed editorial assistance and provided final approval of the manuscript. Dr. David G McCormack (Chapter 2-8, Appendix B) provided ongoing guidance as well as clinical expertise with the interpretation of results as well as with the manuscript drafting and revision, and provided final approval of the manuscripts. Dr. Grace Parraga (Chapters 2-8), as the principal investigator and the author’s supervisor, provided ongoing guidance and contributed to the conception and design, data acquisition and analysis plan and interpretation, drafting and final revisions of manuscript and final approval as well as guarantor of integrity of the data as well as responsible for Good Clinical Practice.

Pulmonary function test measurements were acquired by Sandra Halko and Shayna McKay. Polarization of the $^3$He gas was performed by Andrew Wheatley and Adam Farag. MRI acquisition was performed by Cyndi Harper-Little and Trevor Szekeres.
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I would also like to thank the members of my advisory committee for their ongoing guidance and support throughout my graduate studies. Each of the members of my committee: Dr. Aaron Fenster, Dr. David G McCormack and Dr. Charles McKenzie challenged me with difficult questions, encouraged me to develop and improve my skills and always showed interest and enthusiasm for my research. I am especially grateful for the opportunity I have had to work with Dr. McCormack. I really appreciated and enjoyed all of the meetings we had to discuss our results and manuscripts, and I am very grateful for his insightful comments and clinical expertise that have been invaluable to our research.

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To the graduate students I have worked with past and present, thank you for making my time at Robarts so memorable. I was so fortunate for the opportunity to work with Lindsay Mathew when I started my graduate studies at Robarts. She was an outstanding graduate student and is an outstanding role model and mentor; she provided me with endless
encouragement and support in my early years and I am very grateful to have worked with her. To Laura Wilson and Hassaan Ahmed, we started our graduate studies together and I could not have asked for two better lab mates. We struggled through classes together, kept each other company in the dark room, celebrated each other’s accomplishments and had many good laughs along the way. To Amir Owrangi: thank you for always cheering me up by having a big smile on your face and for your great sense of humor. To Sarah Svenningsen: I have really enjoyed our conversations about science and life, and I look forward to watching you excel in all your future endeavors. Many thanks to all of the other graduate students: Steve Costella for your intelligent and insightful questions, Daniel Buchanan and Nikhil Kanhere for the coffee breaks and fun outside of the lab, Khadija Sheikh for the fun lunch break chats and Damian Pike for the endless entertainment you provide with your unique sense of humor.

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<th>Description</th>
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<td>AD</td>
<td>Abnormal DL(_{CO})</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AP</td>
<td>Anterior-Posterior</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>BW</td>
<td>Bandwidth</td>
</tr>
<tr>
<td>CAT</td>
<td>COPD Assessment Test</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon Monoxide</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>COV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>CS</td>
<td>Center Slice</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CTS</td>
<td>Canadian Thoracic Society</td>
</tr>
<tr>
<td>DL(_{CO})</td>
<td>Diffusing Capacity of the Lung for Carbon Monoxide</td>
</tr>
<tr>
<td>DW</td>
<td>Diffusion-Weighted</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>ERV</td>
<td>Expiratory Reserve Volume</td>
</tr>
<tr>
<td>ES</td>
<td>Effect Size</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>Forced Expiratory Volume in 1 second</td>
</tr>
<tr>
<td>FGRE</td>
<td>Fast Gradient Recalled Echo</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of View</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional Residual Capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
<tr>
<td>G</td>
<td>Gradient amplitude</td>
</tr>
<tr>
<td>GOLD</td>
<td>Global Initiative for Chronic Obstructive Lung Disease</td>
</tr>
<tr>
<td>GOLD-U</td>
<td>GOLD-Unclassified</td>
</tr>
<tr>
<td>(^1)H</td>
<td>Proton</td>
</tr>
<tr>
<td>(^3)He</td>
<td>Helium-3</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
</tr>
<tr>
<td>HU</td>
<td>Housfield Unit</td>
</tr>
<tr>
<td>HU(_{15%})</td>
<td>15(^{th}) Percentile of the frequency distribution of Housfield Units</td>
</tr>
<tr>
<td>IC</td>
<td>Inspiratory Capacity</td>
</tr>
<tr>
<td>ICC</td>
<td>Interclass Correlation Coefficient</td>
</tr>
<tr>
<td>IRV</td>
<td>Inspiratory Reserve Volume</td>
</tr>
<tr>
<td>MAA</td>
<td>Macro-Aggregated Albumin</td>
</tr>
<tr>
<td>MANOVA</td>
<td>Multivariate Analysis of Variance</td>
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<tr>
<td>mMRC</td>
<td>Modified British Medical Research Council</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>mSv</td>
<td>Millisieverts</td>
</tr>
<tr>
<td>N(_2)</td>
<td>Nitrogen</td>
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<tr>
<td>ND</td>
<td>Normal DL(_{CO})</td>
</tr>
<tr>
<td>NDW</td>
<td>Non-Diffusion-Weighted</td>
</tr>
<tr>
<td>P(_A)(_O_2)</td>
<td>Partial Pressure of Oxygen</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>PIPEDA</td>
<td>Personal Information Protection and Accountability Act</td>
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<tr>
<td>PVV</td>
<td>Percent Ventilated Volume</td>
</tr>
<tr>
<td>RA_{950}</td>
<td>Relative Area with Attenuation values below -950 HU</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RV</td>
<td>Reserve Volume</td>
</tr>
<tr>
<td>SaO₂</td>
<td>Arterial Oxygen Saturation</td>
</tr>
<tr>
<td>SDD</td>
<td>Smallest Detectable Difference</td>
</tr>
<tr>
<td>SGRQ</td>
<td>St. George’s Respiratory Questionnaire</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-Noise Ratio</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Tomography</td>
</tr>
<tr>
<td>SRGA</td>
<td>Seeded Region-Growing Algorithm</td>
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<tr>
<td>STPD</td>
<td>Standard Temperature Pressure and Dry</td>
</tr>
<tr>
<td>TCV</td>
<td>Thoracic Cavity Volume</td>
</tr>
<tr>
<td>TLC</td>
<td>Total Lung Capacity</td>
</tr>
<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
</tr>
<tr>
<td>TV</td>
<td>Tidal Volume</td>
</tr>
<tr>
<td>UTE</td>
<td>Ultra-short Echo Time</td>
</tr>
<tr>
<td>VC</td>
<td>Vital Capacity</td>
</tr>
<tr>
<td>VDP</td>
<td>Ventilation Defect Percent</td>
</tr>
<tr>
<td>VDV</td>
<td>Ventilation Defect Volume</td>
</tr>
<tr>
<td>VV</td>
<td>Ventilation Volume</td>
</tr>
<tr>
<td>WA%</td>
<td>Wall Area Percent</td>
</tr>
<tr>
<td>WL</td>
<td>Whole Lung</td>
</tr>
<tr>
<td>^{129}Xe</td>
<td>Xenon-129</td>
</tr>
<tr>
<td>6MWT</td>
<td>6 Minute Walk Test</td>
</tr>
<tr>
<td>6MWD</td>
<td>6 Minute Walk Distance</td>
</tr>
</tbody>
</table>
CHAPTER 1

1 INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD), a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. The objective of Chapter 1 is to provide motivation for the work presented in the remaining chapters of this thesis, as well as to provide an overview of the current standard measurements of pulmonary function, the natural history and progression of lung function decline, the underlying disease mechanisms and finally discuss how nuclear medicine, computed tomography (CT) and magnetic resonance imaging (MRI) have been used to evaluate COPD and provide imaging biomarkers that can be used to evaluate disease progression and treatment response.

1.1 Burden of COPD

According to the World Health Organization estimates in 2004, 64 million people, or 10% of the population, was suffering from COPD. Three million people died of COPD which equaled just over 5% of all global deaths and ranked COPD as the 4th leading cause of death worldwide. By the year 2030, it is predicted that COPD will increase to the 3rd leading cause of death worldwide, as shown in Figure 1-1. It is suggested that the projected increase in the global burden of COPD is due to the aging population and the increasing levels of tobacco smoking in many middle- and low-income countries.

<table>
<thead>
<tr>
<th>Disease or injury</th>
<th>Deaths (%)</th>
<th>Rank</th>
<th>Rank</th>
<th>Deaths (%)</th>
<th>Disease or injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemic heart disease</td>
<td>12.2</td>
<td>1</td>
<td>1</td>
<td>14.2</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>9.7</td>
<td>2</td>
<td>2</td>
<td>12.1</td>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>Lower respiratory infections</td>
<td>7.0</td>
<td>3</td>
<td>3</td>
<td>8.6</td>
<td>COPD</td>
</tr>
<tr>
<td>COPD</td>
<td>5.1</td>
<td>4</td>
<td>4</td>
<td>3.8</td>
<td>Lower respiratory infections</td>
</tr>
</tbody>
</table>

Figure 1-1 The top four leading causes of death in the world in 2004 and the predicted top four leading causes of death in 2030 compared
Adapted from The global burden of disease: 2004 update, World Health Organization.

Similarly, in Canada alone, respiratory disease is ranked the 3rd leading cause of death behind cardiovascular disease and cancer, and among all respiratory diseases that results in death, COPD is responsible for the greatest proportion of all deaths for both men and
women as shown in Figure 1-2. In addition to being a leading cause of death and disability, COPD also imposes significant healthcare costs with exacerbations of COPD, defined as COPD-related symptoms including one or more of the following lasting at least 3 days: cough, wheeze, dyspnea, or sputum production, accounting for the greatest proportion of the total COPD burden on the Canadian healthcare system. The Canadian Institute for Health Information showed that in 2006, COPD accounted for the highest rate of hospital admission among the major chronic illnesses. This rate also included single and repeat hospitalizations indicating that there is a much higher readmission rate for COPD than any other chronic illness including angina, asthma, heart failure, diabetes and epilepsy, as shown in Figure 1-3. A study by Mittmann and colleagues indicated that the average cost of an exacerbation requiring hospitalization in Canada was nearly $10,000, with an average length of stay of 10 days. The authors also estimated that the national cost burden for the direct costs associated with COPD exacerbations ranged from $646 million to $736 million per annum.

Cigarette smoking has long been recognized as the most important risk factor for the development of COPD, greater than that of atmospheric pollution or occupational exposure, although the association between occupational exposure (airborne dusts, fumes and vapors) and COPD has been demonstrated independent of cigarette smoking.
Although there has been less evidence to support the association between second-hand smoke exposure and the risk of developing COPD,\textsuperscript{10,11} more recent studies suggest there is a causal relationship between exposure to second-hand smoke and COPD. There is also a genetic risk factor, alpha-1-antitrypsin deficiency, that has been shown to be associated with an increased risk of COPD.\textsuperscript{12,13} Other COPD risk factors have been suggested,\textsuperscript{1} such as prior tuberculosis, socioeconomic status and asthma, however more work must be done to understand the effect of these and other risk factors on the development of COPD as COPD in those who have never smoked remains poorly understood.\textsuperscript{14}

**Figure 1-3** Repeat Hospitalizations by Condition at First Admission

This graph shows the number of patients with a single hospitalization, one repeat hospitalization and two or more repeat hospitalizations by condition at first admission. Reproduced with permission from the Canadian Institute for Health Information’s (CIHI) publication entitled Health Indicators 2008.\textsuperscript{6}

Recent international epidemiological studies have reported prevalence estimates that suggest that approximately 9-10\% of adults aged 40 years and older have COPD.\textsuperscript{14,15} Importantly, this estimate is much higher than previous estimates\textsuperscript{15} and studies have shown that of these individuals that were determined to have COPD, only 19\% had been
previously diagnosed and were being treated. These studies indicate that the prevalence of COPD is much higher than previously believed and that the majority of the individuals that have the disease are not diagnosed and are not being treated.

In the following section I will describe the structure and function of the lung, introduce the basic mechanisms by which this structure and function is abnormal in COPD, as well as describe the current gold standards for assessing lung function.

1.2 The Respiratory System: Structure and Function

In humans, the respiratory system includes the oral and nasal cavities, the lungs, the conducting airways, the parts of the central nervous system concerned with the control of the muscles of respiration, and the chest wall structures. The respiratory system serves several functions, however, the main function of the respiratory system is to supply the tissues of our body with oxygen for cellular metabolism and to eliminate the carbon dioxide waste that is produced by cellular metabolism, and this process, known as gas exchange, occurs in the lungs. In this section I will discuss the process by which air moves from the environment, through the conducting zone and to the respiratory zone for gas exchange.

1.2.1 The Airways: Conducting Zone

During inspiration, air passes through the nose or mouth into the pharynx. The pharynx branches into two tubes: the esophagus, which passes food to the stomach, and the larynx. Air passes through the larynx into the trachea which then branches into the two main bronchi; one of which enters the left lung and the other the right lung. Within the lungs, air passes through more than 20 generations, or airway branches, and as the airway generation increases the airways become narrower, shorter and more numerous. As shown in Figure 1-4, the conducting zone extends from the top of the trachea (generation 0) to the terminal bronchioles (generation 16). These airways do not contain alveoli and therefore there is no gas exchange. However, following inspiration, some of the inhaled air remains in the conducting zone. The air that resides within the conducting zone at the end of inspiration is known as the anatomic dead space.
The average anatomic dead space is approximately 150 ml in healthy adults, however, this volume has been shown to increase with age.22

1.2.2 The Airways: Respiratory Zone

The terminal bronchioles branch into the respiratory bronchioles (generation 17-19), which marks the beginning of the respiratory zone. Due to the large number of airway branching in the respiratory zone, the total cross sectional area of the airways increases rapidly (Figure 1-5). Accordingly, the respiratory zone constitutes approximately 90% of the lung volume, while the airways and the blood vessels of the conducting zone constitute the remaining 10%.20 The respiratory bronchioles, as shown in Figure 1-4, have few alveoli lining their walls. Finally, the respiratory bronchioles divide into the

Figure 1-4 Schematic of the Human Airways
The conducting zone of the lungs does not participate in gas exchange and extends from generation 0 to generation 16. The respiratory zone is the site of gas exchange and extends from generation 17 to generation 23. Adapted from West JB. Respiratory Physiology: the essentials, Ninth edition.19
alveolar ducts and the alveolar sacs. The number of alveoli increases from the respiratory bronchioles to the alveolar sacs which consist entirely of alveoli. The part of the lung that branches from the respiratory bronchiole and includes the alveolar duct and sac is referred to as an acinus. The acinus represents the functional respiratory unit of the lung where gas exchange occurs.

**Figure 1-5** Schematic showing the rapid increase in the cross sectional area of the airways as the airway generation increases within the respiratory zone. Adapted from West JB. Respiratory Physiology: the essentials, Ninth edition.

### 1.2.3 Ventilation

Ventilation is the process by which gas is brought from the environment to the blood-gas barrier. The volume of gas inhaled and exhaled with each breath, or during one respiratory cycle, is referred to as the tidal volume. Total ventilation is defined as the volume of gas leaving the lung each minute. Therefore, since the average person has a tidal volume of 500 ml and takes approximately 15 breaths/min, the volume of gas entering the lung each minute is 7500 ml/min. However, as discussed above, the air that resides within the conducting zone at the end of inspiration does not participate in gas exchange. Therefore, the volume of gas entering the lung each minute that participates in gas exchange, otherwise known as alveolar ventilation, is approximately 5250 ml/min.
1.2.4 The Alveoli: Site of Gas Exchange

The alveoli are small air sacs at the end of the respiratory bronchioles where gas exchange occurs. It has been estimated that there are approximately 480 million alveoli in the human lungs with a mean size of $4.2 \times 10^6 \mu m^3$, or a diameter of roughly $200 \mu m$. Within each alveoli, oxygen is transported from the alveoli to the pulmonary capillaries by diffusion. The factors that determine the rate of diffusion of O$_2$ from the alveoli to the pulmonary capillaries is described by Fick’s law for diffusion, as shown in equation (1):

$$\text{Diffusion} = \frac{A \cdot D \cdot (P_1 - P_2)}{T}$$

where $A$=surface area of the lung for diffusion, $D$=diffusion coefficient of gas, $P_1$-$P_2$=partial pressure difference of the gas across the alveolar capillary membrane, and $T$=thickness across the alveolar capillary membrane. Therefore, gas diffusion rate is directly proportional to the surface area of the lung, the diffusion coefficient of the gas and the partial pressure difference, and it is inversely proportional to the thickness across the lung membrane.

The total surface area of an average adult’s lungs available for gas exchange is estimated to be approximately 70 m$^2$. The gas in the alveoli follows the basic rule of a pressure gradient and diffuses from an area of high pressure within the alveoli (Partial Pressure of Oxygen, $[P_{A02}] > 100$ mm Hg) to an area of low pressure within the pulmonary capillaries ($P_{A02} = 40$ mm Hg). The gas diffusion is slowed by the alveolar capillary membrane and the thickness of the alveolar capillary membrane of an average healthy adult is approximately 0.2-0.5 μm.

1.3 Established Tests of Lung Function

Pulmonary function tests may be used in a hospital setting or a physician’s office to aid in the diagnosis and management of pulmonary diseases or in research studies. These tests are used to evaluate overall lung function by measuring air flow or volume, symptoms or exercise capacity.
1.3.1 Pulmonary Function Tests

Pulmonary Function tests measure the volume and flow of air during inspiration and expiration as well as provide information regarding gas exchange within the lung. These measurements are used for clinical diagnosis of lung disease, for monitoring disease progression as well as for treatment recommendations in patients with respiratory disease.

1.3.1.1 Spirometry

![Image of spirometry equipment and airflow curve]

**Figure 1-6** Measurement of the Forced Expiratory Volume in 1 second (FEV₁) and the Forced Vital Capacity (FVC)

Body Plethysmograph (MedGraphics Corporation. 350 Oak Grove Parkway St. Paul, MN, USA) is used to measure airflows, lung volumes and capacities (A) and a schematic depicting the airflow curve used to measure FEV₁ and FVC (B).

Spirometry is a physiological test that measures how an individual inhales or exhales volumes of air as a function of time. The two most common spirometric measurements are the forced expiratory volume in 1 second (FEV₁) and the forced vital capacity (FVC). As shown in **Figure 1-6**, these measurements are made while the subject is in the seated position in the Body Plethysmograph chamber. The subject begins with tidal breathing and then is instructed to inhale maximally and then exhale as fast as possible and then continue to exhale as long as possible. Following maximum inhalation, the volume that is exhaled in 1 second is the FEV₁ and the total volume that could be maximally exhaled, following maximum inhalation, is the FVC.
According to the American Thoracic Society (ATS)/European Respiratory Society (ERS) task force, an adequate test requires a minimum of three acceptable maneuvers be performed. An acceptable maneuver is one in which all three phases, defined as 1) maximal inspiration; 2) a “blast” of exhalation; and 3) continued complete exhalation to the end of test, were performed correctly and for the required duration. The maneuvers must also be repeatable with the difference between the largest and the next largest FVC ≤ 0.15 L.

In Canada, the Canadian Thoracic Society (CTS) clinical guidelines specify that spirometry measurements be used to diagnose asthma and COPD, and therefore spirometry should be conducted by trained and qualified personnel, to assure that ATS/ERS spirometry testing standards are met. However, even with technologists trained to meet these standards, spirometry is well-known to be difficult to perform in children and in those with lung disease.

**Figure 1-7** The Volumes and Capacities of the Lung Measured using Body Plethysmography
Adapted from Respiratory Care: Principles and Practice.

### 1.3.1.2 Plethysmography

As shown in Figure 1-7, there are eight static lung volume and capacity measurements that can be determined using body plethysmography. The definition of the lung volumes and capacity measurements are as follows: tidal volume (TV) is defined as the volume of gas inhaled or exhaled during normal breathing; the inspiratory reserve volume (IRV) is the maximum volume of gas that can be inspired from the end of a normal inspiration; the expiratory reserve volume (ERV) is the maximum volume of gas that can be expired...
from the end of a resting expiration; the residual volume (RV) is the volume of gas remaining in the lungs after a maximal expiration; the vital capacity (VC) is the maximum volume of gas that can be exhaled from the lungs after a maximal inspiration or inhaled from a point of maximal exhalation; inspiratory capacity (IC) is the maximum volume of gas that can be inspired from the normal end-expiratory position; functional residual capacity (FRC) is the volume of gas remaining in the lungs at the end of a resting expiration; and, the total lung capacity (TLC) is the volume of gas in the lungs at the end of a maximal inspiration.28

Clearly, many of these static volumes can be measured directly from normal breathing by instructing the subject to perform the four static positions: maximal inhalation, end inspiration, end expiration, and maximal exhalation. However, the gas remaining in the lung after a maximal expiration, known as the residual volume (RV), and the volume of gas in the lung after a normal expiration, known as the functional residual capacity (FRC), must be measured with body plethysmography using Boyle’s law.28 The subject is placed in an air-sealed plethysmograph chamber, as shown in Figure 1-6A, and breathes through a mouthpiece with a shutter. At the end of a normal expiration the shutter closes and the subject is instructed to inhale. As the subject tries to inhale, the pressure changes within the box and at the airway can be measured. Since the box volume is known, the volume of gas in the lungs when the shutter was closed (FRC) can be calculated.

1.3.1.3 Diffusing Capacity

The diffusing capacity of the lung for carbon monoxide (DL$_{CO}$) is another pulmonary function test that provides an indirect measure of diffusion of gases across the alveolar membrane into the blood. The body plethysmograph, as shown in Figure 1-6A, with the attached Medgraphics gas analyzer allows DL$_{CO}$ to be measured. DL$_{CO}$ is measured by instructing the subject to exhale to RV and then perform a maximum inhalation to TLC. As shown in Figure 1-8, during the inhalation the subject inhales a gas mixture that contains a very low concentration of carbon monoxide (CO) (0.3%) and helium (10%) with air; the subject is then instructed to hold their breath for 10 seconds before exhalation.29 The volume of gas exhaled is collected and analyzed. The gas first exhaled
is discarded as it represents the anatomic dead space volume and is defined as 2.2 ml x kg body weight. The remaining volume is analyzed by comparing the exhaled concentrations of CO and helium to the inspired concentrations to determine the amount of CO diffusing across the alveolar membrane.

Figure 1-8 Measurement of the Diffusing Capacity of the Lung for Carbon Monoxide
The subject inhales a mixture of 10% helium and 0.3% CO, holds his/her breath for 10 seconds then exhales. The anatomic dead space volume is discarded and the remaining alveolar sample is analyzed. Adapted from West JB. Respiratory Physiology: the essentials, Ninth edition.

According to the ATS/ERS task force, there should be at least two acceptable tests that meet the repeatability requirement of either being within 3 ml CO standard temperature (273 Kelvin), pressure (101.3 kPa, 760 mmHg) and dry (STPD)-min⁻¹-mmHg⁻¹ of each other or within 10% of the highest value. Factors that affect the DL\textsubscript{CO} include diseases that reduce the volume of pulmonary capillary blood such as anemia, and diseases that affect the area and thickness of the alveolar capillary membrane including emphysema, interstitial lung disease and pulmonary hypertension. Cigarette smoking has also shown to reduce DL\textsubscript{CO} and the ATS/ERS task force recommends that subjects refrain from smoking on the day the test is performed.
Pulmonary function test measurements are usually interpreted by comparing measured values in individual subjects with the values that are predicted for healthy subjects based on age, sex, height and race, and expressing the observed value as a percent of predicted. 

1.3.2 6 minute Walk Test

The 6 minute walk test (6MWT) is a self-paced exercise test that measures the distance a subject can walk on a flat, hard surface in a period of 6 minutes. The 6MWT is thought to reflect the functional exercise level for daily physical activities in subjects with respiratory disease. The 6MWT has been used for measuring the efficacy of inhaled corticosteroids in the treatment of COPD. It has also been used as a measure of functional status for COPD subjects and the 6 minute walk distance (6MWD) has been demonstrated to correlate significantly with mortality.

1.3.3 St. George’s Respiratory Questionnaire

The St. George’s Respiratory Questionnaire (SGRQ) is a questionnaire designed to measure impact on overall health, daily life, and perceived well-being in patients with obstructive airways disease. The questionnaire contains three component scores: symptoms, activity and impact (on daily life), and a total score. Scores range from 0 to 100, with higher scores indicating more severe limitation. In asthmatics and COPD subjects, the test-retest reproducibility has been determined to be high, and significant correlations have been shown between the SGRQ symptom score with frequency of wheeze, the SGRQ activity score with the 6MWD and between the SGRQ impact score with anxiety.

1.3.4 Other Pulmonary Function Tests

The Global initiative for chronic Obstructive Lung Disease (GOLD) recommends the use of the Modified British Medical Research Council (mMRC) questionnaire or the COPD Assessment Test (CAT) for the assessment of COPD symptoms. The mMRC questionnaire is grading system that is used to assess a patient’s level of dyspnea that range from Grade 0 (breathless with strenuous exercise) to Grade 4 (breathless when
dressing or undressing). The mMRC score correlates with standard lung function measurements, the 6MWD and, importantly, predicts mortality better than evaluating disease severity using the FEV1.

The CAT is an 8-item questionnaire that assesses the impact of cough, sputum, dyspnea and chest tightness on health status. The CAT scores range from 0 to 40 with the higher scores indicating a more severe impact of COPD symptoms on health status. CAT scores have been shown to correlate significantly with SGRQ scores in COPD.

Another test that has been recently developed uses four factors that were determined to have the strongest association with one-year morality, called the BODE index: the body mass index (B), the degree of airflow obstruction (O) measured using FEV1 as a percentage of the predicted value, dyspnea (D) measured from the score on the mMRC dyspnea scale, and exercise capacity (E) measured using the 6MWD. The BODE index after lung volume reduction surgery correlates with survival in COPD patients and has been shown to better predict hospitalization for COPD than FEV1 alone.

### 1.4 Lung Function Decline

#### 1.4.1 The Aging Lung

As we age, the most important physiological changes that occur in the lung are, 1) decreased elastic recoil, 2) decreased compliance of the chest wall, and, 3) decreased strength of the respiratory muscles. The decline in elastic recoil of the lung with age is attributed to changes in the lung connective tissue, and the decrease in chest wall compliance is thought to occur due to structural changes within the rib cage, such as decalcification of the ribs, calcification of costal cartilage, changes in rib-vertebral articulations, changes in the shape of the chest, narrowing in intervertebral disk spaces and age-related osteoporosis, which all result in stiffening of the chest. Finally, respiratory muscle strength is known to decrease with age. Respiratory muscle strength has been shown to be related to nutritional status, which is often poor in the elderly. An age-related decrease in respiratory muscle strength is also related to a decrease in muscle mass and a decrease in the number of muscle fibres.
Another important structural change that occurs during aging is the structural changes within lung parenchyma – known as “senile emphysema.” Studies have demonstrated that homogeneous airspace size enlargement occurs over 60 years of age in the normal lungs, without a loss of alveolar attachments and chronic inflammation. It has also been demonstrated that there is thickening of alveolar walls and a reduction in the number of peripheral airways. A consequence of the reduction in supporting tissues around the airways is a tendency for the small airways to collapse at higher lung volumes.

The lung physiological changes that occur during aging result in changes in lung function. Figure 1-9 shows the changes in lung volumes that occur during aging. Early studies have shown that during normal aging, the RV increases while the TLC remains constant. The increase in RV that occurs with age is likely due to the decreased strength of the expiratory muscles and the tendency of the small airways to collapse. There is also an increase in anatomic dead space that occurs during aging and this is thought to be due to calcification of the bronchial cartilage. The DLCO is also been shown to decrease with age and this is likely a result of the decreased alveolar surface area as well as a decrease in the pulmonary capillary blood volume.

In the landmark study by Fletcher and Peto in 1977, airflow obstruction measured using FEV1 was evaluated for 103 men who were non-smokers aged 30-59. As shown in Figure 1-10, FEV1 was shown to decline continuously with age. However, this study was only performed in men and was cross-sectional. Subsequent studies evaluating FEV1 decline with age showed the rate of decline was greater in men than women, and the rate of decline was found to be much higher in longitudinal studies.
As described in the previous section on risk factors for COPD, smoking cigarettes, although not the only risk factor, is the main source of inhaled noxic particles that leads to the development of COPD. However, not all smokers develop airflow limitation; early studies demonstrated that cigarette smoking is associated with inflammation in both the large and small airways and the lung parenchyma in smokers with normal FEV1. Although it is unclear why some smokers develop COPD while others do not, it is known that the inflammatory response in COPD is amplified and persists long after smoking cessation. Irreversible airflow limitation is the defining feature of COPD and is associated with “lesions that obstruct the small conducting airways, produce emphysematous destruction of the lung’s elastic recoil force, or both.” Importantly, the disease that occurs within the airways and the lung parenchyma are independent disease processes that may develop in tandem or in isolation. In this section I will first introduce how COPD is diagnosed using spirometry and the natural history of COPD. Finally, I will discuss the underlying disease mechanisms that are thought to be responsible for the functional lung changes.

1.4.2.1 Diagnosis and Classification of COPD

When COPD is suspected based on symptoms, such as dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors, airflow limitation is measured...
using spirometry and the presence of a post-bronchodilator FEV₁/FVC <0.70 confirms the diagnosis of COPD.¹⁵⁹ As shown in Table 1-1, COPD severity is determined according to the GOLD criteria, which uses specific spirometric cut-points that are obtained after inhalation of a short-acting bronchodilator in order to minimize variability.⁵⁹

**Table 1-1** Classification of Severity of Airflow Limitation in COPD

<table>
<thead>
<tr>
<th>GOLD Stage</th>
<th>Severity</th>
<th>Post-Bronchodilator FEV₁ Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOLD Stage I</td>
<td>Mild</td>
<td>FEV₁ ≥ 80% predicted</td>
</tr>
<tr>
<td>GOLD Stage II</td>
<td>Moderate</td>
<td>50% ≤ FEV₁ &lt; 80% predicted</td>
</tr>
<tr>
<td>GOLD Stage III</td>
<td>Severe</td>
<td>30% ≤ FEV₁ &lt; 50% predicted</td>
</tr>
<tr>
<td>GOLD Stage IV</td>
<td>Very Severe</td>
<td>FEV₁ &lt; 30% predicted</td>
</tr>
</tbody>
</table>

Adapted from the Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (Revised 2011), Global Initiative for Chronic Lung Disease¹

Although this approach has been introduced due to its diagnostic simplicity and ease of use in the primary care setting, the FEV₁/FVC cut off has been acknowledged to potentially result in an over-diagnosis of COPD in the elderly,⁶⁶ as well as under-diagnosis of mild or early stage COPD.⁶⁷ Moreover, there is only a weak correlation between GOLD spirometric severity classification and symptom scores reflecting health-related quality of life.

**1.4.2.2 Natural History of Airflow Obstruction in COPD**

**Figure 1-10** reproduces the landmark study by Fletcher and Peto⁵⁶ that shows the different rates of lung function decline, as measured by FEV₁, for healthy individuals that have never smoked in comparison with those individuals who were current cigarette smokers that exhibit the amplified inflammatory response that is associated with the development of COPD. This study indicates that while the rate of decline of FEV₁ in healthy non-smokers is 42±6 ml/year, this rate is accelerated to 47±3 ml/year in light
smokers and to $66 \pm 4$ ml/year in heavy smokers. Interestingly, this study also showed that the rate of FEV$_1$ decline was slower in individuals that had quit smoking ($32 \pm 4$ ml/year), suggesting that decline in lung function can be slowed by smoking cessation. However, as described previously, this was a cross-sectional study in men.

The more recent longitudinal Lung Health Study$^{68,69}$ also evaluated smokers that were randomized into three groups: a group of smokers who received no intervention, a group of smokers who received smoking cessation intervention, and a group of smokers who received smoking cessation intervention and bronchodilator therapy, and followed the subjects over 11 years. In accordance with the findings by Fletcher and colleagues,$^{56}$ this study found that men who quit smoking at the beginning of the study had a FEV$_1$ decline of 30 ml/year while men who continued to smoke had an accelerated declined of 66 ml/year. In contrast to the study performed by Fletcher and colleagues,$^{56}$ the Lung Healthy Study also evaluated women and showed that women who quit smoking at the beginning of the study had a FEV$_1$ decline of 22 ml/year while women who continued to smoke declined by 54 ml/year.$^{69}$ Interestingly, when decline in FEV$_1$ was expressed as a percentage of the predicted normal value, there were no significant differences between the sexes.$^{69}$
Chronic Bronchitis

Chronic bronchitis is defined as “the presence of cough and sputum production for at least 3 months in each of two consecutive years.” Early work investigating chronic bronchitis attributed the mucous hypersecretion with thickening of the bronchial gland. Reid reported that this thickness was related to gland hypertrophy and that there was a correlation between the amount of sputum produced and gland thickness. It was later shown that chronic bronchitis was associated with bronchial inflammation. Mullen and colleagues demonstrated that smokers with chronic bronchitis had greater inflammation of the cartilaginous airways (> 4 mm in internal diameter) than smokers without chronic bronchitis, and that there was a significant correlation between inflammation of the cartilaginous airways and mucous gland thickness. These data suggest that chronic cough and sputum production was associated with an inflammatory response due to the cigarette smoke in the cartilaginous (or central) airways. Interestingly, it has also been demonstrated that this inflammatory response persists in the central airways of smokers who had quit but continued to report mucus hypersecretion.

Figure 1-10 Lung Function Decline
Lung function declines in never-smokers during aging and declines at an accelerated rate in current smokers. However, lung function declines return to normal rates following smoking cessation. Adapted from Fletcher et. al. (1977).
Much of this early work was performed in smokers with and without airflow limitation measured using FEV₁ and therefore more recent studies have focused on evaluating whether there is a predictive relationship between the presence of chronic cough and sputum production and the future occurrence of COPD. de Marco and colleagues recently evaluated a group of young subjects, aged 20-45, with normal lung function over a period of approximately 9 years. It was shown that at the baseline visit, 9% of subjects reported chronic cough and sputum production and of these subjects that reported persistent symptoms at follow-up, there was a threefold increased risk of developing COPD. Another study demonstrated that chronic cough and sputum production was associated with an increased decline in FEV₁ and subsequent hospitalization due to COPD.

1.4.2.4 Small Airways Disease

Based on the study by Weibel and Gomez, it is known that as the airway generation increases, due to the dichotomous branching pattern of the airways, the number of airways rapidly increases and therefore the total cross-sectional area increases, as shown in Figure 1-5. Although the smaller airways could be considered the major site of resistance in normal lungs based on Poiseuille’s equation that indicates that resistance in a single tube is inversely proportional to the radius of the tube to the 4th power, by inspection of the airways and according to Weibel’s data, we must also consider the number of airways and their total cross-sectional area. Although the radius of the airways decrease as airway generation increases, the number and cross-sectional area of the airways increases. Since these airways are arranged in parallel, the resistances are added as reciprocals and therefore the overall resistance is very small in the small airways.

Early studies by Hogg and Macklem that directly measured small airway pressure using a retrograde catheter confirmed that in healthy subjects, the small airways contributed very little to the total airway resistance. However, they also demonstrated that in subjects with mild and severe emphysema, as well as subjects with bronchiectasis and bronchiolitis that there was a marked increase in the resistance of the small airways (<2 mm in diameter), while there was little or no change in total lung resistance. The authors
observed that the bronchioles were often narrowed and occluded with mucus plugging and defined these changes in the small airways as “small airways disease,” where disease changes may occur without being detected by measuring total airway resistance or by FEV\textsubscript{1}. Taken together, since the total resistance is determined largely by the more central airways, disease may accumulate in the small airways without being detected.

It was later shown in histopathological studies that inflammation was present in the peripheral airways of smokers\textsuperscript{76} and that the most characteristic lesion in the small airways of smokers was respiratory bronchiolitis.\textsuperscript{61} However, these early reports did not perform spirometry in the smokers and therefore the subjects may or may not have had COPD. A more recent study by Hogg and colleagues\textsuperscript{77} evaluated the pathology of the small airways in relation to COPD severity, as measured by the GOLD COPD stages (Table 1).\textsuperscript{1} These authors found that although the degree to which the lumen was filled with mucous was significantly correlated with the severity of COPD, the strongest parameter associated with progression of COPD from GOLD stage 0 to GOLD stage IV was with thickening of the airway wall.\textsuperscript{77} It was also shown that the extent of inflammation was significantly correlated with COPD severity.\textsuperscript{77} Figure 1-11A shows a normal small airway of a non-smoker in comparison with a small airway filled with mucus (Figure 1-11B), a small airway with thickened airway walls and a lumen partially filled with mucus (Figure 1-11C) and a small airway with wall thickening that is thought to restrict airway caliber with lung inflation (Figure 1-11D).

1.4.2.5 Emphysema

Emphysema was first described by Laennec in 1834. He described emphysema of the lungs as “dilation of the air cells” that “may affect both lungs at the same time, one only, or a part of one or of both.”\textsuperscript{78} More recently, the National Heart, Lung, and Blood Institute defined emphysema as “a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by the destruction of their walls, and without obvious fibrosis.”\textsuperscript{79} It is well known that smokers develop two distinct forms of emphysema: centrilobular and panlobular emphysema.\textsuperscript{80-82} Centrilobular emphysema results from dilation and destruction of the respiratory bronchioles, as shown in Figure 1-11E, and has been demonstrated to have a more
uneven pattern of tissue destruction, predominantly occurring in the upper lung lobes. Conversely, panlobular emphysema has a more homogenous pattern and is more likely to be associated with genetic disorders such as alpha-1 antitrypsin deficiency (Figure 1-11F). Moreover, early studies indicated that there was a relationship between cigarette smoking and centrilobular emphysema, but not with panlobular emphysema. Studies in smokers and non-smokers demonstrated that inflammatory cells play a role in the parenchyma tissue destruction in smokers, and that this cigarette smoke-induced inflammation is amplified in subjects with emphysema versus smokers without emphysema.
**Figure 1-11** Small Airways Disease and Emphysema in COPD

A. Normal small airway.  
B. Small airway containing plug of mucus.  
C. Acutely inflamed airway with thickened wall in which the lumen is partly filled with an inflammatory exudate of mucus and cells.  
D. Airway surrounded by connective tissue, which appears as if it might restrict normal enlargement of the lumen and unfolding of the epithelial lining that occurs with lung inflation.  
E. Early lesions of centrilobular emphysema (CLE) that have destroyed central portions of several acini of a single secondary lobule.  
D. More even destruction of the lobule in panacinar emphysema.  
Reproduced with permission from Hogg (2004).
1.5 Imaging Lung Structure and Function in COPD

1.5.1 Chest X-ray

Chest x-ray and x-ray computed tomography (CT) are the two most common lung imaging procedures performed. Chest x-ray is often the first line imaging method because it is widely available, inexpensive, allows visualization of pulmonary structures, including the trachea, carina and major bronchi, mediastinum and hilar regions and the diaphragm, as shown in Figure 1-12, and has a relatively low radiation dose.\(^8\) In the posterior-anterior view, as shown in Figure 1-12A, the patient is upright with the x-ray source positioned so that the x-rays enter through the back (posterior lung) and the anterior surface of the chest is against the film holder. The patient’s hands are placed on their hips with the elbows rolled slightly forward so that the scapulae (or shoulder blades) are removed from the lung field.\(^8\) In the lateral view, as shown in Figure 1-12B, the patient is positioned with their side against the film with their arms over their head so that the arms are not in the lung field.\(^8\) The different lung structures described above can be depicted with a chest x-ray because the x-rays are attenuated differently by the various body tissues. For example, the ribs show up as bright structures because they attenuate the x-rays more than the lung tissue, which are air-filled, and show up as dark regions in the image. This is also the reason why the arms and the scapulae are removed from the lung field.

The radiation dose associated with a typical chest x-ray is approximately 0.01 millisieverts (mSv) which is equivalent to approximately 3 days of background radiation.\(^8\) Although there is very low radiation dose associated with chest x-ray, and identification of lung structures allows abnormalities in the lungs to be detected, such as increased translucency and flattening of the diaphragm which is commonly associated with gas trapping in respiratory diseases, the limitation of chest x-ray is that all anatomic structures are superimposed onto each other. This results in difficulty diagnosing COPD and in particular for the early disease stages where disease-related structural changes may be subtle. While there is some conflicting views on the utility of chest radiography in assessing COPD,\(^2\) and more specifically for the assessment of emphysema, a study comparing chest x-ray with a morphologic diagnosis of emphysema post-mortem...
indicated that a diagnosis of emphysema using chest x-ray was made in only 16% of subjects that were pathologically proven to have mild to moderate emphysema and in only 42% of subjects with moderately severe to severe emphysema.\textsuperscript{88}

1.5.2 X-ray Computed Tomography

Since its introduction in the early 1970s, CT scanning technology has become so advanced that it is now considered by many as the imaging technology of choice for many diseases. As described above, a limitation of conventional x-ray imaging is that it provides a two-dimensional image of a three-dimensional object. CT overcomes this limitation by acquiring the image in slices thereby providing images of three-dimensional objects. Each slice in a CT image is composed of voxels and each voxel is assigned a CT value or Housfield unit (HU) based on the attenuation coefficient relative to water. Therefore, a voxel that contains water will have a value of 0 HU, while air will have a value of -1000 HU and tissues or structures that have a higher attenuation coefficient than water will have a positive HU value; bone has CT values up to 2000 HU.\textsuperscript{85}

Emphysema is characterized by airspace enlargement,\textsuperscript{79} and therefore the air-filled emphysematous regions in the lung will appear dark in CT images. \textbf{Figure 1-13A} shows a central slice CT image for a healthy subject and a COPD subject with emphysema. The
air filled regions, including the trachea as well as the large left apical bullae in the COPD subject with emphysema appear dark in the image. Early pathological correlative studies demonstrated that regions of the lung that visually appeared to be emphysematous on CT images were confirmed as emphysematous in post-mortem evaluations. Later, studies that used radiologist CT grading and pathological grading systems demonstrated that there were strong and statistically significant correlations between CT and pathology emphysema scores, however it was found that CT could not differentiate mild emphysema from normal parenchyma confirmed by pathology in some patients.

Although CT was demonstrated to be very accurate in the determination of the presence and extent of moderate to severe emphysema, with strong and significant correlations found with both pathology and measurements of airflow limitation and diffusion capacity, these early visual scoring systems were subjective and therefore there was considerable inter-observer and intra-observer variability. Because visual assessment was based on the presence of voxels with abnormally low attenuation, the extent of emphysema could be assessed by highlighting voxels within specified ranges of HU called “density masks.” Density mask emphysema scores were demonstrated to be comparable to visual emphysema scores and had strong and significant correlations with pathologic scores, and importantly, eliminated the inter-observer and intra-observer variability introduced by visual assessment. Figure 1-13B shows the CT density mask with all voxels with attenuation values less than -950 HU highlighted in red. Although there are several thresholds that have been introduced, and some contrasting results on which threshold is most appropriate, it has been demonstrated that the low attenuating areas quantified on CT are indicative of the lung tissue destruction that accompanies emphysema.

Although strong and significant correlations between CT measurements of emphysema and diffusion capacity measurements have been reported, there have been only moderated correlations with measurements of airflow. This finding is not surprising given that measurements of airflow limitation (FEV$_1$) are measuring the contributions of the loss of elastic recoil due emphysematous tissue destruction (emphysema) as well as airway narrowing and obstruction (chronic bronchitis and small airways disease). Therefore, it is...
also of great interest to obtain measurements of the airway dimensions from CT. Figure 1-14 shows an airway tree segmentation that was generated in three-dimensions from the CT image. The cross-section of the airway of interest can then be identified and the airway dimensions can be measured. Nakano and colleagues were the first to demonstrate that the percentage of the total airway (airway wall area plus lumen area) that was airway wall (wall area percent, WA%) significantly correlated with airflow limitation, but not DLCO, in COPD smokers independent of emphysema. However, these measurements were based on a large central airway and it is well known, as described above, that the major site of airflow limitation in COPD occurs in the small airways less than 2 mm in diameter. Accordingly, more recent studies have demonstrated that the correlation between CT wall area dimensions with airflow limitation was stronger for higher generation airways.

![Figure 1-13 Coronal CT of a healthy smoker and a COPD ex-smoker](image)

The central slice coronal CT image (A) with the corresponding density mask with all voxels with attenuation values less than -950 HU highlighted in red (B) for a healthy subject and a COPD subject with emphysema.
Although thoracic CT is capable of measuring both emphysema and airways disease in COPD, CT is not without its costs and the field of medicine has observed a considerable increase in the number and type of CT scans performed for clinical investigation where now, in the United States, CT accounts for 24% of all radiation exposure and 50% of all medical radiation exposure. Importantly, the increase in CT scans was also observed in the paediatric population and a study by Mettler and colleagues reported that children aged 0-15 years old accounted for 11.2% of all CT scans performed. This is a concern because the effective dose for a thoracic CT is approximately 8 mSV, which is equivalent to 400 chest x-rays or approximately 4 years of background radiation. In COPD, serial imaging is important for evaluating disease progression and response to treatment or intervention, and therefore the increase in radiation exposure from CT has been a major concern because there is an increased risk of radiation-induced cancer from the cumulative dose related to repeated CT investigations.
1.5.3 Nuclear Medicine

In their seminal study on the site and nature of airway obstruction in COPD, Hogg and colleagues stated that as a result of the obstruction of the small airways in the lung, there is a “strong likelihood that, at some stage, chronic obstructive airway disease causes significant abnormalities in the distribution of ventilation, and presumably in gas exchange.” Therefore, in COPD ventilation may be highly inhomogeneous across the lung or absent in certain lung regions all together. Ventilation scintigraphy can be performed with radioaerosols or radioactive gases for visualization of the regional distribution of ventilation in the lung. Images can be acquired during the gas wash-in phase, at the “steady state” and during the gas wash-out phase. The breathing protocol utilized depends largely on the half-life of the isotope. For example, the short half-life of Krypton-81m of 13 seconds limits its use for evaluating the gas in the wash-in or steady state phase as decay occurs prior to exhalation. Studies have demonstrated that subjects with abnormal pulmonary function tests also have abnormal aerosol deposition patterns, and smokers with reported symptoms and normal pulmonary function measurements often had abnormalities with xenon-133 or aerosol lung imaging, suggesting that functional lung imaging may be more sensitive than spirometry for detecting early disease changes. However, central aerosol deposition is often reported and may be caused by the large particle size or by abnormally rapid or shallow breathing patterns, or both. Taplin and colleagues showed that 10% of subjects having central deposition at one imaging time-point showed normal distribution 1-2 hours later.

The lung attempts to match blood flow to ventilation to maintain optimal efficiency of gas exchange, and therefore subjects with ventilation abnormalities may have perfusion abnormalities as well. Perfusion scintigraphy is often performed using technetium-99m (Tc) labeled macro-aggregated albumin (MAA) injected in a peripheral vein. Studies have shown that there is a significant correlation between perfusion and aerosol lung imaging suggesting that regional perfusion abnormalities are likely related to diminished ventilation within those areas.

While lung scintigraphy acquires two-dimensional projection images, single photon emission tomography (SPECT) allows three-dimensional assessment of ventilation and
perfusion. Studies have recently shown that ventilation/perfusion SPECT with $^{99m}$Tc-labeled carbon (Technegas) is the aerosol of choice for COPD lung imaging, providing more homogeneous distribution within the lungs with less focal deposition in both larger and smaller airways.\textsuperscript{107} It was also recently demonstrated that the extent of ventilation/perfusion abnormalities in COPD subjects was significantly correlated with both spirometric measurements of lung function and with the extent of emphysema measured using CT, but not with COPD-related symptoms.\textsuperscript{108} Although ventilation/perfusion SPECT has been demonstrated to regionally evaluate abnormalities in COPD, it is limited by its low spatial resolution, long image acquisition times and the necessity for inhalation of a radioactive substance.

### 1.6 Magnetic Resonance Imaging

In contrast to other diagnostic imaging methods, magnetic resonance imaging (MRI) does not require the use of x-rays or other ionizing radiation, offering the potential for intensive serial and longitudinal studies in the same patient. MRI is also becoming an important imaging tool because MRI can provide both structural and functional lung information.

#### 1.6.1 Conventional Proton MRI

MRI is an imaging tool that provides unique tissue contrast with high spatial and temporal resolution. However, despite these very practical advantages, MRI of the lung has some unique technical challenges. Figure 1-15 illustrates the lower visibly obvious information content provided by conventional proton ($^1$H) MRI as compared to chest CT for evaluating COPD. Using conventional $^1$H MRI alone, without special attention to echo time, the thoracic cavity appears as a black hole because in the normal lung, tissue density (and consequently $^1$H density) is relatively low in comparison to gas density. Second, the $^1$H MRI lung tissue signal is degraded due to the air-tissue interfaces that introduce microscopic magnetic field inhomogeneities.\textsuperscript{109} Third, the signal is further degraded by respiratory and cardiac motion because of the relatively slow image acquisition time relative to CT. Therefore, the application of conventional $^1$H MRI for
the detection of structural abnormalities in the lung has not progressed beyond the research setting, even though there is great potential for its use.\textsuperscript{110}

To overcome these shortcomings, numerous studies have endeavoured to develop more sensitive $^1$H MRI techniques for lung imaging, including ultra-short echo time (UTE) methods,\textsuperscript{111-113} oxygen-enhanced MRI\textsuperscript{114-124} and Fourier decomposition methods.\textsuperscript{125-129} Although experience is limited in COPD, MRI of cystic fibrosis has been shown to be comparable to CT for identifying morphological changes, such as bronchiectasis, bronchial wall thickening, mucus plugging, air fluid level, consolidation and segmental/lobar destruction.\textsuperscript{130} Although it is clear that MRI lacks the spatial resolution achieved by CT to identify the more subtle morphological changes that are likely to occur in mild disease,\textsuperscript{130} the advantage of MRI is that assessment of lung function is also possible. Oxygen-enhanced MRI allows regional visualization of oxygen diffusion from the alveoli into the capillaries,\textsuperscript{114} and initial studies in COPD demonstrated measurements of oxygen enhancement can differentiate between healthy volunteers and subjects with pulmonary emphysema,\textsuperscript{120} and showed strong and significant correlations with FEV\textsubscript{1}\textsuperscript{120,122} and DL\textsubscript{CO} in subjects with emphysema.\textsuperscript{119,120,122} Fourier decomposition MRI allows lung perfusion and ventilation to be assessed, with no contrast enhancement, and preliminary studies demonstrated that Fourier decomposition MRI is feasible and reproducible in respiratory disease.\textsuperscript{125-129} These previous studies suggest that oxygen-
enhanced MRI and Fourier decomposition MRI have the potential to provide reproducible structural and functional measurements in COPD.

