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Metabolic Imaging of Early Radiation-Induced Lung Injury Using Hyperpolarized 13C-Pyruvate in Rodent Lungs

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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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METABOLIC IMAGING OF EARLY RADIATION-INDUCED LUNG INJURY USING HYPERPOLARIZED $^{13}$C-PYRUVATE IN RODENT LUNGS

Thesis format: Integrated Article

by

Kundan Thind

Graduate Program in Medical Biophysics, CAMPEP stream

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

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

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Abstract

Lung cancer is the leading cause of cancer related death. Radiation therapy is a prominent treatment method but leads to adverse consequences. Radiation-Induced Lung Injury (RILI) is the primary adverse consequence that limits further radiation therapy and develops in 5-37% of the treated patients. RILI proceeds in two distinct phases: a) early and reversible Radiation Pneumonitis (RP), and b) late and irreversible radiation fibrosis. Clinically, Dose Volume Histogram (DVH) parameters derived from radiation therapy planning stage are used to determine outcome and severity of RP but have been demonstrated to possess a very low predictive power. Computed Tomography (CT) is the most commonly used modality for the imaging of RP, but often only detects very late RP that leaves little room for intervention to abort the progress to irreversible radiation fibrosis. Early detection of RP using imaging may allow for interventional treatment and management of the disease and the associated symptoms in a better manner. Improvement in Dynamic Nuclear Polarization (DNP) technology has led to advancement of hyperpolarized 13-Carbon-Magnetic Resonance Imaging (13C-MRI). In this thesis, we present the investigation of early detection of RP with 13C-MRI in an animal model with the use of hyperpolarized 13C-pyruvate. A pilot study demonstrated the proof of concept along with a qualitative histological confirmation. 13C-MRI data and histology data were collected 2 weeks post irradiation of whole thorax in rodents. In the subsequent study, regional and longitudinal 13C-MRI and quantitative histology data were analyzed to demonstrate the early organ-wide response of RP. These data were collected at day 5, 10, 15 and 25 post conformal irradiation of the right rodent lung. Finally, we demonstrate a novel approach to map pH using hyperpolarized 13C-bicarbonate with the use of spiral-Iterative Decomposition of water and fat with Echo Asymmetry and Least squares estimation (IDEAL) pulse sequence. Validation of this approach by comparison to Chemical Shift Imaging (CSI) pH measurement and standard pH measurement with the aid of phantoms along with hyperpolarized 13C-bicarbonate is presented. pH mapping may play a role in the staging and therapeutic intervention of cancer.

Keywords: Hyperpolarized 13C, 13C-pyruvate, 13C-lactate, 13C-MRI, Radiation-Induced Lung Injury, Radiation Pneumonitis, 13C-bicarbonate, 13C-carbon dioxide, pH mapping, cancer pH, IDEAL
Co-Authorship Statement


Kundan Thind was responsible for construction of a $^{13}$C-birdcage coil and a $^1$H-surface coil in addition to the experimental design, testing of protocols, animal preparations, experimental data acquisition and analysis. Dr. Alexei Ouriadov provided assistance in optimization of the RF coils and the pulse sequences. Dr. Eugene Wong and Dr. Matthew Fox assisted with the irradiation procedure. Dr. Lanette Friesen-Waldner assisted with the animal preparation and in polarization of the $^{13}$C-pyruvate. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Eugene Wong and Prof. Jake Van Dyk provided meaningful discussion about the work. Dr. Giles Santyr provided assistance with the manuscript presentation. This work has been published in Magnetic Resonance in Medicine.

Mapping metabolic changes associated with early Radiation-Induced Lung Injury post conformal radiation therapy using hyperpolarized $^{13}$C-pyruvate Magnetic Resonance Spectroscopic Imaging (August 2013, Radiotherapy and Oncology - RO-D-13-00907):

Kundan Thind was responsible for construction of a $^{13}$C-TORO coil system in addition to the experimental design, experimental data acquisition and analysis. Michael Jensen assisted with the irradiation procedure using micro-CT. Dr. Francisco Martinez and Dr. Tim Scholl assisted in the polarization of $^{13}$C-pyruvate for the experiments. Elaine Hegarty performed the animal preparation pre-MRI and obtained histology post-MRI. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Eugene Wong and Prof. Jake Van Dyk provided meaningful discussion about the work. Dr. Giles Santyr provided assistance with the manuscript presentation. This work has been submitted to the Journal of Radiotherapy and Oncology.

Rapid and Accurate Mapping of pH using Spiral-IDEAL with Hyperpolarized $^{13}$C-bicarbonate (to be submitted to Magnetic Resonance in Medicine):
Kundan Thind was responsible for construction of the phantoms, experimental design, experimental data acquisition and analysis. Dr. Francisco Martinez and Dr. Tim Scholl assisted in the polarization of $^{13}$C-bicarbonate for the experiments. Curtis Wiens provided assistance with the IDEAL pulse sequence. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Giles Santyr provided assistance with the manuscript presentation. This work is being prepared for submission to Magnetic Resonance in Medicine.
Acknowledgments

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<td>$^{13}\text{C}$</td>
<td>Carbon-13</td>
</tr>
<tr>
<td>$^{18}\text{F-FDG}$</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>$^{60}\text{Co}$</td>
<td>Cobalt-60</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>CA</td>
<td>Carbonic Anhydrase</td>
</tr>
<tr>
<td>CEST</td>
<td>Chemical Exchange Saturation Transfer</td>
</tr>
<tr>
<td>CSI</td>
<td>Chemical Shift Imaging</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DL$_{\text{CO}}$</td>
<td>Diffusing Capacity of Carbon Monoxide</td>
</tr>
<tr>
<td>DNP</td>
<td>Dynamic Nuclear Polarization</td>
</tr>
<tr>
<td>DVH</td>
<td>Dose Volume Histogram</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>Forced Expiration Volume in One Second</td>
</tr>
<tr>
<td>FID</td>
<td>Free Induction Decay</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of View</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width at Half Maximum</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>Gadolinium-$^{99}\text{mTc}$-Diethylenetriaminepentaacetic acid</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>HEPA</td>
<td>High efficiency particulate air</td>
</tr>
<tr>
<td>HIF-1</td>
<td>Hypoxia Inducible Factor-1</td>
</tr>
<tr>
<td>HNG</td>
<td>Hyperpolarized Noble Gas</td>
</tr>
<tr>
<td>HVL</td>
<td>Half Value Layer</td>
</tr>
<tr>
<td>IDEAL</td>
<td>Iterative Decomposition of water and fat with Echo Asymmetry and Least squares estimation</td>
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<tr>
<td>Lac/Pyr</td>
<td>$^{13}\text{C}$-Lactate to $^{13}\text{C}$-Pyruvate Signal Ratio</td>
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<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MLD</td>
<td>Mean Lung Dose</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>MRSI</td>
<td>Magnetic Resonance Spectroscopic Imaging</td>
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<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>NOE</td>
<td>Nuclear Overhauser Effect</td>
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<tr>
<td>NSCLC</td>
<td>Non-Small Cell Lung Carcinoma</td>
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<tr>
<td>SCLC</td>
<td>Small Cell Lung Carcinoma</td>
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<tr>
<td>NTCP</td>
<td>Normal Tissue Complication Probability</td>
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<tr>
<td>$\text{pO}_2$</td>
<td>Partial Pressure of Oxygen in Arterial Blood</td>
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<td>Positron Emission Tomography</td>
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<td>PFT</td>
<td>Pulmonary Function Tests</td>
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<td>Extracellular pH</td>
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<tr>
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<td>Intracellular pH</td>
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<td>RF</td>
<td>Radiofrequency</td>
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<td>RILI</td>
<td>Radiation-Induced Lung Injury</td>
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<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>RP</td>
<td>Radiation Pneumonitis</td>
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<tr>
<td>SNR</td>
<td>Signal-to-Noise-Ratio</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Transforming Growth Factor Beta One</td>
</tr>
<tr>
<td>TORO</td>
<td>Transmit-Only/Receive-Only</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

This chapter provides the introduction and motivation for the work presented in this thesis. A review of healthy lung physiology, Radiation-Induced Lung Injury and radiopathology of the irradiated lung is provided in sections 1.2, 1.3 and 1.4. Section 1.7 provides an introductory review of basic physics of Magnetic Resonance Imaging (MRI). Section 1.8 describes the hyperpolarization process and biological context for the $^{13}$C-susubstrates used in the thesis. To put the research work within context, clinical and functional imaging methods for Radiation-Induced Lung Injury are introduced in Sections 1.5 and 1.6. Organization of the thesis is provided towards the end of this chapter (section 1.9).

1.1 Overview and Motivation

Recent developments in the hyperpolarization process have led to the imaging of Carbon-13 ($^{13}$C)-labelled substrates and their corresponding downstream metabolites *in vivo* in real time (1), enabling the study of specific enzyme-catalyzed reactions coupled to metabolism. Changes in metabolism precede anatomical and functional changes and can help identify diseases at an earlier time point than conventional imaging. $^{13}$C-MRI has been moderately successful at detection of abnormal metabolism in cancer at a pre-clinical level and is now moving towards clinical application, most recently in the form of a phase I clinical trial for prostate cancer. (2) Lung cancer is responsible for the most cancer related deaths, with radiation therapy as one of the prominent methods of treatment. Up to 37% of the lung cancer patients treated with radiation therapy develop Radiation-Induced Lung Injury (RILI). (3) In this thesis, $^{13}$C-MRI is used to image metabolism in real time in a rat model of RILI. The aim of the work was to detect regional metabolic changes in RILI prior to any functional or anatomical changes. Additional work towards pH mapping with $^{13}$C-MRI was also performed with application towards diseases with abnormal regional pH such as cancer.
1.2 Healthy Lung Physiology

The primary function of the lung is to facilitate gas exchange, specifically absorption of oxygen and elimination of carbon dioxide. The lung enables this process using a large surface area for the contact of air and blood separated by a very thin tissue boundary. (4) The structure of the lung begins with the largest airway called the trachea, which progressively branches twenty-three times into narrower airways until the alveolar level is reached. (5) Alveoli are tiny air sacs consisting of a very thin tissue boundary (or septum) that is the site of gas exchange. Driven primarily by diffusion, gas exchange takes place as the inhaled oxygen is able to diffuse into the capillaries from the alveoli and carbon dioxide is able to diffuse from capillaries to the alveoli. There are on average 480 million alveoli in the human lung (6) and the total air exchange surface area is approximated to 130 m². (4) The interested reader is referred to “Respiratory physiology: The Essentials” by John B. West (7) for comprehensive details on lung function, ventilation and gas exchange.

1.3 Lung Function Tests

A variety of respiratory diseases can lead to the impairment of lung function. Pulmonary Function Tests (PFT) are used to evaluate global function of the diseased lung. The most common PFT is spirometry, which measures airflow as a function of time during both the inspiration phase and the expiration phase of a breathing cycle and is widely considered the gold standard for measuring lung function. (8) Forced Expiration Volume in One Second (FEV₁), the maximum volume of air expired during forced expiration in one second is used to evaluate severity of the disease. In addition, plethysmography is widely used to measure static lung volumes. Spirometry and plethysmography are quick and inexpensive but do not provide any information about gas exchange. Measurement of Diffusing Capacity of Carbon Monoxide (DL_{CO}) provides insight into diffusion of gases across the alveolar membrane by quantifying the alveolar volume and the concentration of carbon monoxide in exhaled gas following inhalation of a known quantity of carbon monoxide gas. (9) Measurement of Partial Pressure of Oxygen in Arterial Blood (p_{a}O₂) is used to identify the global level of hypoxia in the body. A p_{a}O₂ measurement of less than 60 mmHg is considered as clinical hypoxia. (10) All these tests measure global lung function and overall health of the
respiratory system, but lack regional information. Spatial localization of the underlying disease may provide a better insight into the disease pathology. Improved sensitivity may also aid in earlier detection of the underlying disease.

### 1.4 Radiation Therapy of Lung Cancer

#### 1.4.1 Lung Cancer

Lung cancer is responsible for the highest number of cancer deaths in the US population (11) and in the global population. (12) Smoking has been established as one of the largest risk factors globally and is responsible for lung cancer in 80% and 50% of males and females respectively. (13) There is no known screening test for lung cancer. The diagnosis usually comes after presentation of clinical symptoms such as tiredness, lack of energy, shortness of breath, coughing, lack of appetite, hemoptysis, chest pain and dyspnea. (14) The prognosis for lung cancer is very poor with global 5-year survival for lung cancer patients ranging between 6-14% for men and 7-18% for women. (15) Lung cancer can be broadly divided into two major categories with about 85 – 90% of the cases categorized as Non-Small Cell Lung Carcinoma (NSCLC) and 10 – 15% of the cases as Small Cell Lung Carcinoma (SCLC). (16) Internationally accepted staging of lung cancer is based primarily on three categories: T-primary tumour, N-regional lymph nodes, and, M-distant metastasis, known as the (TNM) classification system. T ranges from one to four based on the size and location of the primary tumour within the thorax. N is assigned a value from zero to three based on the metastasis and location of regional lymph nodes within the thorax. M is assigned a one if a distant metastasis is present, zero otherwise. (17) Following the TNM classification of the lung cancer, it is grouped into stages ranging from stage I to stage IV where each overall stage has similar treatment options and survival. For example, stage II could be one of T1N1M0, T2N1M0 and T3N0M0.

#### 1.4.2 Treatment of Lung Cancer

Surgical resection, radiation therapy and chemotherapy are the three most common treatment options for lung cancer patients. Radiation therapy is one of the most prominent methods used for treatment of lung cancer for curative as well as palliative care. It is used in 49.3% of
the NSCLC patient population and 47% of the SCLC patient population. (18) Standard treatment for stages I and II lung cancers is a surgical operation. (19) Adjuvant therapy may be offered to the patient post resection in the form of radiation therapy or chemotherapy. (20, 21) Patients unfit for surgery due to poor PFT are treated with radiation therapy. Curative therapy for stage III lung cancer is provided with chemotherapy in combination with radiation therapy or surgery. Treatment for Stage IV cancer patients is a combination of chemotherapy and palliative radiation therapy. (19, 22)

1.4.3 Radiation-Induced Lung Injury (RILI)

Radiation therapy for the treatment of lung cancer is used with a curative intent, but can lead to adverse consequences. The therapeutic radiation beam is not only damaging to the lung cancer but often the healthy tissue around it as well. As the lung is an extremely radiosensitive organ, (23) irradiation of the healthy tissue leads to unintended consequences known as RILI. This leads to a significant degradation in quality of life of the patient due to symptoms such as dyspnea, cough and fever that accompany decline in lung function. (3) In addition, onset of RILI is the primary criterion that limits the allowable therapeutic dose. RILI occurs in up to 37% of lung cancer patients treated with radiation therapy and the incidence of moderate to severe injury ranges from 10-20%. (24) The three-year survival rate for subjects with severe RILI is a dismal 0%. (25)

RILI occurs in two distinct stages, the Radiation Pneumonitis (RP) phase and the radiation fibrosis phase. A schematic of pathological events post radiation therapy leading to RP and fibrosis is shown in Figure 1-1. RP is the initial acute phase and presents with symptoms of shortness of breath, cough and mild fever that onset 1 – 6 months after the beginning of radiation therapy. (26) RP is marked by inflammation and macrophage accumulation and proliferation, leading to capillary obstruction and septal thickening. (3, 27) If RP is not treated in time with steroids, (26) the persistence of Reactive Oxygen Species (ROS), hypoxia, proliferation of macrophages and cytokines transform RP in to radiation fibrosis. The chronic phase of radiation fibrosis is an irreversible process and typically occurs months to years after the onset of radiation therapy and is marked by progressive chronic dyspnea. (26)
1.4.4 Radiopathology of Irradiated Lung Tissue

Ionizing radiation to the lung inflicts injury primarily to type II pneumocytes and vascular endothelial cells leading to leakage of protein rich plasma into alveolar space resulting in edema and infiltration by inflammatory macrophages. Histological evidence of edema and inflammation during RP is shown in Figure 1-2. These changes cause impairment of gas exchange and reduction in lung compliance. (28-30) At a cellular level, tissue hypoxia is an important contributing factor in mediation of the inflammatory process and the onset of RP. (31) Clinically, p$_a$O$_2$ has been used as an indicator of hypoxia with a measurement of below 60 mmHg deemed as clinical hypoxia (10). Persistence of hypoxia has been reported post radiation therapy (32, 33) and recent studies suggest that it may exacerbate lung injury further. (26) Immunohistochemical analysis in animal models of RILI reveals underlying changes in resident alveolar macrophages, inflammatory cytokines, type II pneumocytes, endothelial cells and fibroblasts. (34, 35) During RP, an inflammatory reaction takes place in response to the radiation damage and as a result leukocytes are recruited from outside the radiation field. (36) Fibroblasts are the first to respond and release cytokines along with chemotactic factors. Circulating monocytes follow to the site of the injury and differentiate into macrophages. (37) Macrophages are a source of ROS and the inflammatory cytokines. The growth factors affect the endothelial cells and may lead to angiogenesis. In addition, mast cells may also be present and lead to synthesis and storage of histamine and cytokines. (35) Persistence of hypoxia and cytokines leads to chronic oxidative stress and tissue remodelling which instead of restoring the healthy lung tissue results in radiation fibrosis.

The onset of hypoxia alters the process of glycolysis in a cell by switching from predominantly aerobic respiration to anaerobic respiration. As the oxygen availability diminishes, anaerobic respiration starts to play a bigger role in the production of energy molecule Adenosine Triphosphate (ATP) in the cell. This phenomenon is known as the Pasteur effect and leads to a higher production of lactate inside the cell. (38) Further evidence on Hypoxia Inducible Factor-1 (HIF-1) that presents with onset of hypoxia suggests that it regulates glycolysis for additional lactate production to ensure cell survival. (39)
Figure 1-1: Radiation therapy leads to tissue damage that induces an inflammatory reaction and macrophage activation and accumulation. Hypoxia mediates these processes and further leads to cytokine and ROS production during Radiation Pneumonitis. These progressions cause additional tissue damage to type II pneumocytes and endothelial cells. If hypoxia and cytokines persist, over time they lead to chronic oxidative stress and tissue remodelling that results in irreversible lung fibrosis. Adapted from reference (26).
Figure 1-2: Haematoxylin and eosin stained sections of healthy lung (left) and irradiated lung (right) from C57BL/6J mice post 12.5 Gy dose at 100× magnification (top) and 180× magnification (bottom) showing edema and inflammation during RP (2 weeks post-irradiation). Arrow 1 shows alveolar wall thickening, arrow 2 shows interstitial edema and arrow 3 shows peribronchial inflammation. Adapted from reference (40).

1.5 Clinical Diagnosis of Radiation-Induced Lung Injury

1.5.1 Epidemiology and Risk Factors

As described earlier, RILI develops in up to 37% of lung cancer patients post treatment with radiation therapy and moderate to severe injury develops in 10-20% of lung cancer patients. (24) Multiple risk factors for RILI have been identified from the existing data in the literature. Some of these include pulmonary dysfunction ($p_{a}O_2 < 80$ mmHg), poor PFT outcome, concurrent chemotherapy and radiation therapy, primary tumour located in lower-lobe of lung, large treatment volume, high dose rate, high dose and elevated levels of
Transforming Growth Factor Beta One (TGFβ1). (25, 41-45) These risk factors can be categorized as either treatment-specific or patient-specific as shown in Table 1-1.

<table>
<thead>
<tr>
<th>treatment-specific</th>
<th>patient-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>• large treatment volume</td>
<td>• poor performance status</td>
</tr>
<tr>
<td>• high dose and dose rate</td>
<td>• smoking history and pulmonary dysfunction (pO2 &lt; 80)</td>
</tr>
<tr>
<td>• concurrent chemoradiation with chemotherapy drugs such as: cisplatin, paclitaxel, docetaxel and mitomycin C</td>
<td>• old age, &gt; 60 years</td>
</tr>
<tr>
<td></td>
<td>• primary tumour located in lower lobe</td>
</tr>
<tr>
<td></td>
<td>• elevation of TGFβ1 post treatment</td>
</tr>
</tbody>
</table>

Table 1-1: Risk factors for development of Radiation Pneumonitis post radiation therapy. Adapted from reference (46)

Despite the identification of risk factors, clear predictive relationships between risk factors and RP have not been established. (47) Although widespread use of 3D-Computed Tomography (CT) has led to improvements in dose planning accuracy whereby high dose is delivered to the target tissue (e.g. tumour) and minimal dose is delivered to the surrounding healthy tissue, RILI is still not predictable. Dose Volume Histogram (DVH) parameters can be helpful and conveniently generated from the 3D-CT plans. Generation of DVH parameters and their implication to RILI are discussed further in the next section.

1.5.2 Dose Volume Histogram Parameters

DVH parameters provide a cumulative dose-volume relationship for single or paired organs in a graphic and mathematical plot. The 3D dose plan from CT is converted to a 2D format that is easier to visualize for dose to the tumour and the critical structures around it. This allows for easier identification of dose threshold to the volume of tumour and the volume of healthy tissue. (46) The major drawback of DVH parameters is the lack of 3D spatial volume and the assumption that all spatial volumes are equal in function. Figure 1-3 shows a representative DVH for treatment of lung tumour with dose-volume plotted as a function of total dose for various tissues as well as the planning target volume i.e. tumour and surrounding tissue.
1.5.3 Signs, Symptoms and Radiographic Findings

Radiation Pneumonitis can manifest as a symptomatic or as an asymptomatic injury. Symptoms of the injury can start as soon as 1 month and as late as 6 months post radiation therapy, but often appear at 2-3 months after the treatment. Dyspnea is the most prominent symptom of RP and can progress into severe respiratory illness. Several other symptoms such as hemoptysis, cough, fever and chest pain can also co-exist. (46) Patients with asymptomatic of RP are often revealed by radiographic findings on plane film chest X-ray at the very late stage of RP. Changes appear as increased ground glass opacity and lack of clear demarcations of normal pulmonary structure following radiation therapy. (52) These changes can range from minimal i.e. slight indistinctness of pulmonary vasculature to complete consolidation of the irradiated area. (53) Computed tomography (CT) indications of very late RP are often observed post radiation therapy. CT evidence for RILI is described as: i) increase in density uniformly across the irradiated volume ii) patchy consolidation not conforming to the irradiated volume iii) discrete, non-uniform consolidation across the irradiated volume, and iv) solid consolidation across the irradiated volume and associated bronchi. (54) Figure 1-4 demonstrates two cases of RILI-CT appearance post radiation therapy treatment that are distinctly different in their appearance and region of occurrence. CT is considered more sensitive than plane film X-ray for early detection of RILI. (54-56) Detection of late RP by X-ray and CT is of little help, as the late detection offers no opportunity to modify the radiation therapy treatment plan.

![Figure 1-4](image_url)

**Figure 1-4:** Left image for a) and b) show axial CT treatment planning image with contours conforming to radiation therapy isocenter. Corresponding right image in a) was taken 5 months after radiation therapy and shows appearance of late RP as irregularly shaped consolidation, non-conformal to dose deposition curves. Right image in b) was taken 6 months post radiation therapy and shows appearance of RP as solid homogeneous consolidation in the irradiated area. Figure adapted from (57).
1.6 Functional Imaging of Radiation-Induced Lung Injury

Imaging modalities such as Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET) and MRI have been used to perform imaging of RILI. Measures of ventilation, perfusion, relaxation times and apparent diffusion coefficients by these modalities can provide a sensitive measure to assess lung anatomy and function.

1.6.1 Nuclear Medicine Imaging Methods

Functional imaging with SPECT can provide ventilation and perfusion maps of the lung post injection of Technetium-99m. (58, 59) Studies have demonstrated a dose-dependent reduction in perfusion and ventilation at 3-4 months post radiation therapy. (60, 61) However, correlations between these metrics and whole lung function assessed by PFT are low and additional work is required to assess regional injury. (62) Additionally, SPECT imaging is limited in resolution, lacks specificity and provides ~3 mSv of extra whole body radiation dose to the patient. (63) PET imaging with Fluorodeoxyglucose ($^{18}$F-FDG) has been performed in lung cancer patients post radiation therapy. The technique is heavily dependent on tissue uptake of $^{18}$F-FDG and is susceptible to a high false positive rate. (64) High uptake of $^{18}$F-FDG is associated with inflammation and tumour growth but no clear separation of cancer and RP has been demonstrated. (65) The tumour response is highly correlated to the detection of inflammatory change by PET. (66) As with SPECT, PET also provides additional radiation dose to the patient and is limited in resolution.

1.6.2 Magnetic Resonance Imaging Methods

MRI uses non-ionizing Radiofrequency (RF) pulses to obtain multiplanar images that are rich in soft-tissue contrast. Functional MRI has been used to assess lung injury post radiation therapy using a variety of methods. Perfusion imaging using Gadolinium-99mTc-Diethylenetriaminepentaacetic acid (Gd-DTPA) has demonstrated consistent contrast enhancement in irradiated dog thorax compared to an unirradiated dog thorax. (67) Significant increases in relaxation times ($T_1$ and $T_2$) have also been shown in rat thorax post irradiation. (68) Hyperpolarized gas imaging ($^{3}$He) with MRI has demonstrated changes in apparent diffusion coefficients in rodent lungs post radiation injury. (69) This technique has been applied in a clinical setting and correlation between severity of radiation injury and percent-ventilated volume has been quantified. (70) While functional MRI methods
demonstrate an improvement over the nuclear medicine imaging methods, they are still based on morphological and functional changes that often occur during the late phase of the injury.

As metabolic changes precede morphological and functional changes, quantification of metabolic signature may help capture early onset of the disease. As such, metabolic imaging of early RP with hyperpolarized $^{13}$C-pyruvate may lead to early diagnosis of the disease using MRI. A review of basic MRI concepts that are applicable to this thesis is provided in the next section.

1.7 Nuclear Magnetic Resonance

The source of detectable signal in Nuclear Magnetic Resonance (NMR) is the precession of an ensemble average of nuclear magnetic moments in the presence of an applied static magnetic field. Interaction of this ensemble, or magnetization, with time-dependent secondary magnetic fields provides an array of information about their spatial position and local environment. The origin of the magnetization and its interaction with an external magnetic field can be described theoretically using either or both of a quantum mechanical approach or a classical approach. The interested reader is referred to (71, 72) for more details. In this section, introductory background material is provided on the origin of nuclear magnetization, behaviour in the presence of external magnetic fields, polarization and chemical shift as a theoretical basis for the thesis work described. The basics of the MRI pulse sequence for Chemical Shift Imaging (CSI), gradient echo imaging and finally, the Dixon approach, central to the methods used in this thesis, are also described.

1.7.1 Nuclear Magnetic Moment

The atomic nucleus is composed of neutrons and/or protons. NMR is derived from nuclei consisting an odd number of neutrons and/or protons. These unpaired neutrons and/or protons confer a property to the nucleus know as spin ($I$). Spin causes the nucleus to behave as a spinning charge enabling the nucleus to acquire a spin angular momentum ($J$). The resulting magnetic moment is given by:

$$\vec{\mu} = \gamma \vec{J} = \gamma \hbar \vec{I},$$  \[1\]
where $\gamma$ is the gyromagnetic ratio of the nucleus under consideration, $J$ is the spin angular momentum, $\hbar$ is the reduced Planck’s constant, and $I$ is the integer half-spin. Gyromagnetic ratios of the two nuclei discussed in this thesis are given in Table 1-2.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>$\gamma$ [MHz rad $^{-1}$ T$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>267.519</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>67.261</td>
</tr>
</tbody>
</table>

**Table 1-2:** Gyromagnetic ratios for Spin $\frac{1}{2}$ Nuclei described in this thesis.

### 1.7.2 Magnetic Moment in the Presence of a Magnetic Field

In the presence of a uniform external static magnetic field, $\vec{B}_0$, the magnetic moment experiences a torque. This torque is described by the following equation:

$$\vec{\tau} = \vec{\mu} \times \vec{B}_0.$$  \[2\]

The principle of conservation of angular momentum requires that this torque be equivalent to the time derivative of the angular momentum, leading to the following equation of motion:

$$\frac{d}{dt} \vec{J} = \vec{\mu} \times \vec{B}_0.$$  \[3\]

Incorporating equation [1] into equation [3] yields:

$$\frac{d}{dt} \vec{\mu} = \vec{\mu} \times \gamma \vec{B}_0.$$  \[4\]

The above differential equation has a well known solution and is given by:

$$\vec{\mu} = \mu_0 e^{-i\omega t},$$  \[5\]

where $\omega$ is the Larmor frequency and is represented by the equation of motion that describes precession, such that:

$$\vec{\omega} = -\gamma \vec{B}_0.$$  \[6\]
The negative sign implies a clockwise direction of rotation when looking into the head of the vector \( \vec{B}_0 \). \( \omega \) can be readily converted to frequency in Hz by the following expression:

\[
\bar{\omega} = 2\pi \bar{f}.
\]

Therefore, the expression for Larmor frequency in Hz is as following:

\[
\bar{f} = -\frac{\gamma}{2\pi} \vec{B}_0.
\]

### 1.7.3 Magnetization and Polarization

In the presence of an external magnetic field, nuclei that possess spin angular momentum tend to align with the magnetic field. The potential energy of a magnetic moment in the presence of an external magnetic field is given by:

\[
E = -\vec{\mu} \cdot \vec{B}_0.
\]

The nuclei in Table 1-2 can exist in either \( I = + \frac{1}{2} \) spin state or \( I = - \frac{1}{2} \) spin state. The spin state of \( + \frac{1}{2} \) corresponds to alignment with the external magnetic field and is referred to as \textit{spin up}, whereas the spin state of \( - \frac{1}{2} \) corresponds to alignment against the external magnetic field and is referred to as \textit{spin down}. Net magnetization arises from a vector sum of spins aligned with the external magnetic field in a unit volume. Following equation [9], the spin states are separated by a net amount of energy given by:

\[
\Delta E = \gamma \hbar B_0.
\]

Alignment of spins is approximated by the Boltzmann distribution. The ratio of nuclei with \textit{spin up} state to nuclei with \textit{spin down} state at a given temperature \( T \) is given by:

\[
\frac{N_u}{N_d} = e^{(-\Delta E/kT)},
\]

where \( N_u \) is the number of spins aligned with the magnetic field and \( N_d \) is the number of spins aligned against the magnetic field. This phenomenon of spin alignment is depicted in Figure 1-5 that shows an aggregate of spins placed in an external magnetic field \( B_0 \). The
excess of \( I = + \frac{1}{2} \) spins aligned with the external magnetic field compared to \( I = - \frac{1}{2} \) gives rise to polarization, which is defined as:

\[
P = \frac{N_1 - N_{-1}}{N_1 + N_{-1}}. \tag{12}\]

Rearrangement of equations [10], [11], [12] provides the following expression for thermal polarization:

\[
P = \frac{e^{\Delta E/kT} - e^{-\Delta E/kT}}{e^{\Delta E/kT} + e^{-\Delta E/kT}} = \tanh \left( \frac{\Delta E}{kT} \right) = \tanh \left( \frac{\hbar \gamma B_0}{2kT} \right). \tag{13}\]

Typically \( 2kT \) is a much bigger quantity than \( \hbar \gamma B_0 \), simplifying the expression for polarization in equation [13] to:

\[
P \approx \left( \frac{\hbar \gamma B_0}{2kT} \right). \tag{14}\]

**Figure 1-5**: An aggregate of spins placed in an external magnetic field.

Magnetization can be expressed in terms of polarization in the following way:

\[
M = N \cdot \mu \cdot P, \tag{15}\]
where $N$ is the number of magnetic dipole moments per unit volume. Combining equations [1], [14] and [15] leads to the following expression for the net magnetization in the presence of an external magnetic field $B_0$:

$$M_0 = N \left( \frac{\hbar \gamma}{2} \right) \left( \frac{\hbar y B_0}{2kT} \right) = \frac{N\hbar^2 \gamma^2 B_0}{4kT},$$  \hspace{1cm} [16]

where $M_0$ refers to equilibrium magnetization.

### 1.7.4 Chemical Shift

The local magnetic field experienced by the nuclear spin is dictated by the local electron distribution. This leads to a phenomenon called chemical shift whereby spins in different chemical environments resonate at slightly different Larmor frequencies. The phenomenon of in vivo chemical shift is depicted in Figure 1-6, where the $^{13}$C-nuclei exhibit a different resonant frequency dependent upon the chemical environment of the host molecule.

The fundamental reason for the difference in chemical shifts is the interaction of the external magnetic field with the variable local electron cloud distribution in the environment where the nuclei of interest reside. For example, protons in water molecules resonate at a different frequency in a tissue environment that primarily consists of muscle compared to a tissue environment that is primarily fat. The electron cloud arranges itself to oppose the external magnetic field, such that the effective magnetic field, $B'_0$, for the local tissue environment is given by:

$$B'_0 = B_0 (1 - \sigma),$$  \hspace{1cm} [17]

where $\sigma$ is the shielding constant that depends on local tissue environment. Chemical shift is usually expressed in parts per million (ppm) of the applied magnetic field, by combining equation [17] with the Larmor equation as:

$$\delta = \frac{\omega_r - \omega_r}{\omega_r} \cdot 10^6,$$  \hspace{1cm} [18]
where $\omega_t$ is the Larmor frequency associated with the local tissue environment and $\omega_r$ is the predetermined reference frequency for a native frequency of $\omega_0$. It should be noted that frequency expressed in $\delta$ is independent of the external magnetic field.

**Figure 1-6**: Metabolite products of $^{13}$C-pyruvate *in vivo* resonate at different frequencies as the $^{13}$C-nuclei experience different chemical shifts due to different chemical environments.

### 1.7.5 Radiofrequency Pulse

RF pulses manipulate the magnetization of a sample to generate the signal in NMR. RF coils are the electric antennae that produce these pulses. Applying a transverse RF pulse to a spin system results in an additional time-varying magnetic field called $\vec{B}_1$ that exerts itself in a direction perpendicular to the main magnetic field $B_0$. This additional magnetic field is a rotating vector with a frequency equal to the Larmor frequency of the nuclei under consideration. As $\vec{B}_1$ is a rotating vector, it is convenient to transform the reference
coordinates from a laboratory frame to the rotating frame about z-axis (direction of $B_0$) with frequency of $\omega_0$. The effective magnetic field $\vec{B}_{\text{eff}}$ can then be described as:

$$\vec{B}_{\text{eff}} = \vec{B}_1 t + \left( \vec{B}_0 - \frac{\omega_0}{\gamma} \right) \hat{k} = \vec{B}_1 t.$$  \[19\]

$\vec{B}_1$ is applied along the x-direction and the result is tipping of the net magnetization to y-direction of the rotating frame of reference. The purpose of $\vec{B}_1$ is to tip, or nutate, the net magnetization by a flip angle $\alpha$, represented as:

$$\alpha = \gamma \int_{0}^{t} B_1(t) \cdot dt,$$  \[20\]

where $t$ denotes the duration of $\vec{B}_1$. Following equation [4], once the aggregate of spins is perturbed from the direction of the static magnetic field of $\vec{B}_0$, the macroscopic behaviour can be represented by the following equation:

$$\frac{d}{dt} \vec{M} = \vec{M} \times \gamma \vec{B}_{\text{eff}} = \vec{M} \times \gamma \vec{B}_1 \hat{t}.$$  \[21\]

If a flip angle of $\alpha = \pi/2$ is used for $\vec{B}_1$, the net magnetization completely aligns in the y-direction. A static frame of reference, rotating frame of reference and the process of tipping magnetization, referred to as the excitation pulse are shown in Figure 1-7.
Figure 1-7: The lab frame (x, y, z) and the rotating frame (x’, y’, z’). After the transformation, application of a RF pulse ($\alpha = \pi/2$) in the x’-axis will lead to tipping of net magnetization in the y’-axis.

After the excitation RF pulse with a flip angle of $\alpha$, the net magnetization slowly returns to equilibrium i.e. re-aligns with $B_0$. This process of relaxation is called longitudinal relaxation or spin-lattice relaxation.

### 1.7.6 Longitudinal and Transverse Relaxation

Spin-lattice relaxation occurs as the excited magnetic dipoles return to equilibrium by interacting with the surrounding lattice. Spin-lattice relaxation is described using an exponential time decay constant $T_1$, called the longitudinal or the spin-lattice relaxation time. The time dependence of the longitudinal component of magnetization, $M_z$ in the presence of longitudinal relaxation is expressed by the following differential equation:

$$\frac{dM_z}{dt} = - \frac{M_z - M_0}{T_1}, \quad [22]$$

where $M_0$ is the equilibrium magnetization and $T_1$ is the longitudinal relaxation time constant. The general solution for equation [22] is:

$$M_z(t) = M(0)e^{-t/T_1} + M_0 \left( 1 - e^{-t/T_1} \right), \quad [23]$$

where $M(0)$ is the longitudinal magnetization at zero time. For an excitation RF pulse of $\alpha = \pi/2$, $M(0) = 0$ and the second component of equation [23], $M_0 \left( 1 - e^{-t/T_1} \right)$ dictates
the re-growth of magnetization to thermal equilibrium such that the magnetization re-grows to \(~63\%\) of the thermal equilibrium in one $T_1$.

Relaxation transverse to $B_0$ or spin-spin relaxation occurs due to local alteration in magnetic fields that the spins experience relative to neighbouring spins. Slight variance in the environment of the spins leads to different local magnetic fields. The dipolar interaction between these spins causes a dephasing of the transverse magnetization and a broadening of the resonant frequency. This relaxation is described by an exponential time decay constant $T_2$, called the transverse relaxation or the spin-spin relaxation time constant. This phenomenon is described by the following differential equation:

$$\frac{dM_{xy}}{dt} = -\frac{M_{xy}}{T_2}, \quad [24]$$

where $M_{xy}$ is the magnetization in transverse plane. The general solution for equation [24] results in:

$$M_{xy}(t) = M(0)e^{-t/T_2}, \quad [25]$$

where $M(0)$ is the transverse magnetization immediately following an excitation RF pulse. Another transverse relaxation time constant called the apparent transverse relaxation time, $T_2^*$, is shorter than $T_2$, due to additional components that cause dephasing of the transverse magnetization. Some of these components include: static $B_0$ inhomogeneities, magnetic susceptibility differences, chemical exchange, diffusion and chemical shift. Exponential time decay with transverse relaxation time constant $T_2^*$ results in a Free Induction Decay (FID). The Fourier transform of a FID is a Lorenzian shape where the width of the peak is dependent on the $T_2^*$ of the FID. This is shown in **Figure 1-8**.
Figure 1-8: Top left shows a FID with an apparent transverse relaxation time constant, $T_2^*$. Top right shows a Lorentzian peak, which is the Fourier transform of the FID. Full-width at Half Maximum (FWHM) of the Lorentzian peak is related to $T_2^*$ such that a longer $T_2^*$ results in a lower FWHM for the Lorentzian peak.

1.7.7 MR Signal and RF Coils

The Bloch equation describes the time evolution of magnetization in the rotating frame of reference, in the presence of an external static magnetic field ($B_0$), a time varying magnetic field ($B_1$) and associated longitudinal and transverse relaxation. The magnetization varies with time as:

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2^*} - \frac{(M_z - M_0)\hat{z}}{T_1},$$

where $T_1$ and $T_2^*$ are the longitudinal and apparent transverse relaxation time constants respectively.

A RF coil is used to transmit the time-varying magnetic field and/or receive the voltage generated by the sample. Based on Faraday’s law of electromagnetic induction, the time varying magnetization generates a corresponding electromotive force or voltage in the RF coil. Since $B_1$ is produced by the RF coil; based on the principle of reciprocity, the sensitivity of a coil can be measured by the transverse field that the coil produces upon application of $B_1$ per unit amount of current produced by the coil. The induced voltage can be expressed as:
\[ V(t) = \frac{d}{dt} \int_{V_s}^{0} dV \cdot (B_1(\vec{r}))_{xy} \cdot M_{xy}(\vec{r}, t), \tag{27} \]

where \( V_s \) represents the volume of the sample over which the integral is calculated. The final voltage obtained contains the Larmor frequency of all the chemical species present in the sample and varies as a function of time as it is modulated by the relaxation mechanisms previously discussed. The final signal obtained is the FID, and is represented as:

\[ S(t) = A \omega_0 \int_{V_s}^{0} dV \cdot (B_1(\vec{r}))_{xy} \cdot M_{xy,0}(\vec{r}) \cdot e^{-t/r_2} \cdot e^{-i\Delta \omega t}, \tag{28} \]

where A is a constant that depends on coil filling, coil geometry, gain and frequency response of the receiver. \( \Delta \omega \) is the range of Larmor frequencies detected by the RF coil and depends on the bandwidth of the RF receive coil and the magnetic gradients applied for spatial encoding.

A RF coil primarily consists of electrical resonant circuits and is used to transmit the time-varying magnetic field \( B_1 \) and/or receive the voltage \( V \), generated by the spin system. The geometry of RF coils is carefully chosen to maximize the Signal-to-Noise-Ratio (SNR) and produce good quality images. The frequency of the electrical resonant circuit of a coil is tuned very closely to the Larmor frequency of interest. This enables the coil to provide efficient transmission of \( B_1 \) and sensitive reception of \( V \) from the spin system. The resonance behaviour of the coil can be approximated by a combination of its inductance and capacitance in the following manner:

\[ \omega = \frac{1}{\sqrt{LC}}, \tag{29} \]

where L is the total inductance of the RF coil and C is the total capacitance of the RF coil respectively. A volumetric (distributed capacitance) design of coils generally known as a birdcage is regarded as the best for \( B_1 \) homogeneity at clinical magnetic field strengths (< 3T). Birdcage coils are versatile and are often used for both transmit and receive operation. The distance between the sample and coil is an important consideration for sensitivity of the
coil. As shown in equation [28], the MRI signal is dependent on the integral over the volume present in the RF coil. In general, the coil sensitivity is inversely proportional to the distance between the coil elements and the sample. This property enables surface coils to attain a higher sensitivity in general as they can be placed fairly close to the sample. The shortcoming of surface coils is the non-uniform transmission of $B_1$. Advantages of a birdcage design (uniform transmission of $B_1$) and a surface coil (higher sensitivity) can be combined to form a Transmit-Only/Receive-Only (TORO) system that offers the best SNR. In this system, the larger birdcage coil is used to provide very uniform $B_1$ field only and a highly sensitive surface coil is used for reception of signal only. As a part of the research for this thesis, a TORO system was developed for in vivo $^{13}$C-MRI. Details on design, construction and operation can be found in the Appendix A-1. It is the highly sensitive TORO system that enabled spatial disease characterization in Chapter 3.