1.1. Hyperpolarized Noble Gas MRI

Over the last two decades, MRI using inhaled hyperpolarized noble gases helium-3 ($^3$He)\textsuperscript{131-144} and xenon-129 ($^{129}$Xe)\textsuperscript{145;146} have been shown to provide structural and functional measurements in healthy volunteers as well as subjects with a range of respiratory conditions. These approaches are based on the serendipitous finding of Albert and colleagues\textsuperscript{145} in 1994 that showed the potential of inhaled hyperpolarized or magnetized noble gas for pulmonary MRI. Albert and colleagues\textsuperscript{145} recognized that polarization of $^3$He and $^{129}$Xe gases could be enormously enhanced through spin-exchange with an optically pumped alkali metal vapour – rubidium.\textsuperscript{147} Albert demonstrated that gas-phase collisions between $^{129}$Xe and the polarized rubidium atoms resulted in the transfer of angular momentum from the rubidium valence electron to the $^{129}$Xe nucleus and thereby increased the signal by $\sim 100,000$ times.\textsuperscript{145} Although the first investigations utilized the isotope $^{129}$Xe,\textsuperscript{145;146} the field quickly transitioned to $^3$He due to the nearly 3-fold higher gyromagnetic ratio ($^3$He: 32.4 MHz/T, $^{129}$Xe: 11.8 MHz/T) and higher enrichment – both of which contribute to a greater MRI signal with $^3$He than $^{129}$Xe. Importantly, excellent subject safety and tolerability across a wide spectrum of volunteers has been reported with both $^3$He\textsuperscript{148} and $^{129}$Xe MRI.\textsuperscript{142;149}

1.1.1. Biomarkers

Hyperpolarized gas MR imaging allows the quantification of several structural/functional components of the lung, commonly referred to as “biomarkers”. The most established measurements are the ventilation defect percent\textsuperscript{150;151} and the apparent diffusion coefficient (ADC).\textsuperscript{152-154} Figure 1-16 shows the static ventilation images and the corresponding ADC maps for a representative subject with normal lung function in contrast with three representative COPD subjects with varying lung function.

1.1.1.1. Ventilation Defect Measurements
Early *in vivo* studies in humans obtained these “spin density” or “static ventilation” images in healthy subjects and demonstrated homogenous signal intensity within the lung, with clear delineation of the diaphragm, heart, chest wall and blood vessels, which appeared as signal voids in the images.\textsuperscript{155-157} The 1st \textsuperscript{3}He MRI investigation in COPD was performed by Kauczor and colleagues\textsuperscript{131} and they demonstrated heterogeneous signal intensity and ventilation abnormalities or “defects,” which were thought to represent local hypoventilation or non-ventilated regions of the lung. Subsequent studies also demonstrated extensive ventilation defects in subjects with severe emphysema that corresponded to defects seen on xenon-133 ventilation (wash-in) scintigraphy.\textsuperscript{132} A larger study evaluating hyperpolarized \textsuperscript{3}He MRI with xenon-133 scintigraphy also found fairly good concordance between the two modalities for depiction ventilation abnormalities in 15 subjects.\textsuperscript{158}

Although the first \textsuperscript{3}He MRI ventilation analyses were based on a radiologist’s interpretation of the ventilated lung regions,\textsuperscript{132;158-160} more recent investigations have utilized scoring and manual volumetric analysis approaches.\textsuperscript{89;150;151;158;160-170} Using these quantitative approaches it was demonstrated that ventilation defect measurements were highly reproducible in COPD for same day evaluations as well as for the images acquired one week later.\textsuperscript{89} Importantly, \textsuperscript{3}He ventilation defect measurements in COPD were also shown to correlate significantly with spirometric measurements of airflow limitation.\textsuperscript{151}

### 1.1.2. Apparent Diffusion Coefficient

Another type of hyperpolarized gas MR image that is commonly acquired uses diffusion-weighted MRI. Hyperpolarized gas diffusion-weighted MRI is sensitive to the self-diffusion of the gas atoms within the lung microstructure. During the diffusion time interval, the hyperpolarized gas atoms diffuse by Brownian motion and the walls of the alveolar structures act as barriers by restricting the gas atoms motion or displacement. Therefore, an “apparent” diffusion coefficient (ADC) can be derived that reflects the level of restriction of the gas atoms within the airways and airspaces of the lung. It is important to note that in contrast to \textsuperscript{3}He, \textsuperscript{129}Xe gas is capable of transmembrane diffusion across the alveolar wall, providing both measurements of alveolar size and
transmembrane diffusion kinetics;\textsuperscript{134-146} in contrast \textsuperscript{3}He gas is biologically inert and therefore it cannot be passively transported across intact biological tissues and membranes.

Importantly, both \textsuperscript{3}He and \textsuperscript{129}Xe ADC measurements have been demonstrated to significantly correlate with age,\textsuperscript{140,171} with standard measurements of pulmonary function\textsuperscript{140,152,172,173} and with CT emphysema measurements.\textsuperscript{169,172-174} The \textsuperscript{3}He ADC has been more extensively evaluated and was shown to be highly reproducible\textsuperscript{89,163,175,176} and significant correlations with histology measurements of airspace size have been demonstrated.\textsuperscript{177} Abnormally elevated \textsuperscript{3}He ADC has also been reported in asymptomatic smokers without COPD,\textsuperscript{174,178} suggesting that \textsuperscript{3}He ADC provides a way to sensitively measure regional lung tissue destruction in early disease.

Another advantage of hyperpolarized gas MR diffusion-weighted imaging is that by changing the diffusion time and gradient strength, different features of the lung microstructure may be evaluated. For example, short-range diffusion experiments are most sensitive to changes in local microstructure (e.g. alveolar destruction), while long-range diffusion experiments are sensitive to the connections between airways and larger lung structures and possibly collateral ventilation in COPD.\textsuperscript{179,180} Moreover, obtaining diffusion-weighted images at multiple $b$-values, measurements of the geometric parameters at the level of the alveoli can be made.\textsuperscript{181-184}
Figure 1-16 Hyperpolarized $^3$He MRI Static Ventilation Image and ADC Map in a Healthy ex-smoker and COPD ex-smokers
Healthy ex-smoker is a 70 yr old male: FEV$_1$=101%pred, FEV$_1$/FVC=0.75; COPD subject 1 (S1) is a 74 yr old male: FEV$_1$=86%pred, FEV$_1$/FVC=0.54; COPD S2 is a 72 yr old female: FEV$_1$=64%pred, FEV$_1$/FVC=0.65; COPD S3 is a 60 yr old female: FEV$_1$=50%pred, FEV$_1$/FVC=0.38.
1.7 Thesis Hypotheses and Objectives

Hyperpolarized $^3$He MRI provides high spatial and temporal resolution images of the lung air spaces in subjects with COPD. These images allow us to directly visualize the lung regions accessed by the hyperpolarized gas during a breath-hold, as well as the regions of the lung not accessed which appear as signal voids and are referred to as “ventilation defects.” The lung micro-structure can also be probed using diffusion-weighted imaging which takes advantage of the rapid $^3$He and $^{129}$Xe atom Brownian motion to generate surrogate measurements of alveolar size. Since the establishment of inhaled gas MRI for pulmonary imaging in 1994, there have been significant advancements in the type and scope of images that can be acquired, however, in order for broader translation of this imaging technology to occur, and for the potential translation of hyperpolarized gas MRI for clinical use, it must be demonstrated that these measurements relate to clinically meaningful outcomes. The overarching objective of this thesis was to generate hyperpolarized gas MRI measurements and measurement tools for the regional quantitative evaluation of hyperpolarized gas MRI with sufficient precision to evaluate COPD disease progression and treatment response. Another important step in the broader translation of this imaging technology is transitioning to $^{129}$Xe MRI, and comparison of hyperpolarized $^{129}$Xe with $^3$He MRI is important in order to realize the potential of $^{129}$Xe MRI for respiratory research. The specific objectives and hypotheses tested in each chapter of this thesis are described below.

To better understand the potential for hyperpolarized $^3$He MRI to provide quantitative longitudinal COPD endpoints, the objective of Chapter 2 was to quantitatively evaluate a small group of COPD ex-smokers and healthy volunteers over 2 years using hyperpolarized $^3$He MRI. As described above, much of our current understanding of the natural history of COPD arises from the landmark study of Fletcher and colleagues$^{56}$ who showed that lung function measured using FEV$_1$ declines as we age, and that this decline is accelerated in smokers with COPD; in ex-smokers, however, they showed that this decline in FEV$_1$ returned to normal rates. Therefore, based on the Fletcher curve prediction, we hypothesized that the longitudinal changes in $^3$He MRI measurements in
the COPD ex-smokers would be similar to those observed in elderly healthy never-smokers.

This previous work evaluating longitudinal changes in $^3$He MRI measurements was performed using manual segmentation approaches, which clearly increases segmentation time and, importantly, introduces the potential for inter- and intra-observer variability. For serial evaluation of $^3$He MRI it is critical that the change measured over time represents physiological change that has occurred and is not due to measurement error. Therefore, the objective of Chapter 3 was to generate a semi-automated segmentation method for $^3$He MRI to enable its use in longitudinal and serial studies, and to compare the reproducibility and spatial agreement of the developed semi-automated segmentation algorithm to manual segmentation. We hypothesized that semi-automated measurements would not be statistically significantly different from manual measurements and have significantly reduced inter- and intra-observer variability.

Using the segmentation algorithm developed in Chapter 3, the objective of Chapter 4 was to evaluate a group of COPD subjects using $^3$He MRI prior to and immediately following bronchodilator therapy. A significant bronchodilator response is currently defined as an increase in post-bronchodilator spirometry, however, based on the findings in Chapter 2 we hypothesized that $^3$He MRI would provide the necessary sensitivity as well as precision to detect any potential regional functional lung changes after bronchodilator therapy in COPD subjects regardless of spirometry based responder classification.

In the same group of COPD ex-smokers evaluated using $^3$He MRI pre- and post-bronchodilator therapy in Chapter 4, the objective of Chapter 5 was to further evaluate the regional effects of bronchodilator administration in COPD using $^3$He MRI ADC measurements. Regional evaluation of tissue micro-structure using $^3$He MRI ADC would provide important insight into the lung alterations that accompany the improvements in regional $^3$He gas distribution after bronchodilator administration that were previously observed. In order to do this we first developed image registration/segmentation methods for quantifying ADC in the lung regions newly ventilated post-bronchodilator. We hypothesized that the regions of the lung that were newly ventilated following
bronchodilator therapy were not more emphysematous than the remaining lung tissue, and that the $^3$He ADC could measure significant reductions in regional gas trapping following bronchodilator therapy.

Despite the unique potential of $^3$He MRI and the previous work demonstrating its potential for use in evaluating COPD outcomes, the broader adoption of hyperpolarized gas MRI requires a transition from $^3$He gas, which has limited and unpredictable global quantities and high cost, to $^{129}$Xe gas, which is substantially more abundant in nature existing in measurable quantities in the atmosphere. The objective of Chapter 6 was to quantitatively compare hyperpolarized $^3$He and $^{129}$Xe MRI acquired within a few minutes in healthy volunteers and subjects with COPD, and to evaluate the correlations between $^3$He and $^{129}$Xe MRI measurements with standard measurements of pulmonary function. We hypothesized that the different properties of $^{129}$Xe gas would result in significant differences in $^{129}$Xe compared to $^3$He gas distribution measurements in COPD but not in healthy volunteers.

To better understand the morphological determinants for the ventilation differences observed between hyperpolarized $^3$He and $^{129}$Xe MRI in COPD in Chapter 6, the objective of Chapter 7 was to evaluate the same group of COPD subjects using the image registration/segmentation methods for quantifying ADC on a regional basis developed in Chapter 5. We hypothesized that emphysematous regions in the lung would more readily fill with $^3$He as compared to $^{129}$Xe gas, leading to decreased $^{129}$Xe MRI ventilation.

While it is important to demonstrate that $^3$He MRI measurements relate to important clinical outcomes, such as disease progression and treatment response, another goal of imaging is to identify early disease changes. The objective of Chapter 8 was to evaluate and compare well-established clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and abnormal DL$_{CO}$ with a group of ex-smokers with normal spirometry and DL$_{CO}$ and ex-smokers with GOLD stage I COPD. We hypothesized that ex-smokers with normal spirometry but abnormal DL$_{CO}$ would have significantly worse symptoms, exercise capacity and $^3$He MRI ADC than ex-smokers with normal DL$_{CO}$.
The objective of Appendix A was to establish imaging measurement reproducibility in adult cystic fibrosis subjects over a short period of time (7 ± 2 days) in the absence of therapeutic intervention using hyperpolarized $^3$He MRI. We hypothesized that $^3$He MRI would provide the necessary and sufficient spatial and temporal sensitivity to detect day-to-day changes in lung function.

A case report of a COPD ex-smoker that was evaluated serially over 4 years using hyperpolarized $^3$He MRI, twice prior to and twice following an acute exacerbation requiring hospitalization is provided in Appendix B. Based on our previous studies demonstrating the high sensitivity of $^3$He MRI for measuring improvements in the absence of FEV$_1$ improvements, we hypothesized that following hospitalization and treatment, visibly obvious improvements in $^3$He gas distribution would be observed prior to improvements in FEV$_1$.

Finally, in the last chapter of this thesis I will provide an overview and summary of the important findings and conclusions of Chapters 2-8. I will also address the study specific limitations as well as general limitations of the hyperpolarized gas MRI studies presented, and provide some potential solutions. Finally, based on the findings and limitations discussed, I will outline a roadmap for future hyperpolarized gas MRI studies.


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CHAPTER 2

To better understand the potential for hyperpolarized $^3$He MRI to provide quantitative longitudinal COPD endpoints, here we quantitatively evaluated a small group of COPD ex-smokers and healthy volunteers over 2 years using hyperpolarized $^3$He MRI.

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2 Longitudinal Hyperpolarized $^3$He Magnetic Resonance Imaging of COPD

2.1 Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality affecting at least 600 million people worldwide. It is the world’s fourth leading cause of death, and the most common chronic, terminal respiratory disease. Due to the heterogeneous nature of COPD, studies of its natural history and progression are complex, typically requiring large study sample sizes and long durations to obtain relevant longitudinal endpoints. Accordingly, much of our current understanding of the natural history of COPD arises from the landmark study of Fletcher and colleagues, and based on the cross-sectional spirometry measurements of the forced expiratory volume in one second (FEV$_1$). The current functional definition of COPD is also based on FEV$_1$ and changes in FEV$_1$ over time are still the most widely-accepted measure of COPD progression. However, a number of limitations of spirometry for the diagnosis, classification and longitudinal monitoring of COPD are motivating the development of new COPD measurements including those derived from non-invasive imaging. For example, high resolution multi-detector x-ray computed tomography (CT) has been recently used to identify phenotypes of both emphysema and airway disease and detects statistically significant COPD changes over relatively short periods of time.
Hyperpolarized helium-3 (\(^3\)He) magnetic resonance imaging (MRI) has recently emerged as another research method for the evaluation of COPD.\(^{16-20}\) In particular, previous work showed that the \(^3\)He apparent diffusion coefficient (ADC)\(^{16,21-23}\) was a sensitive measurement of emphysematous destruction\(^{19,23,24}\) and airspace size\(^{16,17,25,26}\), correlating with pulmonary function (FEV\(_1\) and DL\(_{CO}\))\(^{27}\) as well as histological measurements of lung surface area.\(^{28}\) Importantly, \(^3\)He MRI ADC is also age-dependent,\(^{29}\) and reflects differences in patient anatomical position,\(^{30}\) disease severity\(^{31}\) and smoking history.\(^{32}\) Quantitative focal \(^3\)He MRI ventilation defects have also been shown in COPD,\(^{24,32-34}\) and these reflect differences in subject age\(^{35}\) and disease status.\(^{20}\) To better understand the potential for hyperpolarized \(^3\)He MRI to provide quantitative longitudinal COPD endpoints, we designed a pilot longitudinal \(^3\)He MRI study of COPD. The objective of this study was to quantitatively evaluate a small pilot group of COPD ex-smokers and healthy volunteers over 2 years using hyperpolarized \(^3\)He MRI.

### 2.2 Materials and Methods

#### 2.2.1 Subjects

Twenty subjects were enrolled from the general population of the local tertiary health care center as previously described.\(^{20}\) All subjects provided written informed consent to the study protocol approved by the local research ethics board and Health Canada and the study was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada) and the Health Insurance Portability and Accountability Act (HIPAA, USA). COPD subjects were enrolled who were ex-smokers between the ages of 50-70, with a clinical diagnosis of COPD and were categorized according to the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria,\(^{4}\) with a smoking history of at least 10-pack-years and fewer than three COPD exacerbations within the last 12 months. Exacerbations during the follow-up period were defined as hospitalization for COPD or the patient’s first-time need for antibiotic or prednisone therapy and these were reported from hospital records,\(^{36}\) subject charts and additionally verified in a telephone interview with the subject after follow-up imaging was completed. COPD subjects were excluded during a screening visit if post-salbutamol FEV\(_1\) was greater than 3\%. Healthy elderly volunteers were enrolled (same age range as the COPD subjects)
who had a smoking history of <1 pack–year with no smoking in the previous 25 years, and with no history of previous chronic or current respiratory disease. All tests and imaging were performed at baseline and at 26 months ± 2 (standard deviations).

2.2.2 Pulmonary Function Tests

Spirometry was performed using an *ndd EasyOne* spirometer (*ndd Medizintechnik AG, Zurich, CH*) reporting FEV₁ and forced vital capacity (FVC) with a minimum of three acceptable spirometry maneuvers with the best FEV₁ and FVC selected for analysis according to American Thoracic Society guidelines.³⁷ Whole body plethysmography was performed using a MedGraphics’ Elite Series stand-alone body plethysmograph (MedGraphics Corporation. 350 Oak Grove Parkway St. Paul, MN USA) for the measurement of total lung capacity (TLC), inspiratory capacity (IC), residual volume (RV), and functional residual capacity (FRC).

2.2.3 Imaging

Subjects were screened for MRI and coil compatibility (inner diameter of elliptical coil = 50cm) prior to scanning, and digital pulse oximetry was used to monitor arterial blood oxygenation levels during MR breathhold scanning. A turn-key, spin-exchange polarizer system (*HeliSpin™, GEHC, Durham, NC*) was used to polarize ³He gas to 30-40%, as previously described.³⁸ Doses of hyperpolarized ³He gas (5 mL per kilogram of body weight) were administered in 1 L plastic bags (*Tedlar®, Jensen Inert Products, Coral Springs, FL*) diluted with ultrahigh purity, medical grade nitrogen (*Spectra Gases, Alpha, NJ*). Polarization of the diluted dose was quantified by a polarimetry station (*GENC, Durham, NC*) immediately prior to ³He gas administration to subject.

MRI was performed on a whole body 3.0 Tesla Excite 12.0 MRI system (*GEHC, Milwaukee, WI USA*). ¹H images were acquired prior to ³He MRI during breath-hold of a 1 L ⁴He/N₂ mixture from FRC;³³ diffusion-weighted imaging and ventilation or spin density imaging were also performed during breath-hold of a 1 L ³He/N₂ mixture from FRC.³³ All scanning was completed within approximately 7-10 minutes of subjects first lying in the scanner.
2.2.4 Image Analysis

The signal-to-noise ratio (SNR) for all images acquired was determined by calculating the mean pixel value within a 10 x 10 voxel region of interest (ROI) for four representative ROI within the lung parenchyma, and dividing by the standard deviation of the mean pixel values for noise inside a ROI of the same size at the corners of the image where there was no lung structure. SNR was determined for each slice and then averaged to obtain a single SNR value for each subject and time-point. Images were analyzed in a controlled image visualization environment with room lighting levels equivalently established for all image analysis sessions.

ADC maps were processed using in-house software programmed in the IDL Virtual Machine platform (Research Systems Inc., Denver, CO) as previously described\(^3\) with \(b = 1.6 \text{s/cm}^2\). Spin density images were examined for analysis of ventilation defects in all coronal slices by two expert observers (MK and LM) blinded to subject identity, disease status, and time point, as well as the other observer’s measurements. As previously described,\(^3\) a ventilation defect was identified by each observer independently as any lung region of diminished signal intensity but not those areas of signal loss associated with the pulmonary vascular structures, heart, hilum and mediastinum. Images were reviewed such that \(^3\)He and \(^1\)H images were visible on a digital workstation monitor system (consisting of identical 19 inch flat panel monitors). Manual segmentation of ventilation defects was performed using custom-designed image visualization software which also provided a method for two-dimensional rigid single point image registration (\(^1\)H and \(^3\)He slices) based on the carina, facilitating the manual segmentation of ventilation defects in all slices. Ventilation defect volume (VDV) and thoracic cavity volume (TCV) were recorded following manual segmentation of \(^3\)He and \(^1\)H images respectively, and used to calculate ventilation defect percent (VDP).\(^{16;25;26;40}\) VDV was derived from the manually segmented ventilation defect area for each slice and this was multiplied by the slice thickness; VDV for all slices was summed to obtain a whole lung (WL) VDV. For the center slice (CS), which is the middle slice acquired that clearly shows the carina and two main bronchi, VDV was also manually segmented and
reported. Additionally, WL and CS measurements were recorded for TCV for calculation of both WL and CS VDP, as well as for ADC.

2.2.5 Statistical Methods

For $^3$He MRI ventilation measurements, observer reproducibility was evaluated for 2 different observers using the interclass correlation coefficient (ICC), coefficient of variation (COV) and linear regression ($r^2$) using SPSS 16.00 (SPSS Inc., LEAD Technologies Inc. Chicago, IL, USA). Comparison of baseline and follow-up means were performed using a Wilcoxon matched pairs two-tailed t-test using SPSS 16.00. The relationship between changes in $^3$He MRI measurements and changes in pulmonary function measurements at follow-up were determined using linear regression and Spearman correlation coefficients using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego California, USA). The relationship between the changes in $^3$He MRI VDV and smoking history was also determined using linear regression and Spearman correlation coefficients using GraphPad Prism version 4.00. A Holm-Bonferroni correction was used for multiple paired t-tests and all correlations. The Holm-Bonferroni adjusted p-values were determined by ordering p-values from smallest to largest, with the smallest p-value multiplied by k, where k is the number of hypotheses to be tested. If the resulting modified p-value was less than α (Type I error rate) the hypothesis was rejected. The next smallest p-value was then multiplied by k-1 and the new modified p-value was compared to α. This process was repeated until the modified p-value could not be rejected. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% (p < 0.05). A retrospective sample size ($n$) calculation was also performed to detect a significant change ($\delta$) in FEV$_1$ with $\alpha = 0.05$ and power $\beta = 0.80$ and where SD is standard deviation; accordingly, $Z_\alpha = 1.96$ and $Z_\beta = 0.2$, and $n$ was calculated according to Equation 2-1:

$$n = \frac{2(Z_\alpha + Z_\beta)^2 \cdot SD^2}{\delta^2}$$ (1)
2.3 Results

Demographic characteristics are provided in Table 2-1. All COPD subjects were non-smokers at baseline with a mean smoking history of 47 ± 22 pack-years (range = 11 - 85 pack-years) and mean years not-smoking at baseline of 11 ± 10 years (range = 10 weeks - 34 years). Three subjects experienced a single COPD exacerbation each over the follow-up period.

Table 2-2 shows mean and median WL and CS hyperpolarized $^3$He MRI measurements for the healthy never-smokers and COPD subjects. The absolute change and annualized rates of change of FEV$_1$ and hyperpolarized $^3$He MRI measurements for COPD subjects are provided in Table 2-3. For ADC, annualized rate of change for ex-smokers with COPD was 0.01 cm$^2$/s and that for never-smokers was 0.002 cm$^2$/s. There was no significant association between the changes in image SNR and the changes in ADC ($r$=-.51, $p$=.12) and VDV ($r$=-.17, $p$=.55). Inter-observer reproducibility of $^3$He MRI VDV was previously evaluated for 3 different observers and this was the same as intra-observer variability (COV=10%, unpublished results). For two observers, inter-observer reproducibility was assessed for WL VDV (ICC=.93, COV=38% and $r^2$=.84 ($p$<.0001)) and WL VDP (ICC=.91, COV=43% and $r^2$=0.81 ($p$<.0001)). Figure 2-S1 shows the relationship between ventilation defect measurements for Observer 1 and Observer 2; WL VDV ($r^2$=.84, $p$<.0001) and WL VDP ($r^2$=.81, $p$<.0001). The change at follow-up for Observer 1 and Observer 2 were significantly correlated for WL VDV ($r^2$=.51, $p$=.0004) and WL VDP ($r^2$=.39, $p$=.007). We provide the change in WL VDV and WL VDP for each observer and the mean for both observers in Table 2-S1 within the online supplement, and throughout the main body of the manuscript a single observer’s results are described. Table 2-S2 is also provided in the online supplement and shows a subject listing of baseline and follow-up pulmonary function and $^3$He MRI measurements.

Wilcoxon matched pairs two-tailed t-tests indicated that all $^3$He MRI measurements were significantly different at follow-up for COPD subjects. There was also no significant change observed in FEV$_1$%$_{predicted}$ for the COPD subjects at follow-up. For healthy never-smokers, there was no detectable change in any pulmonary function or imaging measurement. The changes detected in the COPD ex-smokers were significantly
different than the changes measured in the healthy volunteers for WL VDV (p=.04) and WL VDP (p=.01), but not significantly different for WL ADC (p=.96) or FEV1 (p=.21).

Table 2-1 Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>Healthy Never-smokers (n=5)</th>
<th>COPD Ex-smokers (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Age yrs (±SD) [range]</td>
<td>69</td>
<td>72</td>
</tr>
<tr>
<td>Male Sex</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Body Mass Index (±SD)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>FEV1_L (±SD)</td>
<td>2.77 (1.01)</td>
<td>2.62 (0.93)</td>
</tr>
<tr>
<td>FEV1/FVC % (±SD)</td>
<td>110 (23)</td>
<td>107 (23)</td>
</tr>
<tr>
<td>IC % (±SD)</td>
<td>106 (18)</td>
<td>-</td>
</tr>
<tr>
<td>RV % (±SD)</td>
<td>95 (15)</td>
<td>-</td>
</tr>
<tr>
<td>FRC % (±SD)</td>
<td>101 (12)</td>
<td>-</td>
</tr>
<tr>
<td>TLC % (±SD)</td>
<td>105 (12)</td>
<td>-</td>
</tr>
</tbody>
</table>

FEV1= Forced Expiratory Volume in 1s, FVC= Forced Vital Capacity, IC= Inspiratory Capacity, RV= Reserve Volume, FRC= Functional Residual Capacity, TLC= Total Lung Capacity, SD=Standard Deviation

Table 2-2 3He MRI ADC and Ventilation Defect Measurements at Baseline and Follow-up

<table>
<thead>
<tr>
<th></th>
<th>Healthy Never-smokers (n=5)</th>
<th>COPD Ex-smokers (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>WL ADC (±SD) cm^2/s</td>
<td>0.27/0.25</td>
<td>0.29/0.29</td>
</tr>
<tr>
<td>CS ADC (±SD) cm^2/s</td>
<td>0.28/0.27</td>
<td>0.28/0.30</td>
</tr>
<tr>
<td>WL VDV (±SD) L</td>
<td>0.023/0.006</td>
<td>0.020/0.017</td>
</tr>
<tr>
<td>CS VDV (±SD) L</td>
<td>0.003/0.000</td>
<td>0.002/0.001</td>
</tr>
<tr>
<td>WL VDP (±SD) %</td>
<td>0.5/0.2 (0.9)</td>
<td>0.5/0.5 (0.4)</td>
</tr>
<tr>
<td>CS VDP (±SD) %</td>
<td>0.6/0.2 (0.9)</td>
<td>0.4/0.2 (0.4)</td>
</tr>
</tbody>
</table>

ADC=Apparent Diffusion Coefficient, VDV=Ventilation Defect Volume, VDP=Ventilation Defect Percent, CS=Center Slice, WL= Whole Lung, SD=Standard Deviation
**Table 2-3** Annualized changes in pulmonary function and $^3$He MRI measurements for COPD subjects at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Absolute Change</th>
<th>Annualized Rate of Change (/year)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p*</td>
</tr>
<tr>
<td>FEV$_1$ (%)</td>
<td>-1</td>
<td>-0.4</td>
<td>0.97</td>
</tr>
<tr>
<td>WL ADC (cm$^2$/s)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>CS ADC (cm$^2$/s)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>WL VDV (L)</td>
<td>0.4</td>
<td>0.20</td>
<td>0.007</td>
</tr>
<tr>
<td>CS VDV (L)</td>
<td>0.05</td>
<td>0.03</td>
<td>0.003</td>
</tr>
<tr>
<td>WL VDP (%)</td>
<td>7</td>
<td>4</td>
<td>0.0009</td>
</tr>
<tr>
<td>CS VDP (%)</td>
<td>7</td>
<td>3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

FEV$_1$=Forced Expiratory Volume in 1s, ADC=Apparent Diffusion Coefficient, VDV=Ventilation Defect Volume, VDP=Ventilation Defect Percent, CS=Center Slice, WL= Whole Lung

* Wilcoxon matched pairs two-tailed t-tests, ** Holm-Bonferroni adjusted significance values

**Figure 2-1** shows ventilation images, ADC maps and ADC histograms for two representative healthy never-smokers at baseline and follow-up. **Figure 2** shows ventilation images, ADC maps and histograms for two representative COPD subjects at baseline and follow-up.
Table 2-4 shows Spearman correlation coefficients for the changes in FEV1 (absolute and %predicted) with ³He MRI ADC, VDV and VDP for COPD subjects. As shown in Figure 2-3, the change in FEV1 (absolute) showed a significant negative association with the change in CS VDV (r=-.70, p=.02), CS VDP (r=-.70, p=0.03), but not ADC. TCV was calculated for all subjects from the thoracic cavity ¹H MRI and for all subjects, the change in TCV was significantly correlated with the change in TLC measured using plethysmography (r=.81, p=.001). Figure 2-4 shows the relationships between COPD patient smoking history (pack-years smoking) with the changes in ³He MRI VDV and FEV1. There was no significant correlation between pack-years smoking and the change in FEV1 (r=.05, p=.83), and the relationship between pack-years smoking and WL VDV was moderate, but once corrected for multiple tests, this relationship was on the threshold.
of significance (WL VDV \[r=.52, \ p=.02, \ \text{uncorrected}, \ p=.06 \ \text{Holm Bonferroni corrected}\]).

**Table 2-4** Relationship between changes in FEV\(_1\) and \(^3\)He MRI measurement changes for COPD subjects over time

<table>
<thead>
<tr>
<th></th>
<th>Spearman Correlation Coefficients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV(_1) L ((p/p^*))</td>
<td>FEV(_1)%\text{predicted} ((p/p^*))</td>
<td></td>
</tr>
<tr>
<td>WL ADC ((\text{cm}^2/\text{s}))</td>
<td>-0.02 ((0.93/0.93))</td>
<td>0.17 ((0.56/1.0))</td>
<td></td>
</tr>
<tr>
<td>CS ADC ((\text{cm}^2/\text{s}))</td>
<td>-0.06 ((0.84/1.0))</td>
<td>0.15 ((0.60/0.60))</td>
<td></td>
</tr>
<tr>
<td>WL VDV (L)</td>
<td>-0.56 ((0.03/0.09))</td>
<td>-0.53 ((0.04/0.12))</td>
<td></td>
</tr>
<tr>
<td>CS VDV (L)</td>
<td>-0.70 ((0.003/0.02))</td>
<td>-0.67 ((0.007/0.04))</td>
<td></td>
</tr>
<tr>
<td>WL VDP (%)</td>
<td>-0.60 ((0.02/0.08))</td>
<td>-0.55 ((0.03/0.12))</td>
<td></td>
</tr>
<tr>
<td>CS VDP (%)</td>
<td>-0.70 ((0.005/0.03))</td>
<td>-0.70 ((0.005/0.03))</td>
<td></td>
</tr>
</tbody>
</table>

FEV\(_1\)=Forced Expiratory Volume in 1s, ADC=Apparent Diffusion Coefficient, VDV=Ventilation Defect Volume, VDP=Ventilation Defect Percent, CS=Center Slice, WL=Whole Lung

* Holm-Bonferroni adjusted significance values

**Figure 2-2** Representative hyperpolarized \(^3\)He MR VDV and ADC changes during follow-up in COPD

Left panel subject 3004 75 year old male stage III COPD: top panel is baseline and bottom panel is follow-up: (A) ventilation image (B), ADC map (C) and ADC histogram

Right panel subject 2009 61 year old male stage II COPD: top panel is baseline and bottom panel is follow-up: (A) ventilation image (B), ADC map (C) and ADC histogram
Relationship between $^3\text{He}$ MRI measurements and changes in FEV$_1$

Scatterplots show the relationship between changes in FEV$_1$ and changes in center slice (CS) ventilation defect volume (VDV), CS ventilation defect percent (VDP) and WL apparent diffusion coefficient (ADC). The 95% confidence intervals for the regressions are shown as dotted lines. (A) Changes in FEV$_1$ showed a significant negative correlation with changes in CS VDV ($r=-.70$, $p=.02$), and (B) CS VDP ($r=-.70$, $p=.03$). (C) Changes in FEV$_1$ showed no significant correlation with changes in WL ADC.

Relationship between $^3\text{He}$ MRI VDV, FEV$_1$ and smoking history

Scatterplot showing the relationship between smoking history and change in WL VDV ($r=.52$, $p=.02$//p=.06 Holm Bonferroni corrected) and change in FEV$_1$%predicted ($r=.05$, $p=.83$).
2.4 Discussion

Several observations were made in this small longitudinal pilot study. First, we observed that mean $^3$He ADC, VDV and VDP significantly increased during the 26 month follow-up period in the 15 COPD ex-smokers whereas pulmonary function measurements did not significantly change over the same period of time. Based on the previously reported and pioneering epidemiological findings of Fletcher and colleagues, as well as the more recent tiotropium intervention trial, we were not surprised to find that in COPD ex-smokers, FEV$_1$ did not significantly change. Indeed, this study was not powered to detect such changes in FEV$_1$ over a 2 year timeframe; a retrospective power analysis shows that a sample size of approximately 1000 COPD and 1000 healthy volunteers would be required to detect significant change in FEV$_1$ in this study. Unlike the Fletcher curve prediction that longitudinal changes in hyperpolarized $^3$He measurements in the COPD ex-smokers would be similar to those observed in elderly healthy never-smokers, we observed an annualized rate of change in $^3$He MRI ADC of 0.01 cm$^2$/s/y -an order of magnitude greater than the rate previously reported (0.001 cm$^2$/s/y) for healthy non-smokers in a cross-sectional multi-center study at 1.5T, and for the healthy never-smokers reported in this study (0.002 cm$^2$/s/y). Additionally, we observed no significant change in $^3$He MRI ADC, ventilation defect measurements or in pulmonary function measurements for the healthy never-smokers at follow-up. These preliminary longitudinal findings suggest that regional disease markers derived from non-invasive imaging methods can be used in a small number of COPD subjects over short time periods, to quantitatively detect significant changes.

We also showed that the change in $^3$He MRI VDV and VDP indicated a significant inverse correlation with the change in FEV$_1$, whereas the change in ADC showed no such relationship. This result suggests that $^3$He MRI ventilation defect measurements may be more predictive of airflow limitation than $^3$He MRI ADC, which necessitating further testing of this hypothesis. Although we observed statistically significant improvements in FEV$_1$ <20 mL in five COPD subjects, we did not observe a corresponding improvement in $^3$He MR imaging-derived measurements over this time period. On the contrary, for these five subjects we observed a statistically significant increase in $^3$He MRI ADC.
(p=.02) and no change in ventilation measurements. We believe this discordant finding highlights the sensitivity of using both ventilation and ADC measurements in detecting disease changes in COPD. In a related finding, Ohara and colleagues\textsuperscript{15} showed the significant inverse correlation between annual changes in FEV\textsubscript{1} and the CT measurement of wall area percent (WA\% - a surrogate of airway wall thickness) and similar to our results, there was no significant relationship between the change in FEV\textsubscript{1} and emphysema (percent low attenuation areas, %LAA). It is important to note that for five of the 15 COPD subjects evaluated in this pilot analysis, both imaging and spirometry suggested disease progression. This finding generates a number of hypotheses for testing in larger or longer imaging studies of COPD progression evaluating the relationship between exacerbations, treatment changes and changes in quality of life with imaging and pulmonary function measurements. Future COPD studies that include hyperpolarized \textsuperscript{3}He MRI will likely focus on a critical balance between longitudinal time frame and finite numbers of subjects because of the relative complexity of these studies and the prediction for increased costs and decreased availability of \textsuperscript{3}He gas for clinical research.\textsuperscript{44} Nevertheless, the preliminary findings of this pilot hyperpolarized \textsuperscript{3}He MRI longitudinal study provide clear guidance for future COPD imaging studies using established and emerging imaging tools such as optical coherence tomography,\textsuperscript{45} oxygen enhanced and proton MRI methods\textsuperscript{46,47} and hyperpolarized xenon-129 MRI.

Finally, we showed in an exploratory analysis, the potential relationship between smoking history and changes in \textsuperscript{3}He MRI VDV but not with changes in FEV\textsubscript{1}. We note that previous cross-sectional studies suggest ongoing inflammation in ex-smokers with COPD\textsuperscript{3,29} and that smoking history correlated with current inflammatory markers such as eosinophils\textsuperscript{48} and vascular endothelial growth factor\textsuperscript{49} supporting the hypothesis that there is a predictive relationship between pack-years and inflammation post smoking cessation. The results of this exploratory analysis of \textsuperscript{3}He MRI changes and smoking history generate important hypotheses that future studies should explore in more detail.

Although this pilot study reports significant increases in \textsuperscript{3}He MRI measurements in just over two years, we must acknowledge a number of specific limitations of our approach. We recognize that the relatively small group of COPD subjects and healthy never-
smokers that were evaluated, and the relatively short period of follow-up compared to other COPD longitudinal studies\(^5\) certainly limits the applicability of our results. The small sample size necessitates cautious interpretation of the results as well as the future requirement for larger studies to test the hypotheses generated. For example, because of the small number of healthy volunteers and COPD subjects evaluated, significant differences were not detected for pulmonary function measurements in either group. In addition, although we have previously demonstrated that ventilation defects are highly reproducible,\(^20\) the lack of longitudinal information regarding the variability of ventilation defects necessitates that the findings of this study be interpreted with caution.

It is also important to note that although this pilot study showed that ex-smoking COPD patients differed from elderly never-smokers, it was not designed to show the relationship between the significant imaging changes and more established clinical measurements of COPD worsening. To make the important conclusion that \(^3\)He MRI measurements are specifically related to clinical measurements of COPD worsening, future work in longer or larger studies must show that the detected \(^3\)He MRI changes occurred in patients who had measureable clinical changes. Nevertheless, the results suggest that \(^3\)He MRI provides a sensitive method for the detection of the lung structural and functional changes that accompany COPD longitudinally. In future, larger studies that directly compare \(^3\)He MRI and other well-established clinical measurements of COPD progression will be required in order to better understand the relationship between \(^3\)He measurements and other changes that occur in COPD over time. Additionally, a comparison of COPD ex-smokers, healthy smokers and COPD current smokers may help to establish and directly compare rates of lung structural and functional decline. It will also be critical to track patient exacerbations and changes in treatment over longer periods of time to probe potential treatment effects with imaging. We also acknowledge that for this pilot study, we limited the COPD cohort to those with minimal FEV\(_1\) reversibility, although clearly many COPD subjects have varying degrees of FEV\(_1\) reversibility which necessitates further work in such patients. CT images were not prospectively acquired for this study and therefore, our results cannot be directly compared to more established CT measurements such as LAA\(\%\) (a measurement of the extent of emphysema), or WA\(\%\) (a measurement of airway wall thickness). Clearly, a
direct comparison of CT and hyperpolarized $^3$He derived measurements in the same subjects will allow for a better understanding of differences in measurement sensitivity these imaging modalities provide. Previous work has shown significant correlations between ADC and the measurement of the diffusion capacity of carbon monoxide (DL$_{CO}$).\textsuperscript{32} A direct comparison here would have provided another quantitative measure of global emphysematous changes in these subjects. It is also important to note that while Woods and colleagues have previously shown the significant correlation between $^3$He ADC and histological measurements of emphysema,\textsuperscript{28} we have not yet determined the underlying pathology that results in $^3$He ventilation defects we observe, which may be a result of small airway occlusion, mucous plugs, airway wall thickening and inflammation or bullous disease. Related to this is the fact that there is significant information in regions of intermediate signal intensity and in both hyper- and hypo-intense signal regions that have not yet been quantitatively or spatially exploited. The development and validation of $^3$He image analysis techniques that quantify heterogeneous signal information are required in the future to fully characterize the important ventilation information contained in the image. Whether regions of diminished ventilation and emphysema develop independently and the extent to which one leads to the other cannot be ascertained by this preliminary longitudinal study and is yet to be determined in other longitudinal imaging studies. Importantly however, our group previously described the high reproducibility of $^3$He ventilation defects in COPD,\textsuperscript{20} suggesting that the significant changes measured here may be due to changes in lung morphology or function and not due to the variability of the imaging or measurement technique, nor due to short-term changes in small airway diameter or patency.

In summary, in this small pilot study of COPD ex-smokers, we detected significant lung changes using $^3$He MRI that occurred over a relatively short period of time, perhaps before FEV$_1$ changes could be detected or perhaps because such longitudinal changes occurred within the FEV$_1$ silent zones, where disease might have accumulated without detection.
2.5 References


(38) Mathew L, McCall JM, McKay S et al. Hyperpolarized 3He magnetic resonance imaging of ventilation defect volume variability in COPD. Berlin: 2007.


(47) Ohno Y, Iwasawa T, Seo JB et al. Oxygen-enhanced magnetic resonance imaging versus computed tomography: multicenter study for clinical stage classification of


## 2.6 Supplementary Tables

**Table 2-S1** Hyperpolarized $^3$He MRI ventilation measurement observer reproducibility

<table>
<thead>
<tr>
<th>Mean Difference at Follow-up (Significance)</th>
<th>Observer 1</th>
<th>Observer 2</th>
<th>Mean (Observers 1 &amp; 2)</th>
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<tr>
<td>Observer 1</td>
<td>Observer 2</td>
<td>Mean (Observers 1 &amp; 2)</td>
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<tr>
<td>WL VDV (L)</td>
<td>0.30 (0.007)</td>
<td>0.41 (0.02)</td>
<td>0.35 (0.004)</td>
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<td>WL VDP (%)</td>
<td>5.0 (0.002)</td>
<td>6.2 (0.04)</td>
<td>5.6 (0.01)</td>
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</table>

VDV=ventilation defect volume, VDP=ventilation defect percent
Table 2-S2-2 Baseline and follow-up subject listing of pulmonary function test results and $^3$He MRI measurements

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<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Pack-Year (yr)</th>
<th>FEV$_1$/FVC (%)</th>
<th>FEV$_1$/FVC (%)</th>
<th>RV (%)</th>
<th>TLC (L)</th>
<th>ADC ($cm^2/s$)</th>
<th>Dw SNR</th>
<th>TCV (L)</th>
<th>VDV (L)</th>
<th>VDP (%)</th>
<th>SV SNR</th>
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<td>64/37</td>
<td>6.57/4.32</td>
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<td>0.4/2.2</td>
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<td>41/49</td>
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<td>.41/.44</td>
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<td>8.10/7.79</td>
<td>.29/.37</td>
<td>49/18</td>
<td>6.01/5.18</td>
<td>0.04/0.34</td>
<td>0.7/6.3</td>
<td>136/61</td>
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<td>40</td>
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<td>46/42</td>
<td>139/131</td>
<td>5.65/5.74</td>
<td>.60/ .60</td>
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<td>72</td>
<td>30</td>
<td>62/38</td>
<td>48/37</td>
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<td>7.93/-</td>
<td>.40/.40</td>
<td>44/27</td>
<td>6.96/6.57</td>
<td>0.62/2.25</td>
<td>9.0/34.2</td>
<td>90/113</td>
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<td>53/51</td>
<td>116/71</td>
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<td>.49/.48</td>
<td>56/42</td>
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<td>24</td>
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<td>116/124</td>
<td>4.30/5.04</td>
<td>.46/.44</td>
<td>12/17</td>
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<td>48/42</td>
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<td>27/27</td>
<td>6.10/5.62</td>
<td>0.99/0.98</td>
<td>16.2/17.5</td>
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</tbody>
</table>

FEV$_1$=Forced Expiratory Volume in 1s, FVC=Forced Vital Capacity, RV=Reserve Volume, TLC=Total Lung Capacity, ADC=Apparent Diffusion Coefficient, Dw=Diffusion-weighted image, SNR=Signal-to-Noise Ratio, TCV=Thoracic Cavity Volume, VDV=Ventilation Defect Volume, VDP=Ventilation Defect Percent, SV=Static Ventilation Image
Figure 2-S1 Relationship between VDV and VDP for Observer 1 and Observer 2 Scatterplots show the relationship for Observers 1 and 2 whole lung (WL) ventilation defect volume (VDV) and ventilation defect percent (VDP). The 95% confidence intervals for the regressions are shown as dotted lines. (Ai) Observer 1 and 2 VDV ($r^2=.84$, $p<.0001$) and (Aii) Observer 1 and 2 VDP ($r^2=.81$, $p<.0001$). The change at follow-up for Observer 1 and Observer 2 are significantly correlated for VDV ($r^2=.51$, $p=.0004$) (Bi) and VDP ($r^2=.39$, $p=.007$) (Bii).
CHAPTER 3

A limitation of the longitudinal study performed in Chapter 2 was that $^3$He MRI measurements were performed using manual segmentation approaches, which clearly increases segmentation time and, importantly, introduces the potential for inter- and intra-observer variability. Here we generated a semi-automated segmentation method for $^3$He MRI to enable its use in longitudinal and serial studies.

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3 Hyperpolarized $^3$He Magnetic Resonance Imaging Semi-automated Segmentation

3.1 Introduction

Pulmonary hyperpolarized helium-3 ($^3$He) magnetic resonance imaging (MRI) is a functional imaging method that detects ventilation abnormalities in elderly never-smokers\textsuperscript{1} and patients with chronic obstructive pulmonary disease (COPD),\textsuperscript{2-9} asthma,\textsuperscript{10-13} cystic fibrosis (CF),\textsuperscript{14-16} radiation-induced lung injury,\textsuperscript{17,18} and lung transplant recipients.\textsuperscript{19,20} Over the last few years, this approach has been enhanced by improvements in laser optical pumping techniques,\textsuperscript{21} parallel imaging,\textsuperscript{22} single-scan acquisition of ventilation and diffusion-weighted images,\textsuperscript{23} single-scan acquisition of ventilation and $^1$H MRI anatomical images\textsuperscript{24} and dynamic imaging.\textsuperscript{25,26} While this large body of work has advanced the scope and type of functional images that can be acquired, there have been fewer improvements related to image analysis methods.\textsuperscript{27-29} As we move forward with functional noble gas imaging and transition to hyperpolarized xenon-129 ($^{129}$Xe) MRI, a less expensive and more readily available approach, image analysis methods are urgently required for quantitative evaluation across a wide variety of respiratory diseases.

Previous $^3$He functional/ventilation analyses were based on a radiologist’s interpretation of the ventilated lung regions,\textsuperscript{27,30} and quantification of ventilation defects was performed
using scoring$^{1,8,11}$ and volumetric analysis approaches.$^{1,8}$ While in these studies, imaging measurements were correlated with well-established measures of disease, a limitation of manual methods is the inherent reliance upon highly trained observers that increases segmentation time and introduces the potential for inter- and intra-observer variability. Although straightforward automated threshold methods have been used for segmentation of ventilation,$^{27}$ automated segmentation of the $^3$He ventilation defects themselves, previously demonstrated by Tustison et. al.$^{28}$ as a feature that differentiated asthma and healthy subject images, has proven to be much more difficult. Subjects with severe obstructive disease, such as COPD or asthma, have numerous ventilation defects that appear in $^3$He images as signal voids. Hyperinflation is also commonly observed in obstructive lung disease due in part to gas trapping and therefore changes in the thoracic cavity shape vary from patient to patient and within individual patients over time. Thus, segmentation of ventilation defects likely requires an understanding of the relationship between $^3$He functional information with the anatomy of the thoracic cavity derived from proton ($^1$H) MRI. Furthermore, because there appear to be regions with $^3$He signal voids, as well as regions of hypo- and hyper-intensity, segmentation methods are required for the visually different classes of $^3$He MRI signal.

Therefore, our objective was to develop a semi-automated segmentation method by applying two well-established image segmentation approaches, seeded region-growing$^{31}$ for segmentation of the thoracic cavity from $^1$H MRI and K-means clustering$^{32}$ to classify $^3$He MRI pixel intensities as previously investigated,$^{29,33,34}$ to generate $^3$He MRI ventilation measurements. The combination of these well-established and widely used segmentation methods allows for quantification and characterization of the important ventilation information contained in the image. $^3$He MRI segmentation methods must have high intra-observer and inter-observer reproducibility across a variety of respiratory diseases and subjects. Therefore, here we evaluated the reproducibility as well as the spatial and quantitative relationships between manual and semi-automated functional measurements in subjects with asthma, COPD and CF.
3.2 Materials and Methods

3.2.1 Subjects

All subjects provided written informed consent to protocols approved by Health Canada and a local research ethics board. COPD subjects were ex-smokers, with a clinical diagnosis of COPD and categorized according to the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria, with a smoking history of at least 10-pack-years as previously described. Adult asthmatics between 18-50 years of age were enrolled with FEV$_1$%$_{pred}$ > 60% with a physician diagnosis of asthma and adult CF subjects (18-40 years of age) were also enrolled with FEV$_1$%$_{pred}$ > 50%.

3.2.2 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Excite 12.0 MRI system (GEHC, Milwaukee, WI USA) with broadband imaging capability as previously described, and $^3$He MRI was enabled using a single channel, rigid elliptical transmit/receive chest coil (RAPID Biomedical GmbH, Wuerzburg Germany). Pulse oximetry was used to measure arterial oxygen saturation (SaO$_2$) for all subjects during scanning sessions and considered an adverse event occurred when SaO$_2$ decreased below 88% continuously for $\geq$ 15 seconds at any time during the scanning session. Study withdrawal was required when SaO$_2$ decreased to 80% for 10 seconds or longer. It is important to note that we endeavoured to minimize the potential for differences in the levels of inspiration between the breath-hold scans for each subject by: 1) training and practice for all subjects prior to MRI related to the inspiration breath-hold maneuver from functional residual capacity (FRC), and, 2) continuous coaching and monitoring at the MR bedside by a pulmonary function technologist during all inspiration breath-hold scans.

Conventional $^1$H MRI was performed prior to hyperpolarized $^3$He MRI with subjects scanned during 1.0 L breath-hold of $^4$He/N$_2$ using the whole body radiofrequency (RF) coil and $^1$H fast spoiled gradient recalled echo sequence (16s total data acquisition, repetition time (TR) / echo time (TE) / flip angle = 4.7 ms / 1.2 ms / 30°, field-of-view (FOV) = 40 × 40 cm, matrix 256 × 128, 14 slices, 15 mm slice thickness, 0 gap), as previously described.
Prior to $^3$He MRI, a polarizer system (HeliSpin™, GEHC, Durham, NC) was used to polarize $^3$He gas to 30—40% and doses (5 mL/kg body weight) were administered in 1.0 L Tedlar® bags diluted with ultrahigh purity, medical grade nitrogen (Spectra Gases, Alpha, NJ). As previously described, hyperpolarized $^3$He MRI coronal static ventilation images were acquired during breath-hold of a 1L $^3$He/N2 mixture (14s data acquisition, TR / TE / flip angle = 4.3 ms / 1.4 ms / 7°, bandwidth = 31.25, FOV = 40 × 40 cm, matrix 128 × 128, 14 slices, 15 mm slice thickness, 0 gap).

3.2.3 Manual Segmentation

Manual segmentation was repeated five times to estimate observer reliability using the approximation method developed by Walter et. al. All subjects were first randomized and subsequently manually segmented by a single observer blinded to subject identity and disease status, and all subjects measurements were repeated five times each (15 subjects × 5 repetitions = 75 image datasets). To minimize memory bias, at least 24 hours was required between repetition rounds and randomization of subject image datasets was performed between each repetition round. The observer (MK) had three years experience performing manual $^3$He MRI segmentation having been trained by an experience chest radiologist (RER). As previously described, ventilation volume (VV) was recorded following manual segmentation of the ventilated lung regions and ventilation defect volume (VDV) was recorded following manual segmentation of the $^3$He MRI focal ventilation defects, using custom-designed image visualization software, providing a method for two-dimensional rigid single point image registration ($^1$H and $^3$He slices) based on fiducial markers located on the carina. As previously described, a ventilation defect was identified by the observer as any lung region of diminished signal intensity but not including those areas of signal loss associated with the pulmonary vascular structures, heart, hilum and mediastinum.

3.2.4 Semi-automated Segmentation: Overview of Method

Multi-step segmentation software was generated using MATLAB R2007b (The Mathworks Inc., Natick, Massachusetts). All subjects, following randomization, were segmented using the semi-automated method by two observers blinded to subject identity.
and disease status, and measurements were repeated five times each (15 subjects × 5 repetitions × 2 observers = 150 image datasets). To minimize memory bias, at least 24 hours was required between repetition rounds. The observer (blinded) had one year experience developing and performing semi-automated $^3$He MRI segmentation, and the observer (blinded) had 1 month experience performing semi-automated $^3$He MRI segmentation. Figure 3-1 provides a summary of our method. Briefly, $^3$He MR images (Step 1) were segmented automatically using a hierarchical version of K-means clustering algorithm, and $^1$H MR images were segmented automatically using a seeded region-growing algorithm (SRGA) (Step 2). $^1$H MR segmented images were registered to $^3$He MR segmented images (Step 3) to differentiate the ventilation defects from the background and therefore generating a $^3$He voxel cluster map, with clusters ranging from 1 to 5, representing gradations of signal intensity from no signal (Cluster 1, C1) and hypo-intense signal (C2) to hyper-intense signal (C5). This approach was first tested in a training dataset of 5 COPD subjects (5 subjects × 10 slices = 50 image slices) for comparison to manual segmentation. Results from the training dataset indicated that the semi-automated algorithm completed all tasks with image signal-to-noise-ratio (SNR) ≥ 13, and this was the case for nearly all but the most anterior slices for each subject. The combination of 4 initial clusters and re-clustering the initial C1 yielding a total of 5 clusters provided the best qualitative correlation with manual expert observer (chest radiologist) results. We also quantitatively compared K-means with the initial user input of 4 to 10 clusters and the hierarchical K-means approach (4 initial clusters and re-clustering the initial C1) with manual segmentation on the training dataset, and determined that hierarchical K-means provided the highest correlation and lowest Bland-Altman bias with manual segmentation and was subsequently used for the testing dataset (Supplementary Table, Table 3-S1).

### 3.2.5 $^3$He MRI Automated Segmentation: K-means Clustering Algorithm

$^3$He MRI of obstructive (COPD and asthma) and restrictive (CF) lung disease are typically characterized by heterogeneous $^3$He MRI signal intensity that reflect gas distribution heterogeneity during the inspiration breath-hold scan. The expert observer
typically can distinguish between 4 visually apparent classes of $^3$He MRI signal – signal void, hypo-intense (or partial volume), normal intensity, and hyper-intense signal. For this reason, a hierarchical K-means clustering method,\textsuperscript{32} similar to previously described methods for $^3$He MRI segmentation,\textsuperscript{29,33,34} was used to partition the signal intensity frequency distribution histogram into 4 clusters, based on an expert chest radiologist’s interpretation of the clinical meaning of the visible signal intensity differences. Because the first round of K-means clustering resulted in a single cluster C1 that included both signal void and hypo-intense regions (partial volumes and/or partially ventilated volumes), K-means clustering was re-applied to the C1 to separate signal void from the hypo-intense signal regions. This was accomplished in three steps as follows: 1) K-means with four clusters was initially applied to the $^3$He image, 2) K-means with four clusters was then re-applied to C1, 3) the first two clusters from step 2 were merged to represent the background and ventilation defects and the last two clusters from step 2 were merged to represent the hypo-intense signal regions. For both steps 1 and 2, a standard initialization method was performed to produce the initial centroids by dividing the full pixel range of 0-255 into four equal regions: 0-63, 64-127, 128-191, 192-255, and selecting the interval center as the centroid for each cluster.
3.2.6 \textsuperscript{1}H MRI Automated Segmentation: Seeded Region-growing

In conventional \textsuperscript{1}H MRI anatomical images, low \textsuperscript{1}H density in the lung produces a weak MR signal, thus providing sufficient contrast for segmentation methods such as a seeded region-growing algorithm (SRGA)\textsuperscript{31} to segment the contour of the thoracic cavity to differentiate ventilation defects from the edge of the lung. Prior to application of SRGA, all \textsuperscript{1}H images were pre-processed to protect the region-growing algorithm from leaking into regions extending beyond the lung boundary with similar signal intensities. Pre-processing of the \textsuperscript{1}H images was performed using a 2D radially symmetric Gaussian low-pass filter of size $15 \times 15$ with standard deviation 2.0. The images were then converted to a binary mask using a threshold that was selected equal to the half the maximum intensity of the first cluster of K-mean clustering with four clusters. Seed points were then automatically selected in both the left and right lungs of the binary mask by finding the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3-1.png}
\caption{Schematic of \textsuperscript{3}He semi-automated segmentation methodology}
Semi-automated segmentation was accomplished in three steps: 1) hierarchical K-means clustering of \textsuperscript{3}He MR images into 5 clusters where cluster 1 (C1) represented regions of ventilation defect and background, and cluster 2 to cluster 5 (C2-C5) represented gradations of signal intensity/ventilation, 2) \textsuperscript{1}H MRI segmentation using seeded region-growing algorithm, and 3) landmark-based registration of \textsuperscript{1}H and \textsuperscript{3}He MRI segmentation to differentiate the ventilation defects from the background, and therefore generating a \textsuperscript{3}He voxel cluster map.
columns in the image binary mask that contained 20 vertically adjacent pixels. A morphological closing algorithm with a structuring element disk of radius equal to 15 (function \textit{imclose} in MATLAB) was applied to fill areas within the segmented left and right lung separately.

3.2.7 Landmark-based Image Registration

Because of the extensive coaching to minimize the potential for differences in the levels of inspiration between the breath-hold scans for all subjects, $^3$He and $^1$H images were registered using a landmark-based image affine registration approach. Briefly, the center slices, defined as the 2D MR slice that clearly showed the carina and primary bronchi, were first displayed side-by-side. Three to seven fiducial markers were selected on the $^3$He image based on the carina, trachea, and primary bronchi, as well as any other distinguishing features located within the lung or near the lung periphery such as pulmonary vessels and regions near the diaphragm. The same landmarks were selected in the same order on the $^1$H image. Geometric operations consisting of rotation, translation and scaling (same in both x and y directions) were used to transform the $^1$H image to align it with the corresponding landmarks selected in the $^3$He image. The same transformation map was applied to the remaining $^1$H MR image slices. $^3$He VDV was generated using the lowest signal intensity cluster (C1) and $^3$He VV was generated as the sum of the remaining clusters (C2-C5).

3.2.8 Statistical Analysis

A two-way analysis of variance (ANOVA) was performed for comparison of manual and semi-automated volume measurements with segmentation method and repetition (5 repeated measurements) treated as within subject factors using SPSS 16.00 (SPSS Inc., Chicago, IL, USA LEAD Technologies, Inc., Chicago, IL). The relationship between manual and semi-automated segmentation was also determined using linear regression ($r^2$) and Pearson correlation coefficients (r) and the agreement between the methods was determined using Bland-Altman analysis using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego, CA, USA). Intra-observer and inter-observer reproducibility was determined for manual and semi-automated segmentation results using the
coefficient of variation (CV), calculated as the standard deviation (SD) of the five measurements divided by the mean. Two-way random effects single measure intra-class correlation coefficients (ICC) (absolute agreement) were also determined for the five repeated measurements for both manual and semi-automated segmentation using SPSS 16.00. The 95% confidence intervals (CI) for CV and ICC were determined using the Modified McKay’s method using SPSS version 16.00. To compare the reproducibility of manual and semi-automated measurements, approximate tests were performed by examining the overlap in 95% CI. The smallest detectable difference (SDD), defined as the smallest difference that can be measured with prospectively determined confidence not due to measurement error (variability), was calculated using the manual and semi-automated five repeated $^3$He measurements according to Eliasziw et al. and shown in Equation 3-1:

$$SDD \geq z_\alpha \sqrt{2SEM_{int}}$$

(1)

where $z_\alpha$ is 1.96 corresponding to a significance level of $\alpha = 0.05$ and $SEM_{int}$ is the standard error of measurement due to intra-observer variability and was calculated as shown in Equation 3-2:

$$SEM_{int} = \sqrt{\hat{\sigma}^2}$$

(2)

where $\hat{\sigma}^2$ is the intra-observer repeated measures variance. The Dice coefficient, calculated as the area of the intersection of two datasets divided by the average area of the two sets, was determined to measure the agreement or similarity between each of the five repeated measurements for both manual and semi-automated segmentation (5 repetitions = 10 comparisons), as well as between each of the repeated manual and semi-automated segmentation measurements (5 repetitions for manual and semi-automated = 25 comparisons) as shown in Equation 3-3:

$$Dice(A, B) = \frac{|A \cap B|}{|A| + |B|}$$

(3)
where $A$ and $B$ are the two data sets. For semi-automated segmentation, two observers performed all measurements and therefore the mean Dice coefficient for the two observers was used. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

### 3.3 Results

Demographic characteristics are provided in Table 3-1 for 15 subjects in total including five subjects with asthma ($n=3$ males, mean age=36 ($\pm 13$), range=20-53), five subjects with COPD ($n=2$ males, mean age=67 ($\pm 6$), range=61-77) and five subjects with CF ($n=2$ males, mean age=25 ($\pm 9$), range=20-41). COPD subjects were GOLD stage II ($n=1$) and GOLD stage III ($n=4$).

Figure 3-2 shows the centre coronal $^3$He MRI slice, where the trachea and two main bronchi are clearly visible, for each of two representative asthma, COPD and CF subjects; $^3$He ventilation displayed in red registered to the greyscale $^1$H MRI of the thorax, with the segmentation results obtained by manual and semi-automated segmentation. It is important to note that manual segmentation of the $^3$He images required approximately 60 to 90 minutes for all slices per subject, whereas semi-automated $^3$He segmentation required approximately four to eight minutes of supervised computational time for all slices per subject.
**Table 3-1** Subject Demographics

<table>
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<th>Parameter</th>
<th>All (n=15)</th>
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<td>(±SD) [range]</td>
<td>(±SD) [range]</td>
<td>(±SD) [range]</td>
</tr>
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<td>43 (20) [20-77]</td>
<td>36 (13) [20-53]</td>
<td>67 (6) [61-77]</td>
<td>25 (9) [20-41]</td>
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<tr>
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</tr>
<tr>
<td>BMI kg·m(^{-2})</td>
<td>26 (4) [18-30]</td>
<td>27 (3) [21-30]</td>
<td>26 (5) [18-30]</td>
<td>25 (3) [21-29]</td>
</tr>
<tr>
<td>FEV(<em>{1}) %(</em>{pred})</td>
<td>69 (23) [31-108]</td>
<td>91 (16) [72-108]</td>
<td>42 (11) [31-61]</td>
<td>74 (4) [69-79]</td>
</tr>
<tr>
<td>FVC %(_{pred})</td>
<td>87 (12) [65-110]</td>
<td>96 (13) [77-110]</td>
<td>78 (11) [65-90]</td>
<td>87 (1) [85-89]</td>
</tr>
<tr>
<td>FEV(_{1})/FVC</td>
<td>64 (18) [27-86]</td>
<td>77 (8) [65-86]</td>
<td>41 (9) [27-51]</td>
<td>73 (6) [65-80]</td>
</tr>
<tr>
<td>TLC %(_{pred})</td>
<td>114 (21) [94-175]</td>
<td>100 (5) [97-108]</td>
<td>129 (27) [106-175]</td>
<td>111 (12) [94-120]</td>
</tr>
<tr>
<td>IC %(_{pred})</td>
<td>101 (18) [71-138]</td>
<td>113 (15) [98-138]</td>
<td>86 (11) [71-96]</td>
<td>104 (18) [90-128]</td>
</tr>
<tr>
<td>FRC %(_{pred})</td>
<td>121 (44) [65-243]</td>
<td>88 (15) [65-107]</td>
<td>164 (45) [132-243]</td>
<td>109 (23) [88-139]</td>
</tr>
<tr>
<td>RV %(_{pred})</td>
<td>157 (55) [82-285]</td>
<td>108 (29) [82-144]</td>
<td>195 (53) [143-285]</td>
<td>172 (42) [109-197]</td>
</tr>
</tbody>
</table>

SD=Standard Deviation, BMI=Body Mass Index, FEV\(_{1}\)=Forced Expiratory Volume in 1s, %\(_{pred}\)=Percent Predicted, FVC=Forced Vital Capacity, TLC=Total Lung Capacity, IC=Inspiratory Capacity, FRC=Functional Residual Capacity, RV=Reserve Volume, †n=4, ‡n=14

**Table 3-2** shows the mean whole lung \(^3\)He volume measurements for manual and semi-automated segmentation. There was no significant difference between manual VDV and semi-automated VDV (C1) for asthma, COPD and CF subjects (p=.10). There was also no significant difference between manual VV and semi-automated VV (C2-C5) for asthma, COPD and CF (p=.48).
Figure 3-2 Manual and semi-automated segmentation results for representative asthma, COPD and CF subjects. 

$^3$He MRI center slice registered to $^1$H MRI, $^3$He VDV and VV mask generated by manual segmentation, and $^3$He cluster map generated by semi-automated segmentation for two representative asthma, COPD and CF subjects.
### Table 3-2 Manual and semi-automated $^3$He volume measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=15)</th>
<th>Asthma (n=5)</th>
<th>COPD (n=5)</th>
<th>CF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manual</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV L (±SD)</td>
<td>0.92 (0.80)</td>
<td>0.13 (0.15)</td>
<td>1.40 (0.68)</td>
<td>1.23 (0.74)</td>
</tr>
<tr>
<td>VV L (±SD)</td>
<td>4.19 (0.53)</td>
<td>4.37 (0.34)</td>
<td>4.12 (0.73)</td>
<td>4.08 (0.52)</td>
</tr>
<tr>
<td><strong>Semi-automated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV L* (±SD)</td>
<td>0.76 (0.55)</td>
<td>0.26 (0.19)</td>
<td>1.26 (0.45)</td>
<td>0.76 (0.45)</td>
</tr>
<tr>
<td>VV L* (±SD)</td>
<td>4.26 (0.61)</td>
<td>4.43 (0.83)</td>
<td>3.99 (0.90)</td>
<td>4.37 (1.05)</td>
</tr>
<tr>
<td>C2 L (±SD)</td>
<td>0.66 (0.17)</td>
<td>0.51 (0.06)</td>
<td>0.80 (0.07)</td>
<td>0.68 (0.20)</td>
</tr>
<tr>
<td>C3 L (±SD)</td>
<td>1.77 (0.41)</td>
<td>1.64 (0.43)</td>
<td>1.81 (0.35)</td>
<td>1.86 (0.50)</td>
</tr>
<tr>
<td>C4 L (±SD)</td>
<td>1.27 (0.33)</td>
<td>1.55 (0.17)</td>
<td>0.98 (0.32)</td>
<td>1.28 (0.22)</td>
</tr>
<tr>
<td>C5 L (±SD)</td>
<td>0.56 (0.20)</td>
<td>0.73 (0.17)</td>
<td>0.41 (0.16)</td>
<td>0.54 (0.13)</td>
</tr>
</tbody>
</table>

SD=Standard Deviation, VDV=Ventilation Defect Volume, VV=Ventilation Volume, C2=Cluster 2, C3=Cluster 3, C4=Cluster 4, C5=Cluster 5.

*Semi-automated VDV=Cluster 1, Semi-automated VV=Sum of Clusters 2-5

**Figure 3-3** shows the correlations and Bland-Altman plots between manual and semi-automated VDV. Manual VDV was significantly and highly correlated with semi-automated VDV for asthma (r=0.89, p<.0001), COPD (r=0.84, p<.0001) and CF subjects (r=0.89, p<.0001). Bland-Altman analysis indicated that there was negligible bias between manual and semi-automated VDV for asthma (bias=0.01±0.01L), COPD (bias=0.01±0.05L), and CF subjects (0.05±0.04L).

**Figure 3-4** shows the correlations and Bland-Altman plots between manual and semi-automated VV. Manual VV was significantly and highly correlated with semi-automated VV for asthma (r=0.99, p<.0001), COPD (r=0.91, p<.0001) and CF subjects (r=0.84, p<.0001). Bland-Altman analysis indicated that there was negligible bias for manual and semi-automated VV for asthma (bias=-0.003±0.02L), COPD (bias=0.01±0.04L) and CF subjects (-0.03±0.06L).
Table 3-3 shows the intra-observer reproducibility of \(^3\)He volume measurements for manual and semi-automated segmentation for all subjects. Intra-observer reproducibility was significantly higher for semi-automated VDV compared to manual VDV as indicated by the non-overlapping 95% CI for both CV (manual: CV=12%, 95% CI=9%-19%, semi-automated: CV=5%, 95% CI=4%-8%) and ICC (manual: ICC=.98, 95% CI=.96-.99, semi-automated: ICC=1.00, 95% CI=.99-1.00). Intra-observer reproducibility was also significantly higher for semi-automated VV compared to manual VV as indicated by the non-overlapping 95% CI for both CV (manual: CV=4%, 95% CI=3%-7%, semi-
automated: CV=.2%, 95% CI=.2%-4%) and ICC (manual: ICC=.90, 95% CI=.81-.96, semi-automated: ICC=1.00, 95% CI=1.00-1.00).

**Table 3-3 Intra-observer reproducibility of manual and semi-automated $^3$He volume measurements**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=15)</th>
<th>Asthma (n=5)</th>
<th>COPD (n=5)</th>
<th>CF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manual</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>0.98 [0.96-0.99]</td>
<td>0.99 [0.98-1.00]</td>
<td>0.97 [0.90-1.00]</td>
<td>0.96 [0.87-1.00]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>0.90 [0.81-0.96]</td>
<td>0.96 [0.85-1.00]</td>
<td>0.94 [0.68-0.99]</td>
<td>0.83 [0.56-0.98]</td>
</tr>
<tr>
<td><strong>Semi-Automated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [0.99-1.00]</td>
<td>0.99 [0.97-1.00]</td>
<td>0.98 [0.95-1.00]</td>
<td>1.00 [0.98-1.00]</td>
</tr>
<tr>
<td>VV* CV % [95% CI]</td>
<td>0.2 [0.2-0.4]</td>
<td>0.2 [0.1-0.5]</td>
<td>0.2 [0.0-0.1]</td>
<td>0.4 [0.2-1.1]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
</tr>
<tr>
<td>C2 CV % [95% CI]</td>
<td>0.6 [0.5-1]</td>
<td>1 [0.5-2]</td>
<td>0.3 [0.2-1]</td>
<td>1 [0.5-2]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>0.99 [0.97-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
</tr>
<tr>
<td>C3 CV % [95% CI]</td>
<td>0.3 [0.2-0.5]</td>
<td>0.2 [0.1-0.6]</td>
<td>0.2 [0.1-0.3]</td>
<td>0.5 [0.3-1]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [0.99-1.00]</td>
<td>1.00 [0.99-1.00]</td>
</tr>
<tr>
<td>C4 CV % [95% CI]</td>
<td>0.1 [0.1-0.2]</td>
<td>0.1 [0.0-0.2]</td>
<td>0.1 [0.1-0.4]</td>
<td>0.2 [0.1-0.6]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [0.99-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [0.99-1.00]</td>
</tr>
<tr>
<td>C5 CV % [95% CI]</td>
<td>0.1 [0.0-0.1]</td>
<td>0.1 [0.0-0.2]</td>
<td>0.0 [0.0-0.1]</td>
<td>0.1 [0.1-0.2]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
</tr>
</tbody>
</table>

VDV=Ventilation Defect Volume, VV=Ventilation Volume, CV=Coefficient of Variation, ICC=Intraclass Correlation Coefficient, CI=Confidence Interval, C2=Cluster 2, C3=Cluster 3, C4=Cluster 4, C5=Cluster 5.

*Semi-automated VDV=Cluster 1, Semi-automated VV=Sum of Clusters 2-5.
Table 3-4 shows the inter-observer reproducibility of $^3$He volume measurements for semi-automated segmentation for all subjects. For the two observers, CV was low (VDV: CV=7%, 95% CI=5%-11%, VV: CV=.4%, 95% CI=.3%-6%) and ICC was high (VDV: ICC=.96, 95% CI=.76-.99, VV: ICC=1.00, 95% CI=.83-1.00) across all three respiratory diseases evaluated.

**Table 3-4** Inter-observer reproducibility of semi-automated $^3$He volume measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=15)</th>
<th>Asthma (n=5)</th>
<th>COPD (n=5)</th>
<th>CF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC [95% CI]</td>
<td>0.96 [0.76-0.99]</td>
<td>0.95 [0.02-1.00]</td>
<td>0.94 [0.43-0.99]</td>
<td>0.97 [0.41-1.00]</td>
</tr>
<tr>
<td>VV* CV % [95% CI]</td>
<td>0.4 [0.3-0.6]</td>
<td>0.2 [0.1-0.6]</td>
<td>0.2 [0.1-0.7]</td>
<td>0.5 [0.3-1.5]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [0.83-1.00]</td>
<td>0.99 [0.60-1.00]</td>
<td>1.00 [0.79-1.00]</td>
<td>1.00 [0.85-1.00]</td>
</tr>
<tr>
<td>C2 CV % [95% CI]</td>
<td>1 [0.6-1]</td>
<td>1 [1-3]</td>
<td>0.5 [0.3-1]</td>
<td>1 [1-3]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>0.99 [0.54-1.00]</td>
<td>0.92 [0.03-0.99]</td>
<td>0.98 [0.08-1.00]</td>
<td>0.99 [0.25-1.00]</td>
</tr>
<tr>
<td>C3 CV % [95% CI]</td>
<td>0.5 [0.3-0.7]</td>
<td>0.3 [0.2-0.9]</td>
<td>0.3 [0.2-0.8]</td>
<td>0.6 [0.4-1.9]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [0.92-1.00]</td>
<td>1.00 [0.96-1.00]</td>
<td>1.00 [0.78-1.00]</td>
<td>1.00 [0.98-1.00]</td>
</tr>
<tr>
<td>C4 CV % [95% CI]</td>
<td>0.2 [0.2-0.3]</td>
<td>0.1 [0.1-0.2]</td>
<td>0.2 [0.1-0.6]</td>
<td>0.3 [0.9-0.2]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
</tr>
<tr>
<td>C5 CV % [95% CI]</td>
<td>0.1 [0.1-0.2]</td>
<td>0.1 [0.1-0.2]</td>
<td>0.0 [0.0-0.1]</td>
<td>0.1 [0.1-0.4]</td>
</tr>
<tr>
<td>ICC [95% CI]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
<td>1.00 [1.00-1.00]</td>
</tr>
</tbody>
</table>

VDV=Ventilation Defect Volume, VV=Ventilation Volume, CV=Coefficient of Variation, ICC=Intraclass Correlation Coefficient, CI=Confidence Interval, C2=Cluster 2, C3=Cluster 3, C4=Cluster 4, C5=Cluster 5.

*Semi-automated VDV=Cluster 1, Semi-automated VV=Sum of Clusters 2-5

Table 3-5 shows the SDD for measurements obtained by manual and semi-automated segmentation. For manual segmentation, the SDD or the minimum change that could be measured confidently in individual asthma, COPD and CF subjects that were not due to technological or observer measurement variability (measurement error) was 310mL and
480mL for $^3$He VDV and VV, respectively. For the semi-automated method, the SDD or the minimum change that could be measured confidently in individual asthma, COPD and CF subjects was 110mL and 30mL for $^3$He VDV and VV, respectively.

Table 3-5 Smallest detectable difference for manual and semi-automated segmentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=15)</th>
<th>Asthma (n=5)</th>
<th>COPD (n=5)</th>
<th>CF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV L (±SD)</td>
<td>0.31</td>
<td>0.03</td>
<td>0.34</td>
<td>0.42</td>
</tr>
<tr>
<td>VV L (±SD)</td>
<td>0.48</td>
<td>0.20</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>Semi-automated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV* L (±SD)</td>
<td>0.11</td>
<td>0.05</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>VV* L (±SD)</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>C2 L (±SD)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>C3 L (±SD)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>C4 L (±SD)</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>C5 L (±SD)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

VDV=Ventilation Defect Volume, VV=Ventilation Volume, C2=Cluster 2, C3=Cluster 3, C4=Cluster 4, C5=Cluster 5.
*Semi-automated VDV=Cluster 1, Semi-automated VV=Sum of Clusters 2-5

Table 3-6 shows the Dice coefficients for manual and semi-automated segmentation. Dice coefficients (D) were higher for repeated semi-automated segmentation than for repeated manual segmentation measurements for both VDV (manual: D=.71; semi-automated: D=.88) and VV (manual: D=.95; semi-automated: D=1.00). The Dice coefficient for manual and semi-automated VDV was 0.44 and the Dice coefficient for manual and semi-automated VV was 0.91.
Table 3-6 Dice coefficients for manual and semi-automated segmentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=15)</th>
<th>Asthma (n=5)</th>
<th>COPD (n=5)</th>
<th>CF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDV</td>
<td>0.71 (0.09)†</td>
<td>0.69 (0.12)</td>
<td>-</td>
<td>0.74 (0.05)</td>
</tr>
<tr>
<td>VV</td>
<td>0.95 (0.03)†</td>
<td>0.97 (0.00)</td>
<td>-</td>
<td>0.92 (0.02)</td>
</tr>
<tr>
<td>Semi-automated – Semi-automated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV*</td>
<td>0.88 (0.06)</td>
<td>0.82 (0.03)</td>
<td>0.92 (0.04)</td>
<td>0.89 (0.06)</td>
</tr>
<tr>
<td>VV*</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.00)</td>
</tr>
<tr>
<td>Manual – Semi-automated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDV</td>
<td>0.36 (0.20)</td>
<td>0.15 (0.15)</td>
<td>0.52 (0.10)**</td>
<td>0.42 (0.11)</td>
</tr>
<tr>
<td>VV</td>
<td>0.90 (0.06)</td>
<td>0.95 (0.01)</td>
<td>0.88 (0.08)</td>
<td>0.87 (0.04)</td>
</tr>
</tbody>
</table>

*Semi-automated VDV=Cluster 1, Semi-automated VV=Sum of Clusters 2-5, †One repetition of manual segmentation

3.4 Discussion

In contrast to the homogenous $^3$He MRI signal intensity observed in healthy subjects with normal lung function, $^3$He MRI of subjects with respiratory disease results in heterogeneous signal intensity throughout the lung, which may be a result of small airway occlusion, mucous plugs, airway wall thickening and inflammation or bullous disease. Related to this, is the fact that there is significant functional information in the regions of middle-intensity, and both hyper- and hypo-intense signal regions that have not yet been quantitatively or spatially exploited. The development and validation of $^3$He image analysis techniques, or the use of widely available and well established image processing algorithms, that quantify heterogeneous signal information are required to fully characterize the important ventilation information contained in the image, and may enable a better understanding of the physiological changes that occur in disease longitudinally and in response to treatment. Here we evaluated the spatial and quantitative agreement of a semi-automated segmentation method with manual segmentation for the evaluation of $^3$He and $^1$H MRI of asthma, COPD and CF subjects and report: 1) $^3$He manual and semi-automated VDV and VV measurements that were not statistically significantly different, and were significantly correlated with good agreement, 2) significantly higher intra-observer reproducibility for semi-automated compared to manual measurements and high inter-observer reproducibility, 3) smallest detectable differences that were lower for semi-automated measurements, and, 4) high
Dice coefficients for manual and semi-automated VV, indicating excellent spatial overlap.

$^3$He MRI measurements for asthma, COPD and CF subjects generated by manual and semi-automated segmentation were not significantly different and were significantly and strongly correlated and in good agreement. A small but insignificant bias was detected by Bland-Altman analysis that may be due to the manual observers’ tendency to classify hypo-ventilated areas as defects in some subjects and in some image slices and not in others, or the inclusion of regions of signal loss associated with the pulmonary vascular structures, not included with manual segmentation, by the K-means approach. However, it is important to note that the inclusion of pulmonary vascular structures as ventilation defects may not be a critical consideration for serial studies. The improvement in segmentation time afforded by the semi-automated approach and the finding that the lung can be reliably segmented in a number of respiratory diseases, demonstrates the potential for $^3$He MRI segmentation in large scale, multi-centre clinical evaluations.

We also observed that intra-observer reproducibility of the $^3$He measurements was significantly higher for semi-automated segmentation compared to the manual approach, and inter-observer reproducibility was high for the semi-automated method and higher than previously reported manual results. Clearly, segmentation methods that provide high intra-/inter-observer reproducibility are required for future studies involving large subject numbers and multiple research centers such that reliable measurements can be obtained from multiple observers independent of the level of experience. To better understand if the improvements in measurement reproducibility afforded by semi-automated segmentation could be translated to practical use, the SDD was determined for both manual and semi-automated measurements, and the semi-automated method developed here reported a lower SDD as compared to manual results. For serial studies it is important to be confident that the changes measured between imaging time-points are not due to measurement error. Therefore, future $^3$He serial studies should utilize segmentation methods with low measurement error to detect the important functional measurement changes that occur following intervention or during disease progression.
Furthermore, the excellent reproducibility and very low SDD for $^3$He VV suggests that this measurement could be utilized in future studies for the analysis of treatment effects in individual subjects.

Finally, we demonstrated high Dice coefficients between the semi-automated and manual segmentation results. This finding suggests that along with the higher intra- and inter-observer reproducibility of the volume measurements obtained by the semi-automated method, the segmentation results are also highly spatially reproducible. However, we also reported low Dice coefficients between the manual and semi-automated VDV segmentation results for the asthma subjects and moderate Dice coefficients for the COPD and CF subjects. The lack of spatial correspondence between the two methods, particularly in the asthma subjects, is likely due to the small size of the ventilation defects. It should be noted that K-means clustering is a histogram-based segmentation approach, thus very small areas of signal void may be included as defects by the semi-automated method but overlooked due to the small size by the manual observer. Therefore, the excellent spatial and quantitative agreement between manual and semi-automated segmentation suggests that semi-automated or automated segmentation methods are important for future $^3$He imaging studies and should be utilized as we transition to $^{129}$Xe MRI.

Although the semi-automated $^3$He segmentation method is very promising, we must acknowledge that the physiological rationale for selecting the initial cluster number was based on the expert observer’s visual assessment, and not based on a more objective approach. However, we have previously evaluated K-means with the initial user input of 4 to 10 clusters and the hierarchical K-means approach described here, and determined that hierarchical K-means provided the highest correlation and lowest Bland-Altman bias with manual segmentation.

In conclusion, we employed a straightforward combination of previously developed, well-described and widely available methods, namely hierarchical K-means, seeded region-growing and non-rigid registration, to $^3$He MRI to enable semi-automated segmentation of the unique functional information this method provides. Reproducible
and robust segmentation methods that can be applied across many respiratory conditions are urgently needed for larger scale pulmonary functional MRI studies.
3.5 References


(18) Ireland RH, Bragg CM, McJury M et al. Feasibility of image registration and intensity-modulated radiotherapy planning with hyperpolarized helium-3


### 3.6 Supplementary Tables

Table 3-S3-1 Comparison of $^3$He VDV and VV generated by manual segmentation with K-means clustering with 4 to 10 clusters and hierarchical K-means using Pearson correlation coefficients and Bland-Altman analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson Correlation Coefficients</th>
<th>Bland-Altman Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>p-value</td>
</tr>
<tr>
<td>VDV L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Clusters</td>
<td>0.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>5 Clusters</td>
<td>0.79</td>
<td>0.0001</td>
</tr>
<tr>
<td>6 Clusters</td>
<td>0.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>7 Clusters</td>
<td>0.75</td>
<td>0.0001</td>
</tr>
<tr>
<td>8 Clusters</td>
<td>0.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>9 Clusters</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>10 Clusters</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hierarchical K-means</td>
<td>0.84</td>
<td>0.0001</td>
</tr>
<tr>
<td>VV L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Clusters</td>
<td>0.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>5 Clusters</td>
<td>0.89</td>
<td>0.0001</td>
</tr>
<tr>
<td>6 Clusters</td>
<td>0.88</td>
<td>0.0001</td>
</tr>
<tr>
<td>7 Clusters</td>
<td>0.89</td>
<td>0.0001</td>
</tr>
<tr>
<td>8 Clusters</td>
<td>0.91</td>
<td>0.0001</td>
</tr>
<tr>
<td>9 Clusters</td>
<td>0.88</td>
<td>0.0001</td>
</tr>
<tr>
<td>10 Clusters</td>
<td>0.88</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hierarchical K-means</td>
<td>0.91</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

VDV=Ventilation Defect Volume, VV=Ventilation Volume, $r$=Pearson correlation coefficient, CI=95% Confidence Interval. Significance (p<.05).
CHAPTER 4

Using the segmentation algorithm developed in Chapter 3, here we evaluated a group of COPD subjects using $^3$He MRI prior to and immediately following bronchodilator therapy.

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4 Chronic Obstructive Pulmonary Disease: Quantification of Bronchodilator Effects by using Hyperpolarized $^3$He MR Imaging

4.1 Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive expiratory flow limitation that develops due to the combined effects of large and small airway dysfunction, and increased lung compliance due to the permanent destructive changes of emphysema. Gas trapping and dynamic lung hyperinflation are major consequences of decreased expiratory airflow that often lead to many of the disabling symptoms associated with COPD, such as dyspnea and limitation of exercise capacity. After smoking cessation, bronchodilators are the first-line therapy in the symptomatic management of COPD, and assessment of acute response to bronchodilators is often based on spirometry measurements of the forced expiratory volume in 1 s (FEV$_1$). While spirometry provides the most common primary endpoint for clinical trials evaluating therapeutic response, relatively poor correlations have been reported between FEV$_1$ and other clinical measurements of COPD. In other words, symptomatic relief following bronchodilators often occurs with only modest improvements in FEV$_1$, underscoring the apparent discordance between spirometry and COPD symptoms, quality of life and functional measurements.
The discordance between spirometry and COPD symptoms, as well as the limitation of standard measures of expiratory airflow for evaluating the regional nature of bronchodilator response is motivating the evaluation of new methods,\textsuperscript{10,11} including those derived from non-invasive imaging.\textsuperscript{12-15} For example, Computed Tomography (CT) has been used to evaluate changes in airway morphology after administration of salbutamol\textsuperscript{12,13} and tiotropium,\textsuperscript{14,15} and showed greater post-bronchodilator response in COPD patients with mainly airways disease compared to those with mainly emphysema.\textsuperscript{16} Additionally, a recent single photon emission computed tomography (SPECT)-CT and multiple breath nitrogen washout study\textsuperscript{17} reported no significant change in ventilation heterogeneity after tiotropium administration.

Pulmonary magnetic resonance imaging (MRI) using hyperpolarized helium-3 ($^3$He) has emerged as a functional imaging method providing high spatial and temporal resolution\textsuperscript{18-23} images of the lung. Regional ventilation abnormalities are clearly visualized as decreased $^3$He signal in the lung that can be scored or quantified as a ventilation defect percent (VDP)$^{19}$ or percent ventilated volume (PVV)$^{24}$ $^3$He MRI ventilation abnormalities have been previously evaluated in COPD,\textsuperscript{25-30} with measurements that are sensitive to the pathological changes that accompany COPD,\textsuperscript{24} have high same-day and short-term reproducibility,$^{20,21}$ and detect significant longitudinal changes over two years before changes in FEV$_1$ are observed.$^{18}$ Because of previous reproducibility$^{20,21}$ and longitudinal findings,$^{18}$ we hypothesized that $^3$He MRI would provide the necessary and sufficient spatial and temporal sensitivity as well as precision to detect any potential regional functional lung changes after bronchodilator therapy. Therefore, our aim was to evaluate short-acting bronchodilator effects in COPD using hyperpolarized $^3$He MRI, spirometry and plethysmography.

4.2 Materials and Methods

4.2.1 Subjects

All subjects provided written informed consent to the study protocol approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada) and
the Health Insurance Portability and Accountability Act (HIPAA, USA). The use of an onsite $^3$He gas polarizer (HelispinTM; GE Healthcare, Durham, NC) was provided to Robarts Research Institute through an agreement with GE Healthcare for which we pay $100,000 (CDN) annually.

Fourteen subjects with a clinical diagnosis of COPD who were ex-smokers with a smoking history of at least 10-pack-years, and were GOLD stage II-IV were enrolled. Subjects were required to withhold both short-acting and long-acting bronchodilators the morning of the study. Pre-bronchodilator MRI was performed immediately following pulmonary function tests; post-bronchodilator MRI and pulmonary function tests were performed 25 minutes ($\pm$2 minutes) after administration of 400 μg salbutamol (Salbutamol Sulphate USP; Apo-Salvent CFC Free Inhalation Aerosol, Apotex Inc., Toronto, Ontario/Canada) inhaled via a spacer device.

4.2.2 Pulmonary Function Tests

Spirometry was performed using an *ndd EasyOne* spirometer (ndd Medizintechnik AG, Zurich, CH) according to the American Thoracic Society (ATS) guidelines. Static lung volumes and diffusing capacity of carbon monoxide (DL$\text{CO}$) were measured using body plethysmography (MedGraphics Corporation. 350 Oak Grove Parkway St. Paul, MN USA).

4.2.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Excite 12.0 MRI system (GEHC, Milwaukee, WI USA) with broadband imaging capability as previously described$^{21}$ and $^3$He MRI was enabled using a single channel, rigid elliptical transmit/receive chest coil (RAPID Biomedical GmbH, Wuerzburg Germany). It is important to note that we endeavored to minimize the potential for differences in the levels of inspiration between the breath-hold scans for each subject by: 1) training and practice for all subjects prior to MRI related to the inspiration breath-hold maneuver from functional residual capacity (FRC), and, 2) continuous coaching and monitoring at the MR bedside by a pulmonary function technologist during all inspiration breath-hold scans. Because of this extensive coaching, no correction for differences in inspiration levels between breath-hold scans for
individual subjects was required. Pulse oximetry was used to measure arterial oxygen saturation (SaO2) for all subjects during scanning sessions and considered an adverse event occurred when SaO2 decreased below 88% at any time during the scanning session. Study withdrawal was required when SaO2 decreased to 80% for 10 seconds or longer.

Conventional 1H MRI was performed prior to hyperpolarized 3He MRI with subjects scanned during 1.0 L breath-hold of 4He/N2 using the whole body radiofrequency (RF) coil and 1H fast spoiled gradient recalled echo sequence (16s total data acquisition, repetition time (TR) / echo time (TE) / flip angle = 4.7 ms / 1.2 ms / 30°, field-of-view (FOV) = 40 × 40 cm, matrix 256 × 128, 14 slices, 15 mm slice thickness, 0 gap), as previously described.21

Prior to 3He MRI, a polarizer system (HeliSpin™, GEHC, Durham, NC) was used to polarize 3He gas to 30—40 % and doses (5 mL/kg body weight) were administered in 1.0 L Tedlar® bags diluted with ultrahigh purity, medical grade nitrogen (Spectra Gases, Alpha, NJ). As previously described,21 hyperpolarized 3He MRI coronal static ventilation images were acquired during breath-hold of a 1L 3He/N2 mixture (14s data acquisition, TR / TE / flip angle = 4.3 ms / 1.4 ms / 7°, bandwidth = 31.25 kHz, FOV = 40 × 40 cm, matrix 128 × 128, 14 slices, 15 mm slice thickness, 0 gap).

4.2.4 Image Analysis

3He MRI pulmonary ventilation semi-automated segmentation was performed using custom software generated using MATLAB R2007b (The Mathworks Inc., Natick, MA, USA), as previously described.31 Briefly, and as shown in the schematic in Figure 4-1, 3He MR images were evaluated using K-means cluster analysis,32 similar to previously described methods for 3He MRI segmentation,33-35 to classify the 3He MRI voxel intensity values into clusters ranging from 1 to 5, representing gradations of signal intensity from no signal (Cluster 1, C1) and hypo-intense signal (Cluster 2, C2) to hyper-intense signal (Cluster 5, C5), and generating a 3He voxel cluster map (Figure 1B). To obtain the external contour of the thoracic cavity to differentiate ventilation defects (C1) from the edge of the lung, 1H MR images were segmented using a seeded region-growing algorithm36 and registered to the 3He MR ventilation images as previously described.37
VDP was generated using ventilation defect volume (VDV or C1) normalized to the thoracic cavity volume as previously described. For the remaining ventilation clusters, the segmented ¹H thoracic cavity volume was used to generate cluster percent representing a normalized ³He cluster volume for the lung. All measurements were performed by the same observer (MK) blinded to subject and time point with time point randomized to reduce any potential measurement bias. The observer (MK) had three years experience performing manual ³He MRI segmentation and one year experience developing and performing semi-automated ³He MRI segmentation. Reproducibility of the semiautomated method was determined on the basis of the pre- salbutamol intraobserver variability of five repeated measurements for five COPD subjects. The smallest detectable difference (SDD), or the minimum change in ³He measurements that could be measured confidently in individual patients-not due to observer measurement variability, for five of the COPD subjects was 2% for VDP and 0.4%, 1%, 0.6%, and 0.3% for clusters 2–5, respectively.

4.2.5 Statistical Methods

Multivariate analysis of variance (MANOVA) and repeated measures analysis of variance (ANOVA) was performed for comparison of pre- and post-salbutamol pulmonary function and lung volume measurements using SPSS 16.00 (SPSS Inc., Chicago, IL, USA LEAD Technologies, Inc., Chicago, IL).
ANOVA was used to determine the interactions between subjects and treatment, imaging slice and treatment, as well as bronchodilator response group and treatment for all $^3$He MRI measurements using SPSS 16.00. Effect size (ES) calculations allow the magnitude of an effect to be compared between disparate types of measurements. Therefore, for comparison of the magnitude of the treatment effect between pulmonary function and imaging measurements, treatment ES was calculated as the ratio of the mean difference between pre- and post-salbutamol measurements and the pooled standard deviation for all subjects, calculated using Hedges’ $g$ as shown in Equation 4-1:

$$g = \frac{\bar{x}_{post} - \bar{x}_{pre}}{\sqrt{(n_{post} - 1)SD_{post}^2 + (n_{pre} - 1)SD_{pre}^2}} \sqrt{\frac{n_{post} + n_{pre} - 2}{n_{post} + n_{pre}}}$$

where $\bar{x}_{pre}$ and $\bar{x}_{post}$ are the mean pre- and post-salbutamol measurement for all subjects, respectively, $SD_{pre}$ and $SD_{post}$ are the standard deviations of the pre- and post-salbutamol measurements for all subjects, respectively, and $n_{pre}$ and $n_{post}$ are the number of subjects evaluated pre- and post-salbutamol, respectively. The SDD, defined as the smallest difference that can be measured with prospectively determined confidence that is not due to measurement error (variability), was calculated for five repeated pre-salbutamol $^3$He VDP measurements according to Eliasziw et al. and shown in Equation 4-2:

$$SDD \geq z_\alpha \sqrt{2(SEM_{intra})^2}$$

where $z_\alpha$ is 1.96 corresponding to a significance level of $\alpha = .05$ and $SEM_{intra}$ is the standard error of measurement due to intra-observer variability and is calculated as shown in Equation 4-3:

$$SEM_{intra} = \sqrt{\hat{\sigma}_e^2}$$

where $\hat{\sigma}_e^2$ is the intra-observer repeated measures variance. Linear regression ($r^2$) and Spearman correlation coefficients ($r$) were used to determine the relationships between
pulmonary function and $^{3}$He MRI measurements using GraphPad Prism version 4.00 (GraphPad Software Inc version 4.00, San Diego, CA, USA). In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

### 4.3 Results

Demographic characteristics are provided in **Table 4-1** for 14 COPD ex-smokers (GOLD Stage II n=5; GOLD Stage III n=8; GOLD Stage IV n=1).

**Table 4-2** shows mean pulmonary function and $^{3}$He MRI measurements for all subjects and **Table 4-S1** provides a subject listing of all measurements. Statistically significant post-salbutamol changes were observed for FEV$_1$ ($p=.001$, ES=.22), TLC ($p=.04$, ES=-.34), and FRC ($p=.03$, ES=-.10).

**Table 4-1** Subject Demographics

<table>
<thead>
<tr>
<th>All Subjects (±SD) [range]</th>
<th>(n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs</td>
<td>70 (6) [61-77]</td>
</tr>
<tr>
<td>Males</td>
<td>70 (6) [61-77]</td>
</tr>
<tr>
<td>Females</td>
<td>71 (6) [64-77]</td>
</tr>
<tr>
<td>Male Sex</td>
<td>8</td>
</tr>
<tr>
<td>BMI kg·m$^{-2}$</td>
<td>24.9 (3.8) [17.6-31.4]</td>
</tr>
<tr>
<td>FEV$_1$ L</td>
<td>1.23 (0.50) [0.63-2.18]</td>
</tr>
<tr>
<td>FEV$_1$ %pred</td>
<td>45 (12) [28-61]</td>
</tr>
<tr>
<td>FVC L</td>
<td>2.98 (0.83) [1.52-4.44]</td>
</tr>
<tr>
<td>FVC %pred</td>
<td>83 (12) [65-107]</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>41 (10) [22-60]</td>
</tr>
<tr>
<td>TLC L</td>
<td>7.12 (1.27) [4.82-8.96]</td>
</tr>
<tr>
<td>TLC %pred</td>
<td>122 (20) [101-175]</td>
</tr>
<tr>
<td>IC L</td>
<td>2.21 (0.54) [1.39-3.09]</td>
</tr>
<tr>
<td>IC %pred</td>
<td>83 (16) [58-108]</td>
</tr>
<tr>
<td>FRC L</td>
<td>4.89 (1.22) [3.14-7.27]</td>
</tr>
<tr>
<td>FRC %pred</td>
<td>155 (42) [110-243]</td>
</tr>
<tr>
<td>RV L</td>
<td>3.83 (0.94) [2.63-5.25]</td>
</tr>
<tr>
<td>RV %pred</td>
<td>170 (53) [104-285]</td>
</tr>
<tr>
<td>DL$_{CO}$ %pred</td>
<td>43 (19) [20-89]</td>
</tr>
</tbody>
</table>

SD=Standard Deviation, BMI=Body Mass Index, FEV$_1$= Forced Expiratory Volume in 1s, %pred=Percent Predicted, FVC= Forced Vital Capacity, TLC= Total Lung Capacity, IC= Inspiratory Capacity, FRC= Functional Residual Capacity, RV= Reserve Volume, DL$_{CO}$=Carbon Monoxide Diffusion Capacity of the lung.
Table 4-2 Pre- and post-salbutamol measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Salbutamol (n=14)</th>
<th>Post-Salbutamol (n=14)</th>
<th>Significance of Difference (p)</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulmonary Function Measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ L (±SD)</td>
<td>1.23 (0.50)</td>
<td>1.35 (0.57)</td>
<td>0.001⁺</td>
<td>0.22</td>
</tr>
<tr>
<td>FVC L (±SD)</td>
<td>2.98 (0.83)</td>
<td>3.06 (0.85)</td>
<td>0.46⁺</td>
<td>0.10</td>
</tr>
<tr>
<td>TLC L (±SD)</td>
<td>7.05 (1.22)</td>
<td>6.62 (1.26)</td>
<td>0.04⁺</td>
<td>-0.35</td>
</tr>
<tr>
<td>IC L (±SD)</td>
<td>2.15 (0.58)</td>
<td>2.02 (0.66)</td>
<td>0.39⁺</td>
<td>-0.21</td>
</tr>
<tr>
<td>FRC L (±SD)</td>
<td>4.89 (1.22)</td>
<td>4.61 (1.22)</td>
<td>0.03⁺</td>
<td>-0.23</td>
</tr>
<tr>
<td>RV L (±SD)</td>
<td>3.79 (0.91)</td>
<td>3.59 (0.85)</td>
<td>0.23⁺</td>
<td>-0.23</td>
</tr>
<tr>
<td><strong>Hyperpolarized ³He MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDP % (±SD)</td>
<td>28 (7)</td>
<td>24 (9)</td>
<td>&lt;0.0001⁺</td>
<td>-0.50</td>
</tr>
<tr>
<td>Cluster 2 % (±SD)</td>
<td>15 (2)</td>
<td>14 (2)</td>
<td>0.01⁺</td>
<td>-0.50</td>
</tr>
<tr>
<td>Cluster 3 % (±SD)</td>
<td>31 (5)</td>
<td>32 (5)</td>
<td>0.03⁺</td>
<td>0.20</td>
</tr>
<tr>
<td>Cluster 4 % (±SD)</td>
<td>19 (4)</td>
<td>22 (5)</td>
<td>&lt;0.0001⁺</td>
<td>0.66</td>
</tr>
<tr>
<td>Cluster 5 % (±SD)</td>
<td>8 (3)</td>
<td>9 (3)</td>
<td>0.02⁺</td>
<td>0.33</td>
</tr>
</tbody>
</table>

FEV₁= Forced Expiratory Volume in 1s, FVC= Forced Vital Capacity, TLC= Total Lung Capacity, IC= Inspiratory Capacity, FRC= Functional Residual Capacity, RV= Reserve Volume, VDP=Ventilation Defect Percent, ⁺n=13
⁺Repeated measures ANOVA, ‡Two-way mixed design repeated measures ANOVA.