1.7.8 Spatial Localization of Spins

The signal received from the spins must be spatially localized in order to form an image. Spatial localization of the spins is performed by the use of gradient magnetic fields in addition to $B_0$. Gradients can be applied in all three directions such that the net magnetic field is represented as:

$$\vec{B}(x, y, z) = B_0 \hat{k} + \left( G_x x + G_y y + G_z z \right) \hat{k}, \quad [30]$$

where $G_x = \frac{dB_x}{dx}$, $G_y = \frac{dB_y}{dy}$ and $G_z = \frac{dB_z}{dz}$. The combined field points in the z-direction with variations in x, y, and z direction for spatial localization. The application of gradients leads to additional accumulation of phase, which is typically represented in the following way:

$$\vec{k} = \frac{\gamma}{2\pi} \int_0^t dt' \cdot \hat{G}(t'), \quad [31]$$

where $\hat{G}(t')$ is the time varying gradient magnetic field that is turned on for a time $t$. Application of a gradient magnetic field and resulting accumulation of phase is represented by the position of a vector in k-space, $\vec{k}$. k-space is a convenient method to represent the
acquired MRI data and contains spatial frequency components obtained by Fourier transform of the effective spin density.

A slice select gradient is used to spatially localize a 2D slab of the sample. The slice select gradient is turned on simultaneously along with a RF pulse. An axial slice is excited with the thickness $\Delta z$, which is defined by:

$$
\Delta z = \frac{BW_{RF}}{\left(\frac{\gamma}{2\pi}\right)G_z},
$$

where $BW_{RF}$ is the bandwidth of the corresponding RF pulse and $G_z$ is the strength of the slice-select gradient (conventionally assumed to be in the direction of the magnet bore). Phase is accumulated during the time the slice-select gradient is turned on, and an additional gradient is applied in the opposite direction after slice-select gradient to rewind the accumulated phase. This is demonstrated by $G_z$ in Figure 1-9.

Further spatial localization in the 2D slab can be attained using frequency- and phase-encode gradients that can be applied along $G_x$ and $G_y$ after the slice-select gradient. Phase-encode gradients are used to encode columns within the 2D slab selected by the slice-select gradient, as shown in Figure 1-9. The phase-encoding gradient, when applied to a system of spins, enables the accumulation of phase. When the gradient is turned on, it changes the main magnetic field slightly which leads to a slight change in the precession frequency of the spins, and that frequency is a known linear function of spatial position. When the gradient is turned off, the spins return to the original precession frequency but retain the phase accumulated during the gradient. Variable amounts of phase encoding are applied after each consecutive RF pulse to encode voxels in the axial slab to a unique position in the k-space. The acquired signal from each voxel is sampled as a FID and is represented by one point in k-space. The Field of View (FOV) in the direction that phase-encode gradient is applied is given by:

$$
FOV_i = \frac{1}{\left(\frac{\gamma}{2\pi}\right)DG_i \cdot \tau_{PE}},
$$

[33]
where $\Delta G_i$ is the phase-encode gradient step in either $G_y$ or $G_x$ direction that is turned on for a time $\tau_{PE}$.

The frequency-encode gradient is applied along $G_y$ or $G_x$ to encode the spatial localization of spins. It is typically applied during signal read-out along just one direction to encode columns within the selected 2D slab. Use of phase encoding and frequency encoding gradients in a mutually orthogonal direction within the 2D selected slab leads to spatial encoding of the voxels. The FOV in the frequency-encode direction is given by:

$$FOV_i = \frac{1}{\left(\frac{\gamma}{2\pi}\right) G_i \cdot \tau_f}$$  \[34\]

where, $G_i$ is the amplitude of frequency-encode gradient applied for a time $\tau_f$. Formation of the MRI image with the use of gradient magnetic fields is described in the next section. Figure 1-9 also shows a pulse sequence-timing diagram that provides a visual representation of the order of gradients during a gradient echo sequence.

1.7.9 Gradient Echo Imaging

2D gradient echo imaging is a basic MRI pulse sequence used to form an MRI image. Gradient echo imaging starts with a RF pulse turned on simultaneously along with the slice-select gradient to select a 2D slab within a 3D volume as shown in Figure 1-9. The slice-select gradient is applied along $G_z$ such that the thickness of the selected 2D slab is given by equation [32]. Next, a phase-encode gradient is turned on along $G_y$ following the slice select gradient. This enables phase accumulation for a column in the selected 2D slab. Following phase encoding, a frequency-encode gradient is turned on along $G_x$. A pre-phase gradient with negative amplitude is turned on at the same time as the phase-encode gradient. A readout gradient is turned on following the pre-phase gradient during which the data is acquired. The read-out gradient has twice the area of the pre-phase gradient so that the gradient echo occurs at the center of the readout gradient. This phenomenon along with the full pulse sequence is shown in Figure 1-9. The time between RF pulse and the center of readout gradient is called the Echo Time (TE). The time for repetition of the pulse sequence is called the Repetition Time (TR).
Figure 1-9: Pulse sequence diagram for gradient echo imaging (left) and corresponding sample k-space trajectory (right).

1.7.10 Chemical Shift Imaging

Chemical Shift Imaging (CSI) is also known as Magnetic Resonance Spectroscopic Imaging (MRSI) as it enables the acquisition of spectra spatially localized to a volume element, or voxel. It uses phase encoding gradients only to encode the voxels in order to preserve the chemical shift of the constituent chemical species. Spectra from each voxel contain the information about chemical shift of an array of metabolites and their relative magnitude within that voxel. A pulse sequence for 2D-MRSI begins with the application of an excitation RF pulse and a slice-selective gradient $G_z$ simultaneously. Following the excitation RF pulse and slice-selective gradient, phase-encoding gradients are applied in the other two dimensions to encode voxels within the plane of the selected axial slab. Assuming both phase-encode gradients are turned on for a time $\tau$ with different amplitudes $G_x$ and $G_y$ as shown in Figure 1-10, the position in k-space is represented as following:

$$k_{x_i} = a_i \frac{y}{2\pi} (G_x \cdot \tau), \text{ and } k_{y_i} = a_i \frac{y}{2\pi} (G_y \cdot \tau),$$

[35]

where $a_i$ is the amplitude of phase-encode gradients used for each encoding step. The total number of points in each direction in k-space determines the resolution of 2D-MRSI for a
given FOV. The FOV in the x- and y-direction is also a function of phase-encode gradient step and the time that it is turned on for $\tau$, such that:

$$FOV_y = \frac{1}{\left(\frac{\gamma}{2\pi}\right) \Delta G_y \cdot \tau}, \text{and} \ FOV_x = \frac{1}{\left(\frac{\gamma}{2\pi}\right) \Delta G_x \cdot \tau}, \quad [36]$$

where $\Delta G_y$ and $\Delta G_x$ are the x- and y-direction phase-encode gradient steps respectively. Once k-space is fully acquired, a 2D inverse Fourier transform is performed on the k-space data to reveal the MRSI data.

**Figure 1-10:** Pulse sequence for 2D-MRSI and corresponding k-space filling are shown. The RF pulse and the slice select gradient are played out simultaneously and lead to the selection of an axial slab. Phase-encode gradients are applied to encode all voxels, one by one in the selected slab. k-space is organized such that each point in k-space represents a FID. Adapted from reference (73).

1.7.11 Dixon Approach to Image Chemical Species with a Sparse Spectrum

The Dixon approach has been used extensively to image chemical species with a sparse spectra. It is usually based on a gradient echo type of pulse sequence where the reconstruction to formulate final images relies on known chemical shifts between the chemical species. Gradient echo based pulse sequences generally read-out an entire k-space
line after the excitation RF pulse compared to one k-space point in CSI and as a result are significantly faster. The echo times in the gradient echo sequence are selected such that the chemical species with known chemical shifts appear in-phase or out-of-phase with respect to each other. The Dixon approach was first used to separate water and fat that resonate 3.5 ppm apart in proton MRI. A two point Dixon approach to separate water and fat is demonstrated by Figure 1-11. The transverse magnetization of water and fat behave differently due to the difference in their chemical shifts. The net magnetization of fat goes in and out of phase when the frequency of the rotating frame of reference is fixed at the resonant frequency of water. Proper selection of echo times ‘a’ and ‘b’ provide a net image at ‘a’, which is the sum of in-phase water and in-phase fat. Similarly, the total image at ‘b’ is the sum of in-phase water and out-of-phase fat signals. The images at ‘a’ and ‘b’ are added together in a complex fashion to yield a pure water image and subtracted in a complex fashion to yield a pure fat image. (74)
Figure 1-11: Imaging at echo times ‘a’ and ‘b’ results in a net image of in-phase water and in-phase fat at ‘a’ and in-phase water and out-of-phase fat at ‘b’ respectively. These images when added and subtracted together provide a pure water and fat image. Adapted partially from reference (74).

Three echoes can be collected using the Dixon approach to correct for $B_0$ inhomogeneities in addition to separation of water and fat phases. (75) This helps with the reduction of error associated with accurate quantification of the chemical species in a non-uniform $B_0$ field map. The Dixon approach of Iterative decomposition of water and fat with echo asymmetry and least squares estimation (IDEAL) has been used extensively to separate water and fat images, (76) and can be based on a gradient echo pulse sequence approach. (77) It improves upon the traditional Dixon approach by incorporating a parameter called number of signals averaged. This parameter can be optimized by the choice of echo times, which optimizes the overall SNR and quantification of chemical species. (78)

The IDEAL technique has been used with hyperpolarized $^{13}$C-pyruvate to separate pyruvate and the downstream metabolites. (79) A novel application of the IDEAL technique used to
separate $^{13}$C-bicarbonate and $^{13}$C-carbon dioxide images to construct a ratio metric pH map is presented in Chapter 3.

1.8 Hyperpolarized $^{13}$C-pyruvate Magnetic Resonance Metabolic Imaging

MRI signal is dependent on the net magnetization that aligns with the external magnetic field. The magnetization in turn is proportional to the polarization as described in equation [16]. Typically, with an external magnetic field of 3T, using equation [14], the thermal polarization of protons is 10 ppm or 0.001 %. Clinical MRI has been very successful at anatomical imaging as the poor polarization is compensated by the high concentration of protons in vivo. Other nuclei with spin =1/2 ($^{3}$He, $^{129}$Xe, $^{13}$C) can be used with MRI but typically the polarization and the concentration are the limiting factors. For example: $^{13}$C has a natural abundance of only 1.1 % and a gyromagnetic ratio of ¼ compared to protons (Table 1-2) significantly limiting SNR. Improvement in polarization methods has led to the emerging field of hyperpolarized-MRI. Hyperpolarized Noble Gas (HNG) MRI for lung imaging uses $^{129}$Xe and $^{3}$He that are polarized 4-5 orders of magnitude greater than the corresponding thermal equilibrium. (80) Spin exchange optical pumping is typically used for polarization of HNG. (81) Imaging metabolism coupled to a particular enzyme-catalyzed reaction in vivo has been made possible with hyperpolarized $^{13}$C-MRI. (1) The hyperpolarization of $^{13}$C-substrates can be attained either by para-hydrogen induced polarization process (82) or Dynamic Nuclear Polarization (DNP) process. (83) DNP is the more frequently used method and has been used to polarize both $^{13}$C-substrates ($^{13}$C-pyruvate and $^{13}$C-bicarbonate) used for $^{13}$C-MRI in Chapters 2, 3 and 4.

1.8.1 Dynamic Nuclear Polarization Method

DNP is based on polarization of nuclear spins in the solid state. Unpaired electrons are required as one of the ingredients to assist with the process. Overhauser first discovered this phenomenon where highly polarized unpaired electrons under the influence of a microwave irradiation source transfer their polarization to the nuclear spins. (84) The phenomenon came to be known as the Overhauser effect. The interested reader is referred to (85-87) for the exact theory on the quantum mechanical interaction between the electrons and nuclei under
the influence of a microwave irradiation source. The physical conditions best suited for achieving high polarization are low sample temperature, high magnetic field around the sample and irradiation with a microwave source. The sample is prepared as a mixture of unpaired electrons, glycerol and the $^{13}$C-substrate. A homogeneous distribution of electrons is one of the most important considerations for the sample preparation. The source of unpaired electrons is typically an organic free radical. Glycerol is added as a glassing agent to achieve homogeneous distribution of unpaired electrons and to obtain an amorphous solid after cooling the sample. For in vitro and in vivo use, after polarization in the solid state at low temperature, the sample is quickly brought into a liquid state at room temperature. (83) The commercial DNP system (Hypersense, Oxford Instruments, UK) used for research presented in this thesis polarizes $^{13}$C-substrates at very low temperature (1.4 K), high magnetic field (3.35 T) and in the presence of microwave irradiation (~94 GHz). This process is depicted in Figure 1-12. The specific recipes for $^{13}$C-pyruvate and $^{13}$C-bicarbonate preparation and polarization are discussed in Chapters 2 and 3.
Figure 1-12: Illustration above shows homogeneous distribution of electrons within the $^{13}$C-nuclei for efficient polarization. The amorphous solid is irradiated with a microwave source (as shown by arrows ~94 GHz) at high magnetic field and low temperature to achieve desired polarization.

1.8.2 Metabolism of Hyperpolarized $^{13}$C-pyruvate

$^{13}$C-pyruvate is used as a probe to image the metabolism of the endogenous pyruvate. It is quite suitable as it offers a high degree of polarization, good water-solubility and a sufficiently long relaxation time in vivo. Hyperpolarized $^{13}$C-pyruvate has been used extensively to study pre-clinical cancer models. It has also demonstrated useful results in phase I clinical trials in an application towards prostate cancer in humans. (2) In vivo metabolism of intravenously injected hyperpolarized $^{13}$C-pyruvate is demonstrated by Figure 1-13. Injected $^{13}$C-pyruvate is shuttled past the cell membrane and arrives at the intersection of three major metabolic pathways. In healthy cells, most of the pyruvate, which is derived from glucose, is shuttled to the mitochondria where it produces energy by aerobic respiration. Under hypoxic/ischemic conditions, cells switch over to anaerobic respiration where Lactate Dehydrogenase (LDH) readily converts pyruvate to lactate. Pyruvate also undergoes
transamination to form alanine, catalyzed by Alanine Transaminase (ALT) in the cytoplasm. $^{13}$C-pyruvate that is shuttled into mitochondria also leads to irreversible production of $^{13}$C-carbon dioxide, which readily equilibrates with $^{13}$C-bicarbonate, catalyzed by Carbonic Anhydrase (CA).

Imaging endogenous enzyme catalyzed pyruvate to lactate conversion with the use of hyperpolarized $^{13}$C-pyruvate provides insight into the level of anaerobic respiration. The total MRI signal for $^{13}$C-pyruvate and the by-product $^{13}$C-lactate depends on initial polarization, concentration and amount of $^{13}$C-pyruvate injected, in vivo relaxation ($^{13}$C-pyruvate, $^{13}$C-lactate) and pool size of endogenous pyruvate and lactate. To quantify the LDH enzymatic reaction, $^{13}$C-Lactate to $^{13}$C-Pyruvate Signal Ratio (Lac/Pyr) is derived where the total lactate signal to total pyruvate signal is normalized. This factor provides a reliable quantification of the anaerobic metabolism and has been used in Chapters 2 and 4 to capture early RP metabolism in RILI rat model.

The conversion between $^{13}$C-carbon dioxide and $^{13}$C-bicarbonate is highly dependent on pH as bicarbonate is the largest extracellular buffer in vivo. $^{13}$C-bicarbonate can also be polarized by DNP and administered intravenously. As a result, imaging of $^{13}$C-carbon dioxide and $^{13}$C-bicarbonate can provide insight into the pH of the immediate extracellular environment. This concept formed the basis for Chapter 3 in which, a novel pH imaging approach based on the Dixon method is discussed in detail.
Figure 1-13: Injected hyperpolarized $^{13}$C-pyruvate results in $^{13}$C-lactate, $^{13}$C-alanine, $^{13}$C-carbon dioxide and $^{13}$C-bicarbonate. Up-regulation of anaerobic respiration in hypoxic tissue environment leads to a higher production of lactate that can be quantified by the factor, lac/pyr.

1.8.3 Data Acquisition Considerations for Hyperpolarized $^{13}$C-MRI

The net detectable signal from administered $^{13}$C-substrates and the subsequent by-products are a function of their longitudinal relaxation times, concentration and initial polarization of the injected $^{13}$C-substrate. As the polarization in the parent molecule and its metabolites decays fast due to short $T_1$ relaxation times (e.g. $^{13}$C-pyruvate $\sim 40$ s $in$ $vivo$), imaging time is one of the primary constraints for $^{13}$C-MRI. Typically, the CSI sequence used to acquire data for research presented in this thesis took $\sim 12$ s after a wait time of 25 seconds from the start of intravenous injection of $^{13}$C-pyruvate. The wait time is sufficient to allow $^{13}$C-pyruvate to clear out from the vasculature as it is absorbed by the cells and converted into $^{13}$C-lactate. A secondary consideration for pulse sequences is the number of RF pulses used to acquire each dataset. A minimal number of RF pulses is preferred as each RF pulse causes a loss of unrecoverable polarization. The relative amplitude of each RF pulse is also calibrated.
carefully as the amount of polarization lost also depends on the flip angle that each RF pulse generated. For example, the *in vivo* acquisition of CSI dataset in this thesis used 144 RF pulses with a 10-degree flip angle for each RF pulse.

1.9 Thesis Hypothesis and Objectives

The objective of this thesis was to investigate metabolic changes occurring in rat lungs during RP. As metabolic changes precede morphological and functional changes, assessment of metabolic profile could help in early diagnosis of RILI. Hyperpolarized $^{13}$C-MRI with its ability to polarize variety of $^{13}$C-substrates was used to quantify metabolism during RP. $^{13}$C-substrates of $^{13}$C-pyruvate and $^{13}$C-bicarbonate were used to study enzyme-catalyzed reactions. We hypothesize that hyperpolarized $^{13}$C-MRI can be used to detect metabolic changes in early RP which correlate with the known histological transformations. The sub-objectives established for this purpose are included as part of the manuscripts and Appendices that follow.

This chapter has described the motivation for the study of unintended lung injury caused by therapeutic radiation of the lung cancer. An overview of lung cancer, radiation therapy and the pathophysiology of RILI have been described. Clinical diagnosis and emerging imaging modalities for detection of RILI have also been discussed. MRI physics of pulse sequences used for the research in this thesis such as Chemical Shift Imaging, gradient echo imaging and the IDEAL method were explained. Introduction to the DNP process used for polarization of $^{13}$C-substrate and the use of $^{13}$C-pyruvate and $^{13}$C-bicarbonate to probe particular metabolic pathways was described in a biological context.

Chapter 2 describes the validation of detection of metabolic signature in early RP using hyperpolarized $^{13}$C-pyruvate. Six rats were irradiated in the entire thorax region with a cobalt-60 source (dose of 14 Gy, 1 fraction) and imaged two weeks later along with healthy age-matched controls. Results were analyzed for change in lac/pyr in the irradiated cohort compared to the healthy cohort and histology was used as a confirmation for the presence of injury. Kundan Thind was responsible for construction of a $^{13}$C-birdecaige coil and a $^1$H-surface coil in addition to the experimental design, testing of protocols, animal preparations,
experimental data acquisition and analysis. Dr. Alexei Ouriadov provided assistance in optimization of the RF coils and the pulse sequences. Dr. Eugene Wong and Dr. Matthew Fox assisted with the irradiation procedure. Dr. Lanette Friesen-Waldner assisted with the animal preparation and in polarization of the $^{13}$C-pyruvate. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Eugene Wong and Dr. Jake Van Dyk provided meaningful discussion about the work. Dr. Giles Santyr provided overall guidance for the project and assistance with the manuscript presentation.