Figure 4-2 shows two of the central coronal ³He MRI slices, where the trachea and two main bronchi are clearly visible with ³He ventilation displayed in red, registered to the greyscale ¹H MRI of the thorax for each of five representative subjects (S1 and S2=GOLD II, S3 and S4=GOLD III and S5=GOLD IV) before and after salbutamol administration. The change in ³He gas distribution after salbutamol is readily apparent in the right and left apical regions for S1 and S5 and right mid apex for S3, whereas there is little change visible for S2 and S4 for the two slices shown. To ensure that the increased distribution of ³He gas post-salbutamol administration was not due to differences in the volume of polarized gas administered at each time-point, we calculated the total pixel intensity for each lung image, including the trachea and major airways, from the image pixel intensity frequency histograms, as well as the total moles of polarized gas delivered for each subject time-point (data not shown). These estimations showed that the amount of polarized gas administered and inhaled was not significantly different between time-points (p=.87). The signal-to-noise ratio for each pre- and post-salbutamol image pair was also not statistically significantly different (p=.12). To minimize the potential of
introducing intensity variations due to B1-field inhomogeneity, subjects were located in
the same position within the coil between imaging time-points.

**Figure 4-2** $^3$He MRI pre- and post-salbutamol
Pre- and post-salbutamol $^3$He MRI (red) registered to coronal thoracic $^1$H MRI (grey scale) for five representative COPD subjects. Two center slices for GOLD Stage II COPD (S1, S2), GOLD Stage III (S3, S4), and GOLD Stage IV COPD (S5).
Table 4-2 shows mean normalized pre- and post-salbutamol $^3$He measurements and Table 4-S2 (online) shows the mean pre- and post-salbutamol $^3$He volume and signal intensity measurements. There was a significant decrease in VDP following salbutamol administration ($p<.0001$, $ES=-.50$), and there was no relationship between post-salbutamol changes in VDP and image slice (as a region of interest) ($p=.30$), indicating no bias for any measurable changes with respect to image slice. Following salbutamol administration, there was a significant decrease in $^3$He C2 ($p=.01$, $ES=-.50$), and significant increases in $^3$He C3 ($p=.03$, $ES=-.20$), C4 ($p<.0001$, $ES=-.66$) and C5 ($p=.02$, $ES=-.33$). Based on the pre-salbutamol intra-observer variance, the SDD or the minimum change in $^3$He measurements that could be measured confidently in individual subjects - not due to observer measurement variability for five of the COPD subjects (n=1 stage II, n=4 stage III) was 2% for VDP and 0.4%, 1%, 0.6% and 0.3% for C2-5, respectively.

As shown in Figure 4-3, there were significant and moderate Spearman correlations between baseline $^3$He VDP and post-salbutamol changes in FEV$_1$ ($r=-.77$, $p=.001$) and between baseline FEV$_1$%pred and post-salbutamol changes in FEV$_1$ ($r=.56$, $p=.04$). There was also a significant correlation between the changes measured in $^3$He C2 and changes in FEV$_1$ ($r=-.62$, $p=.02$) and FVC ($r=-.59$, $p=.03$).

![Figure 4-3](image)

**Figure 4-3** Correlation between baseline FEV$_1$ %pred and VDP and the post-salbutamol change in FEV$_1$.
BL FEV$_1$%pred is significantly correlated with Δ FEV$_1$ ($r=.60$, $r^2=.36$, $p=.02$, $y=0.006x-0.13$) (A), BL VDP is significantly correlated with Δ FEV$_1$ ($r=-.68$, $r^2=.46$, $p=.008$, $y=-0.01x+0.42$) (B). Dotted lines indicate the 95% confidence intervals.
Recently published ATS and European Respiratory Society (ERS) guidelines define a significant bronchodilator response as an increase in post-salbutamol FEV\textsubscript{1} and/or FVC greater than 200mL and 12\%.\textsuperscript{40} Accordingly, five subjects were classified as bronchodilator responders (BR) and nine subjects were classified as bronchodilator non-responders (BNR). As shown in Tables 4-3, and Table 4-S1, mean reductions in VDP and C2, and corresponding increases in C3-5 were demonstrated for both BR and BNR subjects. As shown in Figure 4-4, VDP was significantly lower for BR compared to BNR at baseline and post bronchodilator (p=.03). As depicted by the parallel slopes for pre- and post-salbutamol measurements shown in Figure 4, there was no significant difference between BR and BNR for post-salbutamol change in \textsuperscript{3}He MRI (VDP, C2-5, p>.05). It is important to point out that there was a single BNR subject that showed a 240ml and 12\% improvement in FEV\textsubscript{1} (just missing the greater than 12\% change in FEV\textsubscript{1} requirement for BR), and when this subject was re-categorized as BR the reported results remained the same. It is also worth noting that there were no significant differences between BR and BNR for baseline FEV\textsubscript{1} (p=.44) or DL\textsubscript{CO} (p=1.00).

![Figure 4-4](image.png)

**Figure 4-4** Mean changes in VDP for BR and BNR.

\textsuperscript{3}He VDP pre- and post-salbutamol for BR (black) and BNR (grey). Means calculated for all subject slices and error bars are 95\% confidence intervals of the mean.
Table 4-3 Subject listing of changes in spirometry and $^3$He MRI measurements after salbutamol administration

<table>
<thead>
<tr>
<th>Subject</th>
<th>Spirometry</th>
<th>$^3$He MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^\Delta$ FEV$_1$</td>
<td>$^\Delta$ FVC</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>%*</td>
</tr>
<tr>
<td>All Subjects</td>
<td>0.12</td>
<td>10</td>
</tr>
<tr>
<td>BR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.14</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>5</td>
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</tr>
<tr>
<td>7</td>
<td>0.18</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>0.27</td>
<td>22</td>
</tr>
<tr>
<td>ALL</td>
<td>0.22</td>
<td>11</td>
</tr>
<tr>
<td>BNR</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.04</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>-0.06</td>
<td>-7</td>
</tr>
<tr>
<td>9</td>
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</tr>
<tr>
<td>11</td>
<td>0.08</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>0.06</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>0.13</td>
<td>9</td>
</tr>
<tr>
<td>14</td>
<td>0.07</td>
<td>4</td>
</tr>
<tr>
<td>ALL</td>
<td>0.06</td>
<td>5</td>
</tr>
</tbody>
</table>

*%=Percent change from baseline absolute value in litres
BR=Bronchodilator Responder, BNR=Bronchodilator Non-Responder

Significant bronchodilator response defined as an increase in FEV$_1$ and/or FVC greater than 200mL and 12% from pre-salbutamol values according to ATS/ERS $^{40}$.

### 4.4 Discussion

In this pilot functional MRI evaluation of bronchodilator effects in COPD, we made a number of observations and report the following: 1) significant post-bronchodilator improvements in both spirometry and $^3$He MRI measurements of gas distribution, and their effect sizes, 2) a significant relationship between $^3$He VDP and changes in FEV$_1$ post-salbutamol, and, 3) changes in $^3$He MRI post-bronchodilator that were not significantly different for BR and BNR subgroups.

First, and as might be expected from previous work that demonstrated modest improvements in lung function in patients with COPD following bronchodilator
therapy, we observed statistically significant changes in mean FEV$_1$, FRC, and TLC. At the same time, significant decreases in $^3$He MRI VDP and C2 and significant improvements in $^3$He MRI C3-5 were measured, suggesting regional gas distribution improvements throughout the lung. Additionally, the $^3$He gas distribution changes occurred in different slices for each subject with no detected bias for specific regions of gas distribution improvements. This finding is in agreement with a recent CT study that demonstrated regionally dispersed airway wall structural alterations post-bronchodilator.

Moreover, when ES was evaluated, which allows the magnitude of the treatment effect to be compared between disparate types of measurements, $^3$He MRI measurements provided greater ES than FEV$_1$. We also observed an overall mean change in VDP and all $^3$He ventilation cluster measurements greater than the SDD indicating that the measured improvement in $^3$He distribution post-salbutamol was not due to measurement error or lack of reproducibility. Although this study did not include a control arm, it is important to note that 11 of the 14 subjects evaluated here previously participated in a reproducibility study approximately 2 years prior to pre- and post-salbutamol imaging. In this previous study, the mean change in VDV for these subjects, approximately 7 minutes after scan, was 1 mL – not a significant or clinically relevant change. For the same 11 subjects reported here, the mean change in $^3$He VDV (C1) was 62 mL. Taken together, these results strongly suggest that the changes following treatment observed here were not due to either scan-rescan variability or the intra-observer variability of the measurement technique. We must also point out that although this is the first reported study demonstrating improvements in $^3$He gas distribution following bronchodilator therapy in COPD, previous $^3$He MRI studies have demonstrated changes in $^3$He gas distribution following bronchial provocation tests with methacholine in asthma and following therapy in cystic fibrosis subjects. Taking together, these imaging-based treatment response studies indicate that functional MRI may be considered for evaluating new respiratory therapies in clinical trials.

Second, we also observed a significant negative correlation between the baseline VDP and changes in FEV$_1$ post-salbutamol, and a significant positive correlation between baseline FEV$_1$ and changes in FEV$_1$. These results suggest that subjects demonstrating the greatest improvement in FEV$_1$ post-salbutamol were those with milder disease. A
recent CT study also evaluated a large number of COPD ex-smokers as part of the National Emphysema Treatment Trial and in accordance with our MRI results, demonstrated that significant bronchodilator reversibility was more likely in patients with higher FEV$_1$. They

Finally, we detected post-bronchodilator improvements in $^3$He VDP and gas distribution that were not significantly different for the five BR and nine BNR subjects. Based on this imaging finding, it appears that even COPD patients without a clinically relevant improvement in FEV$_1$ show improved regional gas distribution, post-bronchodilator. In fact, both groups showed the same mean improvement in $^3$He MRI measurements. This important finding suggests that measurements derived from non-invasive imaging detect functional lung changes that enable a better understanding of the COPD lung and its regional response to therapy which may be important for drug development and drug treatment trials in COPD. Future functional MRI studies with larger sample sizes will help to confirm and extend these important findings.

We acknowledge that this pilot study is limited by the small number of subjects studied and the fact that the analysis was restricted mainly to subjects with stage II and III COPD. Another limitation is the lack of a true reference standard for the measurement of treatment effects in COPD, such as dyspnea scores and measurements of exercise tolerance for direct comparison to $^3$He MRI measurements to evaluate the clinical meaning of the imaging changes. In other words, whether improvements in gas distribution are related to clinical or symptomatic improvement remains to be established in a larger study. We note that subjects were provided training for inspiration breath-hold scanning, they performed practice breath-holds and received coaching during breath-hold MRI. Clearly, reproducible and comparable breath-hold volumes is critically important for acute and chronic therapy repeated studies. We also acknowledge that although $^3$He MRI ventilation defect measurements have been previously reported to have high same-day reproducibility$^{20}$ in the majority of subjects included in this study, the prospective inclusion of a control group would have strengthened the conclusion that changes post-salbutamol observed here were directly attributable to bronchodilator treatment. Another important consideration is the high cost and limited access of $^3$He gas, however, it is
important to note that hyperpolarized $^{129}$Xe MRI is a less expensive and more readily available approach.

In conclusion, our results suggest that noninvasive pulmonary functional $^3$He MRI provides a way to measure acute treatment effects by quantifying $^3$He gas distribution before and after therapy.
4.5 References


(26) de Lange EE, Altes TA, Patrie JT et al. The variability of regional airflow obstruction within the lungs of patients with asthma: assessment with


### 4.6 Supplementary Tables

**Table 4-S4-1** Subject listing of pre- and post-salbutamol pulmonary function and hyperpolarized $^3$He MRI measurements

| Subject | Pre-Salbutamol | | | | | | Post-Salbutamol | | | | | |
|---------|----------------|------------|----------------|------------|----------------|-----------------|-------|----------------|------------|----------------|------------|----------------|-------|
|         | FEV$_1$ (L)   | FVC (L)    | TLC (L)        | IC (L)     | FRC (L)       | RV (L)          | VDP (%) | C2 (%)         | C3 (%)     | C4 (%)         | C5 (%)     | FEV$_1$ (L)   | FVC (L)    | TLC (L)        | IC (L)     | FRC (L)       | RV (L)       | VDP (%) | C2 (%) | C3 (%) | C4 (%) | C5 (%) |
| 1       | 1.26          | 3.22       | 7.7            | 2.56       | 5.15          | 4.02            | 27     | 14             | 32         | 19             | 7          | 1.4            | 3.68        | 7.37            | 2.71       | 4.67          | 3.82         | 29     | 13     | 31     | 20     | 7     |
| 2       | 1.13          | 3.13       | 7.45           | 1.39       | 6.06          | 4.1             | 38     | 10             | 20         | 20             | 12         | 1.15           | 3.12        | 7.56            | 1.34       | 6.22          | 4.63         | 38     | 10     | 20     | 20     | 11    |
| 3       | 1.16          | 3.18       | 7.59           | 1.85       | 5.57          | 4.02            | 23     | 14             | 37         | 20             | 6          | 1.34           | 3.6         | 7.74            | 1.93       | 5.81          | 3.99         | 25     | 14     | 38     | 20     | 4     |
| 4       | 0.63          | 2.31       | 7.66           | 1.69       | 5.97          | 5.25            | 36     | 15             | 31         | 13             | 5          | 0.67           | 2.06        | 6.7             | 1.31       | 5.39          | 4.36         | 38     | 14     | 30     | 12     | 5     |
| 5       | 2.18          | 3.65       | 7.88           | 3.08       | 4.8           | 2.95            | 26     | 15             | 26         | 23             | 11         | 2.5            | 4.01        | -              | -          | -             | -           | 11     | 13     | 28     | 31     | 16    |
| 6       | 1.04          | 2.69       | 5.8            | 2.03       | 3.77          | 3.14            | 34     | 13             | 29         | 17             | 7          | 1.04           | 2.37        | 5.25            | 1.73       | 3.52          | 2.77         | 37     | 13     | 27     | 17     | 6     |
| 7       | 0.72          | 1.52       | 4.82           | 1.39       | 3.43          | 3.09            | 23     | 15             | 32         | 20             | 9          | 0.9            | 1.76        | 4.71            | 1.61       | 3.1           | 2.76         | 23     | 16     | 32     | 20     | 8     |
| 8       | 0.88          | 2.23       | 6.51           | 2.09       | 4.42          | 3.92            | 31     | 18             | 33         | 14             | 5          | 0.82           | 2.16        | 6.41            | 1.97       | 4.44          | 4.08         | 25     | 15     | 36     | 18     | 6     |
| 9       | 2.06          | 4.06       | 8.19           | 3.09       | 5.1           | 4.06            | 13     | 14             | 38         | 25             | 10         | 2.3            | 4.41        | 7.53            | 3.06       | 4.48          | 3.08         | 11     | 14     | 36     | 27     | 12    |
| 10      | 1.22          | 2.28       | 5.3            | 2.17       | 3.14          | 2.86            | 26     | 20             | 32         | 17             | 6          | 1.49           | 2.77        | 4.9             | 2.23       | 2.67          | 2.16         | 15     | 16     | 37     | 23     | 9     |
| 11      | 0.85          | 3.87       | 8.96           | 1.7        | 7.27          | 5.11            | 31     | 15             | 35         | 14             | 5          | 0.93           | 3.98        | 8.31            | 1.77       | 6.54          | 4.66         | 23     | 15     | 30     | 23     | 9     |
| 12      | 0.71          | 2.27       | 7.6            | 1.75       | 5.85          | 5.11            | 37     | 17             | 28         | 13             | 5          | 0.77           | 2.19        | 5.9             | 1.09       | 4.82          | 3.52         | 17     | 16     | 38     | 23     | 7     |
| 13      | 1.4           | 2.92       | 5.61           | 2.39       | 3.22          | 2.63            | 20     | 14             | 35         | 23             | 8          | 1.53           | 2.99        | 5.49            | 2.26       | 3.23          | 2.52         | 17     | 13     | 34     | 25     | 12    |
| 14      | 1.92          | 4.44       | 7.59           | 2.93       | 4.66          | 2.81            | 29     | 13             | 25         | 22             | 10         | 1.99           | 3.68        | 8.19            | 3.19       | 5.01          | 4.3          | 24     | 14     | 32     | 22     | 8     |

FEV$_1$= Forced Expiratory Volume in 1s, FVC= Forced Vital Capacity, TLC= Total Lung Capacity, IC= Inspiratory Capacity, FRC= Functional Residual Capacity, RV= Reserve Volume, VDP= Ventilation Defect Percent, C2= Cluster 2, C3= Cluster 3, C4= Cluster 4, C5= Cluster
Table 4-S4-2 Pre- and post-salbutamol $^3$He volume and signal intensity measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Salbutamol</th>
<th>Post-Salbutamol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (L)</td>
<td>SI ($\times 10^{12}$)</td>
</tr>
<tr>
<td>VDV (±SD)</td>
<td>1.76 (0.54)</td>
<td>0.48 (0.22)</td>
</tr>
<tr>
<td>Cluster 2 (±SD)</td>
<td>0.92 (0.16)</td>
<td>1.18 (0.20)</td>
</tr>
<tr>
<td>Cluster 3 (±SD)</td>
<td>1.93 (0.53)</td>
<td>2.63 (0.69)</td>
</tr>
<tr>
<td>Cluster 4 (±SD)</td>
<td>1.16 (0.34)</td>
<td>5.25 (0.89)</td>
</tr>
<tr>
<td>Cluster 5 (±SD)</td>
<td>0.48 (0.18)</td>
<td>8.73 (1.11)</td>
</tr>
</tbody>
</table>

SI=Signal Intensity, VDV=Ventilation Defect Volume
CHAPTER 5

In the same group of COPD ex-smokers evaluated using $^3$He MRI pre- and post-bronchodilator therapy in Chapter 4, here we further evaluated the regional effects of bronchodilator administration in COPD using $^3$He MRI ADC.

The contents of this Chapter have been previously published in the Journal of Applied Physiology and permission to reproduce the article was not required by The American Physiological Society.


5 Evaluating Bronchodilator Effects in Chronic Obstructive Pulmonary Disease using Diffusion-weighted Hyperpolarized Helium-3 Magnetic Resonance Imaging

5.1 Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive expiratory flow limitation that develops as a result of the lung’s inflammatory response to inhaled toxic gases and particles, primarily from tobacco smoke. Airflow limitation may be caused by a number of factors such as lumen occlusion via mucus plugs, structural alteration of the airway wall resulting in encroachment of the airway wall into the airway lumen and/or a loss of elastic recoil within the lung parenchyma due to emphysematous tissue destruction that leads to reduced tethering forces stabilizing the airways at forced expiration. The prolongation of the time constants for lung emptying results in numerous physiological and functional consequences including hyperinflation and gas trapping as well as ventilation abnormalities that lead to many of the disabling symptoms associated with COPD, such as dyspnea and limitation of exercise capacity.

Administration of short-acting beta-agonist bronchodilators is first-line therapy in the symptomatic management of COPD and this is thought to decrease airway smooth muscle tone thereby improving lung emptying during expiration. While improvements in airflow limitation and reductions in lung hyperinflation following bronchodilator administration have been previously shown, there is still little understanding about
the regional nature of bronchodilator response in COPD. In this regard, pulmonary magnetic resonance imaging (MRI) using hyperpolarized helium-3 ($^3$He) provides high spatial and temporal resolution *in vivo* images of $^3$He gas distribution within lung in COPD subjects. In COPD ex-smokers we previously observed significant improvements in the regional distribution of $^3$He gas after bronchodilator administration, however, it was unclear whether these regional improvements were related to physiological or symptomatic improvements. The measurement of the $^3$He apparent diffusion coefficient (ADC) has been demonstrated to be highly reproducible and is sensitive to changes in the lung microstructure and airspace size, correlating with spirometry, diffusing capacity of carbon monoxide (DL$_{CO}$), multi-slice CT measurements of emphysema, as well as lung surface area measurements. $^3$He MRI ADC measurements are also age-dependent, and reflect GOLD classification of disease severity, smoking history, and detects gas trapping within the dependent lung regions in COPD.

We hypothesized that the improvements in $^3$He gas distribution we previously observed after bronchodilation in COPD may be the direct source of symptomatic relief following therapy. To help test this hypothesis, here we aimed to evaluate hyperpolarized $^3$He MRI ADC as a surrogate of the extent of emphysematous tissue destruction within the newly ventilated lung regions (post-bronchodilator) and to evaluate regional gas trapping following bronchodilator administration. We evaluated lung microstructural measurements using regional $^3$He MRI ADC in COPD before and after bronchodilator administration, after developing image registration/segmentation methods for quantifying ADC in the lung regions newly ventilated post-bronchodilator.

### 5.2 Materials and Methods

#### 5.2.1 Subjects

All subjects were enrolled in a $^3$He MRI study of the acute effects of salbutamol administration in which written informed consent to the protocol approved by the local research ethics board and Health Canada was provided, and the study was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada)
and the Health Insurance Portability and Accountability Act (HIPAA, USA). COPD subjects were enrolled who were ex-smokers between the ages of 50-85, with a clinical diagnosis of COPD and were categorized according to the global initiative for chronic obstructive lung disease (GOLD) criteria\(^1\) with a smoking history of at least 10 pack-years. Subjects were required to withhold short-acting bronchodilators the morning of their study visit. Pre-bronchodilator MRI was performed immediately following pulmonary function tests, and post-bronchodilator MRI was performed 25 minutes (±2 minutes) after administration of 400 μg salbutamol inhaled via a spacer device. Digital pulse oximetry was used to measure arterial oxygen saturation (SaO\(_2\)) during scanning sessions and a hypoxic adverse event was defined as a decrease in SaO\(_2\) below 88% at any time during imaging. Study withdrawal was required when SaO\(_2\) decreased to 80% for 10 s or longer.

### 5.2.2 Pulmonary Function Tests

Spirometry was performed using an ndd EasyOne spirometer (ndd Medizintechnik AG, Zurich, CH) reporting forced expiratory volume in 1 second (FEV\(_1\)) and forced vital capacity (FVC) with a minimum of three acceptable spirometry maneuvers with the best FEV\(_1\) and FVC selected for analysis according to the American Thoracic Society (ATS) guidelines. Total lung capacity (TLC), inspiratory capacity (IC), functional residual capacity (FRC) and residual volume (RV) were measured using body plethysmography (MedGraphics Corporation, 350 Oak Grove Parkway St. Paul, MN USA).

### 5.2.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Excite 12.0 MRI system (GEHC, Milwaukee, WI USA) with broadband imaging capability as previously described.\(^3\) All helium imaging employed a whole body gradient set with maximum gradient amplitude of 1.94 G/cm and a single channel, rigid elliptical transmit/receive chest coil (RAPID Biomedical GmbH, Wuerzburg Germany). The basis frequency of the coil was 97.3 MHz and excitation power was 3.2 kW using an AMT 3T90 RF power amplifier (GEHC, Milwaukee WI USA). Subjects were positioned supine in the MR scanner and SaO\(_2\) was used to monitor arterial blood oxygenation levels during all MRI maneuvers. For both \(^1\)H
and $^3$He MRI, subjects were instructed by a pulmonary function technologist to inhale a gas mixture from a 1.0 L Tedlar bag (Jensen Inert Products, Coral Springs, FL) from FRC, and image acquisition was performed under breath-hold conditions—a period of 8 to 15s.

Coronal proton ($^1$H) MRI was performed prior to hyperpolarized $^3$He MRI with subjects scanned during breath-hold after inspiration of a 1.0 L $^4$He/$^2$N gas mixture using the whole body radiofrequency (RF) coil and $^1$H fast spoiled gradient recalled echo sequence (16s total data acquisition, repetition time (TR) / echo time (TE) / flip angle = 4.7 ms / 1.2 ms / 30°, field-of-view (FOV) = 40 x 40 cm, matrix 256 x 128, 14 slices, 15 mm slice thickness, 0 gap), as previously described.35

Prior to $^3$He MRI, a polarizer system (HeliSpin™, GEHC, Durham, NC) was used to polarize $^3$He gas to 30—40 %. As previously described,36 hyperpolarized $^3$He MRI diffusion-weighted images were acquired in breath-hold after inspiration of a 1.0 L $^3$He/$^2$N mixture (does 5 mL/kg body weight) using a fast gradient-echo method (FGRE) with centric k-space sampling. Two interleaved images were acquired (14s total data acquisition, TR/TE/flip angle = 7.6 ms/3.7 ms/8°, FOV = 40 x 40 cm, matrix 128 x 128, 7 slices, 30 mm slice thickness), with and without additional diffusion sensitization (G = 1.94 G/cm, rise and fall time = 0.5 ms, gradient duration = 0.46 ms, Δ = 1.46 ms, b = 1.6 s/cm$^2$).

5.2.4 Image Analysis

$^3$He MRI ADC analysis was performed using MATLAB R2007b (The Mathworks Inc., Natick, MA, USA). To ensure ADC was generated for voxels corresponding to ventilated lung regions, k-means cluster algorithm,36 previously developed for $^3$He MRI segmentation,22;37;38 was applied to the non-diffusion-weighted images to obtain a binary mask for each slice. The resulting binary masks were applied to the corresponding non-diffusion-weighted images, and the ADC maps were generated on a voxel-by-voxel basis according to Equation 5-1:
\[ ADC = \frac{1}{b} \ln \left( \frac{S_0}{S} \right) \]  

where \( S_0 \) is the segmented non-diffusion-weighted image, \( S \) is the diffusion-weighted image and \( b = 1.6 \text{ s/cm}^2 \).

An overview of the \(^3\text{He}\) MRI analysis methodology is provided in Figure 5-1. To minimize the potential for differences in the levels of inspiration between pre- and post-salbutamol imaging, extensive coaching was performed between the breath-hold scans for all subjects. Pre- and post-salbutamol ADC maps were registered using landmark-based image registration. Briefly, the center slice defined as the slice that clearly showed the carina and primary bronchi, was identified in the non-diffusion-weighted images for each imaging time-point and displayed side-by-side. Fiducial markers were identified on both the pre- and post-salbutamol \(^3\text{He}\) images based on the carina and a translation operation was used to transform the post-salbutamol image for registration with the corresponding landmark in the pre-salbutamol image. The resultant transformation was then applied to the \(^3\text{He}\) ADC maps for all slices. The trachea and visible major airways were removed semi-automatically using a region of interest tool (function \texttt{roipoly} in MATLAB) permitting ADC calculation within the lung parenchyma.
Whole lung (WL) ADC values were determined for each slice and then averaged to obtain a single ADC for each subject. Following registration of the pre- and post-salbutamol ADC maps, the lung regions of interest (ROI) with $^3$He signal at both time-points were identified as the intersection of the pre- and post-salbutamol maps; mean ADC within those ROI was generated for each slice and then averaged to obtain a previously ventilated ADC ($ADCP$) for both pre-salbutamol and post-salbutamol conditions. Lung ROI with $^3$He signal at both time-points were used as a binary ventilation mask on the post-salbutamol ADC maps to calculate mean ADC in newly ventilated ROI post-salbutamol ($ADCN$). To ensure that ADC estimates were generated for the newly ventilated lung regions post-salbutamol, and to minimize the contribution of patient movement or breath-hold mismatch between imaging time-points, we used a morphological closing algorithm on the post-salbutamol ADC maps (function `imclose` in

Figure 5-1  $^3$He MRI Image Analysis Methodology
Subject is 74 yrs old female with Stage II COPD (FEV$_1$ =47%$^{\text{pred}}$, FVC=92%$^{\text{pred}}$, FEV$_1$/FVC=39%, DLCO=20%$^{\text{pred}}$). $^3$He ADC maps pre- and post-salbutamol, binary ventilation mask of lung regions of interest (ROI) with $^3$He signal at both time-points following registration of pre- and post-salbutamol ADC maps, $^3$He ADC maps for the previously ventilated ($ADCP$) lung ROI pre- and post-salbutamol, and $^3$He ADC maps for newly ventilated ($ADCN$) lung ROI post-salbutamol with the CT density masks to outline areas with attenuation values less than -950 HU. CT images were acquired 19 months following pre- and post-salbutamol imaging.
MATLAB) with a disked-shaped structuring element with radius=1. The resultant binary masks were applied to the ADC maps of the newly ventilated regions post-salbutamol, and mean $\text{ADC}_N$ was determined.

### 5.2.5 Statistical Methods

All statistical analyses were performed using IBM SPSS Statistics 19.0 (IBM Inc., Chicago, IL). Multivariate analysis of variance (MANOVA) and repeated measures analysis of variance (ANOVA) were performed for comparison of pre- and post-salbutamol pulmonary function measurements. Statistical comparisons of mean pre- and post-salbutamol WL ADC and $\text{ADC}_P$ measurements were performed using a two-way mixed design repeated measures ANOVA with imaging time-point (pre- and post-salbutamol) treated as the within-subject factor and subject treated as the between-subjects factor. Comparison between $\text{ADC}_P$ and $\text{ADC}_N$ post-salbutamol were also performed using a two-way mixed design repeated measures ANOVA with region of interest (previously ventilated or newly ventilated post-salbutamol) treated as the within-subject factor and subject treated as the between-subjects factor. Anatomical differences in ADC within the regions of the lung ventilated at both time-points were quantified by determining the mean ADC in the most posterior slice and by calculating the absolute difference in ADC between the most anterior and the most posterior slice ($\Delta \text{AP}$). Comparisons between mean ADC in the most posterior slice and $\Delta \text{AP}$ pre- and post-salbutamol were determined using a two-tailed Wilcoxon signed rank t-test. Spearman correlation coefficients were used to determine the relationship between ADC and pulmonary function measurements. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

### 5.3 Results

All subjects completed both scanning sessions and there were no serious or severe breath-hold related or other adverse events reported, nor were there any other adverse events that required subjects to withdraw from the study. Table 5-1 shows study subject demographics and mean pulmonary function measurements ($n=3$ Stage II, $n=6$ Stage III, and $n=1$ Stage IV) before and after salbutamol administration. Statistically significant
post-salbutamol changes were observed for FEV$_1$ (p=.007), TLC (p=.02), FRC (p=.02) and RV (p=.02).

**Table 5-1 Subject Demographics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Salbutamol (±SD) [range] (n=10)</th>
<th>Post-Salbutamol (±SD) [range] (n=10)</th>
<th>Significance of Difference (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs</td>
<td>70 (6) [61-77]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male Sex</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI kg·m$^{-2}$</td>
<td>25 (4) [18-30]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FEV$_1$ L</td>
<td>1.11 (0.42) [0.63-2.06]</td>
<td>1.23 (0.49) [0.67-2.30]</td>
<td>0.007</td>
</tr>
<tr>
<td>FVC L</td>
<td>2.67 (0.71) [1.52-4.06]</td>
<td>2.80 (0.86) [1.76-4.41]</td>
<td>0.20</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>0.41 (0.09) [0.27-0.53]</td>
<td>0.45 (0.12) [0.32-0.70]</td>
<td>0.08</td>
</tr>
<tr>
<td>TLC L</td>
<td>6.68 (1.21) [4.82-8.19]</td>
<td>6.20 (1.12) [4.71-7.74]</td>
<td>0.02</td>
</tr>
<tr>
<td>IC L</td>
<td>2.10 (0.49) [1.39-3.09]</td>
<td>1.99 (0.60) [1.09-3.06]</td>
<td>0.23</td>
</tr>
<tr>
<td>FRC L</td>
<td>4.56 (1.11) [3.14-5.97]</td>
<td>4.21 (1.04) [2.67-5.81]</td>
<td>0.02</td>
</tr>
<tr>
<td>RV L</td>
<td>3.81 (0.89) [2.63-5.25]</td>
<td>3.31 (0.75) [2.16-4.36]</td>
<td>0.02</td>
</tr>
</tbody>
</table>

BMI=Body Mass Index, FEV$_1$=Forced Expiratory Volume in 1s, FVC=Forced Vital Capacity, TLC=Total Lung Capacity, IC=Inspiratory Capacity, FRC=Functional Residual Capacity, RV=Reserve Volume

*Repeated measures ANOVA

**Figure 5-2** shows representative $^3$He ADC maps pre- and post-salbutamol for a single subject and arrows identify visually obvious alterations in gas distribution. **Figure 5-3A** shows mean pre- and post-salbutamol $^3$He MRI ADC measurements for all subjects. There was no significant difference detected for WL ADC post-salbutamol (p=.516). As shown in **Table 5-2**, no significant correlations were detected between the change in WL ADC post-salbutamol and the change in pulmonary function measurements. However, there was a significant correlation between pre-salbutamol WL ADC and the change in IC (r=-.68, p=.03).
Table 5-2 Correlation between the post-salbutamol change in ADC and the change in pulmonary function measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spearman Correlation Coefficients (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WL ADC (cm$^2$/s)</td>
</tr>
<tr>
<td>TLC L</td>
<td>0.515 (0.133)</td>
</tr>
<tr>
<td>IC L</td>
<td>0.139 (0.707)</td>
</tr>
<tr>
<td>FRC L</td>
<td>0.636 (0.054)</td>
</tr>
<tr>
<td>RV L</td>
<td>0.479 (0.166)</td>
</tr>
</tbody>
</table>

Figure 5-4 shows for two representative COPD subjects the WL pre- and post-salbutamol ADC maps and the newly ventilated ADC$_N$ maps post-salbutamol. There was no statistically significant difference between ADC$_N$ and ADC$_P$ post-salbutamol ($ADC_P=.506\pm.072$cm$^2$/s, $ADC_N=.504\pm.082$ cm$^2$/s, $p=1.00$), nor were there any significant interactions between the ADC$_N$ and ADC$_P$ post-salbutamol ($p=.18$), indicating no significant change in ADC within the newly ventilated lung areas following administration of salbutamol.
For ADC$_p$ measurements, there was a statistically significant decrease detected post-salbutamol (p=.013) (Figure 3A). As shown in Figure 5-3B, the mean ADC in the most posterior slice was significantly lower post-salbutamol (p=.049). Additionally, ΔAP was significantly higher post-salbutamol (p=.020), indicating that there was a difference in the mean ADC in the AP direction following bronchodilator therapy (Figure 3C). As shown in Table 5-2 and Figure 5-5, Spearman correlation coefficients indicate that the post-salbutamol change in ADC$_{p}$ was significantly correlated with the change in TLC ($r=.782$, $p=.011$), FRC ($r=.806$, $p=.007$) and RV ($r=.733$, $p=.020$).
Figure 5-4 Regional $^3$He MRI ADC
WL center slice $^3$He ADC maps pre-and post-salbutamol, $^3$He ADC maps for newly ventilated ($\text{ADC}_N$) lung ROI post-salbutamol, and CT density masks to outline areas with attenuation values less than -950 HU, for a 74 yrs old female and a 64 year old male both with Stage III COPD. CT images were acquired 15 months following pre-and post-salbutamol imaging for the 74 yr old subject and 28 months following pre-and post-salbutamol imaging for the 64 yr old subject.
We previously evaluated COPD ex-smokers using hyperpolarized $^3$He MRI before and after bronchodilator administration and provided evidence of significant and visually obvious regional improvements in $^3$He gas distribution following bronchodilator therapy.$^{22}$ Here, we extend these previous findings and explore the relationship of regional hyperpolarized $^3$He ADC measurements in the pre- and post-bronchodilator lung ROI. We aimed to determine if the newly ventilated regions were different than lung ROI participating in ventilation before bronchodilation and based on previous histological findings,$^{31}$ we used the $^3$He MRI ADC as a surrogate measurement of tissue microstructure. We made a number of observations and report the following: 1) mean WL ADC did not change post-salbutamol, 2) mean ADC in newly ventilated lung regions post-salbutamol was not different than mean ADC in previously ventilated lung regions, 3) mean ADC in previously ventilated lung regions was significantly different (improved) post-salbutamol, and, 4) ADC anterior-posterior gradients significantly improved post-salbutamol.

First, we observed no significant change in WL ADC measurements following salbutamol administration, and this finding suggests that the alveolar structures that are

**Figure 5-5** Correlations between the post-salbutamol change in $^3$He ADC$_p$ and pulmonary function measurements. $\Delta$ADC$_p$ was significantly correlated with $\Delta$TLC ($r=.782$, $p=.011$, $r^2=.387$, $p=.055$, $y=26.99x-0.30$) (A), $\Delta$FRC ($r=.806$, $p=.007$, $r^2=.604$, $p=.008$, $y=23.13x-0.19$) (B) and $\Delta$RV ($r=.733$, $p=.020$, $r^2=.379$, $p=.039$, $y=28.91x-0.31$) (C). Dotted lines represent the 95% confidence intervals for the regressions.
probed by the $^3$He gas post-salbutamol are not more emphysematous than the other lung ROI probed by the $^3$He gas pre-salbutamol. In other words, this finding indicates that the bronchodilator was delivered primarily to the more normal lung regions. However, the $^3$He ADC has been previously demonstrated to be sensitive to gas trapping in the dependent lung regions$^{33}$ that may lead to a reduction in the ADC measurement post-bronchodilator. Because we observed no change in WL ADC post-salbutamol we developed methods to determine regional ADC as a way to tease apart the potential combinations of reduced hyperinflation and gas redistribution to lung ROI with greater emphysematous destruction.

We applied these image processing methods and post-salbutamol observed no significant difference between mean ADC in the newly ventilated lung ROI ($ADCN$) and mean ADC in the previously ventilated ROI ($ADCP$), indicating that the lung ROI that participated in gas distribution following administration of salbutamol were not more emphysematous than lung ROI participating in gas distribution before salbutamol administration. These findings must be interpreted with an understanding of the limitations of the relatively large voxel size (3.2mm x 3.2mm x 30mm) in this analysis and these images that reflect the contributions of approximately 70,000 alveoli, estimated based on the mean size of a single normal alveolus to be $4.2\times10^{-6}$ cm$^3$ as previously described.$^{39}$ Additionally, the contribution of subject motion between imaging time-points and the volume of gas within the lungs during the breath-hold imaging may have contributed to measurement uncertainty. Nevertheless, we have previously evaluated the ADC measurement within one week with no treatment (scan, 7-day rescan)$^{19}$ and determined that the estimated standard uncertainty (standard deviation) of both the WL ADC and the center slice ROI ADC measurement in Stage II and Stage III COPD subjects was 0.001cm$^2$/s, indicating that the ADC measurement in subjects with moderate to severe disease was remarkably reproducible. A previous study evaluating asthma subjects using $^3$He MRI and multi-detector CT demonstrated that there was a regional association between $^3$He MRI ventilation abnormalities and CT measurements of gas trapping.$^{40}$ Although it is plausible in the current study that emphysematous tissue destruction may have reduced the tethering or stabilization of the airways at forced expiration$^5$ resulting in trapped gas
and ventilation defects in severe COPD subjects, our findings suggest that defect regions that become ventilated in response to bronchodilators do not reflect more severe emphysema. Moreover, these results indicate that the bronchodilator acted on the obstructed small airways directly, allowing improvements in gas trapping and regional hyperinflation, and not on airways obstructed due to surrounding emphysema. As shown in Figure 5-3, both emphysematous and healthy regions of the lung participated in gas distribution post-salbutamol. We must acknowledge that the very small number of newly ventilated voxels in the post-salbutamol images may lead to biased ADC values due to a low signal-to-noise ratio (SNR), although this would have generated a bias towards higher ADC and this was not observed in the current study. To minimize the potential for bias, we used a morphological closing algorithm to remove the contribution of small isolated voxels to the mean ADC value and an SNR threshold greater than 15 for ADC generation was used as previously described.

We also observed a statistically significant reduction (improvement) in mean ADC\textsubscript{p} post-salbutamol. This small but significant improvement may be explained by both a reduction in the physical size of the airspaces due to the increased distribution of the \textsuperscript{3}He gas (thereby effectively diluting the 1L dose) and bronchodilator-induced reduction in lung hyperinflation. To determine whether the significant change in ADC\textsubscript{p} was related to a reduction in lung hyperinflation, we compared the anatomical distribution of ADC values pre- and post-salbutamol. As previously described, airspaces are less compressible in COPD compared to healthy subjects in the dependent lung likely because of gas trapping. We detected a statistically significant increase in ΔAP and decreased mean ADC\textsubscript{p} in the most posterior slice post-salbutamol, suggesting that bronchodilator administration improved the expiratory time constants which in turn allowed the dependent lung regions to empty more completely. Moreover, as demonstrated in our previous study, there was no bias for any measurable changes in the distribution of \textsuperscript{3}He gas post-salbutamol with respect to image slice, indicating that the reduction in mean ADC\textsubscript{p} within the dependent lung regions was not due to increased gas distribution in these regions. The significant correlation detected between the change in ADC\textsubscript{p} and changes in TLC, FRC and RV post-salbutamol lends support to the hypothesis that the reduction in ADC\textsubscript{p} post-salbutamol was related to a reduction in gas trapping.
We recognize that this work was limited by the small number of subjects and the fact that the analysis was restricted mainly to subjects with moderate to severe COPD. Therefore, caution should be exercised in extrapolating these results to the general COPD population and more specifically to patients with mild and very severe disease. An important limitation of $^3$He MRI is that large regions of the lung are not ventilated or are poorly ventilated preventing calculation of the ADC within these regions, and this may be due to small airway occlusion, mucous plugs, airway wall thickening and inflammation, severe emphysema or bullous disease. We also acknowledge that without the inclusion of dyspnea scores and measurements of exercise tolerance for comparison to $^3$He MRI, the clinical meaning of the imaging changes remains unclear. In other words, the changes in ADC measures post-salbutamol are related to clinical or symptomatic improvement remains to be established in a larger study. The lack of validation by an imaging reference method, such as computed tomography (CT) or proton MRI with ultra-short echo times, is also a limitation of this work. Clearly, the direct comparison of functional and structural $^3$He MRI, proton UTE MRI, CT and symptomatic measurements will allow for a better understanding of the improvements in $^3$He gas distribution observed after bronchodilator administration. It must also be noted that $^3$He MRI ADC provides structural information not only at the scale of the alveoli, probing emphysematous tissue destruction, but also at the scale of the alveolar ducts and the respiratory and terminal bronchioles, thus restriction to diffusion will differ in each of these structures and alveoli diffusion will be more restricted than diffusion in the bronchioles. An important point related to this is the issue of collateral ventilation. In patients with emphysema there is substantial collateral ventilatory pathways, including the pores of Kohn, canals of Lambert and the interbronchial communications of Martin. Although with the very short diffusion times utilized in this study where the atoms are likely to be confined to the alveoli and acinar structures, measurements of $^3$He diffusion over longer times and distances are likely sensitive to collateral ventilation paths, which are significant in patients with emphysema. A better understanding of collateral ventilation and collateral pathways is required and noninvasive imaging measurements such as $^3$He MRI ADC may be useful for treatment planning, such as bronchoscopic airway bypass, and evaluating treatment effects. Reproducible and comparable breath-hold volumes are critically
important for acute and chronic therapy repeated studies and therefore training and instruction for inspiration breath-hold scanning was performed during breath-hold MRI. Although $^3$He MRI has limited access and high cost which has thus far restricted translation of this functional imaging method, $^{129}\text{Xe}$ MRI is a less expensive and more readily available approach with which these reported findings can be directly tested.

In summary, this study demonstrated that $^3$He MRI provides a noninvasive approach for the evaluation of regional structure-function measurements after bronchodilator administration in COPD subjects. Lung micro-structure in ROI that participate in gas distribution only after bronchodilator administration was not more emphysematous than ROI participating in gas distribution before bronchodilator use. We also demonstrated that regional ADC reflect significant changes in the anterior-posterior gradient following bronchodilator therapy and this is possibly related to a reduction in regional gas trapping. The regional evaluation of hyperpolarized $^3$He MRI ADC provides insights into regional lung microstructure for lung ROI that participate in $^3$He gas distribution after bronchodilator administration.
5.5 References


(38) Heydarian M, Kirby M, Wheatley A et al. Two and three-dimensional segmentation of hyperpolarized 3He magnetic resonance functional imaging. 2011.


CHAPTER 6

The limited and unpredictable global supply of $^3$He necessitates the transition to $^{129}$Xe gas, however a direct comparison of hyperpolarized $^3$He and $^{129}$Xe MRI acquired in the same subjects is required in order for us to better understand the potential of $^{129}$Xe MRI in respiratory research. Here we quantitatively compared $^3$He and $^{129}$Xe MRI acquired in the same healthy volunteers and subjects with COPD.

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6 Hyperpolarized $^3$He and $^{129}$Xe MR Imaging in Healthy Volunteers and Patients with Chronic Obstructive Pulmonary Disease

6.1 Introduction

Magnetic resonance imaging (MRI) using hyperpolarized noble gases, such as helium-3 ($^3$He) and xenon-129 ($^{129}$Xe), provides a way to acquire high spatial and temporal resolution pulmonary images. Hyperpolarized $^3$He MRI has dominated for the evaluation of gas distribution and tissue abnormalities in healthy volunteers and in patients with chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, radiation-induced lung injury, and lung transplant. Numerous studies have shown that $^3$He MRI in COPD is highly reproducible, sensitive to early lung micro-structural changes, and significantly correlates with established measurements of pulmonary function, multi-slice computed tomography (CT) measurements and histology measurements of emphysema. Furthermore, longitudinal $^3$He MRI of COPD has highlighted the sensitivity of the method to progressive worsening and shown regional improvements post-bronchodilator.

Unfortunately, despite the unique potential of $^3$He MRI, clinical translation has not occurred in part because of limited and unpredictable global quantities and high cost. $^{129}$Xe gas, on the other hand, is substantially more abundant in nature existing in
measurable quantities in the atmosphere and it is relatively inexpensive. Although hyperpolarized $^{129}$Xe MRI is technically challenging because of its nearly 3-fold lower gyromagnetic ratio and lower enrichment, considerable improvements in $^{129}$Xe gas polarization and imaging methods$^{37}$ have been achieved since the first clinical studies were reported.$^{38-40}$ Recently, the tolerability of a 1.0L inhaled dose of $^{129}$Xe and diffusion-weighted MRI measurements were reported in COPD and healthy subjects.$^{39,40}$ These important results suggested that $^{129}$Xe MRI may be very useful for examining structural and functional abnormalities in COPD and also generated numerous hypotheses to test. In this investigation, we hypothesized that the different properties of $^{129}$Xe gas would result in significant differences in $^{129}$Xe compared to $^3$He ventilation defect percent (VDP) in COPD but not in healthy volunteers. Accordingly, here our objective was to quantitatively compare hyperpolarized $^3$He and $^{129}$Xe MRI acquired within a few minutes in healthy volunteers and subjects with COPD, and to evaluate the correlations between $^3$He and $^{129}$Xe MRI measurements with spirometry and plethysmography.

### 6.2 Materials and Methods

#### 6.2.1 Subjects

All subjects provided written informed consent to the study protocol approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada) and the Health Insurance Portability and Accountability Act (HIPAA, USA). COPD subjects were ex-smokers 50-85 years of age, with a smoking history of at least 10 pack-years. Pack-year was defined as the number of cigarette packs smoked per day multiplied by the number of years smoked. Healthy volunteers were enrolled who had no history of previous chronic or current respiratory disease. For all subjects, mean age was 71±8 yrs, and mean age for men was 71±9 yrs and for women 72±6 yrs.

#### 6.2.2 Pulmonary Function Tests

Spirometry was performed using an *ndd EasyOne* spirometer (ndd Medizintechnik AG, Zurich, CH) according to the American Thoracic Society (ATS) guidelines.$^{41}$ Static lung volumes and diffusing capacity of carbon monoxide (DL$_{CO}$) were measured using body

6.2.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Discovery 750MR (General Electric Health Care, Milwaukee, WI) MRI system with broadband imaging capability as previously described.6 Subjects were instructed to inhale a gas mixture from a 1.0 L Tedlar® bag (Jensen Inert Products, Coral Springs, New Jersey, USA) from functional residual capacity (FRC), and image acquisition was performed in 8-15s under breath-hold conditions. It is important to note that we endeavored to minimize the potential for differences in the levels of inspiration between the breath-hold scans for each subject by: 1) training and practice for all subjects prior to MRI related to the inspiration breath-hold maneuver from FRC, and, 2) continuous coaching and monitoring at the MR bedside by a pulmonary function technologist during all inspiration breath-hold scans.