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Chapter 3 expands on the findings in Chapter 2. The objective for this Chapter was to capture metabolic profile in early RP in a regional and longitudinal manner and correlate the changes to histology. To capture regional metabolic changes, MRI hardware was improved by construction of a TORO coil system that enabled an increase in SNR of 3-4x over the transmit-receive RF coil configuration. This has been described in Appendix A-1. Twelve rats were irradiated in a conformal manner to the right lung using a micro-CT system. $^{13}$C-data was collected at day 5, 10, 15 and 25 post irradiation from three irradiated and three healthy age-matched control animals. Macrophage count from the histology of rat lungs was obtained and correlated with changes in lac/pyr. Kundan Thind was responsible for construction of the $^{13}$C-TORO coil system (Appendix A-1) in addition to the experimental design, experimental data acquisition and analysis. Michael Jensen assisted with the irradiation procedure using micro-CT. Dr. Francisco Martinez and Dr. Tim Scholl assisted in the polarization of $^{13}$C-pyruvate for the experiments. Elaine Hegarty performed the animal preparation pre-MRI and obtained histology post-MRI. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Eugene Wong and Dr. Jake Van Dyk provided meaningful discussion about the work. Dr. Giles Santyr provided overall guidance for the project and assistance with the manuscript presentation.

This work has been submitted to the journal of Radiotherapy and Oncology. (August 2013: RO-D-13-00907)

Chapter 4 describes a novel method to map pH using hyperpolarized $^{13}$C-bicarbonate with the IDEAL pulse sequence. Maintenance of pH balance is one of the most essential homeostatic processes in vivo, disruption of which could lead to severe adverse affects. The
disruption could be caused by onset of cancer, metabolic and respiratory acidosis and alkalosis. In particular, onset of severe RILI could lead to hypoxic tissue environment that would result in a build-up of lactic acid. Accumulation of lactic acid in the tissue results in an acidic tissue environment. Non-invasive pH mapping could potentially help with diagnosis of RILI and other diseases that result in pH imbalance. The IDEAL pulse sequence was used to map $^{13}$C-bicarbonate and its downstream metabolite of $^{13}$C-carbon dioxide. These form an acid-base pair and the ratio of $^{13}$C-bicarbonate to $^{13}$C-carbon dioxide aids in the construction of the pH map. Kundan Thind was responsible for construction of the phantoms, experimental design, experimental data acquisition and analysis. Dr. Francisco Martinez and Dr. Tim Scholl assisted in the polarization of $^{13}$C-bicarbonate for the experiments. Curtis Weins provided assistance with the IDEAL pulse sequence. Dr. Albert Chen was pivotal in providing numerous discussions and the technical guidance for the study. Dr. Giles Santyr provided overall guidance for the project and assistance with the manuscript presentation. This work will be submitted to the journal of Magnetic Resonance in Medicine.

Chapter 5 discusses the results from the three preceding manuscripts and their contribution towards metabolic imaging in the lung for early diagnosis of RP. The discussion includes the merits of early assessment, shortcomings of the studies and future work.

The Appendix A-1 provides additional detail on the design and construction of the TORO coil system and appendix A-2 provides details on statistical tests used in the thesis.
1.10 References


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Chapter 2: Detection of Radiation-induced Lung Injury using Hyperpolarized $^{13}$C Magnetic Resonance Spectroscopy and Imaging


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2.1. Introduction

Lung cancer was the most commonly diagnosed cancer in males in 2008 globally, and fourth most commonly diagnosed cancer in females. It was also responsible for the largest number of cancer related deaths in 2008. (1) Radiation therapy is as an important treatment method for lung cancer, (2) but can lead to RILI, as the lung is an extremely radiosensitive organ. (3) RILI begins with inflammation of the lung referred to as RP, which is the most common dose-limiting factor for thoracic radiation therapy and develops in as many as 37% of patients receiving radiation for lung cancer. (2, 4, 5) Anatomical and functional changes associated with RILI include capillary damage, obstruction of airways and thickening of the septa, resulting in ventilation-perfusion mismatch. RILI can present with shortness of breath, cough, or fever and may require oxygen therapy, ventilator support or even cause death in extreme cases. (6) If changes associated with RILI could be detected, a given radiation therapy plan could potentially be altered to reduce adverse effects. Furthermore, medical intervention in patients developing RP maybe be possible if the injury process can be detected at an early stage. (7)

At the cellular level, immunohistochemical analysis of the irradiated lung reveals macrophage infiltration and activation of inflammatory cytokines. (8, 9) It has also been shown that lung hypoxia is an important contributing factor in the mediation of the inflammatory process and onset of the RP phase of RILI. (10) Decreased $p_aO_2$ has also been reported following irradiation (11, 12), an indicator of hypoxia. (13)
CT is currently the most common modality used to detect RILI in the clinic. CT can measure lung tissue density changes associated with late-stage RILI, but provides no functional information. (9) Technetium-99m SPECT can provide regional ventilation and perfusion changes with the onset of RILI but lacks specificity. (14) Dynamic contrast-enhanced MRI with Gd-DTPA has been used to detect changes post-irradiation in dog lungs. (15) MRI has also been able to measure significant changes in relaxation times \(T_1\) and \(T_2\) post-irradiation in rodent lungs. (16) Hyperpolarized \(^3\)He MRI has also been able to detect significant changes in the Apparent Diffusion Coefficient (ADC) values in rodent lungs post irradiation. (17) These imaging techniques show improvement over global \(p_{a}O_2\) measurements but are primarily based on late-stage morphological and functional changes (e.g., fibrosis). Imaging biomarkers sensitive to early metabolic changes associated with RP (e.g., inflammation and hypoxia) at the cellular level could provide an approach for earlier detection and treatment of RILI.

Recent advances in technology have enabled increases in the MRI signal from \(^{13}\)C-enriched substrates by a factor of more than \(10^5\) using the DNP and dissolution method. Moreover, the signal intensity is enhanced for both the initially hyperpolarized substrate as well as many of its metabolic products. (18-20) Specifically, pyruvate enriched to \(^{13}\)C at the C-1 position of the molecule, can be hyperpolarized to a high via DNP and has sufficiently long \(T_1\) for subsequent imaging \textit{in vivo}. This enhanced polarization provides sufficient signal for high spatial and temporal resolution imaging of pyruvate and its metabolic products. (21-24) Several studies have demonstrated hyperpolarized \(^{13}\)C to be useful for studying changes in metabolic exchange/conversion of pyruvate to lactate, alanine and bicarbonate \textit{in vivo}. (25-30) The feasibility of using hyperpolarized [1-\(^{13}\)C] pyruvate and lactate signals to measure metabolic changes associated with lung ischemia has been shown \textit{ex vivo}. (31) Measuring changes in hyperpolarized [1-\(^{13}\)C] pyruvate and lactate signals may be a useful approach for non-invasive detection of RILI at an early stage \textit{in vivo}. Specifically, hypoxia during RP is expected to increase the level of anaerobic respiration to meet the constant energy needs of the tissue, which in turns leads to a higher level of lactate. We hypothesize that lactate-to-pyruvate signal ratio in the lung as measured by hyperpolarized \(^{13}\)C Magnetic Resonance Spectroscopy (MRS) and MRSI will increase following thoracic irradiation due to the onset of hypoxia and up-regulation of anaerobic respiration and will correlate with a decrease in
In this study, lactate-to-pyruvate signal ratios were measured using hyperpolarized $^{13}$C spectroscopy and imaging in rats two weeks post whole-thorax irradiation (n = 6) and compared to lactate-to-pyruvate signal ratios obtained from healthy, age-matched rats (n = 6). RILI induced inflammation and hypoxia were confirmed using microscopy of bronchoalveolar lavage (BAL) specimens and by measurement of $p_aO_2$ using blood sampling respectively. Dynamic spectroscopy was first performed over the entire thorax and kidney region to reveal the time course and total pyruvate and lactate levels. 2D-CSI was then performed at the optimal time window to quantify regional pyruvate and lactate signal intensities specifically in the heart and in the lung.

### 2.2. Methods

#### 2.2.1 Animal Irradiations

All procedures followed animal care protocols approved by the University of Western Ontario (ACVS) and were consistent with procedures approved by the Canadian Council on Animal Care (CCAC). To induce RILI, Sprague Dawley rats (weight = 350 ± 50 g) were irradiated with a dose of 14 Gy delivered uniformly using a $^{60}$Co source at the London Regional Cancer Program center. A 4 cm diameter collimator was used to deliver the radiation to the entire thorax. Animals were anesthetized using isoflurane delivered with a nose cone and were secured in a polyvinyl box that served as a build up layer for the $^{60}$Co beam. The setup is shown in Figure 2-1.
A pilot study involving eight separate rats irradiated with either 12 or 18 Gy was used to assess the appropriate whole lung radiation dose to be used in the final cohort study. A single fractionation of 14 Gy was chosen as a compromise in order to induce RILI while ensuring survival following irradiation. To provide a uniform dose, 7 Gy was delivered with the animal in the supine position and 7 Gy was delivered with the animal in the prone position. Animals were returned to their cages and monitored daily for two weeks following the irradiation. A total of twenty rats were irradiated, of which six were used for $^{13}$C dynamic MRS and CSI of of the thorax, five were used for $^{13}$C dynamic MRS of the kidneys, five were used for assessment of $p_aO_2$ and four were used to measure blood lactate concentration (as described further below). An equal number of age-matched healthy Sprague Dawley rats were used as controls for each measurement performed. Details for each sub-study are described below.

### 2.2.2 Blood Lactate and $p_aO_2$ Measurement

In order to confirm the presence of RILI, $p_aO_2$ levels were measured in an age-matched, irradiated cohort ($n = 5$) two weeks following irradiations and in a healthy cohort ($n = 5$). The two-week time point was chosen to yield maximum effect, based on the inflammatory and biochemical time course of RILI in a similar rat model shown in previous work by Calveley.
et al. (8) Animals were initially anesthetized in an isoflurane induction chamber (Vet-Equip) at a flow rate of 0.8 L / min using a concentration of 5% isoflurane and were then transferred to a nose cone where the isoflurane concentration was reduced to 2% and a loading dose of ketamine hydrochloride was given at a dose of 50 mg/kg. A 26 gauge catheter was inserted into the right tail vein to facilitate prolonged anesthesia depth by delivery of a mixture of propofol and ketamine at a ratio of 10:1 using an infusion pump with a dose rate of 50 mg/kg/hr. An incision was made across the neck in order to expose the right carotid artery, which was then isolated and cannulated with PE-50 tubing to allow arterial blood sampling and monitor blood pressure. A 0.75 mL blood sample was then extracted and inserted into a CG4+ iStat cartridge for blood oxygenation analysis (iStat blood gas analyzer, Abbott Laboratories).

In order to investigate potential systemic (ie. blood) RILI effects, that may influence the hyperpolarized $^{13}$C metabolic imaging studies, blood lactate concentration levels were measured directly from blood samples obtained in another age-matched cohort (n = 4) two weeks following irradiation as well as a healthy cohort (n = 4). Animals were initially anesthetized in an isoflurane induction chamber (Vet-Equip) at a flow rate of 0.8 L / min using a concentration of 5% isoflurane and were then transferred to a nose cone where the isoflurane concentration was reduced to 2%. A 26-gauge catheter was inserted into the tail artery to draw 0.5 ml of blood. This blood sample was then extracted and inserted into a CG4+ iStat cartridge for blood lactate concentration analysis (iStat blood gas analyzer, Abbott Laboratories, Mississauga, ON).

2.2.3 Hyperpolarized $^{13}$C pyruvate Preparation and Administration

A mixture containing ~30 µl of [1-$^{13}$C]-labelled pyruvic acid (CIL, Cambridge, MA) and 15mM trityl radical (OX63, Oxford Instruments, UK) was hyperpolarized using a commercially-available DNP system (Hypersense, Oxford Instruments, UK) following a previously described protocol (24). In brief, the DNP system utilizes low temperatures (1.4 K), high magnetic field strength (3.35 T) and microwave irradiation (94.15 GHz) to polarize the unpaired electrons in the radical that transfer the magnetization to $^{13}$C in the pyruvate molecule via the Nuclear Overhauser Effect (NOE) in the solid state. The hyperpolarized sample was rapidly dissolved in ~5.5 ml buffered (40 mM Tris) solution containing 80 mM Sodium Hydroxide, 0.1g/L Disodium EDTA dehydrate and 50mM Sodium Chloride to
effectively yield a 80 mM hyperpolarized pyruvate with a nominal pH of 7.4 upon dissolution.

MRI and spectroscopy were performed approximately two weeks (14 days ± 2 days) following irradiation. The experiments extended over four days to permit all twenty-two (11 irradiated and 11 control) animals to be studied at approximately the same time post-irradiation. The mean mass of the irradiated and healthy cohorts at the time of imaging was 410 ± 50 g. Each rat was randomly chosen and measurements were made in a blinded fashion. On the day of measurements, the rats were anaesthetized using isoflurane. A catheter was inserted in the tail vein for injection of hyperpolarized $^{13}$C-pyruvate. The extension tube connecting the catheter to the hyperpolarized sample was 24-gauge and 90 cm in length. This tube made it possible to quickly inject the hyperpolarized sample while minimizing the dead space. Since the hyperpolarized pyruvate signal relaxes relatively quickly ($T_1 \approx 40$ s) in vivo, MR spectroscopy data acquisition was started (as described below) immediately following bolus injection of 2.5 mL of 80 mM [1-$^{13}$C] pyruvate in the tail vein of the rats (~12 s). MRI data were acquired following a time delay (~ 25 s) to maximize lactate signal in the lung. This optimal time delay was based on the observed plateau in lactate signal measured with dynamic spectroscopy (see below).

2.2.4 Magnetic Resonance Spectroscopy and Imaging

MRI and spectroscopy were performed using a 3T MRI system (MR750, GEHC, Waukesha, WI) equipped with multinuclear hardware and software. The rat was supported in the bore of the magnet in the prone position using a custom tray equipped with a warming pad to maintain body temperature. A surface coil centered on the rat chest was fastened underneath the tray to acquire $^1$H images for localization purposes. The tray and surface coil were inserted into a custom-built, rat-sized quadrature transmit/receive bird-cage coil tuned to the $^{13}$C Larmor frequency of 32.12 MHz. During MR measurements, the rats were kept anaesthetized with isoflurane (2 - 2.5%) provided by a nose cone delivery system. Heart rate (320 - 360 beats per minute), breathing rate (40-60 breaths per minute) and core body temperature (37.0 - 37.8 °C) were monitored during the entire procedure (Small Animal Instruments Inc., Stony Brook, NY).
(A) $^1$H imaging: A gradient echo pulse sequence was used to acquire $^1$H images of the rat chest and kidney in the coronal and axial planes. The imaging parameters were as follows: FOV of 12 cm $\times$ 12 cm, matrix of 128 $\times$ 128, TE = 2 ms and TR = 34 ms, flip angle = 30 degree. These images were used to position the $^{13}$C data acquisition slabs and FOV since the boundary of the lung was well visualized as a signal void compared to the other thoracic tissues (e.g. heart).

(B) $^{13}$C Dynamic Spectroscopy: Based on the $^1$H coronal images, an axial slab (thickness = 8.5 mm) was selected in medial regions of the lung, including the heart. Spectroscopic data were acquired from this slab in the irradiated cohort (n = 6) and healthy cohort (n = 6) using a ‘pulse-acquire’ pulse sequence with TR of 1 s following the injection of hyperpolarized $^{13}$C-pyruvate. In total, 128 acquisitions were performed using flip angle = 10 degree, bandwidth = 5 kHz and 2048 points for a total scan time of 128 s. This dynamic spectroscopy data allowed measurements of total $^{13}$C signal in the slab as well as the time evolution of the pyruvate and lactate signals in order to time the spectroscopic imaging data acquisitions (described in section (C) below).

To investigate possible indirect RILI effects outside the thorax, dynamic spectroscopy was performed over the kidney region in a separate age-matched irradiated cohort (n = 5) and healthy cohort (n = 5). An axial slab of thickness 10 mm was acquired in the region of the kidney, using $^1$H coronal images as a reference. $^{13}$C data were acquired with TR = 1 s, flip angle = 10 degree, bandwidth = 5 kHz and 2048 points for a total scan time of 128 s.

(C) $^{13}$C Spectroscopic Imaging: Spectroscopic imaging was performed using a CSI technique from the same cohorts used in the $^{13}$C dynamic MRS studies of the thorax in irradiated rats (n = 4) and healthy rats (n = 4). A FOV of 6 cm $\times$ 6 cm was chosen corresponding to the same axial slab as described in section (B) above. A 16 $\times$ 16 matrix was prescribed with a nominal voxel size of 3.75 $\times$ 3.75 $\times$ 8.5 mm$^3$. CSI data were acquired using a similar pulse-acquire approach as described above (flip angle of 10 degrees, bandwidth of 5 kHz and 256 points) with a repetition time of 60 ms between each phase-encoding step for a total scan time of approximately 16 seconds. Based on the $^{13}$C dynamic spectroscopy
measurements (above), spectroscopic imaging was initiated following a delay of 25 seconds from the start of the bolus injection to maximize the lactate signal.

**D) Data processing and analysis:** SAGE™ software (GEHC, Waukesha, WI) was used for processing and visualization of the $^{13}$C data. For the dynamic spectroscopy protocol, the time domain data from each transient was apodized with a 2.5 Hz Gaussian filter then Fourier transformed. The spectra were then individually phased and baseline corrected. The lactate-to-pyruvate signal ratio was computed by dividing the total pyruvate signal by the total lactate signal intensities obtained from the entire thorax or the kidney slab of the rat by integrating each metabolite peak at each time point and summing the peaks from 0 to 79 seconds, after which the signal intensities were too small to measure.

For the $^{13}$C spectroscopic imaging data, the time domain data were apodized with a 10 Hz Gaussian filter and Fourier transformed in both the time domain and the two spatial dimensions. The metabolite peaks were phased and baseline corrected for every voxel. The 16x16 grid of spectra was overlaid on the axial $^1$H image and four voxels were chosen centered on the lung region and four voxels centered on the heart. The relative positions of these four voxels were kept consistent for both the heart and lung in each animal. The lactate-to-pyruvate signal ratio for each voxel was computed by dividing the total pyruvate signal intensity by the total lactate signal intensity obtained in a given voxel by integrating each metabolite peak. Two-tailed, paired T-tests were performed for the lactate-to-pyruvate ratios corresponding to the entire slab, as well as the lung and the heart for the irradiated cohort and the healthy cohort. A p-value of less than 0.05 was considered significant. Appendix A-2 contains more details on the two-tailed paired T-test and the underlying assumptions.

### 2.2.5 Microscopy of BAL Specimens

Following MR measurements, BAL specimens were collected from the lungs of all the rats. 10 mL of saline was injected into the lungs using an endotracheal tube. The saline was washed in and washed out three times to obtain a preliminary BAL specimen. This was repeated again to obtain a second specimen and combined with the first to obtain a final lavage specimen from the lungs. The BAL sample was then spun down at 1732 rpm for 10 minutes, while keeping the temperature at 8 °C to separate the pellet of cells from the
supernatant fluid using a centrifuge (GS-15R, Beckman Coulter, Mississauga, ON). The pellet of cells was re-suspended in formalin and a smear placed on slides using a cytocentrifuge (Shandon Elliott Cytospin, Shandon Southern Instruments, Camberley, Surrey, England). The cell smears were subsequently stained with hematoxylin and eosin (H&E) to identify cell nuclei (blue) from surrounding cytoplasm (pink). The slides were visualized optically with a Zeiss AXIO Imager microscope (Carl Zeiss Canada ltd., Toronto, ON, Canada). The cell micrographs for healthy and irradiated animals were compared to provide a qualitative assessment of inflammation due to RILI.

2.3. Results

Figure 2-2 shows typical cell micrographs from a representative irradiated rat as well as a healthy rat. At 40× magnification, more macrophages are evident in BAL specimens obtained from the irradiated cohort compared to BAL specimens obtained from the healthy cohort. At 100× magnification, monocytes, foamy macrophages and neutrophils are clearly seen in the BAL specimen from the irradiated cohort. Furthermore, some irregularities in the cell nucleus shape are also evident in the cells from the BAL of irradiated rats. These results qualitatively confirm the inflammatory response (i.e. RP) associated with RILI in the irradiated rats at the two-week time point.
Figure 2-2: Typical micrographs of H&E stained BAL specimens from (a) representative healthy rat and (b) representative irradiated rat (magnification of 40×). The BAL specimens from the irradiated rats contained significant macrophages compared to the healthy group (shown by arrows). (c) BAL specimen from the representative irradiated rat seen at magnification of 100×. Foamy macrophages (FM), monocytes (M) and neutrophils (N) can be distinguished.

The $p_aO_2$ results are summarized in Figure 2-3. In this figure, the mean value of $p_aO_2$ is shown with the error bars representing plus or minus one standard deviation based on the entire cohort. A statistically significant decrease in $p_aO_2$ ($p < 0.01$) was observed in the irradiated cohort ($54.2 \pm 12.28$ mmHg) compared to the healthy cohort ($84.74 \pm 9.98$ mmHg). The low $p_aO_2$ measured in the irradiated cohort compared to the healthy cohort quantitatively confirms the presence of RILI, presumably causing tissue hypoxia.
**Figure 2-3:** Arterial partial pressure of oxygen from cohort of healthy and irradiated rats at two weeks.