Conventional ¹H MRI was performed prior to hyperpolarized ¹²⁹Xe and ³He MRI with subjects scanned during 1.0L breath-hold of ultrahigh purity, medical grade nitrogen (N₂) (Spectra Gases, Alpha, NJ) using the whole body radiofrequency (RF) coil and ¹H fast spoiled gradient-recalled-echo sequence as previously described.6 Theoretical diffusion coefficients for the ³He/N₂ and ¹²⁹Xe/⁴He 50/50 mixtures were generated adopting assumptions previously described.42

Hyperpolarized ³He MRI was enabled using a linear bird-cage transmit/receive chest coil (RAPID Biomedical GmbH, Wuerzburg Germany). A turnkey system (HeliSpin™) was used to polarize ³He gas to 30—40% and doses (5mL/kg body weight) were administered in 1.0L Tedlar® bags diluted with N₂. Hyperpolarized ³He MRI coronal static ventilation images and diffusion-weighted (DW) images were acquired during breath-hold of a 1.0L ³He/N₂ mixture as previously described.6

Hyperpolarized ¹²⁹Xe MRI was enabled using a custom-made, unshielded quadrature-asymmetric bird-cage coil model tuned to 35.34MHz similar to previous approaches43 and as previously described.44 ¹²⁹Xe gas (86% enriched) was polarized to 10—60% using
a turn-key polarizer (XeBox-E10, Xemed LLC, New Hampshire, USA). Doses of hyperpolarized $^{129}$Xe gas were dispensed directly into the pre-rinsed 1.0L Tedlar® bags pre-filled with $^4$He to generate a 50/50 mixture. Polarization of the diluted dose was quantified by a Polarimeter (GEHC, Durham, NC). $^{129}$Xe MRI coronal static ventilation images were acquired using a 3D fast gradient-recalled-echo (FGRE) sequence with centric phase-encoding ordering in the y direction and normal sampling in the z direction during breath-hold of the 1.0 L $^{129}$Xe/$^4$He mixture (14s data acquisition, TE/TR/flip angle = 1.50 ms/6.7 ms/variable flip angle, bandwidth = 15.63 kHz, FOV = 40 × 40 cm, matrix 128 × 128, 14 slices, 15 mm slice thickness, 0 gap). DW images were obtained using a 2D FGRE sequence with centric phase-encoding ordering. Two interleaved images (16s total data acquisition, TE/TR/flip angle = 10 ms/13.5 ms/9°, bandwidth = 31.25 kHz, FOV = 40 x 40 cm, matrix 128 x 80, 7 slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitization with $b = 12$ s/cm$^2$ (maximum gradient amplitude (G) = 2.90 G/cm, gradient rise and fall time = 0.5 ms, gradient separation = 2 ms, gradient duration = 2.0 ms, diffusion time = 5 ms). The diffusion time of 5ms for $^{129}$Xe MRI was selected based on the theoretical background for optimal gradient sequence parameters$^{45}$ and previous findings demonstrating its sensitivity to alveolar enlargement.$^{46}$ All scanning was completed within approximately 5 minutes of subjects first lying in the scanner. Also, based on the calculations for the theoretical diffusion coefficients for $^3$He and $^{129}$Xe and the diffusion times used, the characteristic diffusion length for $^3$He (~490μm) is comparable to $^{129}$Xe (~460μm), indicating similar spatial length scales are being investigated.

CT was performed on a 64-slice Lightspeed VCT scanner (GE Healthcare, Milwaukee, WI USA) using a detector configuration of 64×0.625 mm, 120 kVp, 100 effective mA, tube rotation time of 500 ms and a pitch of 1.0. A single spiral acquisition of the entire lung was acquired from the apex to the base with subjects in the supine position and in breath-hold after inhalation of a 1.0L Tedlar® bag of N$_2$ from FRC. Reconstruction of the data was performed using a slice thickness of 1.25 mm with a standard convolution kernel.
6.2.4 Image Analysis

$^{3}$He and $^{129}$Xe MRI semi-automated segmentation was performed, similar to approaches that have been previously described for quantification of lung volumes, using custom software generated using MATLAB R2007b (The Mathworks Inc., Natick, MA, USA), as previously described. $^{3}$He MRI of COPD is typically characterized by heterogeneous signal intensity that reflects gas distribution heterogeneity during the inspiration breath-hold scan. To compare the distribution of both $^{3}$He and $^{129}$Xe gases within the lung, we segmented the $^{3}$He and $^{129}$Xe images based on the pixel signal intensity. Briefly, $^{3}$He and $^{129}$Xe MRI static ventilation images were segmented using a K-means approach that classified voxel intensity values into five clusters ranging from signal void (cluster 1, C1 or ventilation defect volume (VDV)) and hypo-intense (cluster 2 or partial volume) to hyper-intense signal (cluster 5), and therefore generating a gas distribution cluster-map. For delineation of the ventilation defect boundaries, a seeded region-growing algorithm was used to segment the $^{1}$H MR images of the thoracic cavity for registration to the cluster-map and $^{3}$He and $^{129}$Xe VDP were generated by VDV normalized to the thoracic cavity volume. $^{3}$He and $^{129}$Xe non-diffusion-weighted (NDW) images were also segmented using the same approach where NDW images were segmented using K-means and registered to the corresponding $^{1}$H MRI slices for calculation of VDP. Apparent diffusion coefficient (ADC) analysis was performed as previously described. The signal-to-noise ratio (SNR) for all $^{3}$He and $^{129}$Xe static ventilation and NDW and DW images were determined by calculating the mean voxel value within a 5 x 5 cm$^2$ voxel region of interest (ROI) for four representative ROI within the lung parenchyma, and dividing by the standard deviation of the voxel values for noise inside for four representative ROI of the same size within the image background where there was no lung structure. The ROI within the lung parenchyma and the image background were selected independently for each slice, with exception of diffusion-weighted imaging where the ROI was selected independently for each NDW image slice, and the coordinates of the lung ROI were applied to the diffusion-weighted image slice. SNR was determined for each slice and then averaged to obtain a single SNR value for each subject. CT measurements were performed using MATLAB R2007b; the relative area...
with attenuation values below -950 HU (RA950) and the 15th percentile (HU15%) were generated from the frequency distribution of Hounsfield units.

### 6.2.5 Statistical Methods

Multivariate analysis of variance (ANOVA) and a one-way ANOVA were performed using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). A paired two-tailed t-test was used for statistical comparison for normally distributed data and a two-tailed Wilcoxon signed rank test was used for statistical comparison for non-normally distributed data for tests between 3He and 129Xe VDP, ADC, and SNR using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego, CA, USA). Normality was determined using a Shapiro-Wilk test using IBM SPSS Statistics 20.0. A two-way mixed-effects repeated measures ANOVA was used to determine the interactions for VDP measured from both 3He and 129Xe MRI and imaging slice using SPSS 20.0. The agreement between 3He and 129Xe VDP was evaluated using Bland-Altman plots generated using GraphPad Prism version 4.00. Linear regression ($r^2$) and Pearson correlation coefficients ($r$) were used to determine the relationships between and imaging and other measurements using GraphPad. Correlation coefficients were compared by calculating the Fisher’s $z’$ transformation for each $r$ as shown in Equation 6-1:

$$z’ = \frac{1}{2} (\log(1 + r) - \log(1 - r))$$

(1)

where $r$ is the correlation coefficient for 3He MRI ($r_{3He}$) and 129Xe MRI ($r_{129Xe}$). The Z value was then calculated using Equation 6-2:

$$Z = \frac{z_{3He} - z_{129Xe}}{\sqrt{\frac{1}{n - 3}}}$$

(2)

where $z_{3He}$ is the $z’$ of $r_{3He}$, $z_{129Xe}$ is the $z’$ of $r_{129Xe}$ and $n$ equals the number of subjects compared. A Holm-Bonferroni correction was used for multiple paired t-tests and all correlations. The Holm-Bonferroni adjusted p-values were determined by ordering p-values from smallest to largest, with the smallest p-value multiplied by k, where k is the number of hypotheses to be tested. If the resulting modified p-value was less than $\alpha$
(Type I error rate) the hypothesis was rejected. The next smallest p-value was then multiplied by k minus 1 and the new modified p-value was compared to α. This process was repeated until the modified p-value could not be rejected. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% (p < 0.05).

6.3 Results

All imaging procedures and maneuvers were well-tolerated and there were no serious or severe adverse events reported. There was a single adverse event reported by a single COPD subject (headache seven hours after completion of MRI that resolved without treatment) and this was judged not-related to \( ^3 \)He or \( ^{129} \)Xe gas inhalation; the details of the safety and tolerability of \( ^{129} \)Xe MRI for this study are reported elsewhere\(^{54} \) and tolerability of a 1.0L \( ^{129} \)Xe dose (versus the 50/50 mixture used here) was previously reported.\(^{40} \) Table 6-1 shows subject demographics and pulmonary function measurements for eight healthy volunteers and 10 COPD subjects (GOLD Class I n=1; GOLD Class II n=6; GOLD Class III n=2; GOLD Class IV n=1).\(^{55} \) The two subject groups were significantly different with respect to FEV\(_1\), FEV\(_1\)/FVC, RV, RV/TLC, IC, FRC and DL\(_{CO}\); there were no significant differences between the groups with respect to age, sex or BMI.
Table 6-1 Subject Demographics

<table>
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<tr>
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<th>Healthy Volunteers (n=8)</th>
<th>COPD (n=10)</th>
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<td>Age yrs (±SD)</td>
<td>67 (10)</td>
<td>74 (4)</td>
<td>0.10</td>
</tr>
<tr>
<td>Men</td>
<td>63 (11)</td>
<td>75 (4)</td>
<td>-</td>
</tr>
<tr>
<td>Women</td>
<td>71 (7)</td>
<td>73 (6)</td>
<td>-</td>
</tr>
<tr>
<td>Male Sex n (±SD)</td>
<td>4</td>
<td>8</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI kg·m⁻² (±SD)</td>
<td>25.9 (2.2)</td>
<td>25.4 (5.0)</td>
<td>0.79</td>
</tr>
<tr>
<td>Pack-years yrs (±SD)</td>
<td>0 (0)</td>
<td>62 (15)</td>
<td>-</td>
</tr>
<tr>
<td>FEV₁ % pred (±SD)</td>
<td>107 (13)</td>
<td>57 (24)</td>
<td>&lt;0.001</td>
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<tr>
<td>FVC % pred (±SD)</td>
<td>106 (13)</td>
<td>91 (19)</td>
<td>0.08</td>
</tr>
<tr>
<td>FEV₁/FVC (±SD)</td>
<td>0.75 (.04)</td>
<td>0.46 (.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TLC % pred (±SD)</td>
<td>106 (11)</td>
<td>115 (8)</td>
<td>0.09</td>
</tr>
<tr>
<td>RV % pred (±SD)</td>
<td>105 (19)</td>
<td>159 (46)</td>
<td>0.007</td>
</tr>
<tr>
<td>RV/TLC (±SD)</td>
<td>0.39 (0.10)</td>
<td>0.53 (0.14)</td>
<td>0.03</td>
</tr>
<tr>
<td>IC % pred (±SD)</td>
<td>120 (25)</td>
<td>85 (31)</td>
<td>0.02</td>
</tr>
<tr>
<td>FRC % pred (±SD)</td>
<td>95 (13)</td>
<td>141 (35)</td>
<td>0.002</td>
</tr>
<tr>
<td>DL_CO % pred (±SD)</td>
<td>103 (13)</td>
<td>41 (17)†</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

SD=Standard Deviation, BMI=Body Mass Index, FEV₁= Forced Expiratory Volume in 1s, %pred=Percent Predicted, FVC= Forced Vital Capacity, TLC= Total Lung Capacity, RV= Reserve Volume, IC= Inspiratory Capacity, FRC= Functional Residual Capacity, DL_CO=Carbon Monoxide Diffusion Capacity of the lung. †n=7.

Significance of difference (p<.05) determined using a multivariate analysis of variance. Fisher’s exact test was performed for categorical variables.

**Figure 6-1** shows the two central coronal $^3$He and $^{129}$Xe MRI slices, where the trachea and two main bronchi are clearly visible, with $^3$He gas distribution displayed in red and $^{129}$Xe gas distribution displayed in purple registered to the grey-scale $^1$H MRI of the thorax for each of three representative healthy volunteers and three COPD subjects. For the COPD subjects, regions of signal void are observed in $^{129}$Xe MRI not qualitatively apparent in $^3$He MRI; these regions are readily observed in the right mid apex for COPD S1 and the right and left apical regions for COPD S2 and S3 for the two slices shown in Figure 6-1.
Figure 6-1. $^3$He and $^{129}$Xe MRI static ventilation images of healthy volunteers and subjects with COPD. $^3$He and $^{129}$Xe MRI of the two coronal centre slices, where the trachea and two main bronchi are clearly visible, with $^3$He gas distribution displayed in red and $^{129}$Xe gas distribution displayed in purple registered to the greyscale $^1$H MRI of the thorax for each of three representative healthy volunteers (S1: 75 yr old female, FEV$_1$=93%$_{\text{pred}}$, FEV$_1$/FVC=70%; S2: 57 yr old male, FEV$_1$=95%$_{\text{pred}}$, FEV$_1$/FVC=72%; S3: 51 yr old male, FEV$_1$=120%$_{\text{pred}}$, FEV$_1$/FVC=83%) and three COPD subjects (S1: 77 yr old female, FEV$_1$=50%$_{\text{pred}}$, FEV$_1$/FVC=20%; S2: 68 yr old female, FEV$_1$=59%$_{\text{pred}}$, FEV$_1$/FVC=53%; S3: 71 yr old male, FEV$_1$=107%$_{\text{pred}}$, FEV$_1$/FVC=58%).
Figure 6-2 shows the strong and statistically significant correlations between whole lung (WL) $^3$He and $^{129}$Xe VDP ($r = .91$, $p < .0001$) and $^3$He and $^{129}$Xe ADC ($r = .97$, $p < .0001$). Although $^3$He and $^{129}$Xe VDP were significantly correlated, Bland-Altman analysis indicates that there was a 9±8% bias (95% limit of agreement: -25%–7%) for higher VDP for $^{129}$Xe MRI. Table 6-2 shows mean $^3$He and $^{129}$Xe MRI gas distribution and ADC measurements. $^{129}$Xe VDP was statistically significantly greater than $^3$He VDP for the COPD subjects ($p = .0003$, uncorrected; $p = .03$, Holm-Bonferroni corrected), and $^{129}$Xe signal intensity cluster C3 ($p = .002$, uncorrected; $p = .002$ Holm-Bonferroni corrected) was significantly different. For healthy volunteers, there was no significant difference between $^{129}$Xe and $^3$He VDP ($p = .56$), however there was a significant difference in C4 ($p = .008$, uncorrected) although following Holm-Bonferroni correction this difference was no longer significant ($p = .06$). For both groups, there was no relationship between the difference in $^3$He and $^{129}$Xe VDP and image slice ($p = .99$), indicating no bias for differences between $^3$He and $^{129}$Xe VDP for any specific image slice. To better understand the potential impact of any difference in pulse sequence parameters for $^{129}$Xe (3D acquisition) and $^3$He (2D acquisition) MRI gas distribution measurements, we compared $^3$He and $^{129}$Xe VDP measured from NDW images, both acquired using 2D FGRE sequences. No significant difference was observed between $^{129}$Xe VDP (3D) and $^{129}$Xe VDP measured from NDW images for the healthy volunteers and the COPD subjects ($p = .87$) and $^3$He VDP (2D) and $^{129}$Xe VDP measured from NDW images were not significantly different for healthy volunteers ($p = .50$) however they were significantly different for COPD subjects ($p = .002$). This result is in agreement with the 2D $^3$He and 3D $^{129}$Xe MRI results that showed there was no difference between $^3$He and $^{129}$Xe VDP for healthy volunteers, but for COPD subjects $^{129}$Xe VDP was significantly greater than $^3$He VDP. Mean SNR was significantly lower for $^{129}$Xe MRI (33±17) as compared to $^3$He MRI (56±25, $p < .0001$), however importantly there was no significant correlation for the difference between $^3$He and $^{129}$Xe SNR and the difference between $^3$He and $^{129}$Xe VDP ($r = .26$, $p = .30$).
Table 6-2 \(^3\)He and \(^{129}\)Xe MRI Measurements

<table>
<thead>
<tr>
<th></th>
<th>Healthy Volunteers (n=8)</th>
<th>COPD (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(^3)He MRI</td>
<td>(^{129})Xe MRI</td>
</tr>
<tr>
<td><strong>Gas Distribution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDP % (±SD)</td>
<td>4 (2)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>C2 % (±SD)</td>
<td>10 (1)</td>
<td>11 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3 % (±SD)</td>
<td>36 (5)</td>
<td>26 (10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4 % (±SD)</td>
<td>34 (2)</td>
<td>40 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5 % (±SD)</td>
<td>16 (5)</td>
<td>19 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC cm(^2)/s</td>
<td>0.246 (0.021)</td>
<td>0.053 (0.002)</td>
</tr>
<tr>
<td>(±SD)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VDP=Ventilation Defect Percent, 2=Cluster 2, C3=Cluster 3, C4=Cluster 4, C5=Cluster 5, ADC=Apparent Diffusion Coefficient
SDif*=Significance of difference (p<.05) determined using a two-tailed Wilcoxon signed rank test for gas distribution measurements and a two-tailed paired t-test for ADC measurements.

* Holm-Bonferroni adjusted significance values in parentheses
† n = 3
As shown in Table 6-2, WL hyperpolarized $^3$He and $^{129}$Xe ADC was significantly different for the healthy volunteers ($p=.002$) and the COPD subjects ($p<.0001$). Mean SNR for $^3$He MRI DW and NDW was 36±16 and 64±29, respectively, and for $^{129}$Xe MRI DW and NDW were 11±6, and 28±19, respectively.

Table 6-3 Relationships between $^3$He and $^{129}$Xe MRI with pulmonary function measurements

<table>
<thead>
<tr>
<th></th>
<th>$^3$He VDP %</th>
<th>$^{129}$Xe VDP %</th>
<th>$^3$He ADC cm$^2$/s</th>
<th>$^{129}$Xe ADC cm$^2$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$<em>1$%$</em>{pred}$</td>
<td>-.84</td>
<td>-.89</td>
<td>-.75</td>
<td>-.67</td>
</tr>
<tr>
<td></td>
<td>(&lt;.0001/-.0001)</td>
<td>(&lt;.0001/-.001)</td>
<td>(.0007/-.002)</td>
<td>(.01/-.01)</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>-.84</td>
<td>-.95</td>
<td>-.86</td>
<td>-.77</td>
</tr>
<tr>
<td></td>
<td>(&lt;.0001/-.001)*</td>
<td>(&lt;.0001/-.001)</td>
<td>(&lt;.0001/-.001)</td>
<td>(.002/-.004)</td>
</tr>
<tr>
<td>DL$<em>{CO}$%$</em>{pred}$</td>
<td>-.83</td>
<td>-.92</td>
<td>-.95</td>
<td>-.93</td>
</tr>
<tr>
<td></td>
<td>(.0001/-.001)†</td>
<td>(&lt;.0001/-.001)†</td>
<td>(&lt;.0001/-.001)†</td>
<td>(&lt;.0001/-.001)‡</td>
</tr>
</tbody>
</table>

Significance of difference ($p<.05$) between $^3$He and $^{129}$Xe MRI correlation coefficients for each $r$ were calculated using the Fisher’s z transformation. *$p=.01$

** Holm-Bonferroni adjusted significance values

†$n=15$, ‡$n = 13$, ¥$n = 10$

Table 6-3 shows Pearson correlations for $^3$He and $^{129}$Xe VDP and ADC with pulmonary function measurements. There were significant and similar correlations for $^3$He and $^{129}$Xe MRI VDP with FEV$_1$, although the relationship between VDP and FEV$_1$/FVC was significantly stronger for $^{129}$Xe MRI ($p=.01$). There were also significant and similar correlations for $^3$He and $^{129}$Xe MRI ADC and DL$_{CO}$ (Table 6-3). Figure 6-3 shows the significant and strong relationships for $^3$He and $^{129}$Xe ADC with RA$_{950}$ ($r=.90$, $p=.0005$) and HU$_{15\%}$ ($r=-.91$, $p=.0003$) for COPD subjects only.

Theoretical diffusion coefficients were generated as previously described$^{42}$ for $^3$He/$N_2$ and $^{129}$Xe/$^4$He and are summarized in Table 6-S1. The $^3$He-$N_2$ diffusion coefficient in air was 0.826cm$^2$/s, whereas for $^{129}$Xe-$^4$He, the diffusion coefficient in air was 0.211cm$^2$/s and for air, the self-diffusion coefficient of air was 0.218cm$^2$/s.
We evaluated \(^3\)He and \(^{129}\)Xe MRI acquired within approximately five minutes in healthy subjects with and without COPD, and made a number of observations: 1) significant and strong correlations were observed between \(^3\)He MRI VDP, although visually and quantitatively \(^{129}\)Xe MRI VDP was worse than \(^3\)He MRI VDP in COPD, but not in healthy subjects, 2) significant and strong correlations were observed that were similar for \(^3\)He and \(^{129}\)Xe VDP with FEV\(_1\), but significantly stronger between \(^{129}\)Xe VDP and FEV\(_1\)/FVC, and, 3) a significant and strong correlation was observed between \(^3\)He and \(^{129}\)Xe ADC, both of which showed similar and significant correlations with DL\(_{CO}\) and CT measurements of emphysema.

**Figure 6-3** Relationships between \(^3\)He and \(^{129}\)Xe MRI ADC with CT measurements

A) CT HU\(_{15}\) was significantly correlated with \(^3\)He ADC (r=-.91, p=.0003, r\(^2\)=.83, p=.0003, y=-225x-839) and \(^{129}\)Xe ADC (r=-.91, p=.0003, r\(^2\)=.82, p=.0003, y=-1815x-803).

B) CT RA\(_{950}\) was significantly correlated with \(^3\)He ADC (r=.90, p=.0005, r\(^2\)=.80, p=.0003, y=105x-32) and \(^{129}\)Xe ADC (r=.87, p=.001, r\(^2\)=.76, p=.001, y=821x-47). Dotted lines represent the 95% confidence intervals of the regression line.

### 6.4 Discussion

We evaluated \(^3\)He and \(^{129}\)Xe MRI acquired within approximately five minutes in healthy subjects with and without COPD, and made a number of observations: 1) significant and strong correlations were observed between \(^3\)He and \(^{129}\)Xe MRI VDP, although visually and quantitatively \(^{129}\)Xe MRI VDP was worse than \(^3\)He MRI VDP in COPD, but not in healthy subjects, 2) significant and strong correlations were observed that were similar for \(^3\)He and \(^{129}\)Xe VDP with FEV\(_1\), but significantly stronger between \(^{129}\)Xe VDP and FEV\(_1\)/FVC, and, 3) a significant and strong correlation was observed between \(^3\)He and \(^{129}\)Xe ADC, both of which showed similar and significant correlations with DL\(_{CO}\) and CT measurements of emphysema.
First, in COPD subjects, $^{129}$Xe MRI gas distribution was qualitatively more regionally heterogeneous than $^3$He MRI. This was not the case in healthy volunteers, where $^{129}$Xe and $^3$He MRI showed homogeneous gas distribution and very low VDP that was not significantly different. The visually obvious differences between $^{129}$Xe and $^3$He were also quantitatively different with $^{129}$Xe VDP significantly greater (worse) than $^3$He VDP in COPD subjects, but not healthy volunteers. Importantly, these differences could not be attributed to differences in the pulse sequences used, nor was there a bias detected for specific anterior-posterior slices dominating this result. This unexpected result leads to the simple question: why are these measurements different in COPD? Some explanations might derive from the different gases themselves. In COPD, significant airflow limitation is thought to occur in the small conducting airways $<$2mm in diameter$^{56}$ increasing airway resistance with the potential for regional and preferential $^{129}$Xe gas limitation to the distal airways. Another important consideration relates to the terminal and respiratory bronchioles, where diffusion dominates, and the lower diffusion coefficient of $^{129}$Xe relative to $^3$He may result in slower gas movement into the distal diseased lung regions. Another important consideration is the role of collateral ventilation in COPD. The lower atomic mass and higher diffusivity of $^3$He relative to $^{129}$Xe may allow for regions of the lung that are not ventilated to gradually fill with $^3$He over the time course of the breath-hold scan as has been shown recently by Marshall et. al.$^{57}$ Our finding that ventilation defect percent was greater for $^{129}$Xe MRI than $^3$He MRI, both with $^{129}$Xe 3D and 2D image acquisition, suggests that the lower diffusivity of $^{129}$Xe may slow the process of delayed/collateral ventilation beyond a realistic single breath-hold time. To try to better understand the properties of the two gases inhaled, we generated theoretical diffusion coefficients as previously described$^{42}$ for $^3$He diluted with N$_2$ and air and $^{129}$Xe diluted with $^4$He and air and compared these to the theoretical self-diffusion coefficient of air. Taken together, these results indicated that air has a similar estimated diffusion coefficient as $^{129}$Xe diluted with $^4$He+air (within 3%), and both diffusion coefficients are much lower than the estimated diffusion coefficient of $^3$He diluted with N$_2$+air. Although the exact etiology that may explain the differences between the $^{129}$Xe and $^3$He MRI gas distributions in COPD are not yet established, it is possible that the differences in diffusion coefficients might provide part of the reason for
these observed differences and that different gas mixtures may be helpful in probing
different airway and parenchymal abnormalities. For example, the larger $^{129}$Xe VDP in
COPD might reflect the fact that airway narrowing is less easily penetrated by $^{129}$Xe/$^4$He.

Second, we reported significant and similar correlations between $^3$He and $^{129}$Xe VDP
with spirometry for all subjects, however the correlation coefficient was significantly
stronger for $^{129}$Xe VDP with FEV$_1$/FVC. We note that during expiration in COPD,
airway narrowing is caused by the combination of many factors, including small-airway
wall thickening and obliteration, and collapse of airways secondary to the loss of lung
tissue within the lungs. FEV$_1$/FVC is reduced in subjects with severe COPD and asthma,
and has been reported to be sensitive to airway narrowing and bronchoconstriction. The
finding that the $^{129}$Xe VDP correlation with FEV$_1$/FVC was stronger than $^3$He VDP lends
support to the notion that the differences in diffusion coefficients of the gases might be
helpful in probing different airway and parenchymal abnormalities.

Finally, we also reported strong and significant correlations between $^3$He and $^{129}$Xe ADC
in the same subjects and similar correlation coefficients between $^3$He and $^{129}$Xe ADC
with DL$_{CO}$ and CT measurements. The relationship between $^{129}$Xe ADC and spirometry
measurements for b=12s/cm$^2$ has been previously reported in COPD subjects and
healthy volunteers and similar correlation coefficients for FEV$_1$/$^%$pred and FEV$_1$/FVC
were observed here. However, we reported slightly higher ADC values for both healthy
volunteers and COPD ex-smokers and this may be due to the differences in the inspired
gas mixtures between the two different studies. We note that in the previously published
study, a 1.0L $^{129}$Xe dose was used and as shown in Table E1, the estimated diffusion
coefficient of $^{129}$Xe in air was 0.138cm$^2$/s in comparison to $^{129}$Xe diluted with $^4$H and air
that was used in this study with an estimated diffusion coefficient of 0.211cm$^2$/s. In
addition, the strong correlations between $^{129}$Xe with $^3$He ADC and the strong and similar
correlations between both $^{129}$Xe and $^3$He ADC with CT measurements of emphysema
suggest that $^{129}$Xe diffusion-weighted imaging with b=12s/cm$^2$ is sensitive to lung micro-
structural abnormalities. The comparable results we observed with $^3$He and $^{129}$Xe ADC
suggest that both methods are probing similar spatial dimensions which is important since
the choice of diffusion-weighted gradient can influence the measured ADC. We must
also note that the COPD subjects investigated here showed varying degrees of emphysema, with DLCO ranging from 17\%_{\text{pred}} to 67\%_{\text{pred}}, indicating that $^{129}$Xe MRI as employed in this study provided a way to measure varying degrees of emphysema. Future studies comparing $^{129}$Xe ADC with different b-values to $^{3}$He ADC and CT emphysema measurements, as well as comparing the $^{129}$Xe ADC anterior-posterior gradients with different b-values, which has been previously shown using $^{3}$He MRI to change following treatment in COPD,\textsuperscript{36} are required to determine the differences the diffusion-weighting provides for probing the lung microstructure.

We recognize that there were a small number of subjects used in this study and that most of the subjects evaluated had moderate to severe COPD. We must also acknowledge that because all of these measurements and tests were performed in the same small subject group, extrapolation of these results to a general COPD population cannot be confirmed until studies with larger sample sizes are performed. Another limitation is the difference in pulse sequences used for $^{3}$He and $^{129}$Xe MRI. However, results obtained using NDW images, both acquired using 2D FGRE sequences, were in agreement with the 2D $^{3}$He and 3D $^{129}$Xe MRI acquisition results indicating that there was no difference between $^{3}$He and $^{129}$Xe VDP for the healthy volunteers, however for COPD subjects $^{129}$Xe VDP was greater than $^{3}$He VDP.

In summary, in healthy volunteers and COPD subjects there were strong correlations between $^{129}$Xe and $^{3}$He ADC but significant differences in $^{129}$Xe and $^{3}$He gas distribution in COPD reflecting differences in the gases as well as physiological/anatomical abnormalities in COPD not seen in healthy volunteers.
6.5 References


(43) De ZN, Chhina N, Teh K et al. Asymmetric quadrature split birdcage coil for hyperpolarized 3He lung MRI at 1.5T. Magn Reson Med 2008; 60(2):431-438.


6.6 Appendix

Experimental measurements of the diffusion coefficient of $^{129}$Xe (83% enriched) and air have been previously reported by Kaushik et. al., however the theoretical diffusion coefficient of $^{129}$Xe diluted with $^4$He and air has not been reported. Using the assumptions described in Chen et. al., we estimated the diffusion coefficient of $^{129}$Xe diluted with $^4$He and air and the diffusion coefficient of $^3$He diluted with $N_2$ and air as well as the diffusion coefficient of the main gases ($N_2$ and $O_2$) and air.

The diffusion coefficient of gases A and B may be estimated using the following Equation 6-3

$$D_{AB}^0 = \frac{3}{8} \sqrt{\frac{(kT)^3}{\pi}} \sqrt{\frac{M_A + M_B}{2M_AM_B}} \frac{f_D}{p\sigma_{AB}^2\Omega_D},$$

(1)

where $M_A$ and $M_B$ are the respective molecular weights of gas A and B, $k$ is Boltzmann’s constant, $T$ is the absolute temperature, $P$ is the pressure, $\Omega_D$ is the collision integral for diffusion between molecules, $\sigma_{AB}$ is the characteristic length (mean free path of a molecule) and $f_D$ is a correction term and can be considered to equal 1.

Table 4 shows the estimated self-diffusion coefficients of $^3$He, $^4$He, $^{129}$Xe, $N_2$ and air, as well as the estimated diffusion coefficient of $^{129}$Xe diluted with $^4$He and air and in addition, the diffusion coefficient of $^3$He diluted with $N_2$ and air. The estimated self-diffusion coefficient of air at 310K and 1 atm was 0.218 cm$^2$/s. The estimated diffusion coefficient of $^{129}$Xe diluted with $^4$He and air was 0.211 cm$^2$/s and for $^3$He diluted with $N_2$ and air the diffusion coefficient was 0.826 cm$^2$/s.
Table 6-S6-1 Theoretical Diffusion Coefficients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$^3\text{He}$</th>
<th>$^4\text{He}$</th>
<th>$^{129}\text{Xe}$</th>
<th>$\text{N}_2$</th>
<th>$\text{Air}$</th>
<th>$^{129}\text{Xe}$-$^4\text{He}$-$\text{Air}$</th>
<th>$^3\text{He}$-$^4\text{He}$-$\text{Air}$</th>
<th>$^3\text{He}$-$\text{N}_2$-$\text{Air}$</th>
<th>$^{129}\text{Xe}$-$\text{Air}$</th>
<th>$^{129}\text{Xe}$-$\text{Air}$</th>
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<tbody>
<tr>
<td>$M$ (g/mol)</td>
<td>3</td>
<td>4</td>
<td>129</td>
<td>28</td>
<td>28.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>$\sigma$ (Å)</td>
<td>2.551</td>
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<td>4.047</td>
<td>3.798</td>
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<td>3.1745</td>
<td>3.131</td>
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<td>$\varepsilon/k$ (K)</td>
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<td>61.7984</td>
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<tr>
<td>$kT/\varepsilon$</td>
<td>30.34</td>
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<td>1.342</td>
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<td>11.1971</td>
<td>10.9377</td>
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<td>0.6225</td>
<td>1.257</td>
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<td>0.7282</td>
<td>0.7310</td>
<td>1.0264</td>
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<tr>
<td>$D^0$ (cm$^2$/s)</td>
<td>2.04</td>
<td>1.77</td>
<td>0.061</td>
<td>0.216</td>
<td>0.218</td>
<td>0.211</td>
<td>0.826</td>
<td>0.858</td>
<td>0.138</td>
<td></td>
</tr>
</tbody>
</table>

Note: $\varepsilon$ = energy parameter
CHAPTER 7

To better understand the morphological determinants for the ventilation differences observed between hyperpolarized $^3$He and $^{129}$Xe MRI in COPD in Chapter 6, here we evaluated the same group of COPD subjects using diffusion-weighted MRI.

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7 Pulmonary Ventilation Visualized using Hyperpolarized Helium-3 and Xenon-129 Magnetic Resonance Imaging: Differences in COPD and Relationship to Emphysema

7.1 Introduction

In chronic obstructive pulmonary disease (COPD), irreversible airflow limitation is a consequence of increased time constants for lung emptying related to airway narrowing/occlusion as well as loss of elastic recoil due to emphysematous tissue destruction. $^1$ Magnetic resonance imaging (MRI) using hyperpolarized helium-3 ($^3$He) provides high spatial and temporal resolution images of pulmonary ventilation and ventilation abnormalities $^2$-$^4$ and with the recent advances in polarization physics, $^5$-$^8$ hyperpolarized xenon-129 ($^{129}$Xe) MRI now also provides high resolution images of pulmonary ventilation and transmembrane diffusion. $^6$-$^9$-$^{20}$ The lung micro-structure can also be probed using diffusion-weighted MRI, which takes advantage of the rapid $^3$He and $^{129}$Xe atom Brownian motion to generate apparent diffusion coefficients (ADC), $^{11}$-$^{12}$; $^{16}$; $^{21}$-$^{24}$ now a well-established surrogate measurement of alveolar dimensions. $^{25}$ In a small group of COPD subjects it was also recently demonstrated that there were significantly greater ventilation abnormalities measured using $^{129}$Xe as compared to $^3$He gas and this was not observed in healthy age-matched subjects. $^{26}$ Moreover, in this previous work, some COPD subjects had greater $^3$He-$^{129}$Xe ventilation
differences than others, suggesting that patient-specific, disease-related mechanisms might be responsible for this difference.

We still do not have a clear understanding of the etiology of noble gas MRI ventilation defects, nor their relationship to patient symptoms, exercise capacity or other outcomes; it is also unclear what the structural determinants or mechanisms are behind the differences between $^3$He and $^{129}$Xe ventilation in COPD. A large number of studies have investigated the convective and diffusive gas transport within the lung using multiple-breath washout studies$^{27-30}$ and it is clear from this previous work that there are many factors affecting ventilation distribution within the lung including the inhaled gas physical properties. For example, gas density and viscosity affect flow resistance and this itself is dependent on airway lumen dimensions.$^{31}$ In the small airways (<2mm in diameter) where flow is fully laminar, viscosity provides the main influence on flow resistance. However, in the large airways where turbulent flow dominates, resistance is mainly affected by gas density. The major site of airflow limitation in COPD occurs within the small conducting airways$^{32}$ and therefore it is predicted that the gas viscosity would be the most important gas physical property affecting flow resistance. However, both the greater density and viscosity of $^{129}$Xe gas (pure $^{129}$Xe gas has approximately 40 times greater density and 1.5 times greater viscosity than pure $^3$He gas)$^{33,34}$ may result in greater resistance to flow regardless of the location of airway abnormalities, and therefore may contribute to greater $^{129}$Xe ventilation abnormalities in comparison to $^3$He MRI in COPD. Another potential mechanism responsible for $^3$He-$^{129}$Xe ventilation differences in COPD is the presence of emphysema and the effects of collateral ventilation.$^{35}$ In emphysema, lung tissue compliance is increased and resistance to flow through obstructed airways is higher than through collateral pathways.$^{36,37}$

To provide a better understanding of the clinical or physiological meaning of MRI-derived ventilation abnormalities, we investigated the relationship between emphysema and airway wall morphology with differences observed between hyperpolarized $^3$He and $^{129}$Xe MRI gas distribution. As a first step, here we evaluated a small group of COPD subjects$^{26}$ and hypothesized that emphysematous lung regions would more readily fill with $^3$He as compared to $^{129}$Xe gas under the same physiological conditions. In other
words, lung regions that could not be accessed by $^{129}$Xe gas would be more emphysematous, leading to decreased $^{129}$Xe MRI ventilation. To test this hypothesis, we evaluated regional $^3$He ADC using image registration/segmentation methods and x-ray computed tomography (CT) measurements of airway wall thickness and emphysema to probe the structure-function relationships in lung regions accessed by both gases and those that were accessed only by $^3$He gas.

### 7.2 Materials and Methods

#### 7.2.1 Subjects

We enrolled 10 COPD subjects in a hyperpolarized $^3$He and $^{129}$Xe MRI study and they provided written informed consent to a protocol approved by the local research ethics board and Health Canada, which was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada) and the Health Insurance Portability and Accountability Act (HIPAA, USA). COPD subjects were ex-smokers 50-85 years of age and were categorized according to the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria, with a smoking history of at least 10 pack-years.

#### 7.2.2 Pulmonary Function Tests

Spirometry was performed using an EasyOne spirometer (ndd Medizintechnik AG, Zurich, CH) according to the American Thoracic Society (ATS) guidelines. Static lung volumes and the diffusing capacity for carbon monoxide ($DL_{CO}$) were measured using a whole body plethysmograph (MedGraphics Corporation, 350 Oak Grove Parkway St. Paul, MN USA). All spirometry and plethysmography measurements were performed approximately 1 hour following administration of 400 μg salbutamol inhaled via a spacer device.

#### 7.2.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Discovery 750MR (General Electric Health Care, Milwaukee, WI) MRI system. Subjects were instructed to inhale a gas mixture from a 1.0 L Tedlar® bag (Jensen Inert Products, New Jersey, USA) from functional residual capacity (FRC), and image acquisition was performed during a 8-15s
breath-hold. To minimize the potential for differences in the levels of inspiration between $^3$He and $^{129}$Xe MRI, extensive coaching was performed prior to the imaging sessions to ensure subjects could inspire the entire bag and throughout the duration of all imaging sessions. To ensure that each inhalation was performed from FRC, the subjects were instructed to perform two tidal breaths prior to inhalation from the bag. The order of $^3$He and $^{129}$Xe MRI acquisition was also randomized for each subject.

$^3$He gas was polarized to 30—40% (HeliSpin™) and doses (5mL/kg body weight) were administered in 1.0L Tedlar® bags diluted with medical grade nitrogen ($N_2$) (Spectra Gases, Alpha, NJ). $^3$He MRI diffusion-weighted images were acquired using a fast gradient-recalled-echo (FGRE) sequence immediately following inhalation of the $^3$He/$N_2$ gas mixture during breath-hold conditions. Two interleaved images were acquired (14s total data acquisition, repetition time (TR)/echo time (TE)/flip angle = 7.6 ms/3.7 ms/8°, field of view (FOV) = 40 x 40 cm, matrix 128 x 128, 7 slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitization with $b = 1.6$ s/cm² (gradient amplitude (G) = 1.94 G/cm, rise and fall time = 0.5 ms, gradient duration = 0.46 ms, diffusion time = 1.46 ms). $^{129}$Xe gas was polarized to 10—60% (XeBox-E10, Xemed LLC, New Hampshire, USA) and doses (50/50 $^{129}$Xe/$^4$He) were administered in 1.0L Tedlar® bags. $^{129}$Xe MRI diffusion-weighted images were acquired using a FGRE sequence immediately following inhalation of the $^{129}$Xe/$^4$He gas mixture. $^{129}$Xe gas was diluted with $^4$He instead of $N_2$ to try to reduce the differences in the physical properties of the inhaled $^3$He and $^{129}$Xe gas mixtures and better mimic the mixture of gaseous oxygen in air. Two interleaved images (16s total data acquisition, TE/TR/flip angle = 10 ms/13.5 ms/1°, FOV = 40 x 40 cm, matrix 128 x 80, 7 slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitization with $b = 12$ s/cm² (G = 2.90 G/cm, rise and fall time = 0.5 ms, gradient duration = 2.0 ms, diffusion time = 5 ms). A low dose CT was performed on a 64-slice Lightspeed VCT scanner (GEHC, Milwaukee, WI USA) as previously described.

### 7.2.4 Image Analysis

Hyperpolarized $^3$He and $^{129}$Xe MRI ADC maps were generated from diffusion-weighted and non-diffusion-weighted images as previously described using MATLAB R2007b.
To ensure ADC was generated for voxels corresponding to ventilated lung regions, the non-diffusion-weighted images were segmented to obtain a binary mask for each slice. The resulting binary masks were applied to the corresponding non-diffusion-weighted images, and the ADC maps were generated on a voxel-by-voxel basis. An overview of the image analysis methodology adapted from 38 is provided in Figure 7-1. $^3$He and $^{129}$Xe ADC maps were registered using landmark-based image registration. The trachea and visible major airways were removed semi-automatically permitting ADC calculation within the lung parenchyma. A single ADC value was calculated for each subject by averaging the mean ADC for each slice to obtain a whole lung slice-average (WL) ADC. Following registration of the $^3$He and $^{129}$Xe ADC maps, the lung regions of interest (ROI) within the ADC maps accessed by both gases were identified as the intersection (regions of overlap) of the $^3$He and $^{129}$Xe ADC maps; mean ADC within those ROI was generated for each slice and then averaged to obtain a mean $^3$He ADC value accessed by both gases ($\text{ADC}_{\text{HX}}$). Lung ROI accessed by both $^3$He and $^{129}$Xe were used as a binary mask on the $^3$He ADC maps to calculate mean ADC in $^3$He only ROI ($\text{ADC}_{\text{HO}}$). It is important to note that no lung regions were accessed only by $^{129}$Xe gas. $^3$He and $^{129}$Xe ventilation volume (VV) 41 was generated by summing the voxels in the segmented non-diffusion-weighted image following the removal of the trachea and visible major airways.
CT airway and emphysema analysis was performed using VIDA’s Pulmonary Workstation 2.0 (VIDA Diagnostics, Inc., Coralville, IA). Wall area percentage (WA%) and lumen area (LA) was measured for the 3rd to 7th generation airways. The extent of emphysema was estimated using lowest 15th percentile point of the CT lung density histogram (HU15%).

7.2.5 Density and Viscosity Estimates

The density and viscosity of the inspired \(^{29}\)Xe gas diluted with \(^{4}\)He and the inspired \(^{3}\)He gas diluted with \(\text{N}_2\) as well as the density and viscosity of the pulmonary gases were estimated. The density of gases may be estimated using **Equation 7-1**:

\[
\rho = \frac{MP}{RT}
\]
where $M$ is the molar mass, $P$ is the pressure, $R$ is the universal gas constant and $T$ is the absolute temperature. The density of a mixture of gases A and B may be estimated using Equation 7-2:

$$\rho_{AB} = \frac{\rho_AV_A + \rho_BV_B}{V_A + V_B}$$

where $\rho_A$ and $\rho_B$ are the densities of gases A and B, and $V_A$ and $V_B$ are the volumes of gases A and B. The viscosity of gases may be estimated using the Chapman-Enskog viscosity equation, as previously described and shown below in Equation 7-3:

$$\mu = 26.69 \left(\frac{MT}{\sigma_C^2 \Omega_D}\right)^{\frac{1}{2}}$$

where $M$ is the molecular weight, $T$ is the absolute temperature, $\Omega_D$ is the collision integral for diffusion between molecules and $\sigma_C$ is the collision diameter. The viscosity of a mixture of gases A and B may be estimated using Equation 7-4:

$$\mu_{AB} = \frac{X_A \mu_A M_A^{\frac{1}{2}} + X_B \mu_B M_B^{\frac{1}{2}}}{X_A M_A^{\frac{1}{2}} + X_B M_B^{\frac{1}{2}}}$$

where $X_A$ and $X_B$ are the mole fractions of gases A and B, $M_A$ and $M_B$ are the molecular weights of gases A and B and $\mu_A$ and $\mu_B$ are the respective viscosities of gases A and B. This equation was selected because it can represent with reasonable accuracy the viscosity of a gas mixture at low or moderate pressures. For the viscosity calculations of the $^3$He-N$_2$ mixture, we used the average volume of $^3$He gas given to each subject (390mL) to determine the mole fraction which was approximately 40/60, and for the $^{129}$Xe-$^4$He mixture it was 50/50. We also calculated the characteristic diffusion length ($L_i$) of the gases and gas mixtures using Equation 7-5:

$$L_i = \sqrt{2D_0 \Delta}$$

where $D_0$ is the diffusion coefficient and $\Delta$ is the diffusion time.
Density and viscosity calculations for the $^3$He-$\text{N}_2$ and $^{129}$Xe-$^4\text{He}$ gas mixtures administered at 310K and 1 atm are provided in Table 7-1. The estimated density of $^3$He-$\text{N}_2$ mixture and $^{129}$Xe-$^4\text{He}$ mixture was 0.61kg/m$^3$ and 2.65kg/m$^3$, respectively, and the estimated viscosity of $^3$He-$\text{N}_2$ mixture and $^{129}$Xe-$^4\text{He}$ mixture was 1.99x10$^{-4}$P and 2.55x10$^{-4}$P, respectively. The estimated characteristic diffusion length for the $^3$He-$\text{N}_2$ mixture and $^{129}$Xe-$^4\text{He}$ mixture was 460μm and 490μm, respectively. The calculations for the estimated diffusion coefficient of $^{129}$Xe diluted with $^4\text{He}$ and air and the diffusion coefficient of $^3\text{He}$ diluted with $\text{N}_2$ and air were reported elsewhere$^{26}$ and shown in Table 7-1.

Table 7-1 Density and Viscosity Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$^3\text{He}$</th>
<th>$^4\text{He}$</th>
<th>$^{129}\text{Xe}$</th>
<th>$\text{N}_2$</th>
<th>Air</th>
<th>$^{129}\text{Xe}$-$^4\text{He}$</th>
<th>$^3\text{He}$-$\text{N}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (g/mol)</td>
<td>3</td>
<td>4</td>
<td>129</td>
<td>28</td>
<td>28.85</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>σ (Å)</td>
<td>2.551</td>
<td>2.551</td>
<td>4.047</td>
<td>3.798</td>
<td>3.711</td>
<td>3.505</td>
<td>3.175</td>
</tr>
<tr>
<td>Ω$_D$</td>
<td>0.623</td>
<td>0.623</td>
<td>1.257</td>
<td>0.869</td>
<td>0.888</td>
<td>0.843</td>
<td>0.728</td>
</tr>
<tr>
<td>$D^0$ (cm$^2$/s)</td>
<td>2.04</td>
<td>1.77</td>
<td>0.061</td>
<td>0.216</td>
<td>0.218</td>
<td>0.211*</td>
<td>0.826*</td>
</tr>
<tr>
<td>$L_1$ (μm)</td>
<td>770†</td>
<td>720†</td>
<td>250‡</td>
<td>460‡</td>
<td>470‡</td>
<td>460‡</td>
<td>490†</td>
</tr>
<tr>
<td>μ x 10$^{-4}$ (P)</td>
<td>2.033</td>
<td>2.321</td>
<td>2.593</td>
<td>1.984</td>
<td>2.064</td>
<td>2.552</td>
<td>1.993</td>
</tr>
<tr>
<td>ρ (kg/m$^3$)</td>
<td>0.118</td>
<td>0.157</td>
<td>5.071</td>
<td>1.101</td>
<td>1.140</td>
<td>2.651</td>
<td>0.6095</td>
</tr>
</tbody>
</table>

M=molecular weight, σ=collision diameter, Ω$_D$=collision integral for diffusion between molecules, $D^0$=diffusion coefficient, $L_1$=characteristic diffusion length, μ=viscosity, ρ=density.

* The diffusion coefficient of $^{129}$Xe diluted with $^4\text{He}$ and air and the diffusion coefficient of $^3\text{He}$ diluted with $\text{N}_2$ and air (21).

† diffusion time=1.46ms, ‡diffusion time=5ms

7.2.6 Statistical Methods

A two-way mixed-effects repeated measures analysis of variance (ANOVA) was used to determine the interaction between $^3\text{He}$ and $^{129}$Xe VV as well as the interaction between $^3\text{He}$ ADC for lung ROI ventilated by both gases (ADC$_{\text{HX}}$) and by $^3\text{He}$ gas only (ADC$_{\text{HO}}$) using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Linear regression ($r^2$) and Pearson correlation coefficients ($r$) were used to determine the relationships for the difference between $^3\text{He}$ and $^{129}$Xe VV with CT HU$_{15\%}$, spirometry and ADC$_{\text{HO}}$ using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego, CA, USA). In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% (p < 0.05).
7.3 Results

Table 7-2 shows subject demographics and pulmonary function measurements for all 10 COPD ex-smokers (GOLD Class I n=1; GOLD Class II n=6; GOLD Class III n=2; GOLD Class IV n=1).

Table 7-3 shows a subject listing of mean $^3$He and $^{129}$Xe WL ADC and VV, as well as regional ADC measurements. As previously described in the same group of subjects using static ventilation images, $^3$He VV derived from non-diffusion-weighted images was significantly greater than $^{129}$Xe VV ($^3$He VV=5.17L, $^{129}$Xe VV=4.34L, $p<.0001$). There was a significant interaction for VV between inhaled gas ($^3$He and $^{129}$Xe) and subject ($p=.04$) and this finding may indicate that some COPD subjects had greater $^3$He and $^{129}$Xe ventilation differences than others. Importantly, the difference between $^3$He and $^{129}$Xe SNR was not significantly correlated with the difference between $^3$He and $^{129}$Xe VV ($r=-.25$, $p=.48$).

Figure 7-2 shows $^3$He and $^{129}$Xe ADC maps for all slices for two representative subjects. Visually obvious differences in ventilation between $^3$He and $^{129}$Xe MRI were apparent in the subject with higher mean ADC for all slices, but to a much lesser extent in the subject with moderate ADC values.
Table 7-2 Subject listing of pulmonary function measurements

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>BMI (kg·m⁻²)</th>
<th>FEV₁ (%pred)</th>
<th>FVC (%pred)</th>
<th>FEV₁/FVC (%pred)</th>
<th>TLC (%pred)</th>
<th>RV (%pred)</th>
<th>RV/TLC (%pred)</th>
<th>IC (%pred)</th>
<th>FRC (%pred)</th>
<th>DLCO (%pred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>F</td>
<td>19.8</td>
<td>50</td>
<td>76</td>
<td>0.50</td>
<td>114</td>
<td>156</td>
<td>0.63</td>
<td>86</td>
<td>135</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>M</td>
<td>26.5</td>
<td>59</td>
<td>82</td>
<td>0.52</td>
<td>102</td>
<td>145</td>
<td>0.52</td>
<td>89</td>
<td>112</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>M</td>
<td>23.1</td>
<td>52</td>
<td>104</td>
<td>0.36</td>
<td>114</td>
<td>151</td>
<td>0.51</td>
<td>69</td>
<td>153</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>M</td>
<td>30.6</td>
<td>77</td>
<td>104</td>
<td>0.54</td>
<td>114</td>
<td>140</td>
<td>0.45</td>
<td>128</td>
<td>102</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>M</td>
<td>20.8</td>
<td>26</td>
<td>66</td>
<td>0.29</td>
<td>111</td>
<td>229</td>
<td>0.77</td>
<td>27</td>
<td>189</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>M</td>
<td>18.4</td>
<td>34</td>
<td>94</td>
<td>0.26</td>
<td>132</td>
<td>221</td>
<td>0.63</td>
<td>54</td>
<td>201</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>F</td>
<td>23.7</td>
<td>59</td>
<td>86</td>
<td>0.53</td>
<td>115</td>
<td>121</td>
<td>0.45</td>
<td>121</td>
<td>110</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>76</td>
<td>M</td>
<td>29.6</td>
<td>35</td>
<td>84</td>
<td>0.31</td>
<td>123</td>
<td>205</td>
<td>0.60</td>
<td>70</td>
<td>168</td>
<td>44</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>M</td>
<td>32.1</td>
<td>75</td>
<td>82</td>
<td>0.68</td>
<td>105</td>
<td>134</td>
<td>0.42</td>
<td>92</td>
<td>117</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>M</td>
<td>29.8</td>
<td>107</td>
<td>135</td>
<td>0.58</td>
<td>115</td>
<td>86</td>
<td>0.27</td>
<td>109</td>
<td>121</td>
<td>42</td>
</tr>
<tr>
<td>ALL</td>
<td>74</td>
<td></td>
<td>25.4</td>
<td>57</td>
<td>91</td>
<td>0.46</td>
<td>115</td>
<td>159</td>
<td>0.53</td>
<td>85</td>
<td>141</td>
<td>41</td>
</tr>
</tbody>
</table>

SD=Standard Deviation, BMI=Body Mass Index, FEV₁= Forced Expiratory Volume in 1s, %pred=Percent Predicted, FVC=Forced Vital Capacity, TLC= Total Lung Capacity, RV= Reserve Volume, IC= Inspiratory Capacity, FRC= Functional Residual Capacity, DLCO=Carbon Monoxide Diffusion Capacity of the lung. †n=7
Table 7-3: 3He and 129Xe MRI WL and Regional ADC for all subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>WL 3He MRI VV (L)</th>
<th>WX 3He MRI ADC (cm²/s)</th>
<th>WL 129Xe MRI VV (L)</th>
<th>WX 129Xe MRI ADC (cm²/s)</th>
<th>Regional 3He ADC HX (cm²/s)</th>
<th>Regional 3He ADC HO (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.25</td>
<td>0.608</td>
<td>3.70</td>
<td>0.095</td>
<td>0.600</td>
<td>0.645</td>
</tr>
<tr>
<td>2</td>
<td>5.13</td>
<td>0.312</td>
<td>5.09</td>
<td>0.058</td>
<td>0.306</td>
<td>0.329</td>
</tr>
<tr>
<td>3</td>
<td>6.03</td>
<td>0.534</td>
<td>4.99</td>
<td>0.086</td>
<td>0.511</td>
<td>0.620</td>
</tr>
<tr>
<td>4</td>
<td>5.16</td>
<td>0.332</td>
<td>4.90</td>
<td>0.057</td>
<td>0.320</td>
<td>0.381</td>
</tr>
<tr>
<td>5</td>
<td>6.06</td>
<td>0.575</td>
<td>4.80</td>
<td>0.092</td>
<td>0.572</td>
<td>0.600</td>
</tr>
<tr>
<td>6</td>
<td>5.86</td>
<td>0.622</td>
<td>4.43</td>
<td>0.087</td>
<td>0.610</td>
<td>0.643</td>
</tr>
<tr>
<td>7</td>
<td>5.05</td>
<td>0.503</td>
<td>4.39</td>
<td>0.086</td>
<td>0.508</td>
<td>0.474</td>
</tr>
<tr>
<td>8</td>
<td>6.24</td>
<td>0.479</td>
<td>4.50</td>
<td>0.080</td>
<td>0.466</td>
<td>0.508</td>
</tr>
<tr>
<td>9</td>
<td>3.10</td>
<td>0.290</td>
<td>2.85</td>
<td>0.057</td>
<td>0.274</td>
<td>0.297</td>
</tr>
<tr>
<td>10</td>
<td>4.83</td>
<td>0.527</td>
<td>3.77</td>
<td>0.085</td>
<td>0.529</td>
<td>0.536</td>
</tr>
<tr>
<td>ALL</td>
<td>5.17‡</td>
<td>0.478</td>
<td>4.34‡</td>
<td>0.078</td>
<td>0.470†</td>
<td>0.503†</td>
</tr>
</tbody>
</table>

WL=Whole lung, VV=Ventilation Volume, ADC=Apparent Diffusion Coefficient, ADC_HX=lung regions of interest with signal at both 3He and 129Xe time-points, ADC_HO=lung regions of interest with signal during the 3He breath-hold only.

‡ Denotes p-value < 0.0001 for comparison between WL 3He and 129Xe MRI VV
† Denotes p-value < 0.0001 for comparison between Regional 3He ADC_HX and 3He ADC_HO

Figure 7-3 shows the 3He and 129Xe ADC maps as well as the ADC map for the ROI ventilated only by 3He for a subject with moderately severe COPD. As shown in Table 7-3, regional analysis showed that mean ADC for those regions only accessed by 3He gas (ADC_HO=0.503±0.119 cm²/s) was significantly greater than mean ADC for those regions accessed by both 3He and 129Xe gas (ADC_HX=0.470±0.125 cm²/s, p<.0001). Moreover, to ensure that 129Xe ventilation abnormalities in regions of emphysema were not due to signal attenuation within those regions in the diffusion-weighted images, we compared ventilation measured from the non-diffusion-weighted images (non-diffusion-weighted 129Xe VV=4.27±0.76L) and the ADC maps and determined there was no significant ventilation difference (ADC map 129Xe VV=4.31±0.69L, p=.80). To determine whether airways leading to regions accessed only by 3He gas might be abnormally thickened or obstructed we compared the lung side with the greatest difference between 3He and 129Xe ventilation with the lung side with the lower 3He-129Xe difference and observed no significant difference in WA% (ΔWA%=1%, p=.64) or LA (ΔLA=6.7 mm², p=.33).
In Figure 7-4, the differences between $^3$He and $^{129}$Xe VV are shown and these were significantly correlated with CT HU$_{15\%}$ ($r=-.65$, $p=.04$) and mean $^3$He ADC$_{HO}$ ($r=.70$, $p=.02$). However, there was no significant correlation for the difference between $^3$He and $^{129}$Xe VV with CT WA$\%$ ($r=-.34$, $p=.33$) for all airway generations or for each of the 3$^{rd}$ to 7$^{th}$ generation airways individually. There was also a significant correlation for the
difference between $^3$He and $^{129}$Xe VV with FEV$_1$/FVC ($r = -.79$, $p = .007$), but not with FEV$_1$ ($r = -.48$, $p = .16$) or DLCO ($r = -.37$, $p = .41$).

**Figure 7-3** Regional $^3$He and $^{129}$Xe ADC

$^3$He and $^{129}$Xe ADC maps with the ADC map for the ROI ventilated only by $^3$He registered to the binary ADC map for those regions accessed by both $^3$He and $^{129}$Xe.
Hyperpolarized noble gas MRI has been developed over the last two decades because it has the potential for clinical translation, providing quantitative microstructural and dynamic pulmonary functional information and measurements. However, while $^3$He MRI allows the acquisition of very high quality images, the global availability and costs are motivating a transition to hyperpolarized $^{129}$Xe MRI. Recently, a pilot study performed with both $^3$He and $^{129}$Xe MRI showed there were visually obvious and quantitatively greater gas distribution abnormalities for $^{129}$Xe MRI in COPD subjects but not healthy elderly never-smokers. The reasons for the difference were not clear and so in the current evaluation we investigated emphysema as a contributor to the observed differences in $^3$He and $^{129}$Xe ventilation. Accordingly, here we report: 1) significantly greater $^3$He ADC in regions only accessed by $^3$He gas ($^{3}$He ADC$_{HO}$) as compared to regions accessed by both gases ($^{3}$He ADC$_{HX}$), and, 2) a significant relationship between the

![Figure 7-4](image)

**Figure 7-4** Correlation for the difference between $^3$He and $^{129}$Xe VV and CT HU$_{15\%}$ Threshold

A. The difference between $^3$He and $^{129}$Xe VV was significantly correlated with the extent of emphysema as assessed by CT using HU$_{15\%}$ threshold ($r=-0.65$, $p=0.04$, $r^2=0.42$, $p=0.04$, $y=-34.4x-919.2$).  
B. The difference between $^3$He and $^{129}$Xe VV was significantly correlated with mean ADC in the $^3$He only regions of interest (ADC$_{HO}$) ($r=0.70$, $p=0.02$, $r^2=0.50$, $p=0.02$, $y=3.1x-0.7$).

Dotted lines represent the 95% confidence intervals.

### 7.4 Discussion

Hyperpolarized noble gas MRI has been developed over the last two decades because it has the potential for clinical translation, providing quantitative microstructural and dynamic pulmonary functional information and measurements. However, while $^3$He MRI allows the acquisition of very high quality images, the global availability and costs are motivating a transition to hyperpolarized $^{129}$Xe MRI. Recently, a pilot study performed with both $^3$He and $^{129}$Xe MRI showed there were visually obvious and quantitatively greater gas distribution abnormalities for $^{129}$Xe MRI in COPD subjects but not healthy elderly never-smokers. The reasons for the difference were not clear and so in the current evaluation we investigated emphysema as a contributor to the observed differences in $^3$He and $^{129}$Xe ventilation. Accordingly, here we report: 1) significantly greater $^3$He ADC in regions only accessed by $^3$He gas ($^{3}$He ADC$_{HO}$) as compared to regions accessed by both gases ($^{3}$He ADC$_{HX}$), and, 2) a significant relationship between the
difference in $^3$He and $^{129}$Xe VV and the extent of emphysema assessed by CT but not with CT airway wall thickness (WA%) measurements.

We observed that mean $^3$He ADC in pulmonary ROI accessed only by $^3$He gas were elevated relative to the mean $^3$He ADC in the remaining lung. This finding suggests that more emphysematous or damaged lung was more readily filled with $^3$He as compared to $^{129}$Xe gas for the duration of the breath-hold imaging performed in this study. We acknowledge, however, that SNR was lower for $^{129}$Xe than with $^3$He MRI, and therefore there is the potential for regions of the lung with reduced signal intensity to appear as ventilation defects in low SNR $^{129}$Xe images. However, for this investigation, we observed that the difference between $^3$He and $^{129}$Xe SNR was not significantly correlated with the difference between $^3$He and $^{129}$Xe VV. Moreover, to ensure that $^{129}$Xe ventilation abnormalities in regions of emphysema were not due to signal attenuation within those regions in the diffusion-weighted images, we compared ventilation measured from the non-diffusion-weighted images and the ADC maps and determined there was no significant ventilation difference. We think, therefore, that it is unlikely that this finding is due to SNR alone and another possible explanation for this observation is that the highly diffusive and less dense and viscous $^3$He gas may readily access the slower-filling emphysematous lung during the inhalation period and the breath-hold interval. Alternatively, the $^3$He gas may fill emphysematous lung regions via collateral channels. Collateral channels have been identified in emphysema and although resistance to flow through collateral channels is high in the normal lung, in emphysema resistance to flow through collateral channels is lower than through obstructed airways. Collateral ventilation has been demonstrated with $^3$He MRI and more recently, indirect, non-airway dependent or collateral ventilation was directly visualized using $^3$He MRI in COPD within a single breath-hold. For $^{129}$Xe gas, however, it is likely that there are longer time constants for gas filling in emphysematous regions; $^{129}$Xe gas with its lower diffusion coefficient would diffuse much more slowly into these emphysematous regions than $^3$He and therefore have a much shorter diffusion distance due to the short breath-hold duration, which may contribute to lower $^{129}$Xe signal intensity in regions of significant emphysema. Accordingly, we calculated the characteristic diffusion length for the $^{129}$Xe/$^4$He and $^4$He/N$_2$ gas mixtures and showed that
the characteristic diffusion length for the $^{129}$Xe atoms is smaller than that for the $^3$He atoms. Regardless of the exact mechanisms that are in play, the finding of significant emphysema in lung regions probed only with $^3$He gas suggests that these lung regions cannot be penetrated by $^{129}$Xe gas in the same timeframe.

Importantly, we previously showed that the mixture of $^{129}$Xe/$^4$He gas administered in this study better approximates the self-diffusion coefficient of oxygen mixed with air in the lung,\textsuperscript{26} suggesting that $^{129}$Xe MRI ventilation may be a better estimate of ground truth pulmonary ventilation. The $^3$He/$N_2$ gas mixture has a lower density and viscosity than air, and studies have previously demonstrated that inspiring oxygen mixed with helium reduces airway resistance by decreasing turbulent flow.\textsuperscript{47} This interesting finding suggests that inhaled $^3$He gas and its mixtures may be less sensitive to peripheral airway obstruction. Clearly, there are advantages to utilizing inhaled gases such as $^{129}$Xe and mixtures thereof that may have increased sensitivity to airway obstruction and emphysema for targeted treatments and interventions. On the other hand, the increased density/viscosity and lower diffusion coefficient of $^{129}$Xe may limit signal from those regions of the lung most affected by emphysema or receiving collateral flow. Clearly, both gases provide application-specific advantages.

Importantly, we did not detect a significant correlation for the difference in $^3$He and $^{129}$Xe ventilation and WA%. However, we cannot ascertain whether this might be the case regionally, wherein specific airways leading to regions accessed only by $^3$He gas might be abnormally thickened or obstructed. In other words, it is possible that the whole lung estimates of WA% might not be representative of regional differences. We investigated this possibility by regionally comparing the lung side with the greatest difference between $^3$He and $^{129}$Xe ventilation with the lung side with the lower $^3$He-$^{129}$Xe difference and observed no significant difference in WA% or lumen area. However, we must also acknowledge that for some subjects, the lung region that showed a $^3$He-$^{129}$Xe ventilation difference was very small and this may have also contributed to the lack of significant relationship observed with WA%.
A large number of studies have investigated convective and diffusive gas transport within the lung using multiple-breath washout studies\textsuperscript{27-30} and modeling\textsuperscript{48} and it is evident from these previous studies that the inhaled gas physical properties are important factors affecting ventilation distribution within the lung. Clearly there is significant work to be done to better understand how the different properties of \textsuperscript{3}He and \textsuperscript{129}Xe gas affect ventilation distribution and most importantly, what information these differences in ventilation distribution provide us regarding the sensitivity of the different gases and their mixtures for detecting airway and ventilation abnormalities. Directly visualizing the distribution of the gases using mixtures of \textsuperscript{3}He and \textsuperscript{129}Xe with well-controlled viscosities and densities during a breath-hold in the same subjects using time-resolved imaging sequences, as was recently performed with \textsuperscript{3}He MRI\textsuperscript{46}, could certainly be utilized to test some of our important hypotheses regarding delayed/collateral ventilation within emphysematous lung regions in COPD subjects. Moreover, while several modeling studies have aimed to evaluate gas transport in the lung using \textsuperscript{3}He and \textsuperscript{129}Xe,\textsuperscript{49,50} more studies of this nature are required to fully understand the role of the gas properties on regional ventilation distribution with noble gas MRI as well as to determine the gas mixture that best approximates the distribution of typical room air within the lung.

We recognize that this work was limited by the small number of subjects evaluated meaning that extrapolation of these results to a general COPD population cannot be confirmed until studies with larger sample sizes are performed. This study was also limited by the different diffusion times and b-values that were utilized for \textsuperscript{3}He and \textsuperscript{129}Xe image acquisition, and therefore we may be probing different lung structures. Previous studies have demonstrated that greater diffusion time results in greater diffusion distances and there is therefore the potential to probe a fundamentally different spatial scale.\textsuperscript{51} However, significant and strong correlations have been previously observed between \textsuperscript{3}He and \textsuperscript{129}Xe ADC,\textsuperscript{26} suggesting that both methods are probing similar spatial dimensions. In addition, the acquisition of clinical measurements, such as dyspnea scores and measurements of exercise tolerance, for direct comparison to \textsuperscript{3}He and \textsuperscript{129}Xe MRI measurements may have aided in determining the clinical meaning of these differences. Future studies should aim to evaluate the differences between \textsuperscript{3}He and \textsuperscript{129}Xe MRI in
terms of their relationships with clinical measurements as well as the relative sensitivities for detecting improvements following treatment or interventions.

In summary, we used regional hyperpolarized $^3$He and $^{129}$Xe ADC image registration/segmentation methods to quantify ADC in the lung regions accessed by both gases and the lung regions accessed by $^3$He gas only. We reported a significantly greater mean ADC in the lung regions accessed by $^3$He gas only than in the remaining lung tissue, and a significant relationship between the difference in $^3$He and $^{129}$Xe VV and emphysema. In COPD, the lower resistance to flow within emphysematous lung regions is a potential determinant of the differences observed in $^3$He and $^{129}$Xe ventilation defects.
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CHAPTER 8

While it is important to demonstrate that $^3$He MRI measurements relate to important clinical outcomes, such as disease progression and treatment response, another goal of imaging is to identify early disease changes. Here we evaluated well-established clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and abnormal DLCO as well as a group of ex-smokers with normal spirometry and DLCO and ex-smokers with GOLD stage 1 COPD.

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8 On the Role of abnormal DLCO in Ex-smokers Without Airflow Limitation: Symptoms, Exercise Capacity and Hyperpolarized Helium-3 Magnetic Resonance Imaging

8.1 Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by chronic, progressive expiratory flow limitation that develops as a result of the lung’s inflammatory response to inhaled toxic gases and particles, primarily from tobacco smoke. In COPD, airflow limitation is caused by both small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), however, the relative contributions of these pathologies vary from person to person.

When COPD is suspected based on symptoms, such as dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors, airflow limitation is measured using spirometry and severity is determined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. This approach, however, has been acknowledged to potentially result in an over-diagnosis of COPD in the elderly, as well as under-diagnosis of mild or early stage COPD.

The COPDGene study recently reported low forced expiratory volume in 1 second (FEV1) and normal FEV1/forced vital capacity (FVC) in ex-smokers with significant symptoms and decreased six-minute walk distance (6MWD) and defined these patients as
GOLD-Unclassified (GOLD-U). Until now, ex-smokers with GOLD-U or those with “non-obstructive” or “pure” emphysema without airflow limitation have been systematically excluded from COPD studies. With respect to non-obstructive emphysema, there have been a few case reports and pilot studies that described significant smoking history, severe symptoms and abnormal diffusing capacity for carbon monoxide (DL_CO) in patients concomitant with normal expiratory airflow. A recent study also reported that otherwise normal asymptomatic smokers with abnormal DL_CO showed evidence of endothelial microparticles in the circulation – a marker of early lung destruction associated with emphysema. Although abnormal DL_CO in ex-smokers is a valuable marker of lung function impairment, even in the absence of airflow limitation, the relationship between DL_CO with other functional markers (i.e., symptoms and exercise limitation) is not well-understood. We hypothesized that subjects with abnormal DL_CO without airflow limitation would have imaging evidence of early or mild emphysema with measurable functional consequences.

Multi-detector computed tomography (CT) and hyperpolarized helium-3 (3He) magnetic resonance imaging (MRI) have been used independently to measure emphysema and airways disease as distinct phenotypes in COPD. In particular, hyperpolarized 3He MRI apparent diffusion coefficients (ADC), provide a way to sensitively measure regional lung tissue destruction, -the hallmark of emphysema. Abnormally elevated 3He ADC have previously been reported in asymptomatic smokers without COPD, although the relationship between 3He MRI ADC in early disease with symptoms and other physiological measurements has never been reported and their functional impact is not known. To better understand the consequences of early or mild disease in ex-smokers, here we evaluated well-established clinical, physiological as well as emerging imaging measurements in ex-smokers with normal spirometry but abnormal DL_CO as well as ex-smokers with GOLD stage I COPD and those with normal spirometry and DL_CO.
8.2 Materials and Methods

8.2.1 Study Subjects

All subjects provided written informed consent to the protocol approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (Canada) and the Health Insurance Portability and Accountability Act (USA). Ex-smokers were recruited from a local tertiary care centre and by advertisement. Thirty-eight subjects were enrolled who were ex-smokers without a diagnosis of COPD and 15 ex-smokers were enrolled with a previous diagnosis of GOLD stage I COPD, all of whom were 60-85 years of age, with a smoking history ≥ 10 pack-years. The subjects without a diagnosis of COPD had no history of previous chronic or current respiratory disease and were classified according to ATS/ERS recommendations on the approximate lower limits of normal for DL_{CO} such that normal DL_{CO} ≥ 75%_{pred} and abnormal DL_{CO} < 75%_{pred}.

8.2.2 Spirometry, Plethysmography and other Tests

Spirometry was performed using an EasyOne spirometer (ndd Medizintechnik AG, Zurich, CH) according to ATS guidelines. Lung volumes were measured using body plethysmography and DL_{CO} was assessed using the attached gas analyzer (MedGraphics Corporation. 350 Oak Grove Parkway St. Paul, MN, USA). The St Georges Respiratory Questionnaire (SGRQ) was administered and a standard six minute walk test (6MWT) was performed.

8.2.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla Discovery 750MR (General Electric Health Care, Milwaukee, WI) MRI system. \(^{22}\) \(^{3}\)He gas was polarized to 30—40% (HeliSpin™) and doses (5mL/kg body weight) were administered in 1.0L Tedlar® bags diluted with medical grade nitrogen (N\(_2\)) (Linde, Ontario, Canada). \(^{3}\)He MRI diffusion-weighted images were acquired using a fast gradient-recalled-echo sequence immediately following inhalation of the \(^{3}\)He/N\(_2\) gas mixture during breath-hold conditions. \(^{22}\) Two interleaved images were acquired (14s total data acquisition, repetition time (TR)/echo
time (TE)/flip angle = 7.6 ms/3.7 ms/8°, field of view (FOV) = 40 x 40 cm, matrix 128 x 128, 7 slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitization with \( b = 1.6 \text{ s/cm}^2 \) (gradient amplitude (G) = 1.94 G/cm, rise and fall time = 0.5 ms, gradient duration = 0.46 ms, diffusion time = 1.46 ms).

CT was performed on a 64-slice Lightspeed VCT scanner (GEHC, Milwaukee, WI USA) (64×0.625 mm, 120 kVp, 100 effective mA, tube rotation time=500 ms, pitch=1.0). A single spiral acquisition was acquired in breath-hold after inhalation of 1.0L of N\(_2\) from functional residual capacity. Reconstruction was performed (1.25 mm) using a standard convolution kernel.

To minimize the potential for differences in the levels of inspiration between \(^3\)He MRI and CT, extensive coaching was performed prior to the imaging sessions to ensure subjects could completely inspire the contents of the 1.0L bag. The order of \(^3\)He MRI and CT acquisition was randomized for each subject.

### 8.2.4 Image Analysis

Regions of signal void were quantified as the \(^3\)He ventilation defect percent (VDP). \(^3\)He apparent diffusion coefficient (ADC) maps were also generated as previously described. Regional differences in ADC were evaluated in the anterior-posterior (AP) direction. The AP gradient (AP\(_g\)) was the slope of the line of best fit that described the change in ADC as a function of distance in centimeters. Analysis of CT was performed using the Pulmonary Workstation 2.0 (VIDA Diagnostics, Inc., Coralville, IA). Wall area percent (WA\%) was measured for the segmental and subsegmental airways and the relative area with attenuation values below −950 HU (RA\(_{950}\)) was generated.

### 8.2.5 Statistical Methods

A multivariate analysis of variance was performed using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Univariate comparisons were performed using an unpaired two-tailed t-test and Welch’s correction was used when the F-test for equal variances was significant using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego, CA, USA). A Fisher’s exact test was performed for categorical variables. Linear regression
(r^2) and Pearson correlation coefficients (r) were used to determine correlations using GraphPad Prism version 4.00. Results were considered significant when the probability of making a Type I error was less than 5% (p < 0.05).

8.3 Results

We enrolled 53 ex-smokers, 38 subjects without a diagnosis of COPD and 15 subjects diagnosed with stage I COPD. Of the 38 ex-smokers without COPD, half had normal DL_Co without airflow obstruction (ND, n=19) and the other half had abnormal DL_Co without airflow obstruction (AD, n=19). Table 8-1 shows subject demographic data as well as the pulmonary function, SGRQ, 6MWD, CT and 3He MRI measurements for all subjects categorized according to spirometry and DL_Co results.

Subjects with abnormal DL_Co without airflow obstruction (AD) were not significantly different from ex-smokers with normal DL_Co (ND) and stage I COPD subjects with respect to age, BMI, pack-years, years since smoking cessation, the change in SpO_2 after the 6MWT, CT WA% and 3He VDP. However, there were significantly more female AD subjects than ND (p=.02) and stage I COPD (p=.01) subjects.

Figure 8-1 shows the central coronal 3He MRI static ventilation image and 3He MRI ADC map for subjects with ND, AD and stage I COPD. As shown in Table 8-1, AD subjects had significantly worse 3He ADC (0.30±0.03cm^2/s, p=.01), 6MWD (341±95m, p=.008) and SGRQ total score (29±21, p=.01) compared with ND subjects, but no significant difference for RA950 (p=.53). In comparison with stage I COPD, AD subjects had a significantly reduced 6MWD (341±95m, p=.005), FVC (93±12%pred, p=.001), RA950 (1.6±1.1, p=.0008) and ADC (0.30±0.03cm^2/s, p=.02), and a significantly greater FEV1/FVC (80±7%, p<.0001) and no significant difference for SGRQ total score (p=.59).

Figure 8-2A shows the mean ADC on a slice-by-slice basis in the anterior to posterior direction for ND, AD and stage I COPD subjects. For AD ex-smokers, the ADC gradient in the anterior-posterior direction (ADC AP_G) was significantly lower than for ND (p=.02) and not significantly different from COPD subjects (p=.20). Figure 8-2B shows the significant correlation between ADC AP_G and the 6MWD (r=-.51, p=.0002).
Figure 8-3 shows the correlations between $^{3}$He ADC and CT RA_{950} with DL_{CO}, SGRQ and 6MWD. There was a significant correlation between $^{3}$He ADC and DL_{CO} ($r=-0.55$, $p<0.0001$) and SGRQ ($r=0.34$, $p=0.02$), but not 6MWD ($r=-.17$, $p=0.24$), and as shown in Figure 8-2B, the ADC AP_{G} was significantly correlated with 6MWD. RA_{950} was significantly correlated with DL_{CO} ($r=-0.31$, $p=0.03$); but not SGRQ ($r=0.24$, $p=0.10$); or 6MWD ($r=0.0013$, $p=0.99$).
Table 8-1  Clinical, functional and radiographic measurements of asymptomatic ex-smokers with normal DL\textsubscript{CO} and abnormal DL\textsubscript{CO} and with GOLD stage I COPD

<table>
<thead>
<tr>
<th>ND (n=19)</th>
<th>AD (n=19)</th>
<th>Stage I COPD (n=15)</th>
<th>Significance of Difference (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age yrs (±SD)</td>
<td>71 (7)</td>
<td>74 (7)</td>
<td>77 (5)</td>
</tr>
<tr>
<td>Female Sex</td>
<td>3</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>BMI kg/m\textsuperscript{2} (±SD)</td>
<td>29.5 (3.4)</td>
<td>28.6 (4.0)</td>
<td>28.4 (4.0)</td>
</tr>
<tr>
<td>Pack yrs (±SD)</td>
<td>25 (12)</td>
<td>32 (23)</td>
<td>49 (36)</td>
</tr>
<tr>
<td>Yrs since quit (±SD)</td>
<td>26 (9)</td>
<td>24 (14)</td>
<td>21 (14)</td>
</tr>
<tr>
<td><strong>Pulmonary Function Tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1} %\textsubscript{pred} (±SD)</td>
<td>107 (13)</td>
<td>99 (12)</td>
<td>95 (13)</td>
</tr>
<tr>
<td>FVC %\textsubscript{pred} (±SD)</td>
<td>98 (12)</td>
<td>93 (12)</td>
<td>108 (14)</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC (±SD)</td>
<td>80 (6)</td>
<td>80 (7)</td>
<td>63 (5)</td>
</tr>
<tr>
<td>IC %\textsubscript{pred} (±SD)</td>
<td>112 (17)</td>
<td>103 (22)</td>
<td>103 (17)</td>
</tr>
<tr>
<td>RV %\textsubscript{pred} (±SD)</td>
<td>103 (17)</td>
<td>107 (25)</td>
<td>114 (29)</td>
</tr>
<tr>
<td>TLC %\textsubscript{pred} (±SD)</td>
<td>101 (10)</td>
<td>101 (15)</td>
<td>109 (13)</td>
</tr>
<tr>
<td>RV/TLC %\textsubscript{pred} (±SD)</td>
<td>101 (13)</td>
<td>104 (16)</td>
<td>103 (18)</td>
</tr>
<tr>
<td>DL\textsubscript{CO} %\textsubscript{pred} (±SD)</td>
<td>89 (9)</td>
<td>59 (13)</td>
<td>68 (19)</td>
</tr>
<tr>
<td><strong>6MWT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre 6MWT Sp\textsubscript{O\textsubscript{2}} % (±SD)</td>
<td>97 (2)</td>
<td>95 (2)</td>
<td>95 (2)</td>
</tr>
<tr>
<td>Δ 6MWT Sp\textsubscript{O\textsubscript{2}} % (±SD)</td>
<td>0 (2)</td>
<td>0 (2)</td>
<td>-1 (3)</td>
</tr>
<tr>
<td>Distance m (±SD)</td>
<td>430 (99)</td>
<td>341 (95)</td>
<td>417 (41)</td>
</tr>
<tr>
<td><strong>SGRQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms (±SD)</td>
<td>18 (17)</td>
<td>36 (30)</td>
<td>36 (22)</td>
</tr>
<tr>
<td>Activity Score (±SD)</td>
<td>19 (21)</td>
<td>41 (24)</td>
<td>36 (25)</td>
</tr>
<tr>
<td>Impact Score (±SD)</td>
<td>6 (11)</td>
<td>17 (18)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Total Score (±SD)</td>
<td>12 (14)</td>
<td>29 (21)</td>
<td>25 (17)</td>
</tr>
<tr>
<td><strong>CT Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA\textsubscript{950} (±SD)</td>
<td>1.36 (1.25)</td>
<td>1.60 (1.06)</td>
<td>5.50 (3.16)</td>
</tr>
<tr>
<td>WA% (±SD)</td>
<td>57 (4)</td>
<td>59 (2)</td>
<td>58 (2)</td>
</tr>
<tr>
<td><strong>3He MRI Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC cm\textsuperscript{2}/s (±SD)</td>
<td>0.27 (0.03)</td>
<td>0.30 (0.03)</td>
<td>0.36 (0.08)</td>
</tr>
<tr>
<td>VDP % (±SD)</td>
<td>6 (3)</td>
<td>7 (4)</td>
<td>9 (5)</td>
</tr>
</tbody>
</table>

ND=Normal \textsubscript{DLCO}, AD=Abnormal \textsubscript{DLCO}, COPD=Chronic Obstructive Pulmonary Disease, BMI=Body Mass Index, FEV\textsubscript{1}=Forced Expiratory Volume in 1sec, FVC=Force Vital Capacity, IC=Inspiratory Capacity, RV=Residual Volume, TLC=Total Lung Capacity, DL\textsubscript{CO}=Diffusion capacity of the Lung for Carbon monoxide, 6MWT=6-min Walk Test, Sp\textsubscript{O\textsubscript{2}}=Peripheral Oxygen Saturation, SGRQ=St. George’s Respiratory Questionnaire, RA\textsubscript{950}=Relative Area with attenuation values below −950 HU, HU\textsubscript{15}=lowest 15\textsuperscript{th} percentile from the frequency distribution of HU, WA%=Wall area percent, ADC=Apparent Diffusion Coefficient, VDP=Ventilation defect percent. *n=16, ‡n=17, ¥n=18, †n=14.
Figure 8-1 $^3$He MRI static ventilation images and $^3$He ADC maps for a representative ND and two representative AD and COPD stage I ex-smokers.

ND subject is a 70 yr old male FEV$_1$=101%$_{\text{pred}}$, FEV$_1$/FVC=0.75, DLCO=113%$_{\text{pred}}$, $^3$He ADC=0.26 cm$^2$/s, CT RA$_{950}$=1.25. AD subject 1 is a 74 yr old male FEV$_1$=89%$_{\text{pred}}$, FEV$_1$/FVC=0.77, DLCO=41%$_{\text{pred}}$, $^3$He ADC=0.31 cm$^2$/s, CT RA$_{950}$=1.52; AD subject 2 is a 74 yr old male FEV$_1$=95%$_{\text{pred}}$, FEV$_1$/FVC=0.85, DLCO=63%$_{\text{pred}}$, $^3$He ADC=0.29 cm$^2$/s, CT RA$_{950}$=0.52. GOLD stage I COPD subject 1 is a 74 yr old male FEV$_1$=86%$_{\text{pred}}$, FEV$_1$/FVC=0.59, DLCO=45%$_{\text{pred}}$, $^3$He ADC=0.37 cm$^2$/s, CT RA$_{950}$=6.14; GOLD stage I COPD subject 2 is a 78 yr old male FEV$_1$=118%$_{\text{pred}}$, FEV$_1$/FVC=0.62, DLCO=71%$_{\text{pred}}$, $^3$He ADC=0.38 cm$^2$/s, CT RA$_{950}$=5.52.
Figure 8-2  Regional $^3$He MRI ADC Anterior-Posterior Gradients ($\Delta P_G$) for ND, AD and stage I COPD subjects and Correlation between $^3$He ADC $\Delta P_G$ with 6MWD

A. Mean $\Delta P_G$ was statistically significantly different for AD and ND subjects (AD: $\Delta P_G$=-3.55x10$^{-4}$ ± 4.85x10$^{-4}$ cm$^2$/s/cm, ND: $\Delta P_G$= -7.03x10$^{-4}$ ± 3.03x10$^{-4}$ cm$^2$/s/cm, p=0.02), but not between the AD and Stage I COPD subjects (COPD: $\Delta P_G$=-5.58x10$^{-4}$ ± 3.73x10$^{-4}$ cm$^2$/s/cm, p=0.20). Error bars represent the ADC standard deviation for each image slice.

B. $^3$He $\Delta P_G$ ADC was significantly correlated with 6MWD ($r$=-0.51, $p$=0.0002, $r^2$=0.26, $p$=0.0002, $y$=-0.02x+4.4). Dotted lines represent the 95% confidence intervals of the regression.
Figure 8-3 Correlation between $^3$He ADC and CT RA$_{950}$ with D$_{LCO}$, SGRQ and 6MWD for ND, AD and stage I COPD subjects.

A. $^3$He ADC was significantly correlated with D$_{LCO}$ ($r=-0.55$, $p<0.0001$, $r^2=0.31$, $p<0.0001$, $y=-0.0018x+0.44$), SGRQ ($r=0.34$, $p=0.02$, $r^2=0.12$, $p=0.02$, $y=0.0012x+0.28$), but not 6MWD ($r=-0.17$, $p=0.24$, $r^2=0.03$, $p=0.24$, $y=-0.00013x+0.36$).

B. CT RA$_{950}$ was significantly correlated with D$_{LCO}$ ($r=-0.31$, $p=0.03$, $r^2=0.09$, $p=0.02$, $y=-0.040x+5.42$), but not with SGRQ ($r=0.24$, $p=0.10$, $r^2=0.06$, $p=0.10$, $y=-0.034x+1.71$) and 6MWD ($r=0.0013$, $p=0.99$, $r^2<0.0001$, $p=0.99$, $y=0.00003x+2.5$).

Dotted lines represent the 95% confidence intervals of the regression.
8.4 Discussion

To better understand the relationship between lung structural markers, symptoms and physiological measurements in ex-smokers, we evaluated 53 ex-smokers including 38 subjects who did not have a diagnosis of COPD and 15 subjects with stage I COPD, and observed: 1) 19/38 ex-smokers showed normal spirometry and CT but abnormal DL\textsubscript{CO} and 19/38 ex-smokers showed normal spirometry, CT and DL\textsubscript{CO}, 2) subjects with abnormal DL\textsubscript{CO} had significantly worse 6MWD compared to stage I COPD ex-smokers and significantly worse \textsuperscript{3}He ADC, SGRQ and 6MWD compared to subjects with normal DL\textsubscript{CO}, and, 3) subjects with abnormal DL\textsubscript{CO} had significantly smaller \textsuperscript{3}He MRI ADC anterior-posterior gradients compared to subjects with normal DL\textsubscript{CO}.

We were surprised that half of the ex-smokers without COPD showed abnormal DL\textsubscript{CO} and significantly worse \textsuperscript{3}He ADC, but with normal CT, which based on previous studies,\textsuperscript{14;15} was an unexpected result. Although we were not able to confirm significant disease other than emphysema that could account for these findings, we note that a previous evaluation\textsuperscript{14} of 10 younger asymptomatic smokers (mean age=47 years, range=23-73) showed that 3 of 5 subjects aged 60yr or older also reported DL\textsubscript{CO} < 75\%\textsubscript{pred}. In ex-smokers, abnormal DL\textsubscript{CO} is thought to reflect diminished lung surface area available for gas exchange, although DL\textsubscript{CO} also reflects the volume of blood in the pulmonary capillaries and thickness of the alveolar capillary membrane,\textsuperscript{27} related to bronchiectasis and interstitial lung disease.\textsuperscript{28} Abnormally low DL\textsubscript{CO} is also consistent with pulmonary vascular disease,\textsuperscript{29} and such patients exhibit normal spirometry, dyspnea upon exertion\textsuperscript{30} and a decline in oxygen saturation with exertion.\textsuperscript{31} In the current study, AD subjects did not show reduced oxygen saturation during the 6MWT nor did they report a history of pulmonary vascular disease, so there was no evidence to support the notion that pulmonary vascular disease was responsible for the abnormal exercise performance and dyspnea observed here. Although DL\textsubscript{CO} is a very sensitive marker of emphysema in smokers,\textsuperscript{8} reproducibility can be low, and in some cases, low to moderate correlations have been reported between DL\textsubscript{CO} and pathological assessments of emphysema.\textsuperscript{32;33}
Previous work by Woods and Hogg\textsuperscript{34} compared \textsuperscript{3}He ADC with histology measurements of emphysema in explanted lungs and showed that ADC values could be used to distinguish normal from emphysematous lung tissue with greater precision than the mean linear intercept measurement from histology samples. Another previous study in COPD showed that while \textsuperscript{3}He ADC correlated significantly with CT measurements (i.e., RA\textsubscript{950}), stronger correlations were observed for \textsuperscript{3}He ADC and DL\textsubscript{CO} than for RA\textsubscript{950} and DL\textsubscript{CO}.\textsuperscript{35}

In asymptomatic smokers, \textsuperscript{3}He ADC was shown to correlate with DL\textsubscript{CO}, but there was no significant correlation between DL\textsubscript{CO} and CT RA\textsubscript{950}.\textsuperscript{14} Finally, abnormally elevated \textsuperscript{3}He ADC values were previously observed in never-smokers exposed to significant second-hand-smoke\textsuperscript{36} as compared to never-smokers with no such exposure. Taken together, these previous findings support the observation here that elevated \textsuperscript{3}He ADC in ex-smokers with abnormal DL\textsubscript{CO} may reflect mild emphysema not detected by CT. Our observations are also consistent with previous reports\textsuperscript{5-8,37} and the identification of mild emphysema using histology that was not predicted using preoperative CT.\textsuperscript{38,39} While we cannot rule out the presence of small airways disease in subjects with AD, there was no significant difference between the AD and ND subjects for \textsuperscript{3}He VDP and CT WA\textsubscript{p}, both of which provide estimates of airways disease. Taken together, these results suggest that \textsuperscript{3}He ADC is sensitive to very mild emphysema in subjects with abnormal DL\textsubscript{CO} who have no CT evidence of airways disease or emphysema.

Concomitant with significantly elevated \textsuperscript{3}He ADC, we observed significantly worse 6MWD in AD as compared to COPD and ND ex-smokers. This is an important finding and the first to provide evidence of a relationship between \textsuperscript{3}He MRI ADC reflective of early or mild emphysema and exercise capacity. It is also important to note that the ratio of female/male ex-smokers with AD was 11/8 (1.4) and for ND, this ratio was 3/16 (0.2). Although the current study was not powered to evaluate sex differences, previous evidence suggests that female sex is significantly associated with early-onset COPD.\textsuperscript{40,41} However, previous studies have also shown that emphysema dominates in males as compared to females,\textsuperscript{42} whereas here, the sex ratio was reversed. We note that imaging was performed at a fixed volume and because there were more females in the AD group (who potentially had smaller lungs), we investigated the relationship between lung size and \textsuperscript{3}He ADC and observed no correlation for \textsuperscript{3}He ADC with height (r=-.36, p=.18), TLC
(r=.33, p=.21) or thoracic cavity volume (r=-.20, p=.45). Therefore the elevated ADC in the AD subjects observed here was not related to lung size, and cannot explain the preponderance of female subjects in the AD subgroup. Consistent with our findings, the 6MWD in COPD was also previously shown to be lower for FEV$_1$-matched females versus males.$^{43}$

We took advantage of the fact that $^3$He MRI diffusion-weighted images were acquired in the supine position and measured compression of the dependent lung due to gravity. Several sites have reported smaller $^3$He ADC in the dependent lung (or posterior slices) relative to the non-dependent lung,$^{25,44,45}$ likely due to gravitational compression of the parenchyma. In COPD subjects,$^{25,45}$ this anterior-to-posterior difference is significantly smaller and this is thought to be due to regional gas trapping that counteracts gravitational compression of the dependent regions. Here, we observed that these gradients were significantly smaller in AD subjects compared to ND subjects suggesting that regional gas trapping was greater in the AD subgroup.

Finally, we showed that $^3$He ADC was significantly correlated with SGRQ and that $^3$He ADC AP gradients were significantly correlated with the 6MWD. The significant relationships between $^3$He ADC with respiratory symptoms and exercise capacity suggest that in early emphysema, symptomatic changes can go unnoticed in older patients even when standardized tests report significant changes in health-related quality of life and exercise capacity. While elevated $^3$He ADC in asymptomatic ex-smokers was previously described,$^{14,15}$ the imaging-to-exercise capacity and imaging-to-symptoms correlations observed here in very early emphysema are novel findings. The unexpected finding of $^3$He ADC anterior-posterior gradient correlations with 6MWD also provides more evidence about the role of mild emphysema and regional gas trapping that may together lead to exercise limitation even in early disease. AD ex-smokers also reported SGRQ that was not significantly different from the stage I COPD ex-smokers, and worse than ND subjects, which supports previous reports of compromised health-related quality of life$^{46}$ and reduced work capacity in very early disease.$^{47}$
This study was limited by the relatively small number of subjects evaluated, although we note that this is the single largest prospective study that directly compared CT, symptoms, exercise capacity and $^3$He MRI in ex-smokers with and without airflow obstruction. We admit that we were surprised to find such a large proportion of asymptomatic ex-smokers without airflow limitation and abnormal DL$_{CO}$ in this study. This finding raises the important question as to whether this subgroup is atypical or perhaps this is a unique finding because “asymptomatic” ex-smokers are rarely administered SGRQ or the 6MWT. Importantly, the selection criteria, manner and location for subject recruitment are those we have previously used for the recruitment of older ex-smokers, and typical of other studies. It is possible that in this unique subgroup, patients were less likely to recognize and report symptoms. Our results certainly raise many intriguing questions regarding whether these subjects are unusual or whether we have simply uncovered a group of older ex-smokers with both unrecognized mild emphysema and functional limitations.

In summary, we evaluated 38 ex-smokers without airflow limitation and 15 ex-smokers with COPD. In the absence of spirometry or CT abnormalities, half of the ex-smokers without COPD showed abnormal DL$_{CO}$ and abnormally elevated $^3$He ADC consistent with early or mild emphysema. These subjects had significantly and markedly worse 6MWD and SGRQ compared to ex-smokers with normal ADC and DL$_{CO}$, and worse 6MWD than subjects with COPD. These findings provide a better understanding of abnormal DL$_{CO}$ in ex-smokers without COPD.
8.5 References


CHAPTER 9

9 Conclusions and Future Directions

In this final chapter, I will provide an overview and summary of the important findings and conclusions of Chapters 2-8. I will also address the study specific limitations as well as general limitations of the hyperpolarized gas MRI studies presented and provide some potential solutions. Finally, based on the findings and limitations discussed, I will outline a roadmap for future hyperpolarized gas MRI studies.

9.1 Overview and Research Questions

COPD is currently ranked the 4th leading cause of death worldwide,1 and despite the fact that the global burden of many other leading causes of deaths are declining,1 COPD remains largely under-diagnosed and under-treated.2,3 When COPD is suspected based on symptoms, such as dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors,4 airflow limitation is measured using spirometry and the presence of a post-bronchodilator FEV1/FVC <0.70 confirms the diagnosis of COPD.4,5 However, this approach is limited for several reasons. First, although it is utilized due to diagnostic simplicity and ease of use in the primary care setting, the fixed FEV1/FVC cut off has been acknowledged to potentially result in an over-diagnosis of COPD in the elderly,6 whom are expected to have a decline in lung function due to the normal aging process.7 Second, spirometry may be insensitive to mild or early disease changes.8 It is well known that the airways < 2mm in diameter are the major site of airway obstruction in COPD,9 and therefore disease may accumulate and progress to more severe states before detection with FEV1. Third, chronic airflow limitation in COPD may be caused by small airways disease (obstructive bronchiolitis) or parenchyma destruction (emphysema) or some mixture of both of these underlying disease mechanisms. Therefore, FEV1 alone does not provide any information regarding the underlying disease mechanism responsible for the lung function impairment. Finally, FEV1 measures the overall disease burden, and does not provide any information regarding which regions of the lung are most severely affected. Clearly, it is important to identify the disease in its earliest possible stages for smoking cessation interventions to slow disease progression.
to identify the underlying disease mechanisms and target the regional disease. Therefore, it is because of these limitations that there is a continued need for the development of sensitive imaging approaches for regional and surrogate measurements of COPD.

Chest x-ray was the first imaging modality introduced for the evaluation of COPD and although it is widely available, inexpensive, and allows visualisation of pulmonary structures, because chest x-ray is a planar imaging modality, it is not useful to establish a diagnosis of COPD and has been shown to be insensitive to early disease stages where disease-related structural changes may be subtle. Although there is no question that CT is superior to conventional x-ray imaging because it provides high resolution anatomical images and in three-dimensions, it provides no information regarding lung function and the radiation exposure from thoracic CT has been a major concern. Although CT is not routinely recommended, it is useful in the differential diagnosis in COPD where concomitant diseases are present. Currently, the only clinical means for imaging lung ventilation is with lung scintigraphy, however, it is limited by the need to inhale a radioactive substance and has low spatial resolution. Therefore, a high resolution imaging method that does not involve the use of ionizing radiation that can evaluate both lung structure and function is needed.