**Figure 2-4:** Lactate concentration measured from direct sampling of blood from irradiated and healthy rats. The lack of any significant difference confirms the absence of systemic spread of injury.
The blood lactate concentration levels are summarized in Figure 2-4. Mean lactate concentration is shown with error bars representing the standard deviation within the cohort. No statistically significant ($p = 0.69$) increase in blood lactate levels was observed in the healthy cohort ($1.255 \pm 0.247 \text{ mmol/L}$) compared to the irradiated cohort ($1.325 \pm 0.214 \text{ mmol/L}$). Lack of a significant difference between blood lactate concentration levels between the two cohorts confirms the absence of any systemic (ie. blood) increase in lactate due to RILI.

Figure 2-5: Representative normalized spectra showing sum of metabolites from time 0 to 79 seconds over the entire slab from (a) healthy rat and (b) irradiated rat. Note the increase in the level of lactate in the irradiated rat.

Figure 2-5 shows the summation of spectra from the hyperpolarized $^{13}$C dynamic MRS experiment from time 0 to 79 seconds corresponding to the entire slab in the lung region for a representative healthy rat as well as a irradiated rat. Figure 2-6 shows representative $^{13}$C-CSI data overlaid on the corresponding $^1$H image. The lactate-to-pyruvate signal ratio from the $^{13}$C dynamic MRS data from the entire thorax was observed to increase in the irradiated cohort ($0.128 \pm 0.041$) compared to the healthy cohort ($0.061 \pm 0.019$), representing a statistically significant increase of approximately 110% in the entire thorax ($p < 0.01$).
Figure 2-6: Axial proton image with localized $^{13}$C spectra magnitude overlaid on top with highlighted voxels of interest in lung and heart. Clear pyruvate and lactate peaks are observed in the heart and the lung region.

The lactate-to-pyruvate signal ratios corresponding to the lung, heart and kidney tissues for both the healthy and irradiated cohorts are shown in Table 2-1. This table reports the mean values and the error bars represent plus or minus one standard deviation based on the entire cohort. The lactate-to-pyruvate signal ratios in the lung tissue were observed to increase in the irradiated rats (0.280 ± 0.054) compared to the healthy rats (0.178 ± 0.046), representing a statistically significant increase of approximately 57% ($p < 0.02$). Similarly, lactate-to-pyruvate signal ratio in the heart tissue also showed a statistically significant increase of approximately 107% ($p < 0.01$) in the irradiated cohort (0.504 ± 0.083) compared to the healthy cohort (0.244 ± 0.035).
Table 2-1: lactate-to-pyruvate signal ratio measured in the entire thorax (summed from 0 to 79 seconds) as well as the lung, heart and kidney regions for healthy and irradiated rat cohorts. The data is represented as the mean ± standard deviation based on the respective cohorts. (Note: the lung and heart data for two of the rats had insufficient signal-to-noise ratio and are not included in the table and the kidney data shown above is from separate but identically irradiated and age matched healthy animal cohorts).

These are depicted in Figure 2-7. In the cohort of irradiated and control rats $^{13}$C dynamic MRS data from which a slab through the kidneys was acquired, no statistically significant difference ($p = 0.50$) in lactate-to-pyruvate signal ratios between the healthy ($0.215 \pm 0.100$) and irradiated cohorts ($0.215 \pm 0.054$) was observed.

![Figure 2-7: Relative lactate-to-pyruvate signal ratio in the heart tissue and the lung tissue displayed.](image)

2.4. Discussion

To our knowledge this is the first time that hyperpolarized $^{13}$C-pyruvate spectroscopic...
imaging has been applied *in vivo* to assess lung metabolism. Specifically, lactate-to-pyruvate signal ratios are shown to be sensitive to metabolic changes associated with irradiation of the thorax in rats. The lactate-to-pyruvate signal ratio increased significantly in irradiated rats compared to age-matched, healthy rats. This increase in lactate-to-pyruvate signal ratio was demonstrated both for the entire thorax and regionally in the heart tissue and lung tissue. These increases are consistent with the expected changes in metabolism accompanying early RILI, namely anaerobic glycolysis due to underlying hypoxia. These results are also consistent with RP confirmed by micrographs of BAL smears reflecting a qualitative increase in the numbers of inflammatory cells in the lung. Furthermore, the absence of any change in lactate-to-pyruvate signal ratio in the kidney region nor any change in lactate levels in the blood of similarly irradiated rats compared to healthy age-matched rats confirms that the effects of injury are confined to the thorax region where radiation was directed and thus is a direct measure of RILI.

A larger increase in lactate-to-pyruvate signal ratio was measured in the heart region of the irradiated animals compared to the healthy animals as compared to the lung region. This could have resulted from two possible factors. Firstly, the heart region is also irradiated in addition to the lung region, presumably leading to direct cardiac damage due to radiation. Secondly, due to the known compromise in lung function caused by the radiation as verified by \( p_aO_2 \) measurements and cell micrographs, it is possible that the heart rate increased to compensate for low blood oxygenation to maintain the same amount of absolute oxygen delivery to the cells. Increase in workload on the heart and decrease in \( p_aO_2 \) leading to lower \( p_aO_2 \) in the coronary arteries could also potentially contribute to a higher lactate-to-pyruvate signal ratio observed in the heart region compared to the lung region.

Hypoxia is expected in early RILI due to damage to blood vessels, especially in the lung, which is a particularly radiosensitive organ. While not measured directly and independently in this study, changes in lung perfusion due to RILI may also contribute to the increases in lactate-to-pyruvate signal increases observed. The sensitivity of blood vessels to high radiation doses (i.e. hypofractionation) such as used in this study has been previously reported in the literature (6). Hypoxia was confirmed in this study by direct measurement of significant \( p_aO_2 \) decreases in the irradiated rat cohort (54.2 ± 12.28 mmHg) compared to the healthy group (84.74 ± 9.98 mmHg). It has been well established clinically that a \( p_aO_2 \) value
of less than 60 mmHg can lead to tissue hypoxia. (13) Decreases in $p_aO_2$ following irradiation of lung tissue has been previously reported. (11, 12) These results suggest that enhanced lactate-to-pyruvate signal ratio may reflect early onset of RILI and could be correlated with the concurrent phase of RP. The advantage of this technique over clinical standards such as $p_aO_2$ measurement is that it provides a localized measurement related to radiation injury and can potentially reveal regional variation in the severity of the injury. This is an important consideration from a clinical perspective as most treatment plans for lung cancer employ regional (i.e. conformal) radiation delivery. This study is a first step toward a longitudinal study of the effects of radiation dose, conformity and fractionation scheme that may help elucidate the mechanisms of RILI from a regional metabolism perspective. Other hyperpolarized $^{13}$C substrates such as $^{13}$C-bicarbonate may also be useful to measure regional changes in pH (32) associated with RILI and could provide information complementary to changes in pyruvate metabolism.

Although data acquisition and post-processing were kept consistent within individual data sets in this study, variations could have been introduced by subjective tasks between data sets. In particular, phasing and baseline correction for spectra were performed manually. In this study, signals were localized to regions of the lung or heart only, therefore partial volume contamination from surrounding tissues was likely. The use of absolute fiducial markers for choice of 2D-CSI voxels within the heart and lung regions may help reduce variable partial volume effects in future. Sampling of BAL from the lungs and processing was also subjective in nature and could also have contributed to the uncertainty in cell micrographs. SNR could be improved by the use of even more sensitive surface coils, which would also aid in improving spatial resolution and/or performing 3D localization in the lung with the use of Echo Planar Spectroscopic Imaging (EPSI) (33, 34).

In summary, hyperpolarized $^{13}$C-pyruvate spectroscopic imaging can detect increases in lactate-to-pyruvate signal ratio in the lung and heart tissue of rats as early as two weeks following whole-thorax irradiation. This is consistent with hypoxic tissue conditions and radiation pneumonitis, as confirmed by decreased $p_aO_2$ and increases in inflammatory cells respectively in the irradiated animals compared to healthy rats. This study is an important first step in quantifying regional RILI by modifying fractionation schemes and/or conformal radiation therapy and/or applying adjuvant therapies for the mitigation of RP. Concurrent
development of human hyperpolarized $^{13}$C approaches could pave the way for the clinical use of this regional technique in future.

2.5. Acknowledgements

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2.6. References


Chapter 3: Mapping metabolic changes associated with early Radiation-Induced Lung Injury post conformal radiation therapy using hyperpolarized $^{13}\text{C}$-pyruvate Magnetic Resonance Spectroscopic Imaging


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3.1 Introduction

Lungs cancer was the most prevalent type of cancer among men and the fourth most prevalent type of cancer among women globally in 2008. It was also responsible for the highest number of cancer related deaths in 2008. (1, 2) Radiation therapy is a primary treatment method for lung cancer (3) but leads to additional adverse consequences as the lungs are one of the more radiosensitive organs. (4) One of the major side effects of thoracic radiation therapy is RILI, which begins with inflammation of the lungs known as RP and can proceed to irreversible lung fibrosis in the later stages. (3) RILI occurs in up to 37% of lung cancer patients receiving radiation therapy (3, 5, 6), with moderate to severe RILI in about 20% of patients. (7) Underlying anatomical and functional changes in RILI include thickening of septa, capillary damage and obstruction of airways leading to ventilation-perfusion mismatch. These changes lead to symptoms such as shortness of breath, cough, fever and the need for oxygen therapy and ventilation support. (8, 9)

Early detection of RP using imaging could potentially improve outcomes by appropriate modification of the radiation therapy treatment plan and/or administration of corticosteroids to help reduce inflammation. (10) Chest X-ray and CT imaging are currently the most commonly used methods for detection of RILI in the clinic. These techniques detect changes in lung density and can be useful for evaluation of fibrosis associated with late stage RILI stage, but generally provide no functional information. (11) SPECT using technetium-99m can provide regional ventilation and perfusion changes associated with the onset of RILI but
lacks specificity. (12) 3D-CT has been used to provide lung DVH parameters to predict the development of RP. (13) The two most common DVH parameters used as predictors of RILI are $V_{\text{dose}}$ (percentage of CT-defined total lung volume receiving greater or equal than the threshold dose) and mean lung dose (average dose of the CT-defined total lung volume). However, these parameters have very low predictive powers. (3)

Despite the low proton signal afforded in the lungs, MRI has been used for detection of RILI. Dynamic contrast-enhanced MRI with Gd-DTPA has been used to detect perfusion changes post-irradiation in dog lungs. (14) MRI has also been able to measure significant changes in relaxation times ($T_1$ and $T_2$) post-irradiation in rodent lungs. (15) More recently, hyperpolarized $^3$He-MRI has been used to detect regional changes in ADC and gas exchange associated with RILI in rodent lungs post irradiation. (16) Although these MRI developments show promise, they are still predominantly based on morphological and functional changes, which generally occur too late in RILI to mitigate. Imaging biomarkers sensitive to early metabolic changes associated with RP at the cellular level could provide an approach for earlier detection of RILI.

At a cellular level, immunohistochemical analysis of RILI animal models reveals underlying changes in alveolar macrophages, inflammatory cytokines and type II pneumocytes. (17, 18) It is known that inflammatory cells are recruited from outside the radiation field following irradiation. (19) These infiltrating macrophages are a source of cytokines that are associated with the onset of inflammation in early RP. (20) RILI has also been shown to occur both in and out of the radiation field following conformal radiation therapy (21, 22), with the base of lung showing higher radiosensitivity than the apex. (21-24) Conformal radiation therapy of the base of the left and right lung leads to biphasic cytokine expression with the first wave occurring 24 hours post irradiation and the second wave occurring 5-15 days post irradiation, (25) along with DNA damage that occurs from a period of 3 days up to a period of 4 weeks post irradiation. Additionally, tissue hypoxia in the lungs plays a role in mediation of inflammatory process in RP. (26) Decreased $p_{\text{a}}\text{O}_2$, which is used as an indicator of hypoxia (27) has been reported following radiation therapy. (28, 29)

Advances in hyperpolarized MRI technology, specifically DNP, have enabled increases in the signal from $^{13}$C-substrates of a factor of up to 100,000, enabling in vivo detection with
Hyperpolarized endogenous liquid $^{13}$C-substrates can be safely injected into the bloodstream of animals and humans and the increased signal allows regional detection of the distribution of the injected $^{13}$C-labelled compounds and their metabolic products in real time. The pyruvate molecule enriched with $^{13}$C at the C-1 position has been used extensively since it can be polarized to a high degree and has a sufficiently long $T_1$ for in vivo imaging (~40 s). High resolution spatial and temporal imaging of the conversion of $^{13}$C-pyruvate to its metabolic products, $^{13}$C-lactate, $^{13}$C-bicarbonate and $^{13}$C-alanine, has been observed in vivo. Specifically, changes in $^{13}$C-lactate to $^{13}$C-pyruvate signal ratio (lac/pyr) have been used to measure lung ischemia ex vivo. Our research has previously demonstrated changes in lac/pyr in a rat model of RILI at two weeks post whole thorax irradiation. A longitudinal study of regional changes in metabolism following conformal radiation therapy would be a useful next step in the understanding the progression of RILI, specifically early detection of RP.

The purpose of this work was to quantify regional lac/pyr using hyperpolarized $^{13}$C MRSI in a rat model of RILI involving conformal radiation therapy of the lungs, 5, 10, 15 and 25 days post-irradiation. These results were compared to those obtained in healthy age-matched rats and correlated with quantitative (i.e. cell counting) histological analysis of the lungs following imaging. The potential origin of lac/pyr changes in RP is discussed in terms of changes in cell populations, specifically macrophages.

### 3.2 Methods

#### 3.2.1 Animal Irradiation

All procedures followed animal care protocols approved by the Western University (ACVS) and were consistent with procedures used by the Canadian Council on Animal Care (CCAC). To induce RILI, 12 Sprague Dawley rats (200 ± 50 g) were irradiated with a total dose of 18.5 Gy (two equal fractions of 9.25 Gy, 24 hours apart). The treatment plan consisted of two $14\times14\text{mm}^2$ opposed beams, incident on the dorsal and ventral surfaces.

Irradiation was performed using a micro-RT system based on a modified micro-CT (eXplore CT 120, GE Healthcare, Milwaukee WI USA), similar to a system previously
described. (40-42) For the micro-RT application, the micro-CT system was upgraded with the addition of a more powerful x-ray generator and computer-controlled variable aperture collimator. Image-guidance was performed using both planar fluoroscopy and micro CT scanning. The therapeutic beam had energy of 140kVp with a Half Value Layer (HVL) of 6 mmAl. It was filtered by 4.5 mmAl to harden the beam for deeper penetration. The beam was pulsed at approximately 10% duty cycle, with pulse duration of 125 ms at 63 mA. The resulting dose rate was approximately 0.15 Gy/min including additional cooling breaks.

Delivery of each fraction required approximately 1.5 hours, with 1 hour needed for irradiation and the remainder for imaging and setup. The animals were imaged with fluoroscopy prior to therapy to localize the right lung. The collimator jaws were set to place a 14x14mm² field on the lower-medial right lung at end expiration such that the diaphragm and heart were shielded. **Figure 3-1** shows the simulated conformal dose distribution in the lungs and heart region of rat thorax for a single fraction (9.25 Gy).

**Figure 3-1:** Calculated dose distributions shown in (a) axial plane, (b) coronal plane and (c) sagittal planes. The yellow contour around the right lung represents the isodose line corresponding to 9 Gy. Most of the heart region and left lung received minimal radiation dose (< 0.5 Gy). The dose delivery was gated to the breathing cycle for accurate dose deposition.

During the irradiation procedures, the rats were anesthetized using isoflurane initially in an induction chamber at 5%, and then transferred to a nose cone at 1.5 – 2.5% at a gas flow rate
of 1.0 L/min medical oxygen. Lacrilube® (Allergan Inc, Markham, Canada) was applied gently to both eyes to reduce the effect of dehydration. Animals were placed supine on the micro-RT couch and were kept warm during the irradiation using a heating pad. A small animal monitoring system (Small Animal Instruments Inc., Stony Brook, NY) was used to monitor core body temperature and detect the breathing cycle to trigger the beam for gated delivery of the radiation. Breathing rate was kept consistent at 50-60 breaths per minute by regulating the amount of isoflurane and dose was delivered only during the expiration phase of the breathing cycle for better dose registration. Animals were returned to high efficiency particulate air (HEPA) filtered cages after the procedure. An equal number of age-matched healthy Sprague Dawley rats were also housed in HEPA filtered cages and were used as controls for MRI, blood analysis and histological measurements.

3.2.2 Blood Lactate and $p_a$O$_2$ Measurements

In order to investigate systemic effects of conformal radiation therapy, blood lactate concentration levels were measured in the irradiated cohort ($n = 3$) and corresponding age-matched healthy cohort ($n = 3$) at day 5, 10, 15 and 25-post irradiation. $p_a$O$_2$ levels were also measured in each animal to quantify hypoxia post irradiation, using 60 mmHg as a clinical threshold to classify hypoxic tissue environment. (27) To obtain the arterial blood, animals were initially anesthetized in an isoflurane induction chamber (Vet-Equip) at a flow rate of 0.8 L / min using a concentration of 5% isoflurane and were then transferred to a nose cone where the isoflurane concentration was reduced to 2%. A 26-gauge catheter was inserted into the tail artery and a 0.75 mL blood sample was then extracted and inserted into a CG4+ iStat cartridge for blood lactate concentration and $p_a$O$_2$ measurement (iStat blood gas analyzer, Abbott Laboratories, Mississauga, ON). Two-tailed paired T-tests were performed on blood lactate concentration and $p_a$O$_2$ level between the healthy and irradiated cohorts for each time point. Appendix A-2 contains more details on the two-tailed paired T-test and the underlying assumptions.

3.2.3 Hyperpolarized $^{13}$C-pyruvate Preparation, Administration and MRI

Approximately 30 µl of [1-$^{13}$C]-labelled pyruvic acid (CIL, Cambridge, MA) was mixed with 15mM trityl radical (OX63, Oxford Instruments, UK) and was hyperpolarized using a commercial DNP system (Hypersense, Oxford Instruments, UK). The hyperpolarization
protocol has been described in detail previously. (43) During dissolution, the hyperpolarized sample was readily dissolved in ~5.5 ml buffered (40 mM Tris) solution containing 80mM Sodium Hydroxide, 0.1g/L Disodium EDTA dehydrate and 50mM Sodium Chloride to yield a 80 mM hyperpolarized pyruvate with a nominal pH of 7.4.

MRI was performed on the irradiated cohort (n = 3) and corresponding age-matched healthy cohort (n = 3) at day 5, 10 ± 1, 15 ±1 and 25 ± 1 post irradiation for a total of 12 irradiated rats and 12 healthy rats. For each cohort, rats were randomly chosen and measurements were performed in a blinded fashion. The rats were anesthetized using initial bolus of 5% isoflurane at a flow rate of 0.8 L / min and were then transferred to a nose cone where the isoflurane concentration was reduced to 2%. After blood lactate concentration and \( p_aO_2 \) measurements, a catheter was inserted in the tail vein for injection of hyperpolarized \(^{13}\)C-pyruvate. The extension tube connecting the catheter to the hyperpolarized sample was 24-gauge and 90 cm in length.

3.2.4 Magnetic Resonance Spectroscopy and Imaging

MRI and MRSI were performed using a 3T MRI system (MR750, GEHC, Waukesha, WI) equipped with multinuclear hardware and software as described previously. (39) The animal was supported in the prone position with a custom-built tray equipped with a warming pad to maintain body temperature. They tray included a nose cone that supplied 2 – 3% isoflurane at 0.8 L/min flow rate to maintain anaesthesia during the experiment. A custom surface coil resonant at the \(^{13}\)C Larmor frequency (32.12 MHz) was wrapped around the animal thorax. The tray was then inserted into a custom-built, rat-sized quadrature dual-tuned birdcage coil resonant at both the \(^1\)H Larmor frequency and \(^{13}\)C Larmor frequency. The birdcage coil was used to transmit/receive signal for \(^1\)H MRI and to transmit \(^{13}\)C signal only. The \(^{13}\)C surface coil was used to receive signal for \(^{13}\)C MRSI. This TORO setup for reception of \(^{13}\)C-signal enhanced the \(^{13}\)C-SNR by up to 4 times and is discussed in more detail in Appendix A-1. Heart rate (320-360 beats per minute), breathing rate (40-60 breaths per minute) and core body temperature (37.0-37.8 °C) were monitored during the entire MRI experiment (Small Animal Instruments Inc., Stony Brook, NY).

\(^1\)H MRI: A gradient echo pulse sequence was used to acquire \( T_1 \)-weighted \(^1\)H images of the rat thorax in the coronal and axial planes. The imaging parameters were as follows: FOV =
12 cm × 12 cm, matrix = 128×128, TE = 2 ms and TR = 34 ms, flip angle = 30 degree, slice thickness = 3 mm. These images were used to localize the $^{13}$C data acquisition volume of interest in the lungs as the boundary of the lungs was well visualized as a signal void compared to the other tissues (e.g. heart and liver).

$^{13}$C MRSI: MRSI was performed using a CSI technique described previously.(39) An axial FOV of 6 cm × 6 cm was chosen centred on the lungs, avoiding liver and large regions of the heart. A 12 × 12 matrix was prescribed in the FOV with a slice thickness of 8 mm. CSI data were acquired using a pulse-acquire approach (flip angle of 10 degrees, bandwidth of 5 kHz and 256 points) with a repetition time of 80 ms for a total scan time of approximately 12 seconds. Spectroscopic imaging was initiated following a delay of 25 seconds from the start of the bolus injection to maximize the lactate signal. The optimum lactate window was based on previous $^{13}$C dynamic spectroscopy experience with a similar RILI rat model. (39)

MR data processing and analysis: SAGE™ software (GEHC, Waukesha, WI) was used for processing and visualization of the $^{13}$C data overlaid on $^1$H localization images. The $^{13}$C spectroscopic imaging data were apodized with a 10 Hz Gaussian filter in the time domain, zero-filled 1× in the time dimension and both spatial dimensions. Fourier transformations were then performed in both the time domain and the two spatial dimensions to obtain a 16 × 16 grid of spectra with each voxel dimension of 3.75 mm × 3.75 mm × 8 mm. The metabolite peaks were phased and baseline corrected for every voxel. The 16 × 16 grid of spectra was overlaid on the axial $^1$H image and twelve voxels were chosen: four centred on the left lung, four on the right lung and four on the heart. The relative positions of these four voxels for each organ were kept consistent for each animal. The measure of lac/pyr for each group of four voxels was computed by dividing the total lactate signal intensity in the four voxels by the total pyruvate signal intensity in the four voxels, obtained by summing the appropriate integrated metabolite peak in each voxel. Two-tailed, paired T-tests were performed for the lac/pyr measurements corresponding to the left lung, the right lung and the heart between the irradiated cohort and the healthy cohort for each time point. A p-value of less than 0.05 was considered significant. A mixed analysis of variance with repeated measures at each time point was also run on the data to determine any within- or between-subject interactions using
SPSS software. (IBM, Markham, Ontario). Appendix A-2 contains more details on the two-tailed paired T-test and mixed analysis of variance including the underlying assumptions.

3.2.5 Histology Preparation and Analysis

Following MRI, the rats were euthanized using an injection of 540 mg/kg rat mass of sodium pentobarbital (Euthanyl Forte, Bimeda-MTC, Cambridge, Canada) and the lungs extracted for histological analysis. The lungs were fixed by intratracheal infusion of 8 mL of 10% neutral-buffered formalin. The fixed lungs specimens were embedded in paraffin, cut into 5 μm thick tissue sections and stained with H&E. Using a Zeiss Axio Imager microscope (Toronto, Ontario, Canada), five representative images, at a magnification of 40 times and a FOV of 240 μm by 320 μm, of the caudal lobe of right lung and from the basal left lobe were taken using a Retiga EXi Digital CCD camera (Q imaging, Vancouver, BC, Canada) attached to the microscope. Areas selected contained predominantly alveolar duct and alveoli, avoiding any major airways and blood vessels. The infiltrating macrophages were counted manually in each image to obtain macrophage density and were used as a gauge for injury during RP. The criteria for counting macrophages were consistent for all images and were defined as larger cells with clear boundaries and darker cytoplasm. Figure 3-2 shows a representative H&E stained section of the right lung at day 25 for both a healthy and an irradiated rat with the macrophages marked by black arrows.
Figure 3.2: Section of H&E slide from lower right lung of a healthy (unirradiated) rat at day 25 (a) and from lower right lung of an irradiated rat at day 25 post-irradiation (b). There is a clear increase in the number and size of macrophages as marked by the black arrows in the irradiated lungs compared to the unirradiated lungs.

3.3 Results

Figure 3.3 shows a typical CSI grid overlaid on the corresponding $^1$H image for a representative RILI rat, including the voxels chosen for analysis of lac/pyr in left lung, right lung and heart region respectively. Figure 3.4 provides a summary of the average lac/pyr measurements obtained from these regions in both the irradiated and healthy cohorts at day 5, 10, 15 and 25-post irradiation. Both the right lung and the left lung from the irradiated cohort show a statistically significant increase in lac/pyr compared to the right lung and left lung from the healthy cohort respectively at each time point. This confirms the response to radiation both at the site of the irradiation (radiation therapy to medial right lung) as well as the unirradiated (i.e. contralateral) left lung. The enhanced lac/pyr in the contralateral lung was evident at all four time points and did not vary substantially from one time point to another. Elevated lac/pyr in the right lung of irradiated cohort was consistent across all time points.
Figure 3-3: $^{13}$C MRSI overlaid on $T_1$-weighted $^1$H axial image. The lung boundary is clearly visualized by as signal void. $^{13}$C measurements were made in twelve voxels, four each from the left lung, right lung and the heart region are indicated as a), b) and c) respectively. Voxel positions with respect to the $^1$H image were kept consistent for all animals.
Figure 3-4: Measurement of lac/pyr plotted for the right lung, left lung and heart regions in the healthy and irradiated cohorts at all four time points post irradiation. Statistically significant increases in lac/pyr (** p < 0.01, * p < 0.05) are apparent in the right lung and left lung of the irradiated cohort compared to right lung and left lung of the healthy cohort or the heart region at all time points post irradiation.

Additionally, mixed analysis of variance demonstrated a statistically significant decrease in lac/pyr at day 25-post irradiation in the right lung of irradiated cohort compared to lac/pyr at day 10-post irradiation in the right lung of the irradiated cohort (p < 0.05), suggesting the initial phase of radiation induced effects beginning to subside. Table 3-1 summarizes lac/pyr (mean ± first standard deviation) from the right lung, left lung and heart for irradiated and healthy cohort for all time points.
Table 3-1: Summary of lac/pyr from the right lung, left lung and heart regions and macrophage count from right lung and left lung of the irradiated and healthy cohorts at each time point post conformal radiation therapy. Data is represented as mean ± standard deviation.

Measured lac/pyr was not statistically significant higher in the heart region of the irradiated cohort compared to the heart region of healthy cohort at day 5, 10 and 15-post irradiation. At day 25, lac/pyr was lower in the heart region of the irradiated cohort than the heart region of the healthy cohort (0.21 ± 0.01 vs. 0.25 ± 0.01, n = 3, p < 0.05). Analysis of the histology
from the heart region at day 10 and day 15-post irradiation confirmed that they did not show any signs of injury.

Analysis of blood lactate concentration levels did not show statistically significant changes between the irradiated and healthy cohorts at any time point post irradiation. Measurement of blood lactate from all irradiated and healthy cohorts (non time point specific) was $1.22 \pm 0.3$ mM ($n = 12$) and $1.23 \pm 0.31$ mM ($n = 12$) respectively. Additionally, blood $p_aO_2$ level measurement for all irradiated and healthy cohorts (non time point specific) was $80 \pm 15$ mmHg ($n = 12$) and $78 \pm 14$ mmHg ($n = 12$) respectively. $p_aO_2$ did not drop below normal (60 mmHg) for any irradiated cohorts and there was a lack of a statistically significant change in $p_aO_2$ level between irradiated cohorts and healthy cohorts at any time point post irradiation.

Table 3-1 summarizes the macrophage count for right lung and left lung from irradiated and healthy cohorts at each time point. Figure 3-5 shows the time courses for average lac/pyr and macrophage count for all time points for both the right and left lung of both cohorts. Macrophage counts were observed to increase in both the left and right lung of the irradiated cohort compared to the healthy cohort, following a trend similar to lac/pyr at each time point. Figure 3-6 shows a scatter plot of the lac/pyr measurements versus macrophage count for the right and left lung of all the rats for all time points. Non-parametric analysis of these data provided Spearman coefficients ($r$) of $0.86$ ($p < 0.01$) and $0.85$ ($p < 0.01$) respectively for the right and left lung. The strong correlation between lac/pyr and macrophage count suggests that the enhanced lac/pyr seen in the irradiated cohort is likely due to inflammation associated with RP, potentially up-regulation of macrophage activity.
Figure 3-5: Time evolution of the average lac/pyr in healthy and irradiated cohorts in the right lung (a) and left lung (b) for the healthy and irradiated cohorts. The corresponding macrophage counts are shown in (c) and (d). A similar time trend can be seen for both lac/pyr and macrophage count.
3.4 Discussion

To our knowledge this is the first time that hyperpolarized $^{13}$C-pyruvate MRSI has been used to assess the early phases of RILI longitudinally following conformal radiation therapy, specifically RP. In this rat model of RILI involving conformal irradiation of the right lung, measures of lac/pyr were shown to be elevated in both right and left lung at all time points (5, 10, 15 and 25 days) following irradiation. This is consistent with previous observations in a similar rat model, which showed that cytokines and DNA damage persist 5 to 15 days and 3 days to 4 weeks post irradiation respectively. (25) The statistically significant decrease in lac/pyr in the right irradiated lung observed at day 25 compared to day 10 may be evidence of reduced inflammation from the receding initial phase of RP. This seems to be supported by the reduction in measured macrophage activity at the later time points (Figure 3-5).

Additionally, lac/pyr was also shown to detect the response of the entire lungs to conformal radiation therapy (medial right lung) both at the site of irradiation but also on the unirradiated contralateral side (left lung). This observation is in agreement with previous findings, which have demonstrated that conformal radiation therapy of the lungs results in injury response of the lungs both in and out of the radiation field (21, 22).
Measurements of lac/pyr did not show altered heart metabolism at any time point. This was confirmed by the analysis of the histology from the heart region. Analysis of the lactate concentration and $p_aO_2$ levels of the blood also showed no statistically significant difference between the healthy and irradiated cohorts for any time points. Lack of change in both lac/pyr in the heart region and in blood lactate levels between the two cohorts demonstrates that the injury response was only in the lungs with no systemic effects. This confirms that the irradiation conformed to the right lung, sparing other surrounding tissues. As expected, this was not seen in our previous studies involving whole-thorax irradiation, which resulted in a significant increase in lac/pyr in the heart tissue and increases in blood lactate levels at 2 weeks post-irradiation. (39)

The lack of a $p_aO_2$ decrease (below 60 mmHg) in any of the rats at any time point and the lack of a statistically significant change in $p_aO_2$ level between healthy and irradiated cohorts confirms the response to radiation was confined to the lungs. Unlike our previous finding of decreased $p_aO_2$ in the blood following whole thorax irradiation (41), the conformal irradiation of the right lung does not appear to compromise the lung function and could be attributed to the compensatory effects of the unirradiated lung, enabling adequate oxygen supply to the body. In addition to the conformal radiation therapy, the lower dose rate of the radiation beam used in this study may also have reduced the level of tissue injury.

**Figure 3-5** shows that the macrophage count in both the left and right lung of the irradiated cohort was higher than the corresponding left and right lung of the healthy cohort. Previous studies have shown that macrophages are recruited from outside the radiation field post irradiation (19) and that these infiltrating macrophages are a source of cytokines associated with onset of inflammation in early RP. (20) As such, macrophage count is a useful quantitative indicator of the inflammation in early RILI. Additionally, the error bars (single standard deviation) on macrophage count as shown in **Figure 3-5** c) and d) do not overlap between the healthy and irradiated cohort at any time point in either the left lung or the right lung. This trend is similar to lac/pyr trends observed in the healthy and irradiated cohorts (**Figure 3-5** a) and b)). The hypothesis that lac/pyr changes may be a reflection of increased macrophage activity associated with RP is further supported by the strong correlation
between macrophage count and lac/pyr for the right lung (r = 0.86, p < 0.01) and the left lung (r = 0.85, p < 0.01). This hypothesis also seems to be supported by previous evidence linking increases in lac/pyr with inflammation in other disease (arthritis) models. (44) Increasing the sample size (n > 3) should result in more powerful statistics and better quantification of lac/pyr and macrophage count to further support this hypothesis. Staining for particular macrophage type and following the evolution of type II pneumocyte population post irradiation could provide further insight into the progression of RILI.

In summary, early metabolic changes (over days) can be mapped using hyperpolarized $^{13}$C-pyruvate MRSI in a rat model of RILI using conformal radiation therapy. Specifically, lac/pyr is observed to increase in irradiated and unirradiated lung regions consistent with histological changes associated with radiation pneumonitis. Increases in lac/pyr are highly correlated with macrophage count in both the irradiated and unirradiated lung suggesting that part of the enhanced lac/pyr may be caused by an increase in the number and activity of resident and infiltrating inflammatory macrophages. Future studies could focus on the use of hyperpolarized $^{13}$C-pyruvate MRSI to measure the effects on radiation pneumonitis arising from varying conformal radiation therapy (dose, dose rate, fractionation scheme etc.) and intervention during the course of the injury for mitigation of RILI. Furthermore, application of this technique in a clinical environment should also be possible with the recent availability of hyperpolarized $^{13}$C-pyruvate for human use.

### 3.5 Acknowledgements

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3.6 References


Chapter 4: Rapid and Accurate Mapping of pH using Spiral-IDEAL with Hyperpolarized $^{13}$C-bicarbonate 


An updated version of the following chapter will be submitted to Magnetic Resonance in Medicine as a Technical Note.

4.1 Introduction

Many fundamental physiological mechanisms in the human body depend on pH balance, thereby making pH regulation one of the most important homeostatic processes (1). Two major endogenous buffers that regulate pH are the phosphate buffer and the bicarbonate buffer. Specifically, the bicarbonate buffer is responsible for 86% of the extracellular buffering capacity. (2) Physiologically stable pH is measured at 7.35 – 7.45 for the human body. (3) Disruption of pH balance, local or systemic can result from a variety of disorders such as cancer, metabolic acidosis, chronic respiratory acidosis, stroke, cardiac failure, inflammation and infection. These disorders cause major changes in physiology such as altered energy metabolism, perfusion changes, failure of Na$^+$/H$^+$ transporters and lower lung tidal volumes (4). As a result of these changes, enhanced anaerobic glucose metabolism leads to accumulation of lactic acid and in combination with poor perfusion and reduced bicarbonate buffering capacity, lead to an acidic extracellular pH (pHe) (5). Although measurement of blood pH using an electrophysiological probe can indicate a systemic problem, it cannot point to the local site of the disorder (4). Furthermore, as metabolic changes precede functional and anatomical changes, it is important that spatially-localized changes in pHe be mapped in diseases to improve sensitivity, for example in cancer, where pHe change is linked strongly to prognosis and outcome (4). Significantly lower pHe (~6.7) in the tumour microenvironment has been reported in the literature. (6)

Several MRI and MRS techniques have been used to map pH using both exogenous substances and endogenous compounds. $^{31}$P-MRS has been used to estimate intracellular pH
(pHi) using endogenous inorganic phosphate (5). Exogenous $^{19}$F compounds have been used to quantify pH in vivo using $^{19}$F-MRS. A fluorinated derivative of vitamin B6, 6-fluoropyridoxol has been shown to measure both pH and pHi in rodent tumours (7). A major drawback of this technique is the instability of the fluorinated compounds. Exogenous substances such as Imidazole with pH sensitive $^1$H resonances have been used to quantify pHe with the aid of $^1$H-MRS (8, 9). However, the rather small chemical shift (0.7 ppm) over the entire titration range makes this technique less viable at lower field strengths. The $^1$H-MRI magnetization transfer technique referred to as Chemical Exchange Saturation Transfer (CEST) has also been used to measure pH in based on endogenous amide groups in mouse brain under ischemia and with gliomas (10, 11). A more specific CEST technique has been used with exogenous substrates such as 5,6-dihydrouacil to measure pHi as well (12). Limitations to CEST techniques include high concentration requirement for the CEST agent and the need for strong MRI radiofrequency (RF) pulses in order to enhance the saturation effect. Similar to CEST, pH dependent relaxation agents such as Gd(DOTA)-4AmP$^5^-$ have been used to measure renal pHe (13) but are highly dependent on favourable pharmacokinetics. Overall, the above MRI methods are limited by inherently low signal-to-noise ratio (SNR).

One of the more recent pHe measurement techniques utilizes hyperpolarized $^{13}$C-bicarbonate and Chemical Shift Imaging (CSI) approaches. With advances in Dynamic Nuclear Polarization (DNP), polarization of $^{13}$C-substrates can be enhanced by up to 100,000 times compared to thermal polarization (14). In vivo, the carbonic anhydrase (CA) enzyme facilitates equilibration between bicarbonate and carbon dioxide (equation [37]):

$$HCO_3^- + H^+ \underset{CA}{\leftrightarrow} CO_2 + H_2O$$  \[37\]

Gallagher et al. have used the CSI method to quantify in vivo pHe in a mouse tumour model using hyperpolarized $^{13}$C-bicarbonate ($H^{13}CO_3^-$) and its metabolite $^{13}$C-carbon dioxide ($^{13}CO_2$) via the Henderson-Hasselbalch equation (15), based on equation [37]:

$$pH = pK_a + \log_{10}\left(\frac{[H^{13}CO_3^-]}{[^{13}CO_2]}\right)$$  \[38\]

where $H^{13}CO_3^-$ and $^{13}CO_2$ are the signal intensities obtained from the in vivo spectra. Unfortunately, CSI is time-consuming (several minutes) and not the most efficient way to use...
the available non-renewable hyperpolarized magnetization. CSI utilizes a large number of RF pulses (typically equal to the number of voxels), acquires a coarse spatial resolution (16 x 16) and takes a considerable amount of time (~12 s) to acquire the entire scan, thereby limiting SNR due mainly to the short T1 relaxation time of the $^{13}$C-bicarbonate (~10 s) and its metabolite $^{13}$C-carbon dioxide (~10 s) in vivo. Spatial encoding with CSI can be accomplished more quickly using echo-planar read-out approaches (16, 17) and spiral read-out approaches (18, 19). However, echo-planar and spiral CSI still suffer from coarse spatial resolution and long acquisition times (e.g. a 12 x 8 x 16 spatial encoding takes 15.4 s) when suitable spectral resolution is required for substrates such $^{13}$C-pyruvate and its many downstream metabolic products (17)

As the $^{13}$C-bicarbonate and $^{13}$C-carbon dioxide spectrum is sparse in nature (ie. two peaks), the spectral resolution may be traded off for improved spatial resolution. The Dixon approach has proven to be useful to image sparse spectra as it exploits prior information of the chemical shifts between sparse chemical peaks in a spectrum to optimize readout echo spacing for improved efficiency. With this method, n+1 echoes from a sparse spectrum containing n substrate peaks can be used to resolve the spatial distribution of each of the substrates as well as account for B0 field non-uniformities in the volume of interest. A Dixon approach-incorporating IDEAL has been used extensively to separate fat and water images in $^1$H-MRI (20-23). Spiral-IDEAL single shot images with echo time shifts in between excitations has been used to encode the spectral-spatial concentration of hyperpolarized $^{13}$C-pyruvate and its metabolites (24). With this technique, k-space is encoded in an outward spiral fashion following a single excitation RF pulse, which helps preserve the non-renewable polarization of $^{13}$C-metabolites.

The purpose of this work was to implement a spiral-IDEAL method to map pH utilizing hyperpolarized $^{13}$C-bicarbonate. Initial validation of the method was performed in thermally-polarized phantoms by comparing pH measurements using spiral-IDEAL with pH measurements obtained using a conventional single-voxel FID-CSI approach. Both above MRI methods were compared with measurements obtained using a pH meter. The spiral-IDEAL technique was then used to map pH with hyperpolarized $^{13}$C-bicarbonate and its metabolic by-product $^{13}$C-carbon dioxide in vitro. Extension of this technique to in vivo applications is discussed.
4.2 Methods

4.2.1 Construction of Thermally-Polarized Phantoms

Five phantoms with pH ranging from 7.02 to 7.96 were constructed for *in vitro* validation of the spiral-IDEAL approach. 1 g of $^{13}$C-sodium bicarbonate (Sigma-Aldrich, Oakville, ON, CA) was dissolved in 20 ml of deionized H$_2$O. 200 µl ProHance (Bracco Imaging, Anjou, QC, CA) was added to the resulting mixture to yield a T$_1$ of 54 ms to improve polarization. The mixture was then divided into five equal 4 mL volumes and placed in five 8 ml glass vials. An appropriate volume of 1 N HCl was added to adjust the pH of each vial. The mixtures were sealed and stored for two weeks to ensure equilibration of H$_{13}$CO$_3^-$ and $^{13}$CO$_2$. The pH of each mixture was verified by measurement using a standard pH meter (sympHony, VWR, Mississauga, ON, CA).