MRI with the inhaled hyperpolarized gases $^3$He and $^{129}$Xe provides high resolution images of pulmonary structure and function and does not require the use of x-rays or other ionizing radiation. The overarching objective of this thesis was to generate hyperpolarized gas MRI measurements and measurement tools for the regional quantitative evaluation of hyperpolarized gas MRI with sufficient precision to evaluate COPD disease progression and treatment response. The specific research questions investigated here included: 1) Are longitudinal changes in $^3$He MRI measurements in the COPD ex-smokers similar to those observed in elderly healthy never-smokers (Chapter 2)?; 2) Can $^3$He MRI measurements derived from semi-automated segmentation methods be developed that have significantly reduced inter- and intra-observer variability than manual measurements and are not statistically significantly different from manual measurements (Chapter 3)?; 3) Can the semi-automated $^3$He MRI measurements developed in Chapter 3 provide the necessary sensitivity as well as precision to detect any
potential regional functional lung changes after bronchodilator therapy in COPD subjects regardless of spirometry-based responder classification (Chapter 4)?; 4) Are the regions of the lung that were newly ventilated following bronchodilator therapy in the same subjects evaluated in Chapter 4 more emphysematous than the remaining lung tissue, and can $^3$He ADC measure significant reductions in regional gas trapping following bronchodilator therapy (Chapter 5)?; 5) Do the different properties of $^{129}$Xe gas result in significant differences in $^{129}$Xe compared to $^3$He gas distribution measurements in COPD (Chapter 6)?; 6) Are the greater ventilation abnormalities for $^{129}$Xe compared to $^3$He MRI in COPD observed in Chapter 6 spatially correlated to emphysema (Chapter 7)?; and, 7) Do ex-smokers with normal spirometry but abnormal DL$_{CO}$ have significantly worse symptoms, exercise capacity and $^3$He MRI ADC than ex-smokers with normal DL$_{CO}$?

9.2 Summary and Conclusions

In Chapter 2 we quantitatively evaluated a small group of 15 COPD ex-smokers and five healthy volunteers over 2 years using hyperpolarized $^3$He MRI. For COPD subjects, significant increases in $^3$He VDP and ADC were detected whereas there was no significant change in FEV$_1$; healthy never-smokers showed no significant change in imaging or pulmonary function measurements at follow-up. This finding suggests that regional $^3$He MRI measurements provide adequate sensitivity for detecting COPD changes over relatively short time periods, before significant changes in FEV$_1$ can be detected.

In Chapter 3 we aimed to improve intra- and inter-observer variability of $^3$He MRI segmentation by developing a semi-automated segmentation method. The objective of this work was to compare the reproducibility of manual and semi-automated segmentation of $^3$He MRI ventilation measurements in subjects with a range of respiratory conditions (i.e., asthma, COPD and cystic fibrosis). In 15 subjects we found that manual measurements and semi-automated measurements of $^3$He VDV were not significantly different and were strongly and significantly correlated. The semi-automated VDV had high inter-observer reproducibility and intra-observer reproducibility was significantly higher for semi-automated compared to manual measurements. These
findings indicate that $^3$He MRI semi-automated segmentation provides excellent inter-/intra-observer precision with high quantitative agreement with manual measurements enabling its use in longitudinal and serial studies.

In Chapter 4 we evaluated a group of 14 COPD subjects using the $^3$He MRI semi-automated measurements developed in Chapter 3 prior to and immediately following bronchodilator therapy. Post-salbutamol, we observed significant improvements in $^3$He gas distribution measurements for all subjects. Although five subjects were classified as bronchodilator responders and nine subjects were bronchodilator non-responders according to ATS/ERS criteria, there was no significant difference in the magnitude of the $^3$He MRI changes post-salbutamol between responder groups. These results indicate that significant gas distribution improvements occur even in COPD subjects with minimal FEV$_1$ or FVC response to bronchodilator therapy which is relevant to our understanding of the regional and functional effects of bronchodilators.

In Chapter 5 we evaluated the same group of COPD ex-smokers evaluated using $^3$He MRI pre- and post-bronchodilator therapy in Chapter 4 using $^3$He MRI diffusion-weighted imaging. Using image registration/segmentation methods developed for quantifying ADC regionally, we found that the newly ventilated lung regions did not have significantly elevated ADC compared to the lung regions participating in ventilation before bronchodilator administration. We also measured statistically significant differences between ADC in the most anterior and most posterior image slices post-salbutamol, suggesting a reduction in regional gas trapping following bronchodilator therapy. Regional evaluation of tissue microstructure using hyperpolarized $^3$He MRI ADC provides insights into lung alterations that accompany improvements in regional $^3$He gas distribution after bronchodilator administration.

In Chapter 6 we quantitatively compared hyperpolarized $^3$He and $^{129}$Xe MRI acquired within a few minutes in 8 healthy volunteers and 10 subjects with COPD, and evaluated the correlations between $^3$He and $^{129}$Xe MRI measurements with standard measurements of pulmonary function. We found that there were significant and strong correlations between $^{129}$Xe and $^3$He VDP for all subjects, however, for the COPD subjects $^{129}$Xe VDP
was significantly greater than $^3$He VDP. We also found there were similar and strong correlation for $^{129}$Xe and $^3$He VDP with FEV$_1$ and for $^{129}$Xe and $^3$He ADC with DL$_{CO}$. These findings suggest that the differences between $^{129}$Xe and $^3$He MRI VDP in COPD may reflect differences in the properties of the gases and physiological/anatomical abnormalities in COPD that are not seen in healthy volunteers.

In Chapter 7 we aimed to better understand the morphological determinants for the ventilation differences observed between hyperpolarized $^3$He and $^{129}$Xe MRI in COPD found in Chapter 6 using $^3$He MRI diffusion-weighted imaging. In the same 10 COPD ex-smokers in Chapter 6 and using the image registration/segmentation methods for quantifying ADC on a regional basis developed in Chapter 5, we found that the mean ADC in lung regions accessed by $^3$He gas only were statistically significantly greater than the mean ADC for lung regions accessed by both gases. We also found that the difference between $^3$He and $^{129}$Xe ventilation was significantly correlated with the extent of emphysema measured using CT. These findings indicate that the regions of decreased $^{129}$Xe ventilation were spatially and significantly correlated with regions of increased pulmonary emphysema. Therefore, it is most likely that the highly diffusive and less dense and viscous $^3$He gas may readily access the slower-filling emphysematous lung than $^{129}$Xe gas during the inhalation period and the breath-hold interval.

In Chapter 8 we aimed to evaluate well-established clinical, physiological and emerging imaging measurements in 38 ex-smokers without airflow limitation and compared them with 15 ex-smokers with COPD. In the absence of spirometry or CT abnormalities, half of the ex-smokers without COPD showed abnormally elevated $^3$He ADC and abnormal DL$_{CO}$ consistent with early or mild emphysema. These subjects had significantly and markedly worse exercise capacity and symptoms compared with ex-smokers with normal ADC and DL$_{CO}$, and worse exercise capacity than the subjects with COPD. These novel findings provide evidence for the impact of mild or early stage emphysema and a better understanding of abnormal DL$_{CO}$ and hyperpolarized $^3$He MRI in ex-smokers without COPD.
9.3 Limitations

In this section I will discuss some of the most significant limitations of the $^3$He MRI studies presented in Chapters 2-8, although the more detailed description of the study limitations were presented within the Chapters. I will also outline how these limitations may be overcome in future studies. Finally, I will end this section by describing the more general limitations common to each of these Chapters and introduce work that has yet to be done to overcome these limitations.

9.3.1 Study Specific Limitations

In the longitudinal study presented in Chapter 2, we did not acquire more established clinical measurements (ie., symptom scores and exercise tests) and therefore it is unknown whether these subjects had symptomatic worsening or a decline in exercise capacity over the two years that could be attributed to their disease progression. In addition, CT images were also not acquired and therefore our results could not be directly compared to more established CT measurements, such as the percentage of low attenuation pixels (LAA%) as a measurement of the extent of emphysema or WA% as a measurement of airway wall thickness. Clearly, a direct comparison of CT and hyperpolarized $^3$He MRI measurements in the same subjects would allow for a better understanding of the differences in measurement sensitivity these imaging modalities provide. Moreover, the relatively small group of COPD subjects and healthy never-smokers evaluated, and the relatively short period of follow-up certainly limits the applicability of our results. Also, the comparison of current smokers with COPD, ex-smokers with COPD and never-smokers may help to establish and directly compare rates of lung structural and functional decline. Another important limitation of this study was the use of manual segmentation approaches. Manual segmentation introduces inter- and intra-observer variability and therefore there is the potential for the changes measured over time to be due to the measurement error. Although in this study, two independent observers evaluated the measurements at the baseline and the 2 year follow-up time point and both demonstrated statistically significant changes, segmentation methods with high precision are required to ensure that the changes measured over time represent physiological changes. To overcome these limitations, larger studies of current and ex-
smokers with COPD and healthy never-smokers should be followed longitudinally over multiple time-points and evaluated using hyperpolarized gas MRI and CT with automated segmentation approaches, as well as with symptom scores (St. George’s Respiratory Questionnaire) and tests of functional capacity (6 minute walk test).

In Chapter 3, one of the important limitations of Chapter 2 was addressed. We developed a semi-automated $^3$He MRI segmentation algorithm that provided excellent inter-/intra-observer precision with high spatial and quantitative agreement with manual measurements enabling its use in longitudinal studies. However, this segmentation algorithm is not without its own limitations. First, the algorithm is not fully automated and therefore it requires some user intervention to remove the trachea as well as to manually select landmarks for registration. Although this segmentation method has clear advantages over manual segmentation, automating these steps would clearly improve the reproducibility of the technique and reduce segmentation time. One way to improve this technique is the simultaneous acquisition of $^3$He and $^1$H MRI, which would eliminate the registration step and improve reproducibility.

In Chapter 4 and 5 we evaluated COPD subjects pre- and post-bronchodilator and demonstrated significant improvements in gas distribution to regions of the lung that were not more emphysematous than the remaining lung participating in gas distribution before bronchodilator use, however we did not acquire more established clinical measurements to determine whether these improvements in gas distribution were related to improvements in symptoms or exercise capacity. Importantly, although $^3$He MRI in COPD has been shown to have high reproducibility for same-day repeat imaging as well as imaging over one week, the prospective inclusion of a control group would have strengthened the conclusion that the changes post-bronchodilator were directly attributable to bronchodilator treatment. Also, studies have previously demonstrated significant changes in CT airway morphology measured in COPD after administration of a bronchodilator, and therefore acquiring CT in these subjects would have provided support for these findings as well as provide a better understanding of the relative sensitivities these imaging modalities provide for detecting changes following treatment. Also, the relatively small group of COPD subjects and the fact that the analysis was
restricted mainly to subjects with stage II and III COPD certainly limits the applicability of our results. To overcome these limitations, larger studies evaluating COPD ex-smokers randomized to either a control (pre- and post-placebo) or treatment group (pre- and post-bronchodilator) should be performed using hyperpolarized gas MRI and CT as well as symptom scores and tests of exercise capacity.

Although Chapter 6 and 7 had the inclusion of CT measurements which were strengths of these studies, the acquisition of clinical measurements for direct comparison to $^3$He and $^{129}$Xe MRI measurements were not acquired and may have aided in determining the clinical meaning of the differences observed. Future studies should aim to evaluate the differences between $^3$He and $^{129}$Xe MRI in terms of their relationships with clinical measurements as well as the relative sensitivities for detecting improvements following treatment or interventions. Also, there is significant work to be done to better understand how the different properties of $^3$He and $^{129}$Xe gas affect ventilation distribution and most importantly, what information these differences in ventilation distributions provide us regarding the sensitivity of the different gases and their mixtures for detecting airway and ventilation abnormalities. Directly visualizing the distribution of the gases using mixtures of $^3$He and $^{129}$Xe with well-controlled viscosities and densities during a breath-hold in the same subjects using time-resolved imaging sequences, as was recently performed with $^3$He MRI, could certainly be utilized to test some of our important hypotheses regarding delayed/collateral ventilation within emphysematous lung regions in COPD subjects. These studies should be performed in a larger group of COPD subjects with varying stages of disease severity.

In Chapter 8, we prospectively included $^3$He MRI, CT, SGRQ and the 6MWT which was an important strength of this study. However, this study was limited by the relatively small number of subjects evaluated and due to the small subject number we were unable to evaluate sex differences. Larger studies evaluating subjects with abnormal and normal DL$_{CO}$ without airflow limitation is required to evaluate whether emphysema dominates in females in early disease and whether females are more susceptible to develop early on-set COPD. Moreover, it would be very important to longitudinally evaluate these subjects to determine whether the subjects with abnormal DL$_{CO}$ develop airflow limitation.
9.3.2 General Limitations

Although in the last chapter of this thesis we prospectively acquired CT, SGRQ and the 6MWT concurrently with $^3$He MRI, all of the previous studies in this thesis would have been strengthened by the inclusion of these more established measurements. Moreover, although $^3$He ADC and CT measurements have been directly compared and shown to be significantly correlated in COPD subjects\textsuperscript{14} and asymptomatic smokers\textsuperscript{15} there have been limited comparisons between $^3$He ventilation measurements and CT airway wall measurements in COPD\textsuperscript{16}. An important aim of future work should be to directly compare $^3$He MRI functional measurements and CT structural airway measurements in a large group of COPD subjects.

Another important limitation of this work is that we do not have a clear understanding of the etiology of noble gas MRI ventilation defects. It is hypothesized that $^3$He ventilation defects are caused by narrowed or constricted/occluded small airways, however, there is also the possibility that the ventilation abnormalities are due to mucous plugs. Previous studies have demonstrated $^3$He MRI ventilation defect measurements were highly reproducible over 7-days in COPD\textsuperscript{12} while poor $^3$He MRI reproducibility was shown in cystic fibrosis subjects over 7-days\textsuperscript{17} which was thought to be related to defective mucus clearance. $^3$He ventilation abnormalities in COPD may also be related to bullous disease or severe emphysema which would indicate that $^3$He ventilation defects reflect structural changes in the lung parenchyma rather than changes in the small airways. Therefore, investigating the etiology of hyperpolarized MRI ventilation defects is important for our understanding of what this measurement represents in COPD subjects.

9.4 Future Directions

9.4.1 Airway Morphology and Hyperpolarized $^3$He MRI Ventilation Defects

Although $^3$He MRI ventilation abnormalities in subjects with pulmonary disease has been shown to spatially correlate with ventilation abnormalities seen on xenon-133 scintigraphy\textsuperscript{18} and correlate significantly with spirometric measurements of airflow limitation,\textsuperscript{19} we still do not have a clear understanding of the etiology of noble gas MRI
ventilation defects. Depending on the respiratory disease, $^3$He ventilation defects could be caused by narrowed or constricted/occluded small airways, mucous plugs, bullous disease, severe emphysema or any combinations of these pathologies. Therefore, investigating the etiology of hyperpolarized MRI ventilation defects is important for our understanding how this measurement can be used to monitor disease progression and response to treatment.

Clearly, investigating the etiology of hyperpolarized gas MRI ventilation defects will require direct comparison with histology to fully uncover the underlying pathologies. Studies performed by Jason Woods and Jim Hogg$^{20}$ have previously compared the $^3$He ADC with histology in explanted human lungs. Explanted lungs from patients who underwent lung transplantation for advanced COPD and donor lungs that were not used for transplantation were evaluated using $^3$He diffusion-weighted MRI and histology. Woods and colleagues$^{20}$ showed that the $^3$He ADC was significantly correlated with histology measurements (mean linear intercept and the surface area to lung volume ratio). Importantly, normal control lungs could be more clearly separated from emphysematous lungs using the $^3$He ADC compared to histology measurements, suggesting the $^3$He ADC is very sensitive to mild and moderate emphysematous tissue destruction. Studies of this nature are required comparing $^3$He static ventilation imaging and histology in the ventilation defect regions as well as the regions of hypointense signal. In addition, it would be useful to evaluate the airway dimensions using histology in airways proximal to $^3$He ventilation abnormalities to confirm that $^3$He ventilation defects are due to narrowed or constricted/occluded small airways.

However, before comparison between $^3$He MRI and histology is performed, comparisons between $^3$He MRI functional airway measurements (i.e., VDP) and CT structural airway measurements (i.e., WA%) may begin to tease apart the contributions of these different underlying disease changes. Although in COPD, $^3$He ventilation defects may be caused by disease changes in either the lung parenchyma, the airways, or both, asthma is solely an airways disease. In the following sections I will outline research that could be performed using MRI-CT comparisons to better understand the etiology of $^3$He ventilation defects in asthma and COPD.
9.4.1.1 CT Airway Morphology and Hyperpolarized $^3$He MRI Ventilation Defects in Asthma

Asthma is a complex inflammatory disorder of the airways that is characterized by airway hyper-responsiveness and variable airflow obstruction. The primary consequence of airway inflammation in asthmatic patients is airway smooth muscle hyperplasia and/or hypertrophy.\textsuperscript{21} This airway smooth muscle hyperplasia and hypertrophy, as well as epithelial basement membrane thickening, mucous gland and goblet cell hyperplasia, are part of the airway remodeling process that occurs in asthma that contributes to airway wall thickening and to a decrease in lumen diameter.

There have been several studies performed in asthma using CT to investigate the relationship between airway remodeling with functional limitation.\textsuperscript{22-30} These studies have shown strong relationships between CT structural airway measurements with inflammation,\textsuperscript{22,23} spirometry,\textsuperscript{22-27} and air trapping.\textsuperscript{22,28-30} Hyperpolarized $^3$He MRI has also been used to visualize heterogeneous and abnormal gas distribution in asthma\textsuperscript{28,31} and $^3$He MRI gas distribution measurements were shown to significantly correlate with spirometry\textsuperscript{28,32,33} and disease severity.\textsuperscript{32,34}

Therefore, in asthma the hypothesis is that the functional abnormalities observed with $^3$He MRI are related to airways that are remodeled and/or constricted. Using CT, the percentage of the total airway that was airway wall (WA\%) and lumen area can be measured\textsuperscript{35} up to the 6\textsuperscript{th} generation airways.\textsuperscript{36} By registering hyperpolarized $^3$He MRI and CT acquired in the same asthmatic subjects at the same lung volume, the following research questions can be addressed:

1) In asthmatics following $^3$He MRI and CT registration, can the CT airways feeding the regions of the lung where $^3$He MRI ventilation defects are observed be identified?

2) Are the CT-derived airway wall measurements greater in asthmatics proximal to $^3$He MRI ventilation defects compared to non-asthmatics and asthmatics with no ventilation defects?
3) Is there a significant relationship between the extent of airway wall thickening measured using CT with the volume of the $^3$He MRI ventilation defects in asthma?

In a small proof-of-principle study of 18 asthmatics and 5 healthy volunteers, $^3$He MRI and CT in a region-of-interest (ROI) spatially related to $^3$He MRI ventilation defects was acquired, as shown in Figure 1. Since there were no ventilation defects for the healthy volunteers, the CT ROI was selected at random. CT analysis in the ROI showed that WA% was significantly greater ($p=.002$) and lumen area was significantly reduced ($p=.02$) for the asthmatics in comparison to the healthy volunteers. Moreover, the extent of airway wall thickening measured using CT was significantly correlated with the extent of ventilation abnormalities measured using $^3$He MRI ($r=.43$, $p=.04$). These findings provide a better understanding of the underlying airway spatial structure-function relationships in asthma and, importantly, suggest that there is a relationship between airways that are remodeled and/or constricted and $^3$He ventilation abnormalities. However, larger studies are required to confirm these important findings. Also, the acquisition of whole lung CT images will allow for registration of the three-dimensional airway tree to $^3$He MRI for identification of the specific airway that feeds the $^3$He MRI ventilation defect region. The identification of $^3$He MRI functional abnormalities and the airways responsible for these functional abnormalities may help guide therapy or airway interventions in asthmatic subjects, as well as aid in the evaluation of the airway interventions over time.

![Image](3He/1H MR Image Registration CT)

**Figure 9-1** CT and $^3$He MRI in Asthma

A partial CT (as shown registered to the $^3$He MR image) was acquired in a region of interest that was selected based on the location of hyperpolarized $^3$He MRI ventilation defects for quantification of structural abnormalities.
9.4.1.2 CT Airway Morphology and Hyperpolarized $^3$He MRI Ventilation Defects in COPD

As described above, and in contrast to asthma, $^3$He ventilation defects in COPD may be caused by narrowed or constricted/occluded small airways, mucous plugs, bullous disease, severe emphysema or any combinations of these pathologies. Much of our understanding of small airways disease is from the early studies by Hogg and Macklem\textsuperscript{9} that identified the small airways as the major site of airflow resistance in COPD. The authors observed that the bronchioles were often narrowed and occluded with mucus plugging and defined these changes in the small airways as “small airways disease.” More recently, Hogg and colleagues\textsuperscript{37} evaluated the pathology of the small airways in relation to COPD severity, as measured by the GOLD COPD stages.\textsuperscript{5} These authors found that although the degree to which the lumen was filled with mucous significantly correlated with COPD severity, the strongest association with progression of COPD from GOLD state 0 to GOLD stage IV was with thickening of the airway wall.\textsuperscript{37} Therefore, it is hypothesized that in COPD subjects there is a significant relationship between airway wall thickness measured using CT and $^3$He MRI ventilation abnormalities. Since bullous disease and emphysematous tissue destruction may also result in $^3$He gas distribution abnormalities in the lung, we hypothesize that the relationship between airway wall thickness measured using CT and $^3$He MRI ventilation abnormalities would be strongest in subjects without emphysema. By registering hyperpolarized $^3$He MRI and CT acquired in the same COPD subjects at the same lung volume, the following research questions can be addressed:

1) In COPD following $^3$He MRI and CT registration, can the CT airways feeding the regions of the lung where $^3$He MRI ventilation defects are observed be identified?

2) Are the CT-derived airway wall measurements greater in COPD subjects proximal to $^3$He MRI ventilation defects compared to COPD subjects with no ventilation defects in the same lung region?

3) Is there a significant relationship between the extent of airway wall thickening measured using CT with the volume of the $^3$He MRI ventilation defects in COPD?
4) *Is the relationship between airway wall thickness measured using CT and $^3$He MRI ventilation abnormalities stronger in COPD subjects without emphysema compared to COPD subjects with emphysema?*

As part of a larger longitudinal study of 200 ex-smokers with and without COPD, we performed a preliminary analysis in the first 21 subjects comparing the functional abnormalities measured using $^3$He MRI with CT structural airway measurements and found that there was a significant relationship between $^3$He MRI VDV with CT airway wall area (Aaw) for the 5th generation ($r=.72$, $p=.002$) and 6th generation ($r=.56$, $p=.03$) airways, as shown in Figure 2. The quantitative evaluation of $^3$He MRI functional measurements with CT structural measurements in this large group of COPD subjects with varying stages of COPD and with varying extents of emphysema will provide a better understanding of the underlying disease changes responsible for the $^3$He ventilation defects in COPD.

![Figure 9-2 Evaluation of CT and $^3$He MRI in COPD](image)

**Figure 9-2** Evaluation of CT and $^3$He MRI in COPD

A preliminary comparison of $^3$He MRI and CT in COPD subjects (A) showed a statistically significant correlation between $^3$He VDV and CT airway wall area (Aaw) for 5th ($r=.72$, $r^2=.52$, $p=.002$) and 6th ($r=.56$, $r^2=.31$, $p=.03$) generation airways (B).

**9.4.2 Hyperpolarized $^3$He MRI and CT COPD Phenotypes**

Although it is well known that the underlying disease mechanisms in COPD is small airways disease or emphysema, or some combination of both, patient-specific phenotyping continues to be a critical unmet clinical need that may help to guide patient therapy and management. Moreover, spirometry provides only a global measurement of
lung function and therefore the limitation of spirometry for differentiating these underlying pathologies or phenotypes has motivated the use of non-invasive imaging.

Previous studies have demonstrated that these emphysema and airways disease dominant phenotypes can be distinguished by measuring LAA% as a measurement of emphysema and the WA% as a measurement of airways disease.\textsuperscript{35} It has been shown that although the combination of CT emphysema and airways measurements had the strongest correlation with FEV\textsubscript{1}, both measurements were also independently correlated.\textsuperscript{35} However, thickening and narrowing of the small airways < 2 mm in diameter leads to the most important functional limitation in COPD,\textsuperscript{9} and CT is severely limited in that measurement of the small peripheral airways is not possible due to the CT resolution limits.

Hyperpolarized noble gas MR imaging also has the potential to distinguish these distinct underlying phenotypes. The $^3$He ADC has been shown to correlate significantly with histology measurements of emphysema in explanted lungs,\textsuperscript{20} and shown to correlate significantly with CT measurements and DL\textsubscript{CO} in COPD subjects\textsuperscript{14} and in asymptomatic smokers.\textsuperscript{15} Although the exact pathology underlying $^3$He ventilation defects has yet to be determined, as described above, significant correlations with xenon-133 scintigraphy\textsuperscript{18} and FEV\textsubscript{1}\textsuperscript{19} have been observed. It is therefore hypothesized that $^3$He ventilation defects reflect airflow limitation that is caused by small airways disease. Importantly, we have previously demonstrated in a small proof-of-principle study evaluating 20 subjects ranging from stage I to stage IV COPD evidence of either $^3$He VDP or ADC providing the main contribution to $^3$He MRI measured disease, or in other words, these COPD subjects showed evidence of having a single dominant disease phenotype.\textsuperscript{19} It was also suggested that the subjects with an imaging dominant phenotype showed decreased disease severity as measured by FEV\textsubscript{1}. This interesting finding suggests that subjects may develop one disease phenotype in the early disease stages and then progress to a mixed disease phenotype and more severe disease over time.

This very interesting hypothesis should be tested in a larger group of COPD subjects, ranging from stage I to stage IV disease. The acquisition of CT for these subjects would
also allow for the relationship between $^3$He MRI and CT measurements of airways and emphysema phenotypes to be directly compared. Importantly, the relationship between these imaging phenotypes with pulmonary function test measurements, symptom scores, and measurements of exercise tolerance would provide important information regarding the functional effect these underlying disease phenotypes have on the subject. Also, these subjects should be followed longitudinally to determine how and if phenotypic dominance occurs in early disease and progresses to more severe mixed disease over time.

9.4.3 Hyperpolarized $^3$He MRI Phenotypes of COPD: Relationship to Exacerbations

In Appendix B we reported serial hyperpolarized $^3$He MRI over a four year period in a 77 year old COPD ex-smoker prior to and following treatment for an acute exacerbation (AE) of COPD. In this case study, $^3$He MRI showed improvements in gas distribution prior to improvements reflected by FEV$_1$. Importantly, it was also suggested that this subject had a worsening of $^3$He gas distribution 6 months prior to the exacerbation, although there was no change in the subjects clinical status until approximately one month before hospitalization when the subject complained of symptoms. We suggested that $^3$He MRI may have reflected regional pulmonary decline before, or even in the absence of changes in FEV$_1$. From this case report several hypotheses and questions arose related to the time-course of COPD exacerbations that should be tested in a larger study that follows COPD subjects longitudinally. The research questions that should be tested are:

1) Is there a relationship between symptomatic and $^3$He MRI improvements following therapy, before changes in FEV$_1$ are measured?

2) Do COPD patients with specific imaging phenotypes have a propensity for increased exacerbation frequency?

In a study currently underway, we aimed to determine if subjects that experienced an AE prior to or following hyperpolarized $^3$He MRI had a greater propensity for a specific imaging phenotype in comparison to subjects that did not experience an AE, and whether
there was a relationship between these imaging measurements with symptoms and functional limitation. Based on the findings of the case report presented in Appendix B the demonstrated VDP worsening prior to an AE, we hypothesized that subjects that experienced an AE following the $^3$He MRI study would have significantly worse VDP than subjects that did not experience an AE. To date 94 COPD subjects have underwent $^3$He MRI, CT, spirometry/plethysmography, the St. George’s Respiratory Questionnaire (SGRQ) and the six-minute walk test (6MWT). We defined an AE as a worsening of COPD symptoms that required hospitalization and was determined by examining patient records for up to 2 years prior to or following the study visit. Subjects were divided into three groups: no AE, AE prior to study visit and AE following study visit. In this preliminary investigation we found that of the 94 COPD subjects evaluated, 8 subjects experienced a single AE 13±9 months prior to the study visit and 10 subjects experienced a single AE 7±8 months following the study visit. Interestingly, we showed those subjects that experienced an AE following the study visit reported a significantly worse SGRQ score (p=.05), 6MWD (p=.02) and $^3$He VDP (p=.02) than the subjects that did not experience an AE (Figure 3), lending support to the hypothesis that $^3$He VDP may worsen prior to exacerbations in COPD. However, the 2 year follow-up period is not complete for most of the COPD subjects evaluated and therefore it is important to continue to follow these subjects to determine the frequency of exacerbations as well as to determine if $^3$He MRI gas distribution improves following exacerbation treatment. This finding may have important implications for future COPD imaging studies evaluating the association between COPD phenotypes and exacerbation risk. Clearly, predicting those COPD patients that are at the greatest risk for acute exacerbations will enable the targeting of these individuals for preventive therapy, or at least more aggressive monitoring.
A major strength of MRI is that it does not require the use of ionizing radiation and provides regional structural and functional information simultaneously. This is important for all longitudinal studies of lung disease, but this is especially the case in children with chronic life-threatening pulmonary disease. Therefore, hyperpolarized noble gas MRI can be used to measure lung structure and function in young subjects and at multiple time-points. However, despite the fact that the imaging procedure has been demonstrated to be feasible and well-tolerated in healthy non-sedated infants and children\(^3\) and infants and children with asthma and cystic fibrosis (CF),\(^4\) most \(^3\)He and \(^129\)Xe MRI studies to date have been performed in adults and experience in children has been largely limited to CF. There is clearly considerable potential for hyperpolarized gas MRI to be used to evaluate CF and asthma, as well as other chronic lung diseases in the pediatric population, and therefore larger MRI studies in children are required.

**Figure 9-3** \(^3\)He MRI static ventilation images and ADC maps for a representative subject that had an exacerbation and a representative subjects that did not have an exacerbation. Blue arrows indicate the large ventilation defects visually apparent in the subject that experienced an exacerbation.

### 9.4.4 Hyperpolarized Noble Gas MRI of Pediatric Lung Disease

A major strength of MRI is that it does not require the use of ionizing radiation and provides regional structural and functional information simultaneously. This is important for all longitudinal studies of lung disease, but this is especially the case in children with chronic life-threatening pulmonary disease. Therefore, hyperpolarized noble gas MRI can be used to measure lung structure and function in young subjects and at multiple time-points. However, despite the fact that the imaging procedure has been demonstrated to be feasible and well-tolerated in healthy non-sedated infants and children\(^3\) and infants and children with asthma and cystic fibrosis (CF),\(^4\) most \(^3\)He and \(^129\)Xe MRI studies to date have been performed in adults and experience in children has been largely limited to CF. There is clearly considerable potential for hyperpolarized gas MRI to be used to evaluate CF and asthma, as well as other chronic lung diseases in the pediatric population, and therefore larger MRI studies in children are required.
9.4.4.1 Hyperpolarized Noble Gas MRI in Pediatric Cystic Fibrosis

Although hyperpolarized $^3$He MRI experience is limited in the pediatric population and only moderate correlations have been shown between $^3$He MRI and spirometry in pediatric CF subjects, possibly due to the fact that children have difficulty performing the manoeuvres, $^3$He MRI has demonstrated regional changes in gas distribution in children following chest physiotherapy, following treatment with a bronchodilator and after mechanical airway clearance treatment. More recently, dramatic improvements in $^3$He gas distribution were visualized in children with CF following treatment with Ivacaftor, the first investigation to use hyperpolarized gas MRI to assess an investigational CF drug.

Although pulmonary function tests are still the mainstay for monitoring disease status and progression, and FEV$_1$ is still the primary endpoint used and accepted by regulatory authorities for clinical trials of CF therapies, the limitations of standard pulmonary function test measurements and the clear advantages to using sensitive imaging biomarkers for evaluating the efficacy of novel therapies suggest this is likely a target application for hyperpolarized gas MRI. It is clear from these previous $^3$He MRI studies in CF that the advantages of MRI for the quantitative, sensitive and regional evaluation of lung structure and function intensively in serial and longitudinal studies necessitates further investigation of pulmonary MRI in pediatric therapy studies.

9.4.4.2 Hyperpolarized Noble Gas MRI in Pediatric Asthma

Asthma is the most common chronic disease in the pediatric population and the prevalence of asthma continues to rise. Despite advances in drug therapy, a significant proportion of health care expenditure, morbidity and excess mortality are related to a small but significant number of patients with severe asthma symptoms that remain unresponsive to current treatments. The development of new and improved asthma therapies and interventions are required to improve asthma symptoms and reduce the morbidity and mortality associated with asthma. Moreover, the development of new therapies is going to require evaluation using sensitive tools that provide useful information regarding the effects of treatment on lung structure and function.
In preliminary studies utilizing hyperpolarized $^3$He MRI in asthmatics with abnormal spirometry, ventilation defects were observed that resolved and reappeared over a three week period. Other studies using $^3$He MRI in asthmatics also demonstrated $^3$He ventilation defects and showed that these defects increased in number and extent following an exercise challenge and methacholine administration. Importantly, $^3$He ventilation defect measures correlated significantly with spirometry and disease severity. It has also been demonstrated that many of the ventilation defects visible with $^3$He MRI persist or recurred in the same location over time or with repeated bronchoconstriction, suggesting airway abnormalities are regional in nature.

In a study by Fain and colleagues comparing hyperpolarized $^3$He MRI with chest CT in asthmatics, regions of the lung with $^3$He MRI ventilation defects were shown to spatially correlate with regions of hyperlucency on CT. The authors also obtained inflammatory markers using bronchoscopy in these ventilation defect regions and detected an increase in inflammatory cell counts, suggesting these regional ventilation defects are likely related to airway inflammation and obstruction. Another recent study showed that $^3$He ventilation defect measurements correlated significantly with specific airway resistance, symptoms and exhaled nitric oxide, also lending support to the notion that ventilation defects are likely related to inflammation and narrowing/obstruction of the airways.

From this work it is clear that hyperpolarized gas MRI has many potential clinical applications in asthma. The direct visualization of ventilation defects that recur in the same locations following provocation may help guide airway treatments and interventions to the regions within the lung or the specific airways that are hyper-responsive. However, nearly all $^3$He MRI studies have been performed with adult asthma subjects and therefore further studies evaluating regional $^3$He MRI structural and functional measurements at baseline and following provocation and treatment are required in the pediatric population.
9.4.4.3 Hyperpolarized Noble Gas MRI: Lung Growth and Development

Importantly, studies in healthy pediatric subjects showed that $^3$He MRI ADC increases with age, suggesting that diffusion-weighted MRI can be used to assess lung growth and alveolar enlargement. Lung growth was also recently investigated using hyperpolarized $^3$He diffusion-weighed MRI by Butler and colleagues in a 33-year-old woman and demonstrated that this woman had an apparent 64% increase in the number of alveoli in her left lung 15 years following a right-sided pneumonectomy. These very exciting studies suggest that diffusion-weighted MRI has the potential to evaluate lung growth and development in children and in particular, it may be used to evaluate non-invasively and \textit{in vivo} chronic lung diseases in children that affect the development of normal lung microstructure, such as bronchopulmonary dysplasia (BPD). Although diffusion-weighted MRI has yet to be exploited in larger studies of pediatric diseases such as BPD, the promise remains for a non-invasive measurement that may regionally and longitudinally assess normal and abnormal lung growth and development.

We believe that these important studies in children demonstrate the feasibility of hyperpolarized gas MRI to non-invasively measure disease morphological and functional consequences and to explore disease pathophysiology \textit{in vivo} to potentially provide an improved understanding of pediatric lung disease.

9.5 Significance and Impact

Although FEV$_1$ remains the primary outcome measure for assessing COPD progression and response to treatment, it is well recognized that COPD is a multi-component disease and, while FEV$_1$ has its strengths, there is a continued and urgent need for additional pulmonary biomarkers for assessing COPD outcomes. Here, we have demonstrated that regional $^3$He MRI measurements provide adequate sensitivity for detecting two important outcomes in COPD, namely evaluating significant changes over relatively short periods of time and evaluating changes following treatment. Importantly, these $^3$He MRI measurement changes occurred in the absence of FEV$_1$ changes, thus suggesting that $^3$He MR imaging biomarkers may have increased sensitivity to measuring outcomes in
COPD. In this regard, we also identified ex-smokers with $^3$He MRI evidence of emphysema, symptoms and reduced exercise capacity with “normal” spirometry. Taken together, these findings suggest that $^3$He MRI may provide the opportunity for measuring early disease changes in subjects with symptomatic changes, as well as measuring longitudinal and treatment changes, in the absence or possibly before changes in FEV$_1$.

In order to measure these important COPD outcomes, hyperpolarized gas MRI measurement tools for the regional quantitative evaluation with sufficient precision were needed. We developed reproducible and robust hyperpolarized gas MRI registration/segmentation methods for generating measurements of pulmonary gas distribution that could be applied across many respiratory conditions, and also be used for larger scale pulmonary functional MRI studies. We also developed image analysis tools for hyperpolarized gas diffusion-weighted MRI that provided a way to evaluate regional lung microstructure, and provided important insights into pulmonary structure-function on a regional basis. These important findings indicate that hyperpolarized gas MRI can be used to improve our understanding of lung structural and functional changes in COPD.
9.6 References


APPENDIX A

In Appendix A we aimed to establish imaging measurement reproducibility in adult cystic fibrosis subjects over a short period of time (7 ± 2 days) in the absence of therapeutic intervention using hyperpolarized $^3$He MRI.

The contents of this Chapter have been previously published in Academic Radiology and permission to reproduce the article was granted by John Wiley and Sons and is provided in Appendix C.


A Quantitative Evaluation of Hyperpolarized Helium-3 Magnetic Resonance Imaging of Lung Function Variability in Cystic Fibrosis

A.1 Introduction

High resolution computed tomography (HRCT) is currently the gold standard imaging method for providing quantitative morphological measurements of lung abnormalities associated with cystic fibrosis (CF). Scoring systems have been developed,\(^1\) for radiological interpretation of various pulmonary abnormalities, including bronchiectasis, mucous plugging, airway wall thickening, and air trapping. Such scoring systems correlate with chest radiograph scores,\(^1\) pulmonary function tests,\(^2\) and clinical findings\(^3\) and have been shown to be more sensitive than spirometry for detecting disease worsening.\(^4\) Despite these important advantages, the risk associated from repeated radiation exposure, particularly in monitoring disease progression and serial imaging in therapy studies, limits the use of CT in CF. In addition, the relationship between lung functional changes and the structural, anatomical and morphological changes reflected by quantitative HRCT is still not completely understood.

Hyperpolarized Helium-3 ($^3$He) magnetic resonance imaging (MRI) provides high spatial and temporal resolution images of both lung structure and function\(^5-10\) without ionizing radiation. Regional ventilation abnormalities can be clearly visualized as decreased or absent $^3$He signal in the lung that can be evaluated using quantitative scoring systems or
volumetric approaches.\textsuperscript{9,11} These measurements are sensitive to the pathological changes that accompany CF,\textsuperscript{12-18} have high same-day reproducibility in pediatric CF patients,\textsuperscript{18} and changes are clearly visible despite clinically normal spirometry results.\textsuperscript{12} Because of previous same-day reproducibility\textsuperscript{18} and treatment\textsuperscript{12,16,18} studies in CF patients, we hypothesized that $^3$He MRI would provide the necessary and sufficient spatial and temporal sensitivity to detect day-to-day changes in lung function.

There is an urgent requirement for precise and sensitive measurements or surrogates that can be used to determine pulmonary response to treatment and to monitor disease progression; imaging methods may be ideally positioned to provide some of these measurements. Establishing imaging measurement reproducibility over time in the absence of therapeutic intervention is essential for future studies that aim to evaluate treatment changes. Accordingly, the objective of this study was to evaluate lung function over a relatively short period of time (7 ± 2 days) using spirometry, plethysmography and hyperpolarized $^3$He MRI in adult CF patients with moderate lung function. To our knowledge, this is the first reported study that quantitatively evaluates short-term lung functional variability in CF using $^3$He MRI.

A.2 Materials and Methods

A.1.1 Subjects

Study subjects provided written informed consent to the study protocol approved by the local research ethics board and Health Canada. The study was compliant with the Personal Information Protection and Electronic Documents Act (PIPEDA, Canada) and the Health Insurance Portability and Accountability Act (HIPAA, USA). Subjects between the ages of 18 and 45 years, without claustrophobia or MRI contraindications and with baseline FEV$_1$%$_{\text{pred}}$ > 60% at their last clinical visit were enrolled. Study participation was also limited to those CF patients experiencing no more than three previous exacerbations (change in medication or hospitalization for CF) within the last 12 months, as an indication of appropriate disease control. A baseline and 7-day repeat testing paradigm was chosen to differentiate the day-to-day changes common in CF from
longer term alterations in lung physiology that accompany CF lung disease progression. All subjects were evaluated using spirometry and plethysmography 30 minutes prior to MRI and all subjects were evaluated on two separate occasions within 7 ± 2 days. Digital pulse oximetry was used to measure arterial oxygen saturation (SaO₂) for all subjects during scanning sessions. An adverse event was considered to have occurred when SaO₂ decreased below 88% at any time during the scanning session. Study withdrawal was required when SaO₂ decreased to 80% for 10 seconds or longer.

A.1.2 Pulmonary Function Tests

Spirometry was performed using an ndd EasyOne spirometer (ndd Medizintechnik AG, Zurich, CH) reporting forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) with a minimum of three acceptable spirometry maneuvers with the best FEV₁ and FVC selected for analysis according to the American Thoracic Society (ATS) guidelines. Total lung capacity (TLC), inspiratory capacity (IC), residual volume (RV), functional residual capacity (FRC), and diffusing capacity of carbon monoxide (DLCO) were measured using body plethysmography (MedGraphics Corporation, 350 Oak Grove Parkway St. Paul, MN USA).

A.1.3 Image Acquisition

MRI was performed on a whole body 3.0 Tesla (3T) Excite 12.0 MRI system (GEHC, Milwaukee, WI USA) with broadband imaging capability as previously described. All helium imaging employed a whole body gradient set with maximum gradient amplitude of 1.94 G/cm and a single channel, rigid elliptical transmit/receive chest coil (RAPID Biomedical GmbH, Wuerzburg Germany). The basis frequency of the coil was 97.3 MHz and excitation power was 3.2 kW using an AMT 3T90 RF power amplifier (GEHC, Milwaukee WI USA). Subjects were positioned supine in the MR scanner and SaO₂ was used to monitor arterial blood oxygenation levels during all MRI maneuvers. For both ¹H and ³He MRI, subjects were instructed by a pulmonary function technologist to inhale a gas mixture from a 1.0 L Tedlar® bag (Jensen Inert Products, Coral Springs, FL) from
FRC, and image acquisition was performed under breath-hold conditions—a period of 8 to 15s.

Coronal proton ($^1$H) MRI was performed prior to hyperpolarized $^3$He MRI with subjects scanned during breath-hold after inspiration of a 1.0 L $^4$He/$N_2$ gas mixture using the whole body radiofrequency (RF) coil and $^1$H fast spoiled gradient recalled echo sequence (16s total data acquisition, repetition time (TR) / echo time (TE) / flip angle = 4.7 ms / 1.2 ms / 30$^\circ$, field-of-view (FOV) = 40 x 40 cm, matrix 256 x 128, 14 slices, 15 mm slice thickness, 0 gap), as previously described.\textsuperscript{10}

Prior to $^3$He MRI, a polarizer system (HeliSpin\textsuperscript{TM}, GEHC, Durham, NC) was used to polarize $^3$He gas to 30-40%. As previously described,\textsuperscript{10} hyperpolarized $^3$He MRI coronal static ventilation images were acquired in breath-hold after inspiration of a 1.0 L $^3$He/$N_2$ mixture (does 5 mL/kg body weight) (14s data acquisition, TR / TE / flip angle = 4.3 ms / 1.4 ms / 7$^\circ$, bandwidth = 31.25, FOV = 40 x 40 cm, matrix 128 x 128, 14 slices, 15 mm slice thickness, 0 gap).

\subsection*{A.1.4 Image Analysis}

All $^3$He measurements were repeated four times to estimate observer reliability according using the approximation method developed by Walter \textit{et. al.}\textsuperscript{19} All measurements were performed by a single observer blinded to subject and time-point with both subjects and time-points randomized between each of the four repeated measurement trials to reduce any potential memory bias. Additionally, each randomized time-point was analyzed independently and one day apart to minimize observer memory bias.

The signal-to-noise ratio (SNR) for all images acquired was determined by calculating the mean signal intensity value within a 5 x 5 voxel region of interest (ROI) for four representative ROI within the lung parenchyma, and dividing by the standard deviation of the mean signal intensity values for noise inside a ROI of the same size within the image background where there was no lung structure. SNR was determined for each slice and then averaged to obtain a single SNR value for each subject and time-point.
$^3$He MRI pulmonary ventilation segmentation was performed with a semi-automated method generated using MATLAB R2007b (The Mathworks Inc., Natick, MA, USA), as previously described. Briefly, $^3$He MRI ventilation images were evaluated using a k-means cluster algorithm, similar to previously described methods for $^3$He MRI segmentation, to classify the $^3$He MRI pixel intensity values into clusters ranging from 1 to 5, representing gradations of ventilation from unventilated to hyperventilated. To obtain the external contour of the thoracic cavity to differentiate ventilation defects from the edge of the lung, $^1$H MR images were segmented using a seeded region growing algorithm and registered to the segmented $^3$He MR ventilation images as previously described. The segmented $^1$H thoracic cavity volume was used to generate ventilation defect percent (VDP), representing a normalized $^3$He ventilation defect volume (VDV or volume of cluster 1) for the lung.

A.1.5 Statistical Methods

Mean VDV and VDP for baseline and 7-days was calculated using the mean and standard deviation (SD) of four repeated measurements. The smallest detectable difference (SDD), defined as the smallest difference that can be measured with prospectively determined confidence that is not due to measurement error (variability), was calculated using the baseline repeated $^3$He measurements according to Eliasziw et. al. and shown in Equation A1:

$$SDD \geq z_\alpha \sqrt{2SEM_{\text{intra}}}$$ (1)

where $z_\alpha$ is 1.96 corresponding to a significance level of $\alpha = .05$ and $SEM_{\text{intra}}$ is the standard error of measurement due to intra-observer variability and is calculated as shown in Equation A2:

$$SEM_{\text{intra}} = \sqrt{\hat{\sigma}_e^2}$$ (2)

where $\hat{\sigma}_e^2$ is the intra-observer repeated measures variance. Multivariate analysis of variance (MANOVA) was performed for comparison of baseline and 7-day pulmonary
function measurements using SPSS 16.00 (SPSS Inc., Chicago, IL, USA LEAD Technologies, Inc., Chicago, IL). For $^3$He MRI measurements, a three-way mixed design repeated measures analysis of variance (ANOVA) was used to determine the interactions between time-point (baseline and 7-day), repetition (four repeated measurements) and subject using SPSS 16.00. The agreement between time-points for both pulmonary function and $^3$He MRI measurements was evaluated using Bland-Altman plots generated using GraphPad Prism version 4.00 (GraphPad Software Inc, San Diego, CA, USA). Linear regression ($r^2$) and Spearman correlation coefficients (r) were used to determine the relationship between pulmonary function and $^3$He MRI measurements using SPSS 16.00. A sample size ($n$) calculation was also performed to determine the number of subjects required in a controlled trial to detect a significant difference ($\delta$) for VDP between baseline and follow-up with 95% confidence ($\alpha = 0.05$, $Z_\alpha = 1.96$) and 80% power ($\beta = 0.20$, $Z_\beta = 0.84$), according to Equation A3:

$$n = \frac{2(Z_\alpha + Z_\beta)^2 SD_{\text{Diff}}^2}{\delta^2}$$

where $SD_{\text{Diff}}$ is the standard deviation of the difference between baseline and follow-up. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

### A.3 Results

The demographic characteristics for 12 CF subjects enrolled ($n=5$ males, mean age=26±8, BMI=24±4) are provided in Table A1. The first study visit was in September 2007 and the last visit was in January 2010. All subjects completed both scanning sessions and there were no serious or severe breath-hold-related or other adverse events reported, nor were there any other adverse events that required subjects to withdraw from the study. Approximately one year after subject scanning, a single subject (Table A3, subject 1, female, age 24) died due to respiratory complications related to underlying CF.
**Table A1 Subject Demographics**

<table>
<thead>
<tr>
<th>Subjects (±SD) [range] (n= 12)</th>
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<tbody>
<tr>
<td><strong>Age yrs</strong></td>
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<tr>
<td><strong>Male Sex</strong></td>
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<tr>
<td><strong>BMI kg·m⁻²</strong></td>
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<tr>
<td><strong>FEV₁ L</strong></td>
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<tr>
<td><strong>FEV₁%pred</strong></td>
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<tr>
<td><strong>FVC L</strong></td>
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<tr>
<td><strong>FVC %pred</strong></td>
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<tr>
<td><strong>FEV₁/FVC %</strong></td>
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<tr>
<td><strong>FEV₁/FVC %pred</strong></td>
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<tr>
<td><strong>TLC L</strong></td>
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<tr>
<td><strong>TLC %pred</strong></td>
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<tr>
<td><strong>IC L</strong></td>
</tr>
<tr>
<td><strong>IC %pred</strong></td>
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<tr>
<td><strong>FRC L</strong></td>
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<tr>
<td><strong>FRC %pred</strong></td>
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<tr>
<td><strong>RV L</strong></td>
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<tr>
<td><strong>RV %pred</strong></td>
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<tr>
<td><strong>DL₃CO</strong></td>
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<td><strong>DL₃CO%pred</strong></td>
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</table>

BMI=Body Mass Index, DL₃CO=Carbon Monoxide Diffusion Capacity of the lung, FEV₁=Forced Expiratory Volume in 1s, FRC=Functional Residual Capacity, FVC=Forces Vital Capacity, IC= Inspiratory Capacity, %pred=Percent Predicted, RV= Reserve Volume, SD=Standard Deviation, TLC=Total Lung Capacity, ‡n=6, †n=9.