4.2.2 FID-CSI and Spiral-IDEAL pH Mapping

FID-CSI and spiral-IDEAL were performed using a 3T MRI system (MR750, GEHC, Waukesha, WI, USA) equipped with multinuclear hardware and software. FID-CSI and spiral-IDEAL acquisitions were performed on each thermally-polarized phantom independently using a solenoid $^{13}$C coil resonant at a frequency of 32.12 MHz. Imaging parameters for the FID-CSI acquisition were as follows: Gaussian pulse, FOV of 12 × 12 cm, TR of 1 s, bandwidth of 5 kHz, number of points equal to 2048, flip angle of 90 degree, matrix size 1 × 1, number of RF pulses equal to 512, giving a total scan time equal to 8 min and 32 s. (total number of scans equal to 512 as one FID per scan) The spiral-IDEAL sequence acquires a FID prior to acquisition of the echoes, which aids in reconstruction of the spectral information. More details on the spiral-IDEAL sequence can be found in Wiesinger et al. (24) Each excitation pulse is followed by a single-shot outward spiral readout. The echoes are time shifted from excitation to excitation to encode the spectral information. Based on the three echoes and a frequency shift between $^{13}$C-bicarbonate and $^{13}$C-carbon dioxide of 36 ppm, an echo time shift of 292 µs was determined to yield maximum SNR, which was used in the acquisition. A FID was also acquired prior to the echoes in every scan, which aids in the reconstruction. The following additional parameters were used: FOV of 12 × 12 cm, TR of 250 ms, flip angle of 90 degrees, echo time shift of 292 µs, number of RF pulses equal to 256, giving a total scan time equal to 1 min and 4 s.
(total number of scans equal to 64 as one FID and three echoes per scan)

4.2.3 Data Processing

FID-CSI data were processed using SAGE™ software (GEHC, Waukesha, WI, USA). H$^{13}$CO$_3^-$ and $^{13}$CO$_2$ peaks were established to be ~36 ppm apart. A spectral apodization of 5 Hz was used. Peak areas for H$^{13}$CO$_3^-$ and $^{13}$CO$_2$ were calculated for each vial and the ratio was used to compute pH using the Henderson-Hasselbalch equation (equation [38]). The pKa was assumed to be 6.17 (15). For spiral-IDEAL, the spectral information in the FID was used in the reconstruction of metabolite images. The acquired k-space data were filtered and an image-encoding matrix of $128 \times 128$ was calculated based on the spiral-readout (24). The spiral-IDEAL reconstructed metabolite images were further processed off-line using Matlab (MathWorks, Natick, MA) to obtain the pH maps. A spectral apodization of 15 Hz was performed and a pixel-by-pixel ratio of H$^{13}$CO$_3^-$ and $^{13}$CO$_2$ intensities was used to compute a map of pH using equation [38] with an assumed pKa of 6.17. To reduce noise in the pH map, only those voxels with a signal greater than three times the standard deviation of background noise in the $^{13}$CO$_2$ image were used in the $^{13}$CO$_2$ image. Corresponding pixels in the H$^{13}$CO$_3^-$ image were used to compute the final pH map.

4.2.4 Hyperpolarized $^{13}$C-bicarbonate Preparation and Polarization

$^{13}$C-sodium bicarbonate was hyperpolarized using a commercially available DNP system (Hypersense, Oxford Instruments, UK). The $^{13}$C-sodium bicarbonate (Sigma-Aldrich, Oakville, Canada) was initially dissolved in glycerol to yield a concentration of 1.7 M using a magnetic stirrer and hot plate (70 °C) and 18 mM trityl radical (OX63, Oxford Instruments, UK) was added to the mixture (25). To get the final dissolution, 5 ml of phosphate buffer dissolution media with a pH of 7.4 was used to dissolve a 170 µl hyperpolarized sample for a final concentration of 58 mM $^{13}$C-sodium bicarbonate. The absolute $^{13}$C-bicarbonate polarization available following DNP at 3T was measured to be between 2-4%.

4.2.5 In Vitro pH Mapping using Hyperpolarized $^{13}$C-bicarbonate

Immediately following the dissolution, equal amounts of hyperpolarized $^{13}$C-bicarbonate were mixed with two 4 ml 500 mM sodium phosphate-buffered solutions at pH 6.83 and 7.19 (measured using the pH meter) respectively containing 10ug of CA enzyme (Isozyme II from
bovine erythrocytes, 3000W-A units/mg protein, Sigma Aldrige St. Louis, MO, USA). The spiral-IDEAL acquisition was initiated following a delay time of 5 s to allow the $\text{H}^{13}\text{CO}_3^-$ and $^{13}\text{CO}_2$ to equilibrate. The spiral-IDEAL acquisition was setup with the following parameters: field of view of $12 \times 12$ cm, repetition time of 250 ms, three echoes spaced 292 $\mu$s apart, flip angle of 20 degrees, number of RF pulses equal to 100 and total scan time equal to 25 s. (total number of scans equal to 25 as one FID and three echoes per scan) Data processing was performed in an identical fashion as described above for the spiral-IDEAL imaging of the thermally-polarized phantoms.

4.3 Results

Figure 4-1 presents a typical thermally-polarized phantom spectrum showing $\text{H}^{13}\text{CO}_3^-$ and $^{13}\text{CO}_2$ peaks separated by 36 ppm which was obtained using the FID-CSI method. Using equation [38], pH was computed to be 7.48. Using a standard pH meter, the pH for the same solution was measured to be 7.46. The agreement between FID-CSI and actual pH was measured for a range of pH values from 7.03 and 7.98 as listed in Table 1. The difference between pH measurement by the FID-CSI method and the pH meter ranged from 0.27 to 2.26 %.
Figure 4-1: Spectrum obtained from FID-CSI of the vial with pH meter measurement of 7.46 (FOV 12 cm, TR 1s, BW 5k, 2048 pts, 512 averages). pH calculated from above spectrum based on the ratio of peak areas of $^{13}$CO$_3^-$ and $^{13}$CO$_2$ using the Henderson-Hasselbalch equation was 7.48 (equation [38]). All pH measurements using FID-CSI method are listed and compared with standard pH meter measurements in Table 4-1.
**Figure 4-2:** Cross section pH map of the vials acquired using IDEAL (FOV 12 cm, TR 250 ms). Mean pH of the vials ranges from 7.03 to 7.98 which all fall within < 0.5 % of the actual pH (using a pH meter). Standard deviation in the spiral-IDEAL pH measurements ranges from 0.41 for pH of 7.03 to 0.89 for pH of 7.99, as reported in Table 4-1.
Table 4-1: pH measured using the pH meter, spiral-IDEAL method and FID-CSI methods along with differences are displayed in the columns respectively. Mean and standard deviation of the difference in pH between MRI methods and pH meter are shown at the bottom.

Figure 4-2 shows the pH maps derived using spiral-IDEAL acquisitions of the five vials. Quantified mean and standard deviation of pH from the spiral-IDEAL pH maps for each vial are listed and compared to the standard pH meter reading in Table 1. The difference between mean pH measurement by the spiral-IDEAL method and the pH meter ranged from -0.14 to 0.38 %. The standard deviation of the pH maps obtained using spiral-IDEAL range from 0.41 for low pH (7.02) to 0.89 for high pH (7.96).
Figure 4-3: Linear regression fits between pH measured using pH meter on horizontal axis and FID-CSI method on vertical axis ($y = 1.009x$, $R^2 = 0.9765$) and between pH measured using pH meter on horizontal axis and spiral-IDEAL method on vertical axis ($y = 0.9917x$, $R^2 = 0.9830$).

Figure 4-3 demonstrates pH measurement with the pH meter on the horizontal axis and pH measurement with the MRI methods on the vertical axis. The standard deviation of the pH in the spiral-IDEAL pH maps is plotted as the error bars. A strong agreement between pH measured using the FID-CSI method and the pH meter as well as pH measured using spiral-IDEAL and the pH meter is observed. A linear regression fit between pH measurement using pH meter and FID-CSI method ($y = 1.009x$, $R^2 = 0.9765$) appears to be similar compared to
the linear regression fit between pH measured using the pH meter and the spiral-IDEAL approach ($y = 0.9917x$, $R^2 = 0.9830$).

**Figure 4-4** presents the *in vitro* pH maps obtained using spiral-IDEAL with hyperpolarized $^{13}$C-bicarbonate. Spiral-IDEAL pH measurements of $6.82 \pm 0.21$ and $7.16 \pm 0.25$ (mean ± first standard deviation in pH map) compared well to pH meter readings of 6.83 and 7.19 respectively. The high standard deviation of the hyperpolarized pH maps may be attributable to the low polarization level of $^{13}$C-bicarbonate that translates to a low $^{13}$CO$_2$ SNR.

![pH maps](image)

**Figure 4-4:** *In vitro* pH mapping with hyperpolarized $^{13}$C-bicarbonate using the spiral-IDEAL method. Carbonic anhydrase enzyme was added to the phosphate buffer solutions for rapid conversion and equilibration of hyperpolarized $^{13}$C-bicarbonate to $^{13}$C-carbon dioxide. Spiral-IDEAL pH measurements of $6.82 \pm 0.21$ and $7.16 \pm 0.25$ (mean ± first standard deviation in pH map) compared well to pH meter readings of 6.83 and 7.19 respectively.

### 4.4 Discussion

The conventional CSI method has been demonstrated in the literature to measure pH using hyperpolarized $^{13}$C-bicarbonate (15). This was verified here by comparison of the pH measurement using the FID-CSI method and a standard pH meter. In this work, a spiral-IDEAL approach was used based on the known chemical shifts and sparse (i.e. two peak) spectrum associated with $^{13}$C-bicarbonate to rapidly form (8 times faster than CSI) metabolite maps (24). This method was then used to form images of $H^{13}$CO$_3^-$ and $^{13}$CO$_2$ in thermally-
polarized phantoms, which were then used in conjunction with the Henderson-Hasselbalch equation to compute pH maps (equation [38]). Validation of this method as a tool for accurate pH measurement was performed using a standard pH meter, where a strong linear regression fit between pH measurement using spiral-IDEAL and standard pH meter demonstrated the accuracy of this method (Figure 4-3).

The pH in each thermally-polarized phantom was independently measured by FID-CSI, spiral-IDEAL and a pH meter. The scan time for the FID-CSI is proportional to the number of RF pulses used, which in turn is proportional to the matrix size used in the acquisition. Spiral-IDEAL presents a distinct advantage with the single-shot readout such that the number of RF pulses does not increase with the matrix size. For example, if the FID-CSI approach used an 8 x 8 matrix, the total scan time would have been 64 times as much as it has been reported. In comparison, the spiral-IDEAL inherently performs pH mapping with a matrix size of 128 x 128 with the reported scan time. Additionally, use of FID-CSI with hyperpolarized $^{13}$C-bicarbonate and a large matrix size would reduce SNR due to the smaller voxel and significant $T_1$ decay of hyperpolarized $^{13}$C-bicarbonate and $^{13}$C-carbon dioxide due to the longer scan time.

It should be noted that both CSI and spiral-IDEAL techniques are highly sensitive to the concentration of $^{13}$CO$_2$ relative to H$^{13}$CO$_3$. As absolute pH increases, the concentration of $^{13}$CO$_2$ decreases, thereby decreasing $^{13}$CO$_2$ SNR. This introduces a potential for larger error at higher absolute pH compared to lower absolute pH. This phenomenon is observed in Table 1 where standard deviation of pH was reported for the spiral-IDEAL measurement as an estimate of the error in the pH maps. The error was smaller (0.41%) at lower pH (7.02) and higher (0.89%) at higher pH (7.96). Additionally, larger differences from actual pH (pH meter reading) for the MRI methods (FID-CSI and spiral-IDEAL) are observed for the vial with a higher measured pH. The precision and accuracy of the pH maps obtained from spiral-IDEAL would both likely benefit from a higher number of averages in the MRI acquisition of each vial due to the associated increase in $^{13}$CO$_2$ SNR.

In this study, an absolute polarization level of the hyperpolarized $^{13}$C-bicarbonate at 3T following DNP was between 2% and 4%. This was lower than anticipated and prohibited implementation of the technique in vivo in the present study. A lower polarization results in
lower SNR for both $^{13}$CO$_2$ and H$^{13}$CO$_3^-$, but the $^{13}$CO$_2$ SNR affects pH mapping more profoundly as it is the lower concentration metabolite. *In vitro* hyperpolarized $^{13}$CO$_2$ SNR for the spiral-IDEAL pH map with a mean pH of 7.16 was approximately 2. Higher $^{13}$CO$_2$ SNR is required at higher pH as an increase in pH leads to reduction in the absolute concentration of $^{13}$CO$_2$, as dictated by equation [38]. A theoretical increase in *in vitro* pH from 7.16 to 7.4 would result in a two-fold increase in $^{13}$CO$_2$ SNR thereby allowing improved image quality as presented in Figure 4-4. Furthermore, *in vivo* use would result in a reduction in T$_1$ relaxation time of the $^{13}$CO$_2$ in addition to a longer wait time before imaging following the administration of hyperpolarized $^{13}$C-bicarbonate. The longer wait time is required for the lengthy intravenous injection (~10 s) as well as additional transit time for H$^{13}$CO$_3^-$ to move into the extracurricular space (estimated 5-7 s). The T$_1$ relaxation time of $^{13}$CO$_2$ was estimated to reduce from ~30 s *in vitro* to ~10 s *in vivo*. We estimate an *in vitro* $^{13}$CO$_2$ SNR of ~10 with our current imaging parameters to be sufficient for a suitable *in vivo* pH map. Keeping everything else consistent, this requires a $^{13}$C-bicarbonate polarization level of 10-20%, which has been achieved by others. (26)

In summary, rapid and accurate pH mapping was demonstrated in phantoms using hyperpolarized $^{13}$C-bicarbonate with the spiral-IDEAL technique. Comparison of the pH measurement using the spiral-IDEAL technique and FID-CSI method as well as the spiral-IDEAL method and a standard pH meter provide validation for the applicability of this method. Accurate *in vitro* pH mapping with hyperpolarized $^{13}$C-bicarbonate provides further evidence for the usability of this technique. Application of this technique *in vivo* may be possible with the new DNP polarizers that promise higher polarization of $^{13}$C-substrates and improved bicarbonate preparations (e.g. Cesium $^{13}$C-bicarbonate). (15)
4.5 References


Chapter 5: Discussion and Future Work

In this work, new approaches to quantify \textit{in vivo} metabolism using hyperpolarized $^{13}$C-substrates ($^{13}$C-pyruvate and $^{13}$C-bicarbonate) have been investigated. These two substrates were polarized to a high degree (~10,000-100,000 ppm) for the feasibility of \textit{in vivo} imaging at a magnetic field strength of 3T. The higher polarization was required to compensate for the lower gyromagnetic ratio (about $\frac{1}{4}$ of $^1\text{H}$ gyromagnetic ratio – Table 1-2) and lower concentration of the $^{13}$C-substrates compared to $^1\text{H}$. The gyromagnetic ratio of $^{13}$C-substrates dictated the thermal polarization of ~2.5 ppm compared to a thermal polarization of ~10 ppm for $^1\text{H}$ at a magnetic field strength of 3T. Additionally, the higher polarization compensated for the lower concentration of $^{13}$C-substrates (80 mM for $^{13}$C-pyruvate and 60 mM for $^{13}$C-bicarbonate) administered intravenously compared to the concentration of water \textit{in vivo} (estimated at 55 M). \textit{In vivo} metabolism was quantitated regionally by measuring the ratio of lac/pyr following injection of hyperpolarized $^{13}$C-pyruvate. In the first study (Chapter 2), a higher lac/pyr was observed in rodent lungs and heart two weeks post-irradiation of the entire thorax using a $^{60}$Co source (14 Gy, one fraction). Radiation Pneumonitis (RP) was confirmed qualitatively by macrophage infiltration observed in cell micrographs from irradiated rodents. Lack of increase in lac/pyr and lack of macrophage infiltration was observed in the age-matched healthy cohort. This study was improved upon in Chapter 3 by performing conformal radiation therapy, acquisition of regional and longitudinal lac/pyr data and acquisition of quantitative histology. The conformal radiation therapy was delivered using a modified uCT machine to the right rodent lung (18.5 Gy, two fractions). Regional (left lung, right lung and heart) and longitudinal (day 5, 10, 15 and 25 post radiation therapy) acquisition of lac/pyr demonstrated organ wide response of injury in the entire lung. Lack of increase in lac/pyr in the heart region was observed. Infiltrating macrophages were counted in the lower lung regions to obtain quantitative histology that confirmed an organ-wide response to the irradiation (left and right lung) and a lack of injury to the heart region. In Chapter 4, hyperpolarized $^{13}$C-bicarbonate was used to map pH with the spiral-IDEAL technique. As bicarbonate is the largest extracellular buffer \textit{in vivo}, the ratio of bicarbonate to its metabolic product of carbon dioxide provides an estimate of the pH. Validation of the spiral-IDEAL technique was performed using thermal phantoms with pH varying from 7.02 to 7.96. The feasibility of this technique was further demonstrated by the use of
hyperpolarized $^{13}$C-bicarbonate to map pH \textit{in vitro} with the aid of carbonic anhydrase enzyme.

5.1 Radiation Pneumonitis Induced using $^{60}$Co

Chapter 2 describes the study performed to quantify lac/pyr in rodent lungs post-irradiation of the whole thorax using $^{60}$Co. Pilot experiments were initially performed to select a dose that would induce RP in the rodent lungs. Two animals were irradiated in the whole thorax (one fraction) with a dose of 12 Gy, 14 Gy and 18 Gy in each cohort for a total of 6 animals. 12 Gy was deemed to be too low and 18 Gy led to severe adverse symptoms (e.g. weight loss, difficulty in breathing). The dose of 14 Gy was chosen to be adequate to induce RP without inducing severe adverse symptoms.

Uniform irradiation to the rodent thorax was performed by providing 7 Gy of dose in the supine position and 7 Gy of dose in the prone position for a total dose of 14 Gy. Collimation of the $^{60}$Co beam was accomplished using a lead block with a circular opening 4 cm in diameter. Attempts to avoid irradiation to the liver region of the rodents were made by using an animal positioning system. As such, direct exposure of the rodents’ liver to the irradiation beam was avoided; although $^{60}$Co with its large geometric penumbra could have contributed to some dose to the liver region. The thickness of the animal position system (0.75 cm) also ensured a build-up region for the high-energy $^{60}$Co beam (1.2 MeV).

The timeline to perform imaging post-irradiation was based on a similar irradiation technique in the literature. (1) Two weeks post-irradiation, higher lac/pyr was observed in the lungs and the heart region of the irradiated cohort compared to the age-matched healthy cohort. The injury was confirmed by macrophage infiltration observed in cell micrographs. A lower $p_aO_2$ was also observed in the irradiated cohort compared to the healthy cohort. Irradiation of the whole thorax was responsible for the higher lac/pyr in the heart region of the irradiated cohort. Although the heart is less radiosensitive than the lungs, the irradiation of the whole thorax led to a significant increase in the lac/pyr in the heart region. The parameter of lac/pyr was also quantified for the kidneys to estimate systemic effect of the irradiation. Lack of
increase in lac/pyr in the kidneys confirmed the localized response to irradiation was limited to the thorax region, as measured by lac/pyr.

A birdcage $^{13}$C-RF coil and a surface $^1$H-RF coil were used to perform MRI and MRSI. The surface coils are known for their high sensitivity but a lack of uniformity along the direction orthogonal to the surface-plane of the coil. As such they lack $B_1$ uniformity (i.e., intensity) along the depth direction. This can be observed in Figure 2-6 as a gradient in the $^1$H image that forms the background image underlaid below the $^{13}$C-CSI data in Chapter 2. Although $^{13}$C-RF coil was sufficient to obtain $^{13}$C-signal from lungs and the heart region, it lacked the SNR needed to delineate $^{13}$C-signal from the left and right lung. Nevertheless, this study demonstrated the feasibility of hyperpolarized $^{13}$C-pyruvate to detect RP using lac/pyr. A few areas of improvement that were addressed in the study in Chapter 3 included: a) conformal radiation therapy, b) regional acquisition of lac/pyr (left and right lung) using a TORO coil approach and, c) acquisition of quantitative histology.

5.2 Radiation Pneumonitis Induced using Conformal Radiation Therapy

The study presented in Chapter 3 improved upon the findings in Chapter 2. In this study, 12 rodents were irradiated with two fractions of 9.25 Gy each given 24 hours apart in a conformal fashion using a modified uCT system. (2) A higher dose than used in the previous study (Chapter 2) was used to compensate for the low dose rate of the uCT system (~0.15 Gy/min) compared to the $^{60}$Co (~1.1 Gy/min). (3) The $^{13}$C-data was acquired from the left lung, the right lung and the heart region of 3 irradiated rodents and 3 healthy age-matched rodents each at day 5, 10, 15 and 25 post radiation therapy.

The irradiation was conformed to the right lung using two parallel-opposed beams with a FOV of 14 mm x 14 mm. The energy output of the uCT machine was 140 kVp with a half value layer of 6 mm of Aluminum. The procedure was also gated to the breathing cycle of the rodents for accurate dose registration. This ensured minimal dose to the contralateral lung as well as the heart and the liver region. The low energy of the uCT beam led to high dose
deposition in the bones that were present in the irradiation field (ribs, part of spine) due to the photoelectric effect.