**Table A2** shows mean baseline and 7±2 day pulmonary function and ³He MRI measurements and **Table A3** shows a subject listing of all pulmonary function and ³He MRI measurements for all subjects. For baseline repeated measurements, the intra-observer CV for both VDV and VDP was 0.04. Based on the baseline intra-observer variance, the SDD or the minimum change in VDV and VDP that could be measured confidently in individual subjects that was not due to technological or observer measurement variability (measurement error) was 120ml and 2%, respectively. As shown in **Table A3**, 8 of 12 subjects showed changes in both VDV and VDP over 7 days greater than or equal to the SDD.
Table A2 Baseline and 7-day pulmonary function and $^3$He MRI measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (n=12)</th>
<th>7-day (n=12)</th>
<th>Mean Difference [p]*</th>
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<tbody>
<tr>
<td><strong>Pulmonary Function Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV$_1$ (L) (±SD)</td>
<td>2.68 (0.66)</td>
<td>2.69 (0.54)</td>
<td>-0.01 (0.19) [NS]</td>
</tr>
<tr>
<td>FEV$<em>1$%$</em>{\text{pred}}$ (±SD)</td>
<td>72 (14)</td>
<td>72 (12)</td>
<td>0 (6) [NS]</td>
</tr>
<tr>
<td>FVC %$_{\text{pred}}$ (±SD)</td>
<td>87 (12)</td>
<td>86 (10)</td>
<td>0 (4) [NS]</td>
</tr>
<tr>
<td>FEV$_1$/FVC (±SD)</td>
<td>70 (12)</td>
<td>71 (11)</td>
<td>-1 (4) [NS]</td>
</tr>
<tr>
<td>TLC %$_{\text{pred}}$ (±SD)</td>
<td>108 (12)†</td>
<td>113 (16)†</td>
<td>-5 (7) [NS]</td>
</tr>
<tr>
<td>IC %$_{\text{pred}}$ (±SD)</td>
<td>101 (20)†</td>
<td>103 (19)†</td>
<td>-2 (14) [NS]</td>
</tr>
<tr>
<td>FRC %$_{\text{pred}}$ (±SD)</td>
<td>116 (29)†</td>
<td>125 (32)†</td>
<td>-9 (7) [NS]</td>
</tr>
<tr>
<td>RV %$_{\text{pred}}$ (±SD)</td>
<td>168 (41)†</td>
<td>176 (58)†</td>
<td>-8 (37) [NS]</td>
</tr>
<tr>
<td>DL$<em>{\text{CO}}$ (%$</em>{\text{pred}}$) (± SD)</td>
<td>106 (18)‡</td>
<td>111 (13)‡</td>
<td>-5 (8) [NS]</td>
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<tr>
<td><strong>Hyperpolarized $^3$He MRI</strong></td>
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<tr>
<td>VDV L (±SD)</td>
<td>1.06 (0.56)</td>
<td>0.93 (.59)*</td>
<td>0.13 (0.25) [&lt;.0001]</td>
</tr>
<tr>
<td>VDP % (±SD)</td>
<td>20 (10)</td>
<td>17 (10)*</td>
<td>3 (4) [&lt;.0001]</td>
</tr>
</tbody>
</table>

DL$_{\text{CO}}$=Carbon Monoxide Diffusion Capacity of the lung, FEV$_1$=Forced Expiratory Volume in 1s, FRC=Functional Residual Capacity, FVC=Forces Vital Capacity, IC=Inspiratory Capacity, %$_{\text{pred}}$=Percent Predicted, RV=Reserve Volume, SD=Standard Deviation, TLC=Total Lung Capacity, VDP=Ventilation Defect Percent, VDV=Ventilation Defect Volume, ‡n=8, †n=9.

*Significance of difference (p<.05) was determined using a repeated measures ANOVA.

There was no significant difference for pulmonary function measurements (p=.56) between time-points. As shown in Table A2, significant differences were detected after 7-days for both $^3$He VDV (130 ± 250ml, p<.0001) and VDP (3%± 4%, p<.0001), and a significant interaction between the changes measured after 7-days and subject was also observed (p<.0001, Figure A1), indicating that not all subjects showed a change over this time frame. To underscore this point, Figure A1 shows center slice $^3$He ventilation images for three representative subjects at baseline and 7-days with subject numbers that correspond to the subject numbers shown in Table A2. For subject (S1), very little change in $^3$He gas distribution is observed between the time-points, however the change in gas distribution after 7-days is readily apparent in subjects S2 and S3. However, it is important to note that after conservatively comparing the 95% confidence intervals (CI) for baseline and 7-day $^3$He measurements, baseline and 7-day 95% CI overlapped for both VDV (baseline: [95% CI, 0.74L-1.38L], 7-day: [95% CI, 0.60L-1.26L]) and VDP (baseline: [95% CI, 15%-25%], 7-day: [95% CI, 12%-23%]). Image signal-to-noise ratio (SNR) for all baseline and 7-day image pairs was not statistically significantly different.
(p=.47), nor was there a significant relationship between the change in SNR and the change in VDV (r=-.29, p=.35).

**Figure A1** Baseline and 7-day $^3$He MRI static ventilation images for three representative CF subjects.
Shown in Figure A2, Bland-Altman analysis was also performed to evaluate differences between time-points for FEV₁ (mean difference ($\bar{d}$)=0.01L, 95% CI =-0.37-0.39L) and no systematic bias was detected and differences were within the 95% limits of agreement for all but one subject. However, Bland-Altman analysis also showed 7-day differences for $^3$He VDV ($\bar{d}$=-0.13L, 95% CI =-0.63–0.36L) and VDP ($\bar{d}$=-3%, 95% CI =-11–5%) with large 95% limits of agreement between the measurements.

**Figure A2** Bland-Altman plots of the differences between baseline and 7-day measurements. Scatterplots show the differences between baseline and 7-day FEV₁, VDV and VDP for all subjects, against their mean. The mean difference (±SD) was 0.01L (0.19L) for FEV₁ (lower limit=-.37L, upper limit=.39L) (A), -0.13L (0.25L) for VDV (lower limit=-.63L, upper limit=.36L) (B), and -3% (4%) for VDP (lower limit=-11%, upper limit=5%) (C). Solid lines indicate the mean difference and dotted lines indicate the 95% limits of agreement.
Table A3 shows the relationship between the baseline and 7-day pulmonary function and \(^3\)He MRI measurements. Figure 3 also shows the significant and moderate Spearman correlation between FEV\(_1\)%\(\text{pred}\) and VDV (r=-.65, p=.001) and VDP (r=-.68, p<.0001). The relationship between baseline and 7-day measurements (Figure A3) was evaluated and showed a significant correlation for FEV\(_1\)%\(\text{pred}\) (r=-.89, p<.0001), \(^3\)He VDV (r=.85, p=.001), and VDP (r=.94, p<.0001). There were no significant correlations between the baseline and 7-day change in pulmonary function and the change in \(^3\)He measurements.

Table A3 Subject listing of baseline and 7-day pulmonary function and hyperpolarized \(^3\)He MRI measurements

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Mean (± SD)</th>
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<tr>
<td><strong>Pulmonary Function Measurements</strong></td>
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<td><strong>Baseline</strong></td>
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<tr>
<td>FEV(_1) (L)</td>
<td>2.11</td>
<td>3.55</td>
<td>1.70</td>
<td>2.53</td>
<td>2.83</td>
<td>3.58</td>
<td>3.56</td>
<td>2.79</td>
<td>2.22</td>
<td>2.28</td>
<td>1.93</td>
<td>3.04</td>
<td>2.68 (0.66)</td>
</tr>
<tr>
<td>FEV(_1)%(\text{pred})</td>
<td>50</td>
<td>78</td>
<td>54</td>
<td>74</td>
<td>79</td>
<td>81</td>
<td>79</td>
<td>82</td>
<td>69</td>
<td>55</td>
<td>61</td>
<td>96</td>
<td>72 (14)</td>
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<tr>
<td>FVC (L)</td>
<td>3.41</td>
<td>4.87</td>
<td>3.06</td>
<td>2.92</td>
<td>3.55</td>
<td>5.65</td>
<td>4.88</td>
<td>3.52</td>
<td>2.87</td>
<td>5.02</td>
<td>2.96</td>
<td>3.77</td>
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<tr>
<td>FVC%(\text{pred})</td>
<td>65</td>
<td>89</td>
<td>79</td>
<td>75</td>
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<td>103</td>
<td>90</td>
<td>90</td>
<td>74</td>
<td>103</td>
<td>81</td>
<td>103</td>
<td>87 (12)</td>
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<tr>
<td><strong>7-day</strong></td>
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<tr>
<td>FEV(_1) (L)</td>
<td>2.17</td>
<td>3.39</td>
<td>2.2</td>
<td>2.7</td>
<td>2.66</td>
<td>3.46</td>
<td>3.4</td>
<td>2.69</td>
<td>2.36</td>
<td>2.32</td>
<td>1.85</td>
<td>3.04</td>
<td>2.69 (0.54)</td>
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<tr>
<td>FEV(_1)%(\text{pred})</td>
<td>51</td>
<td>74</td>
<td>69</td>
<td>79</td>
<td>75</td>
<td>78</td>
<td>75</td>
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<td>73</td>
<td>56</td>
<td>58</td>
<td>96</td>
<td>72 (12)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.69</td>
<td>4.75</td>
<td>3.39</td>
<td>3.05</td>
<td>3.49</td>
<td>5.31</td>
<td>4.82</td>
<td>3.34</td>
<td>3.05</td>
<td>4.87</td>
<td>2.83</td>
<td>3.76</td>
<td>3.86 (0.85)</td>
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<tr>
<td>FVC%(\text{pred})</td>
<td>70</td>
<td>87</td>
<td>87</td>
<td>78</td>
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<td>96</td>
<td>89</td>
<td>86</td>
<td>79</td>
<td>100</td>
<td>77</td>
<td>102</td>
<td>86 (10)</td>
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<td><strong>(^3)He MRI Measurements</strong></td>
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<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td>VDV (L)</td>
<td>1.70</td>
<td>1.49</td>
<td>1.15</td>
<td>0.52</td>
<td>0.62</td>
<td>1.79</td>
<td>0.87</td>
<td>0.38</td>
<td>0.98</td>
<td>1.38</td>
<td>1.71</td>
<td>0.15</td>
<td>1.06 (0.56)</td>
</tr>
<tr>
<td>VDP (%)</td>
<td>30</td>
<td>24</td>
<td>22</td>
<td>13</td>
<td>13</td>
<td>27</td>
<td>17</td>
<td>10</td>
<td>21</td>
<td>24</td>
<td>34</td>
<td>3</td>
<td>20 (9)</td>
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<tr>
<td><strong>7-day</strong></td>
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<tr>
<td>VDV (L)</td>
<td>1.80</td>
<td>1.16</td>
<td>1.61</td>
<td>0.30</td>
<td>0.46</td>
<td>1.31</td>
<td>0.52</td>
<td>0.33</td>
<td>0.65</td>
<td>1.36</td>
<td>1.51</td>
<td>0.14</td>
<td>0.93 (0.59)</td>
</tr>
<tr>
<td>VDP (%)</td>
<td>30</td>
<td>18</td>
<td>27</td>
<td>7</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>22</td>
<td>31</td>
<td>3</td>
<td>17 (10)</td>
</tr>
<tr>
<td>(\text{FEV}_1=\text{Forced Expiratory Volume in 1s, FVC= Forced Vital Capacity, } %\text{pred}=\text{Percent Predicted, VDP=Ventilation Defect Percent, VDV=Ventilation Defect Volume})</td>
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We generated sample sizes required to detect significant differences in VDP in an interventional study. Instead of assuming there would be no change in VDP over time in the absence of treatment, we used the mean difference over 7 days and the standard deviation of that difference measured in this study to generate sample sizes for the use of VDP. Accordingly, to detect a significant 5%/7%/10% absolute change in VDP for a treatment group would require 60/15/5 subjects per group.
A.4 Discussion

Functional imaging methods such as $^3$He MRI provide a way to perform intensive serial studies in individual patients to monitor therapy effects and disease changes and as well as repeated measurements in patient groups for therapy trials. However, before $^3$He MRI measurements can be used as a complement to established endpoints, measurement sensitivity and precision must be determined. In order to determine and compare the
variability of lung function measurements in CF, we evaluated 12 adult CF subjects with moderate lung function, using spirometry, plethysmography and $^3$He MRI, twice over 7 days. We made a number of important observations and report: 1) the SDD in $^3$He VDV and VDP for individual subjects, 2) highly correlated baseline and 7-day VDV and VDP but significant change in $^3$He MRI VDV and VDP, not reflected by changes in spirometric or plethysmographic measurements, 3) a significant and moderately strong relationship between FEV$_1$ and $^3$He MRI functional measurements but not between the difference between time-points in FEV$_1$ and $^3$He MRI measurement, and 4) sample sizes required to detect significant changes in $^3$He MRI VDP in a controlled clinical study.

First we calculated the SDD for $^3$He VDV and VDP for an individual CF subject that was not due to measurement variability. The relatively low SDD for $^3$He ventilation measurements was likely a consequence of the high precision of the $^3$He MRI measurement and the semi-automated image segmentation method used.

Second, we observed statistically significant changes in mean $^3$He measurements in 7 days, however no significant differences were detected using spirometry or plethysmography (and this study was not powered to detect such changes). Although previous studies have demonstrated high same-day reproducibility in pediatric CF subjects,$^{18}$ significant differences over short time periods in individuals with CF was not unexpected because the movement of mucus plugs and subsequent alterations of airway patency, could certainly result in $^3$He gas distribution changes. However, the statistically significant and visually obvious change in $^3$He measurements we observed must be examined in light of previous reports of excellent 7-day $^3$He MRI reproducibility in COPD.$^7$ The 7-day functional variability finding may reflect the greater contribution of defective mucus clearance to CF physiological variability and is important to consider when planning pulmonary functional imaging studies including $^3$He and $^{129}$Xe MRI that evaluate long-term treatment interventions. The possibility exists that the differences measured, though small, were due to lung structural and functional changes that occurred over a one week period. Certainly serial $^3$He MRI in COPD has revealed changes over a two-year time period with no concomitant change in FEV$_1$. $^6$ The results of the current study in CF indicate that $^3$He MRI sensitively quantified gas distribution changes that
occurred without changes detected in FEV₁ suggesting it is possible to measure physiological changes that occur over short periods of time in CF. Importantly, over half of the individual subjects (8/12) studied experienced a change in VDP and VDV greater than or equal to the SDD, over 7 days and these measurement changes were therefore unlikely due to measurement error. The clinical meaning of this variability may be related to day to day variations in mucus redistribution. As suggested by Bannier et al.,¹² mucus movement over short periods of time can potentially result in many small ventilation defects that traditional scoring or defect counting methods may not have the sensitivity to detect.

In support of the finding of significant changes in mean ³He measurements over 7-days, Bland-Altman plots also identified a bias or improvement in ³He measurements between time-points. This was unexpected as there was no significant difference between SNR for the image pairs to account for this difference and there was approximately a three year gap between the first and last subject imaging sessions, making it unlikely that the bias detected was due to technical or hardware issues at the second time-point for all subjects. Whether the mean improvement in ³He gas distribution was due to the small number of subjects evaluated or related to improved therapy compliance for some subjects because of the 1-week study design cannot be ascertained by this study.

We also reported a significant relationship between FEV₁ and ³He functional measurements and this finding agrees with previous studies that have demonstrated the relationship between FEV₁ and ³He measurements within a single day in both adult CF¹⁵ and in pediatric CF patients.¹⁷,¹⁸ It is still unclear what the clinical meaning that ventilation distribution has in CF because ³He MRI measurements have not been validated using histology. However it is possible that the ventilation measurements reported in this study reflect regional lung function and functional changes that occur on a day-to-day basis in CF.

Finally, to use the current results as a guide for future studies, we estimated sample sizes required to detect significant differences in ³He VDP. Relatively small sample sizes of 60/15/5 subjects were estimated to be required per group to detect significant
5%/7%/10% absolute changes in VDP. This suggests that $^3$He MRI can be considered when small sample sizes are necessitated such as in rare diseases with small patient populations, or in high cost per patient study designs.

We acknowledge that this pilot study is limited by the small number and type of CF subjects studied. The study subjects were recruited to represent CF subjects that would be appropriate candidates for therapy trials based on their FEV$_1$, and therefore caution should be exercised in extrapolating these results to a pediatric population with less severe disease. In addition, the interpretation and full understanding of the clinical relevance and meaning of $^3$He MRI measurements in CF patients is incomplete. For example, the distribution of $^3$He gas within the lungs of CF patients is hypothesized to be related to mucous plugging and perhaps inflammation of the airways causing disruption in regional airflow. However, histological validation or cross modality comparisons, such as with HRCT and the lung clearance index, are urgently required to identify the specific airway pathologies in and around ventilation defects. We must also acknowledge that although we have taken into account the variability of the measurement technique over 7-days, we did not determine the reproducibility of the $^3$He measurements within the same-day, which is required to ensure that the differences observed here over 7-days were not attributable to differences in the breath-hold maneuver, patient effort, or patient positioning between acquisitions. However, a previous study evaluating measurement reproducibility in CF showed high same-day reproducibility, lending strong support to the findings in this study over 7-days. It is also important to note that while volumetric approaches may be more sensitive than visual scoring for quantifying changes in CF patients, certainly a regional analysis of the changes in ventilation distribution, as was recently shown by Woodhouse et al., would increase the sensitivity for detecting change and may be relevant in guiding therapies. Another consideration is the limited $^3$He MRI access, although the development of hyperpolarized $^{129}$Xe MRI a less expensive and more readily available approach, is a promising alternative with which these reported findings can be directly tested.

During progressive disease in CF, it is believed that adaptive remodeling of the lung airways and tissue occurs in response to bacterial/viral/fungal colonization, inflammation
and mucous plug occlusions. Clinical research studies of CF continue to focus on FEV₁, and in some cases high resolution or multi-detector x-ray CT methods to evaluate novel interventions and disease progression. The results of the current study suggest that ³He MRI provides another imaging tool for the assessment of both airway and airspace changes in CF with sufficient precision and sensitivity to physiological lung functional changes for clinical research studies. While much noble gas MRI pulmonary research is transitioning to ¹²⁹Xe MRI, we believe the current results are applicable to its use as well. Moreover, both methods provide a relatively rapid and safe method of visualizing and quantifying both spatial and temporal lung structural and functional dynamics related to pulmonary disease. In conclusion, our results indicated that ³He MRI detected significant changes in gas distribution in CF patients over a short 7-day period and these measurements can be considered as potential intermediate endpoints for evaluating functional lung changes with relatively small sample sizes.
A.5 References


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APPENDIX B

A case report of a COPD ex-smoker that was evaluated serially over 4 years using hyperpolarized $^3$He MRI, twice prior to and twice following an acute exacerbation requiring hospitalization is provided in Appendix B.

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B Hyperpolarized Helium-3 Magnetic Resonance Imaging of Chronic Obstructive Pulmonary Disease Exacerbation

B.1 Introduction

Chronic obstructive pulmonary disease (COPD) is the 4th leading cause of death worldwide and COPD exacerbations constitute a major burden on the health care system, and are associated with disease progression and reduced quality of life. The need for sensitive regional and surrogate measurements of COPD exacerbation risk and response to therapy continues to motivate the development of non-invasive and sensitive imaging approaches, such as hyperpolarized helium-3 ($^3$He) magnetic resonance imaging (MRI). Numerous studies have shown that $^3$He MRI measurements in COPD are highly reproducible, sensitive to early lung micro-structural changes, and significantly correlate with established measurements of pulmonary function, multi-slice computed tomography (CT) measurements and histology measurements of emphysema. Furthermore, longitudinal $^3$He MRI of COPD has highlighted the sensitivity of the method to progressive worsening and shown regional improvements post-bronchodilator and following interventions such as stent supported airway bypass. Unfortunately, there have been few reports of the effect of such novel imaging results on therapeutic and patient care decision making. Here, we report serial hyperpolarized $^3$He
MRI over a four year period in a 77 year old COPD ex-smoker including images acquired prior to and following treatment for an acute exacerbation (AE) of COPD.

**B.2 Case Report**

A 77 year old male with GOLD stage III COPD and a 20 pack-year smoking history, having ceased smoking 10 years prior, provided written informed consent to a longitudinal $^3$He MRI study that was Health Insurance Portability and Accountability Act (HIPAA) compliant and approved by a local ethics board. Hyperpolarized $^3$He static ventilation and diffusion-weighted MRI was performed 25 minutes after 400μg salbutamol administration in breath-hold after inspiration of a 1.0L gas mixture of $^3$He (5mL/kg body weight) and N$_2$ gas from functional residual capacity (FRC) as previously described. $^{15}$ $^3$He gas was polarized to 30—40 % using a polarizer system (HeliSpin™, GEHC, Durham, NC). Static ventilation images were acquired using a fast-gradient-recalled-echo (FGRE) sequence (14s data acquisition, TR / TE / flip angle = 4.3 ms / 1.4 ms / 7°, bandwidth = 31.25, FOV = 40 x 40 cm, matrix 128 x 128, 14 slices, 15 mm slice thickness, 0 gap) and diffusion-weighted images were acquired using a FGRE sequence with centric k-space sampling; two interleaved images were acquired (14s total data acquisition, TR/TE/flip angle = 7.6 ms/3.7 ms/8°, FOV = 40 x 40 cm, matrix 128 x 128, 7 slices, 30 mm slice thickness), with and without additional diffusion sensitization (G = 1.94 G/cm, rise and fall time = 0.5 ms, gradient duration = 0.46 ms, Δ = 1.46 ms, b = 1.6 s/cm$^2$). It is important to note that pulse oximetry was used to measure arterial oxygen saturation (SaO$_2$) during all imaging sessions. No adverse events occurred during any imaging session for this subject and SaO$_2$ remained above 88% at all times. Proton ($^1$H) MRI was also acquired with the same breath-hold volume within 5 min of $^3$He MRI as previously described$^{15}$ to obtain an anatomical thoracic image to enable clear delineation of the thoracic cavity. Registration and semi-automated segmentation of the $^3$He MRI functional and $^1$H MRI anatomical image for each time-point provided a way to generate the lung ventilation defect percent (VDP) as previously described.$^{16,17}$ $^3$He apparent diffusion coefficients (ADC) were generated from $^3$He diffusion-weighted imaging and ADC maps as previously described.$^{13}$ Hyperpolarized $^3$He MRI measurement reproducibility in COPD was previously shown to be high at a number of independent
sites,\cite{4,5,9} and these results clearly provided good support for its use in serial studies of COPD.

Approximately 2.5 years pre-AE, as shown in Table B1, the forced expiratory volume in 1sec (FEV$_1$) was 1.48L (41\%pred), forced vital capacity (FVC) was 3.19L (66\%pred), inspiratory capacity (IC) was 2.42L (64\%pred) and FRC was 3.74L (95\%pred). As shown in Figure B1, $^3$He MRI revealed heterogeneous and abnormal gas distribution (whole lung VDP=16\%) reflective of airway obstruction and Figure B2 provides an ADC map (whole lung ADC=0.34cm$^2$/s) reflective of regional emphysema.

### Table B1 $^3$He MRI and pulmonary function measurements in a single subject pre- and post-exacerbation

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<td></td>
<td>days [months]</td>
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<td><strong>Pulmonary Function Tests</strong></td>
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<tr>
<td>FEV$_1$ (%pred)</td>
<td>41</td>
<td>47</td>
<td>45</td>
<td>54</td>
</tr>
<tr>
<td>FEF$_{25%}$ (%pred)</td>
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<td>19</td>
<td>23</td>
<td>34</td>
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<td>FEF$_{50%}$ (%pred)</td>
<td>-</td>
<td>18</td>
<td>15</td>
<td>17</td>
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<tr>
<td>FVC (%pred)</td>
<td>66</td>
<td>70</td>
<td>68</td>
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<td>FEV$_1$/FVC (%)</td>
<td>46</td>
<td>49</td>
<td>48</td>
<td>51</td>
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<td>TLC (%pred)</td>
<td>78</td>
<td>73</td>
<td>78</td>
<td>74</td>
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<td>IC (%pred)</td>
<td>64</td>
<td>35</td>
<td>44</td>
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<td>FRC (%pred)</td>
<td>95</td>
<td>105</td>
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<td>RV (%pred)</td>
<td>94</td>
<td>76</td>
<td>113</td>
<td>85</td>
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<td>DL$_{CO}$ (%pred)</td>
<td>-</td>
<td>36</td>
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<td><strong>Hyperpolarized $^3$He MRI</strong></td>
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<tr>
<td>VDP (%)</td>
<td>16</td>
<td>29</td>
<td>20</td>
<td>14</td>
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<tr>
<td>ADC (cm$^2$/s)</td>
<td>0.34</td>
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<td>0.34</td>
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FEV$_1$ = forced expiratory volume in one second, FEF$_{25\%}$ = forced expiratory flow at 25\% forced vital capacity (FVC), FEF$_{50\%}$ = forced expiratory flow at 50\% FVC, SVC = slow vital capacity, TLC = total lung capacity, IC = inspiratory Capacity, FRC = functional residual capacity, RV = residual volume, DL$_{CO}$ = carbon monoxide diffusion capacity of the lung, VDP = ventilation defect percent, ADC = apparent diffusion coefficient.

Approximately 6 months pre-AE, $^3$He MRI (Table B1 and Figure B1) showed increased heterogeneity of gas distribution and increased VDP, suggestive of progressive worsening. As shown in Figure B2, the elevated $^3$He ADC 6 months pre-AE was dominated by the contributions of the posterior lung slices. Previous studies\cite{7} evaluating the anatomical distribution of $^3$He ADC in COPD and healthy volunteers have
demonstrated that the anterior-posterior gradient is significantly greater in healthy volunteers than in severe COPD subjects, suggesting dependent lung region gas trapping in contrast to progressive worsening of emphysema. At the same time, measurements of gas trapping such as IC were also worse (Table B1). Although there was worsening of both $^3$He MRI VDP and ADC, there was no change in the subject clinical status between the 29 and 6 months pre-AE time-points. It is also important to note that there was sufficient image signal-to-noise-ratio (SNR) at all time-points (SNR>20).
Hyperpolarized $^3$He MRI pre- and post-exacerbation

Hyperpolarized $^3$He MRI static ventilation images with the ventilation defect percent (VDP) shown below for the three centermost slices for a COPD subject 29 and 6 months prior to hospitalization for an acute exacerbation with pneumonia and 8 days and 16 months following hospitalization. The length of time prior to and following the acute exacerbation is provided in days and months in square brackets. $^3$He MRI shows a heterogeneous and abnormal gas distribution with signal voids or “defects” reflective of airway obstruction 29 months prior to hospitalization that was visually more heterogeneous 6 months prior to hospitalization. Improvements in $^3$He gas distribution were observed 8 days post-exacerbation that persisted 16 months post-exacerbation.
**Figure B2** Hyperpolarized $^3$He MRI ADC of COPD subject pre- and post-exacerbation

A. $^3$He MRI ADC maps for the centermost slice for COPD subject 29 and 6 months prior to hospitalization for an acute exacerbation with pneumonia and 16 months following hospitalization. The subject was unable to perform $^3$He MRI ADC 8 days following hospitalization.

B. $^3$He ADC histograms for each slice in the posterior to anterior direction at the pre- and post-exacerbation time-points.

C. Mean $^3$He ADC was 0.34cm$^2$/s (mean ADC=0.34cm$^2$/s, 95% confidence interval (CI)=0.32cm$^2$/s-0.36cm$^2$/s; linear regression slope=0.01, 95% CI=-0.002-0.02) at 29 months pre-exacerbation and was elevated (mean ADC=0.38cm$^2$/s, 95% CI=0.35cm$^2$/s-0.40cm$^2$/s; slope=-0.01, 95% CI=-0.03-0.008) at 6 months pre-exacerbation. The elevated ADC in the posterior slices suggests dependent lung region gas trapping. At 16 months post-exacerbation, ADC values returned to baseline (mean ADC=0.34cm$^2$/s, 95% CI=0.32cm$^2$/s-0.34cm$^2$/s; slope=0.005, 95% CI=-0.002-0.01).
Twenty-eight days prior to hospitalization, the subject complained of symptomatic worsening of COPD, characterized by increased shortness of breath and cough. He was evaluated by a primary care physician and treated with levofloxacin 500mg for 10 days. Eighteen days later, he attended the emergency department with worsening symptoms and was hospitalized for COPD AE and treated with a course of 50mg of prednisone for 6 days, 500mg of cefuroxime for 14 days and 250mg of erythromycin for 7 days. Four days after discharge from hospital, the subject returned for $^3$He MRI and pulmonary function tests. Although pulmonary function measurements were unchanged compared to 6 months prior (FEV$_1$=45%pred, FEV$_1$/FVC=48%), $^3$He MRI (Figure B1, 8 days post-AE) showed gas distribution and VDP (VDP=20%) improvements. Because of technical difficulties, $^3$He diffusion-weighted imaging was not performed at this time-point and therefore no ADC measurements were acquired.

Approximately 16 months post-AE, visibly obvious improvements in gas distribution persisted and there was improved VDP, and improved FEV$_1$, FVC, IC and FRC. In addition, mean ADC also decreased to values that were the same as nearly 4 years earlier with a more normalized anterior-posterior ADC gradient reflecting diminished gas trapping compared to 6 months pre-AE.

**B.3 Discussion**

In this hyperpolarized $^3$He MRI study we evaluated a single COPD ex-smoker over nearly four years, including scans 6 months prior to and immediately following treatment for an acute exacerbation of COPD. We observed visibly obvious improvements in $^3$He gas distribution immediately following exacerbation treatment, likely while the subject was still recovering, although no improvements were reflected by FEV$_1$. Improvements in $^3$He MRI VDP and ADC were also evident 16 months post-AE and these imaging improvements were mirrored by improved spirometry measurements. Importantly, the change in the $^3$He VDP 6 months prior to the exacerbation compared to all other time-points exceeded the smallest detectable difference of the measurement as previously reported, suggesting that the patient could have been worse 6 months before the exacerbation, although there was no change in the subjects clinical status until
approximately one month before hospitalization when the subject complained of symptoms. It is important to note that the acute exacerbation may have been caused by infection since improvement was seen only after broad-spectrum antibiotic treatment, and therefore $^3$He MRI might not have predicted the exacerbation onset. However, IC was also reduced 6 months prior to the exacerbation, and previous studies have demonstrated that IC can be used to reliably detect changes in end expiratory lung volume during COPD exacerbations.\textsuperscript{18} Taken together this suggests that MRI may have reflected regional pulmonary decline before, or even in the absence of changes in FEV\textsubscript{1}. Moreover, immediately after treatment for a COPD AE, spirometry measurements were relatively unchanged, although MRI showed substantive improvements.

Several hypotheses and questions arise from our results that are related to the time-course of exacerbation treatment such as: 1) Is there a relationship between symptomatic and $^3$He MRI improvements following therapy, before changes in FEV\textsubscript{1} are measured?; 2) Do COPD patients with specific imaging phenotypes have a propensity for increased exacerbation frequency? We note that the potential for imaging surrogate endpoints as markers of future clinical outcomes was recently demonstrated in cystic fibrosis (CF), whereby the relationship between high-resolution computed tomography measurements and CF exacerbations was shown.\textsuperscript{19} Improvements in $^3$He gas distribution have also been demonstrated in the absence of concomitant improvements in spirometry in CF patients following chest physical therapy.\textsuperscript{20} We think these previous results and the current case study highlight the discordance between spirometry and exacerbations and the potential predictive relationship between imaging and clinical outcomes many years later. In particular for hyperpolarized noble gas imaging, high short term reproducibility\textsuperscript{4} coupled with the high sensitivity in longitudinal\textsuperscript{11} and other acute therapy studies,\textsuperscript{12;13} suggest that this may be an ideal imaging approach to study or identify COPD patients at high risk for exacerbation.

We must acknowledge that this study was limited because $^3$He MRI and pulmonary function measurements were not acquired more close in time to the onset of the AE. Clearly, acquiring $^3$He MRI at time-points more closely timed to the clinical course would strengthen the findings of this investigation. The limited and high cost of $^3$He gas
that which has restricted translation to specialized MR physics centers is another important limitation. However, the development of hyperpolarized $^{129}$Xe MRI$^{21,22}$ is a promising alternative with which these findings can be directly tested in a larger COPD patient population.

It is nearly 2 decades since the potential for pulmonary functional imaging using inhaled noble gases was first described,$^{21}$ and it is yet unclear what role this novel imaging tool might play in the management of patients with COPD. Hyperpolarized $^3$He MRI is highly reproducible in COPD$^{4,5,9}$ with a modest smallest detectable difference (SDD=0.16L for VDV)$^{17}$ and this bodes well for serial studies in individual patients to monitor or perhaps predict disease worsening. However, it has not yet been demonstrated how such structure-function measurements can be used to make or alter therapy decisions, nor do we fully understand the clinical meaning of $^3$He or $^{129}$Xe gas distribution measurements or the clinical relevance of their apparent worsening. In this regard, the current case provides evidence that serial $^3$He MRI measurements of airway function (VDP) and parenchyma microstructure can be used to visualize regional changes in COPD subjects, with the potential to modify therapy. However, it must be also noted that for the foreseeable future, this imaging approach cannot be generalized to routine care or screening. Clearly, however, larger scale studies are required to evaluate the effect on COPD outcomes of incorporating pulmonary functional imaging into regular clinical monitoring and visits.
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The University of Western Ontario

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Principal Investigator:

Review Number: 11751

Revision Number:

Protocol Title: A single-center pilot study exploring the utility of magnetic resonance imaging in patients with COPD

Department and Institution: Respiratory, University of Western Ontario

Sponsor: MERCK FROSST

Ethics Approval Date: November 22, 2005

Expiry Date: September 1, 2006

Documents Reviewed and Approved: UWO Protocol, Letters of Information & Consent (main, dated November 10, 2005; and, pharmacogenetic research, dated September 21, 2005), Advertisement


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Principal Investigator:  
Review Number: 13743
Review Date: November 6, 2007
Protocol Title: A single-centre pilot study exploring the utility of magnetic resonance imaging in patients with chronic lung disease
Department and institution: Radiology, Robarts Research Institute
Sponsor:  
Ethics Approval Date: November 27, 2007  
Expiry Date: October 31, 2017
Documents Received for Information: Protocol, October 23, 2007; 1B, 6th ed, 9th Sept 2005

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Principal Investigator:

Review Number: 15830
Review Date: February 10, 2009

Protocol Title: Longitudinal Study of Helium-3 Magnetic Resonance Imaging of COPD

Department and Institution: Diagnostic Radiology & Nuclear Medicine, Robarts Research Institute

Sponsor: INTERNAL RESEARCH FUND-UW

Ethics Approval Date: May 25, 2009
Expiry Date: November 30, 2013


Documents Received for Information: Protocol, January 27, 2006; IB, ed 6, 09 Sep. 05

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines, and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the date noted above. The membership of this REB also complies with the membership requirements for REBs as defined in Division 3 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB’s periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g., change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert
Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Grace Parraga
Review Number: 18131
Review Level: Full Board
Approved Local Adult Participants: 50
Approved Local Minor Participants: 0
Protocol Title: Xenon-129 Magnetic Resonance Imaging of Healthy Subjects: Hardware and Software Development and Reproducibility
Department & Institution: Imaging, Roberts Research Institute
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: August 12, 2011
Expiry Date: August 31, 2016

Documents Reviewed & Approved & Documents Received for Information:

<table>
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<th>Document Name</th>
<th>Comments</th>
<th>Version Date</th>
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</thead>
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<td>Advertisement</td>
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The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

Ethics Office to Contact for Further Information

This is an official document. Please retain the original in your files.

The University of Western Ontario
Office of Research Ethic...
Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Grace Parraga
Review Number: 18730
Review Level: Full Board
Approved Local Adult Participants: 100
Approved Local Minor Participants: 0
Protocol Title: A Single-center Study Evaluating Hyperpolarized 129Xenon Magnetic Resonance Imaging in Subjects with Chronic Lung Disease
Department & Institution: Imaging, Robarts Research Institute
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: August 12, 2011  Expiry Date: August 31, 2016

Documents Reviewed & Approved & Documents Received for Information:

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<tr>
<th>Document Name</th>
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__________________________
Signature

Ethnic Officer to Contact for Further Information

This is an official document. Please retain the original in your files.

The University of Western Ontario
Office of Research Ethics
APPENDIX E - CURRICULUM VITAE

EDUCATION

Sep. 2008 – present
Doctor of Philosophy (Candidate)
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada
Supervisor: Dr. Grace Parraga
Thesis: ‘Hyperpolarized Helium-3 Magnetic Resonance Imaging of Chronic Obstructive Pulmonary Disease’

Bachelor of Science (Gold Medal for Honors Double Major in Applied Mathematics)
Honors Double Major Applied Mathematics and Biology
The University of Western Ontario, London, Ontario, Canada

POSITIONS

Sep. 2008 – present
Research Assistant, Doctoral
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada
Supervisor: Dr. Grace Parraga
Project: ‘Hyperpolarized Helium-3 Magnetic Resonance Imaging of Chronic Obstructive Pulmonary Disease’

Research Assistant, Summer Research Assistantship
Supervisor: Dr. Grace Parraga
Project: ‘Hyperpolarized Helium-3 Magnetic Resonance Imaging of Phenotyping Potential in Chronic Obstructive Pulmonary Disease’

ACADEMIC AWARDS, SCHOLARSHIPS and DISTINCTIONS

Feb. 2013
3rd Annual Canadian Thoracic Society Poster Competition Finalist
Top 30 abstracts out of all Canada-based trainees that submitted to the American Thoracic Society Meeting 2013 selected to compete in poster competition.
National

Jan. 2013
International Society for Magnetic Resonance in Medicine Educational Stipend Award
Awarded to support the attendance of students, postdoctoral and clinical trainees to present abstracts at the scientific meeting.
International
$440 USD

Apr. 2013  CIHR IMPACT Strategic Training Initiatives in Health Research Program
The University of British Columbia
Awarded to high quality clinical and basic science post-doctoral fellows to create the next generation of investigators capable of developing and translating knowledge from bench to bedside with the outcome of improved cardio-pulmonary health status of the Canadian population.
Institutional
$52500

Jun. 2012  Nellie Farthing Fellowship in the Medical Sciences Schulich School of Medicine & Dentistry, The University of Western Ontario
Awarded to a full-time doctoral student in a Schulich School of Medicine & Dentistry graduate program for excellence in research.
Institutional
$3000

Apr. 2012  Three Minute Thesis (3MT) Competition Finalist, The University of Western Ontario
Awarded to the highest ranked graduate students for presentation of their research in 3 minutes or less to a panel of non-specialist judges and peers.
Institutional
$500

Apr. 2012  American Thoracic Society Travel Award from the National Emphysema Foundation Honoring Claude Lenfant
Awarded to support the attendance of fellows or in training members to present abstracts at the scientific meeting.
International
$500 USD

Mar. 2012  International Society for Magnetic Resonance in Medicine Summa Cum Laude Award
Awarded to student members whose abstract scored in the top 3% of all abstracts submitted to the same general category.
International

Feb. 2012  International Society for Magnetic Resonance in Medicine Educational Stipend Award
Awarded to support the attendance of students, postdoctoral and clinical trainees to present abstracts at the scientific meeting.
International
$540 USD

Schulich Graduate Scholarship, The University of Western Ontario  
*Awarded to a full time graduate student for stipend support who has maintained an average of 80% or more.*  
Institutional  
$7647.48

Mar. 2011  
1st Annual Canadian Thoracic Society Poster Competition Finalist  
*Top 29 abstracts out of all Canada-based trainees that submitted to the American Thoracic Society Meeting 2011 selected to compete in poster competition.*  
National

Feb. 2011  
International Society for Magnetic Resonance in Medicine Educational Stipend Award  
*Awarded to support the attendance of students, postdoctoral and clinical trainees to present abstracts at the scientific meeting.*  
International  
$450 USD

Jan. 2011  
3rd Japanese Society of Pulmonary Functional Imaging & 5th International Workshop for Pulmonary Functional Imaging Scientific Presentation Award  
*Awarded in recognition of the excellence of a scientific paper presented orally*  
International

Schulich Graduate Scholarship, The University of Western Ontario  
*Awarded to a full time graduate student for stipend support who has maintained an average of 80% or more.*  
Institutional  
$7376.46

Mar. 2010  
Margaret Moffat Research Day Travel Award, The University of Western Ontario  
*Awarded to the student with the highest ranked poster in the Imaging category*  
Institutional  
$500

May 2010 – May 2013  
Natural Sciences and Engineering Research Council of Canada (PGS-Doctoral)  
National  
$63,000
Sep. 2009 – Sep. 2010  
Schulich Graduate Scholarship, The University of Western Ontario  
*Awarded to a full time graduate student for stipend support who has maintained an average of 80% or more.*  
Institutional  
$7080.87

Jul. 2009  
JR Cunningham Young Investigator Silver Medalist Award Winner  
*Awarded to the top oral presentations in the Young Investigators' Symposium at the Canadian Organization of Medical Physic annual meeting.*  
National  
$300

Jun. 2009  
RSNA Trainee Research Prize Competition Finalist  
*Awarded to the highest ranked abstracts submissions for the RSNA Trainee Research Prize.*  
International

May 2009  
JR Cunningham Young Investigator Finalist  
*Awarded to the top 10 highest scored Young Investigator submissions for the Canadian Organization of Medical Physic annual meeting.*  
National  
$175

May 2009 – May 2010  
Ontario Graduate Scholarship (OGS)  
Provincial  
$15,000

Sep. 2008 – Sep. 2010  
Canadian Institutes of Health Research Strategic Training Program in Vascular Research  
Institutional  
$20,000

Schulich Graduate Scholarship, The University of Western Ontario  
*Awarded to a full time graduate student for stipend support who has maintained an average of 80% or more.*  
Institutional  
$6,683.22

Jul. 2008  
Gold Medal for the Honors Double Major in Applied Mathematics, The University of Western Ontario  
*Awarded to a full time student in the respective Honors program with the highest graduating average.*  
Institutional

May 2008,  
Dean’s Honor List
May 2007  
Awarded to a full time student who has maintained an average of 80% or more.  
Institutional

Sep. 2004  
Western Scholarship of Distinction  
Awarded upon admission to Western students who have an admission average of 85-89.9%.  
Institutional  
$1,500

Sep. 2004  
The Queen Elizabeth II Aiming for the Top Scholarship  
Awarded to students who have shown academic excellence at the high school level.  
Institutional  
$3,500

PUBLICATIONS and PRESENTATIONS

A. Refereed Journal Manuscripts (5 In Press, 14 published, 1 under review, 3 in preparation)

In Press (5)


Published (14)


**Under Review (1)**


**In Preparation (3)**


**B. Published Refereed Conference Papers (2)**


C. **Peer Reviewed Published Conference Abstracts (23)**


8. S Svenningsen, **M Kirby**, A Wheatley, A Farag, A Ouriadov, GE Santyr, DG McCormack and G Parraga. Anatomical Distribution of Hyperpolarized $^3$He and


D. Peer Reviewed Oral Presentations (19) *presenter


16. M Kirby*, A Wheatley, DG McCormack and G Parraga. Development of Image processing methods to quantify Spatial and Temporal Ventilation Dynamics using Hyperpolarized $^3$He magnetic resonance imaging, Society of Photographic Instrumentation Engineers, San Diego, California, USA (02/10)


19. M Kirby, A Wheatley, G Santyr, DG McCormack and G Parraga*. Visualizing and Quantifying Hyperpolarized $^3$He Magnetic Resonance Imaging Ventilation in
COPD pre- and post-Salbutamol. International Summit on Future of Quantitative and Functional Lung Imaging Meeting, Iowa City, Iowa, USA, (10/08)

E. Peer Reviewed Poster Presentations (52)


22. S Svenningsen, **M Kirby**, A Wheatley, A Farag, A Ouriadov, GE Santyr, DG McCormack and G Parraga. Anatomical Distribution of Hyperpolarized $^3$He and $^{129}$Xe MRI Apparent Diffusion Coefficients in Asthma. International Society for Magnetic Resonance in Medicine, Melbourne, Australia (05/12)

23. A Ouriadov, A Farag, **M Kirby**, DG McCormack, G Parraga and G Santyr. Hyperpolarized $^{129}$Xe Apparent Diffusion Coefficient Anisotropy in Chronic Obstructive Pulmonary Disease. International Society for Magnetic Resonance in Medicine, Melbourne, Australia (05/12)


34. **M Kirby**, N. Krowchuk, A Wheatley, DG McCormack, H Coxson, G Parraga. Evaluation of COPD Airway Function and Structure using Hyperpolarized 3He
35. S Svenningsen, **M Kirby**, H Ahmed, N Paterson, G Parraga. Evaluation of Short Term Reproducibility of Hyperpolarized Helium-3 Magnetic Resonance Imaging in Adult Cystic Fibrosis using a Semi-Automated Segmentation Tool. International Society for Magnetic Resonance in Medicine, Montreal, Quebec, Canada (05/11)


38. L Mathew, **M Kirby**, R Etemad-Rezai, D G McCormack, G Parraga. Hyperpolarized $^3$He Magnetic Resonance Imaging Biomarkers of Bronchoscopic Airway Bypass in COPD. 5th International Workshop for Pulmonary Functional Imaging, Awaji, Japan (01/11)

39. SE Costella, S Choy, **M Kirby**, A Wheatley, R Etemad-Rezai, DG McCormack, G Parraga. Hyperpolarized $^3$He MRI Apparent Diffusion Coefficients as a Probe of Airway Function in Asthma After Methacholine Challenge and Recovery. 5th International Workshop for Pulmonary Functional Imaging, Awaji, Japan (01/11)


43. **M Kirby**, L Mathew, A Wheatley, DG McCormack and G Parraga. Inter-Observer Reproducibility of Longitudinal Hyperpolarized Helium-3 Magnetic...
Resonance Imaging of Chronic Obstructive Pulmonary Disease. International Society for Magnetic Resonance in Medicine, Stockholm, Sweden (05/10)


52. **M Kirby**, L Mathew, A Wheatley, DG McCormack and G Parraga. Chronic Obstructive Pulmonary Disease Progression Detected by Hyperpolarized Helium-
F. Invited Presentations (4)


**POST-GRADUATE EDUCATION DEVELOPMENT**


**Graduate Student Supervisor**

**Resident:** Aasim Hasany, 2nd year Internal Medicine, UWO

6 Week Project: ‘Longitudinal Study of Oscillatory Positive Expiratory Pressure (oPEP) in Stable COPD’

*Poster Presentation ImNO: N Kanhere, A Hasany, **M Kirby**, J Suggett, DG McCormack, G Parraga. Hyperpolarized ³He Magnetic Resonance Imaging following Oscillatory Positive Expiratory Pressure Treatment in GOLD stage II and III COPD. Imaging Network Ontario Symposium, Toronto, Ontario, Canada (02/13)*

May 2012 – Aug. 2012

**Graduate Student Supervisor**

**Medical Student:** Lauren Villemaire, 2nd year Medicine, UWO

*Project: ‘Hyperpolarized Helium-3 Diffusion-Weighted Magnetic Resonance Imaging of Cystic Fibrosis’*


*Manuscript in Preparation: **M Kirby**, L Villemaire, H Ahmed, NAM*


**Graduate Student Supervisor**

**Resident:** Yaj Shukla, 2nd year Internal Medicine, UWO

**6 Week Project:** ‘Hyperpolarized $^{129}$Xe Magnetic Resonance Imaging: Tolerability in Healthy Volunteers and Subjects with Pulmonary Disease’


**Jun. 2011**

**3D Slicer Workshop**

**Instructor:** Dr. Ron Kikinis

**Objective:** Introduction to the 3D Slicer environment, as well as to illustrate the ability to integrate with ITK and Python modules.

**Jun. 2011 – Aug. 2011**

**Graduate Student Supervisor**

**Medical Student:** Casey Neron, 2nd year Medicine, UWO

**8 Week Project:** ‘Evaluating the Relationship between Atherosclerosis and Lung Function in Subjects with Smoking Histories’


Sep. 2010 – Apr. 2011  **Graduate Student Supervisor**

*Undergraduate Student:* Sarah Svenningsen, 4th year Medical Biophysics, UWO


Sep. 2010 – Dec. 2010  **Graduate Teaching Assistant**

3505F Mathematical Transform Applications in Medical Biophysics
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada

Sep. 2009 – Dec. 2009  **Graduate Teaching Assistant**

3505F Mathematical Transform Applications in Medical Biophysics
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada

**RELEVANT GRADUATE COURSES**
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada

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<tr>
<th>Sep. 2009 – Apr. 2010</th>
<th>BIOPHYS 9663</th>
<th>MRI Physics</th>
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<td>BIOPHYS 9516</td>
<td>Imaging Principles</td>
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<td>BIOPHYS 9662</td>
<td>Nuclear Magnetic Resonance Imaging</td>
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<td>BIOPHYS 9509</td>
<td>Digital Image Processing</td>
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<th>BIOPHYS 9515</th>
<th>Medical Imaging</th>
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<td>VASCProg 9603</td>
<td>Research Ethics</td>
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<td>BIOPHYS 9513</td>
<td>Scientific Communication</td>
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<td></td>
<td>BIOPHYS 9522</td>
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**PROFESSIONAL MEMEBERSHIPS**
2010 – 2011  Biomedical Engineering Society
Student Member
2009 – present  Canadian Thoracic Society
Student Member
2008 – present  American Thoracic Society
Student Member
2008 – present  International Society of Magnetic Resonance in Medicine
Student Member
2008 – present  Canadian Organization of Physicists in Medicine
Student Member

LEADERSHIP and VOLUNTEER ACTIVITIES
2013  Volunteer, Let’s Talk Science
       Member, Biomedical Imaging Research Centre (BIRC)
       Member, Network of Imaging Students (NOISe)

2012  Volunteer, Canadian Cancer Society, Relay for Life
       Volunteer, Let’s Talk Science
       Member, Biomedical Imaging Research Centre (BIRC)
       Member, Network of Imaging Students (NOISe)

2011  Volunteer, Let’s Talk Science
       Member, Biomedical Imaging Research Centre (BIRC)
       Member, Network of Imaging Students (NOISe)

2010  Member, Biomedical Imaging Research Centre (BIRC)
       Member, Network of Imaging Students (NOISe)

2009  Volunteer, Doors open London, Robarts Research Institute
       Member, Network of Imaging Students (NOISe)

2008  Member, Network of Imaging Students (NOISe)