The $^{13}$C-MRSI data was collecting using the TORO operation, which offered a significantly higher SNR that allowed delineation of the $^{13}$C-data from the left lung and the right lung. The TORO system allowed for rapid switching between the $^1$H-$^{13}$C-birdcage transmit coil and a $^{13}$C-surface receive coil for an SNR improvement of ~4 times over the $^1$H-$^{13}$C-birdcage transmit/receive coil for $^{13}$C-MRI. The construction, setup and enhancement using the TORO operation are discussed in detail in Appendix A-1.

An increase in lac/pyr was observed both in the right lung (site of irradiation) and in the left lung (contralateral lung) at all time points post-irradiation. A lack of increase in lac/pyr in the heart region was also observed at all time points post-irradiation. Blood $p_a$O$_2$ did not change significantly between the irradiated and healthy cohorts at all time points post irradiation. The high dose in the bones did not lead to any adverse effects or change the observed lac/pyr in adjoining lung regions. Macrophages counts in the rodent lungs was significantly higher in both left and right lungs of the irradiated cohorts compared to the healthy cohorts at all time points. It is possible but not likely that the contralateral lung responded directly to the small direct dose that it received during the irradiation procedure. The reason that it is unlikely is due to the very small volume in addition to the very small dose (< 0.5 Gy) that the contralateral lung received during the irradiation procedure, as demonstrated by the dose distribution in Figure 3-1. (3)

The high correlation between the macrophage count and lac/pyr could potentially mean that part of the enhanced lac/pyr may be attributable to the increase in metabolically-active macrophages infiltrating the lung tissue. Further testing is required to verify the above statement. Although the low dose rate of the uCT system did ensure RP in the rodent lungs, a lack of fibrosis at 16 week post-irradiation was observed in the histology. This implied that most of the injury had been repaired and that the healthy lung was restored. This demonstrates that the lac/pyr parameter is quite sensitive to RP in the rodent lungs, as confirmed by the histology. Therefore, a very high dose to ensure severe RP is not a requirement for observation of the injury using lac/pyr. Nonetheless, a dose escalation study would help understand the injury in a better manner.
5.3 pH Mapping using $^{13}$C-bicarbonate with Spiral-IDEAL

The study presented in Chapter 4 presents a novel method to map pH using hyperpolarized $^{13}$C-bicarbonate with spiral-IDEAL. The acid-base pair of bicarbonate and carbon dioxide forms the largest extracellular buffer in the body. (4) The Henderson-Hasselbalch equation can be used to compute the final pH of the bicarbonate-carbon dioxide pair. The spiral-IDEAL pulse sequence utilizes the available magnetization more efficiently than the CSI method. (2) In this work the use of spiral-IDEAL to map pH was validated by comparison of pH mapped by this method to pH measured using conventional single-voxel CSI (5) and a standard pH meter. *In vitro* application using hyperpolarized $^{13}$C-bicarbonate was also validated with a standard pH meter. Although the method itself is feasible, limited $^{13}$C-bicarbonate polarization and concentration prevented us from implementing the technique *in vivo*.

The recipe of dissolving $^{13}$C-bicarbonate in glycerol inherently suffers from a low solubility (1.7 M). (6) In addition, the amount of $^{13}$C-sodium bicarbonate dissolved in glycerol that can be polarized efficiently in the DNP system was limited to 170 ul. Furthermore, the absolute polarization for $^{13}$C-bicarbonate available at the 3T after the dissolution was measured at 2-4%. Lastly, the T$_1$ relaxation time for $^{13}$C-bicarbonate is ~30 s *in vitro* reduces to ~10 s *in vivo*. A combination of the above mentioned factors restricted the *in vivo* implementation of this technique.

An absolute polarization of 2-4% at the 3T for $^{13}$C-bicarbonate resulted in an insufficient SNR of $^{13}$C-carbon dioxide. As discussed in Chapter 4, based on the *in vitro* pH map using hyperpolarized $^{13}$C-bicarbonate with spiral-IDEAL, an increase in $^{13}$C-carbon dioxide SNR from ~2 to ~10 would yield an adequate *in vivo* pH map. This would require the polarization of $^{13}$C-bicarbonate to increase to 10-20% from 2-4%. In addition, other chemical formulations with higher solubility (e.g. $^{13}$C-cesium bicarbonate) could be used to ensure higher injectable concentration to yield a better SNR *in vivo*. As well, newer DNP polarizer technologies promising higher polarizations may allow for *in vivo* pH mapping using hyperpolarized $^{13}$C-bicarbonate with spiral-IDEAL in future.

The spiral-IDEAL method described in Chapter 4 reads out the magnetization associated with the FID first as well as three echoes to form the set of images of $^{13}$C-bicarbonate and
$^{13}$C-carbon dioxide images along with the $B_0$ field correction. (2) Each echo is read out in a single-shot spiral fashion after the excitation RF pulse. The spiral encoding is ensured by the echo time shift from excitation to excitation. The flip angle for the excitation was chosen to be 20 degrees such that it was adequate to form metabolite images while minimizing the SNR variation from echo to echo. Minimal SNR variation across three echoes in each data set ensured proper reconstruction to formulate the metabolite images. The choice of a smaller TR also helped minimize the $T_1$ relaxation effect in the final images. A variable flip angle (VFA) scheme could be implemented to ensure uniform SNR across all echoes in future.

5.4 Future Work

The central questions about the mechanism of RILI motivated quantitation of metabolic changes associated with RP, specifically hypoxic tissue environment, inflammation and macrophage proliferation in this thesis. However, further work is needed in order to delineate the interplay of these mechanistic processes and this will likely be aided by the use of animal models of RILI. Quantification of the metabolic signature during RP based on changing total dose, dose rate and fractionation scheme could be studied. The data could be collected for a longer time span (e.g. zero days to 20 weeks post irradiation procedure) to understand the evolution of the underlying disease. This would include lac/pyr ratio and identification of the type of proliferating macrophages. The hypothesis that part of the lac/pyr enhancement may be a result of highly metabolic infiltrating macrophages could also be tested. Overlay of lac/pyr data over a) the hypoxia map from histology, and b) the macrophage map from histology and comparison of the two data sets would help decode the origin of lac/pyr changes in RP. Additional studies could be performed to profile the inflammatory cytokines, type II pneumocytes endothelial cells and fibroblasts in a similar manner to test the relationship between lac/pyr and the evolution of these physiological parameters. A study could also be performed to evaluate the metabolic signature following administration of radioprotector chemical species such as Superoxide Dismutase post-irradiation. (1) Establishment of the mechanistic nature of the injury in more controlled animal models would result in a better understanding of RP. A deeper level of understanding would benefit the clinical studies, where the injury often progresses in a more complex manner than in the
animal models due to other confounding factors. Some of these confounding factors include the risk factors listed in Table 1-1 under the patient category. Animal models of RILI with low $p_aO_2$ prior to radiation therapy along with a variable conformation of the radiation therapy across the lung may help elucidate these clinical risk factors in a better manner.

Clinical use of hyperpolarized $^{13}$C-pyruvate MRI for detection of RP could aid in real-time monitoring of ongoing radiation therapy of breast cancer, lung cancer and other thoracic cancers that may be susceptible to RILI. The information could be used as feedback for modification of the radiation therapy (or chemotherapy) plan or to determine whether anti-inflammatory drugs would help (e.g. corticosteroids). As every patient may have a slightly different physiological make-up, $^{13}$C-pyruvate MRI could assist with personalized treatment where the metabolism of each patient could be recorded and used in the ongoing therapeutic intervention. The non-ionizing nature of MRI also permits repeated scans for continuous monitoring of a patient, which may not be possible with X-ray based modalities such as PET and CT. Continuous monitoring may be important to track the evolution of both the tumour response and the RP in cancer patients. Cancer cells may begin to demonstrate lower lac/pyr post therapeutic intervention, as demonstrated in the literature with other cancer cell lines, (7) while onset of RP leads to higher lac/pyr post radiation therapy, as demonstrated in this thesis. In addition, hyperpolarized $^{13}$C-bicarbonate could be used in the clinic to map pH of tumours undergoing radiation therapy, as pH plays a vital role in tumour progression and therapy. (8) Successful phase I clinical trials for the use of hyperpolarized $^{13}$C-substrates in detection of prostate cancer (9) may pave the way for use of this exciting technique in a variety of human diseases, including as RP, in the near future.
5.5 References


Appendix A-1: $^{13}$C-SNR enhancement using the Transmit-Only/Receive-Only (TORO) RF coil configuration

Chapter 2 used a switch-tuned $^{13}$C-$^1$H-birdcage RF coil that could be rapidly switched between anatomical imaging mode ($^1$H) and metabolic imaging mode ($^{13}$C) by use of a PN-junction with isolation region diodes. This coil was designed and constructed by Heeseung Lim. In addition, the fast switching mechanism was exploited to operate the birdcage coil as a $^{13}$C-transmit-only coil with a $^{13}$C-surface coil for reception of the $^{13}$C-signal, otherwise known as the Transmit-only/Receive-only (TORO) configuration. The surface coil and the fast switching mechanism between the birdcage coil and the surface coil were designed and constructed by Kundan Thind. Overall, the combined system is capable of TORO operation for $^{13}$C-imaging and spectroscopy and ordinary transmit/receive operation as a $^1$H coil, which results in an enhanced $^{13}$C-SNR.

During the transmit phase of the TORO operation, the transmit coil must be tuned to the resonant frequency while the receive coil is de-tuned and during the receive phase of the TORO operation, the alternate is true. This rapid switching was accomplished by the application of a direct current bias to the diodes in the RF hardware circuit. The bias current tuned the $^{13}$C-$^1$H birdcage transmit coil to resonate at the $^{13}$C-frequency while simultaneously de-tuned the $^{13}$C-surface receive coil. The bias current required for the TORO operation was provided by the MRI system but was not sufficient to bias all the diodes. For a fully functioning TORO operation, the bias current was amplified using an additional fast switching circuit and an external power supply in order to bias all the diodes. The complete circuit diagram is shown in Figure A-1. During the transmit phase, the transmit RF pulse from the scanner (consisting of both an alternating current component at the resonant frequency and a direct current component) was filtered using a tank circuit to remove the alternating current component. This yielded a clean direct current bias that was amplified using a metal oxide semiconductor field effect transistor circuit and an Agilent U8002A power supply. The amplified bias was then used to drive the $^{13}$C-$^1$H-birdcage transmit coil and the $^{13}$C-surface receive coil. The orientation of the diodes on the coils was arranged such that with the onset of the transmit pulse (the direct current bias being in the ‘ON’ state) the birdcage transmit coil was tuned to the $^{13}$C-resonant frequency and the $^{13}$C-surface coil was...
de-tuned. After the transmit RF pulse, the direct current bias would switch to the ‘OFF’ state which de-tuned the $^{13}\text{C}$-birdcage coil (it would switch to $^1\text{H}$ frequency) and re-tuned the $^{13}\text{C}$-surface coil for signal reception.

**Figure A-1:** Detailed outline of the TORO operation. A tank circuit filters the alternating current component from the transmit pulse. The resulting direct current bias was amplified using a metal oxide semiconductor field effect transistor circuit and an Agilent U8002A power supply. The amplified direct current bias was supplied to the $^1\text{H}$-$^{13}\text{C}$-birdcage coil and the $^{13}\text{C}$-surface coil that led to a tuned $^1\text{H}$-$^{13}\text{C}$-birdcage coil (at $^{13}\text{C}$-resonant frequency for efficient transmit) and a de-tuned $^{13}\text{C}$-surface receive coil. After the transmit pulse, the $^1\text{H}$-$^{13}\text{C}$-birdcage coil was de-tuned (switches back to $^1\text{H}$-resonant frequency) and $^{13}\text{C}$-surface coil was re-tuned to $^{13}\text{C}$-resonant frequency for efficient reception. Figure courtesy of Heeseung Lim.

The SNR enhancement was calculated for the TORO operation over the regular transmit/receive with the birdcage $^{13}\text{C}$-$^1\text{H}$-birdcage coil using a thermally-polarized $^{13}\text{C}$-
sodium acetate phantom. 2D $^1$H images were acquired for localization purposes in the coronal plane with a 128 mm × 128 mm FOV and 40 mm slice thickness that resulted in an in-plane resolution of 0.5 mm/voxel. A fast gradient echo sequence was employed with the following parameters: TR = 34 ms, TE = 4.1 ms, flip angle = 90 degree and 16 averages. For $^{13}$C-imaging, the in-plane resolution was reduced to 1.0 mm/voxel over the same FOV and with the same slice thickness as the coronal $^1$H images. Images of the phantoms were acquired using a broad-banded fast gradient echo sequence with TR = 34 ms, TE = 2.3 ms, flip angle = 90 degree and 64 averages. Figure A-2 shows the $^{13}$C-images acquired from the transmit/receive operation of the $^{13}$C-$^1$H-birdcage coil and the TORO setup. The $^{13}$C-SNR was computed to be 4.2 times higher in the TORO operation than the transmit/receive operation of the $^{13}$C-$^1$H-birdcage coil.

Figure A-2: $^{13}$C-2D-coronal image acquired using transmit/receive operation of the $^{13}$C-$^1$H-birdcage coil is shown on the left and a similar image acquired using the TORO operation (with identical sequence parameters) is shown on the right. The TORO operation demonstrates an SNR improvement by a factor of 4.2 times compared to the transmit/receive configuration using the $^{13}$C-$^1$H-birdcage coil. Figure courtesy of Heeseung Lim.
Appendix A-2: Statistical tests used in the thesis

A brief introduction of the paired t-tests and mixed analysis of variance used in this thesis is given below. The interested reader is referred to (1) for detailed reading.

Chapter 2 and 3 used paired two tailed t-tests to verify the significant differences between the healthy and irradiated cohorts. The significance differences are verified using the p value with a value of less than 0.05 generally considered as significant. The test assumes that the samples follow a normal distribution, are independently collected and identically distributed. The paired test consists of samples that are matched in pairs of similar units of measurement. For the thesis, the samples from healthy and irradiated cohorts were paired with administration of irradiation being the only difference between the two cohorts. Two tailed t-tests are less sensitive to directional change compared to one tailed t-tests. Thus verification of directional change by two tailed t-tests instead of one tailed t-tests ensured further validation of the change between healthy and irradiated cohorts.

Mixed analysis of variance was performed in Chapter 3 to test for significant differences between irradiated cohorts over time. In mixed analysis of variance, the difference between two independent cohorts is tested while subjecting the cohorts to repeated measures. The two factors include one that varies between-subjects and the other that varies within-subjects. The test assumes that the samples follow a normal distribution, are independently collected and are identically distributed. Adjustments could be made if any of these assumptions are violated prior to the test.

Some of the cohorts tested may not have met all the assumptions required for the two tailed paired t-tests and mixed analysis of variance due to the small number of samples. Increasing the sample size should ensure validity of the assumptions or corrections required for the assumptions before testing for the significant differences between cohorts.

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<td>K. Thind, A. Chen, L. Friesen-Waldner, A. Ouriadov, T. J. Scholl, M. Fox, E. Wong, J. VanDyk, A. Hope, G. Santyr</td>
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Curriculum Vitae

Education

**Sept. 2009 – present**, CAMPEP PhD candidate in Medical Biophysics,
Western University, London, Ontario
Research in metabolic imaging using hyperpolarized $^{13}$C-MRI applied to
Radiation-Induced Lung Injury model in rat lungs

**Sept. 2005 – April 2009**, Bachelor of Electrical and Biomedical Engineering,
McMaster University, Hamilton, Ontario
Graduated with GPA of 3.9/4

Academic contributions

*Published Articles*

**Magnetic Resonance in Medicine, October 2012.** Published: Detection of Radiation-
induced Lung Injury using Hyperpolarized $^{13}$C Magnetic Resonance Spectroscopy and
Imaging. *Kundan Thind*, *Albert Chen*, *Lanette Friesen-Waldner*, *Alexei Ouriadov*, *Timothy
Scholl*, *Matthew Fox*, *Eugene Wong*, *Jake VanDyk*, *Andrew Hope*, and *Giles Santyr*. doi/10.1002/mrm.24525

**Electrical Engineering Biomedical Capstones, June 2009.**
Wireless post-processing and interfacing ECG, blood pressure and blood oxygen
measurement systems (2009). *Kundan Thind*. EE 4Bi6 Electrical Engineering Biomedical

*Submitted Articles*

metabolic changes associated with early Radiation-Induced Lung Injury post conformal
radiation therapy using hyperpolarized $^{13}$C-pyruvate Magnetic Resonance Spectroscopic
Imaging. *Kundan Thind*, *Michael Jensen*, *Elaine Hegarty*, *Albert Chen*, *Heeseung Lim*,
*Francisco Martinez*, *Jake VanDyk*, *Eugene Wong*, *Timothy Scholl*, *Giles Santyr*.

Metabolic Magnetic Resonance Imaging Characterization of an orthotopic mouse model of
prostate cancer. *Christiane Mallett*, *Heeseung Lim*, *Kundan Thind*, *Yaeo Chen*, *Emilene
Ribot*, *Francisco Martinez*, *Timothy Scholl*, *Paula Foster*. 
Articles being prepared for Submission


**Magnetic Resonance In Medicine**, Construction and application of switch-tuned $^{13}$C-$^1$H birdcage radiofrequency coil. Heeseung Lim, Kundan Thind, Jian-xiong Wang, Francisco Martinez, Timothy Scholl.

**Oral Presentations (Refereed)**

**Canadian Association of Radiation Oncology - Canadian Organization of Medical Physicists, September 2013.**

**10th Annual CIHR Strategic Training Program in Cancer Research and Technology Transfer Research and Education day, June 2013.**
Detecting early metabolic changes in Radiation-Induced Lung Injury using hyperpolarized $^{13}$C-pyruvate post conformal radiation therapy of the lung. Kundan Thind*, Michael Jensen, Elaine Hegarty, Albert Chen, Heeseung Lim, Francisco Martinez, Jake VanDyk, Eugene Wong, Timothy Scholl, Giles Santyr.

**2013 International Pulmonary Imaging Workshop, May 2013.**

**Radiological Society of North America, November 2012.**

**World Molecular Imaging Conference, September 2012.**

**The Third International Workshop on Metabolic Imaging, July 2012.**
Quantification of pH in the rat thorax using hyperpolarized $^{13}$C-bicarbonate.
Kundan Thind*, Francisco Martinez, Albert Chen, Alexei Ouriadov, Timothy Scholl and Giles Santyr.

The International Society for Magnetic Resonance in Medicine, May 2012.

Imaging Network of Ontario, February 2012.

Canadian Organization of Medical Physicists, July 2011.

Imaging Network of Ontario, January 2011.

Canadian Organization of Medical Physicists, June 2010.

Posters (Refereed)

The International Society for Magnetic Resonance in Medicine, April 2013.

The International Society of Magnetic Resonance in Medicine, April 2013.

The International Society of Magnetic Resonance in Medicine, April 2013.

The International Society of Magnetic Resonance in Medicine, April 2013.
Transmit-only receive-only operation of a switch-tuned $^{13}$C-$^1$H radiofrequency coil for improved in vivo $^{13}$C-spectroscopy. Heeseung Lim, Kundan Thind, Francisco Martinez,
The International Society of Magnetic Resonance in Medicine, April 2013.
Timothy Scholl.

Ti Nuclear Magnetic Resonance Dispersion of Hyperpolarized $^{13}$C-Sodium Bicarbonate. Francisco Martinez, Narayan Chattergoon, Kundan Thind, Albert Chen, Timothy Scholl.

The International Society of Magnetic Resonance in Medicine, April 2013.

The International Society of Magnetic Resonance in Medicine, April 2013.

Imaging Network of Ontario, January 2013. (Awarded 3rd place in poster competition)
Use of IDEAL technique with Hyperpolarized $^{13}$C-bicarbonate to map pH. Kundan Thind*, Alexei Ouriadov, Albert Chen, Heeseung Lim, Curtis Wiens, Francisco Martinez, Charles McKenzie, Timothy Scholl, Giles Santyr.


The Third International Workshop on Metabolic Imaging, July 2012.
Quantification of pH in the rat thorax using hyperpolarized $^{13}$C-bicarbonate. Kundan Thind*, Francisco Martinez, Albert Chen, Alexei Ouriadov, Timothy Scholl and Giles Santyr.


The International Society of Magnetic Resonance in Medicine, May 2012.
Switch-Tuned Dual-Frequency Birdcage RF Coil for $^{13}$C and $^1$H Imaging. Heeseung Lim, Kundan Thind, Jian-xiong Wang, Andrew Alejski, Francisco Martinez, and Timothy Scholl.

Cancer Care Ontario, April 2010.

Scholarships and Awards

*Western University, London*

May 2013 – April 2014, *OGS*  
$15000 towards PhD program (declined)

January 2013, *Poster Presentation award at ImNO*  
$100 for 3rd place in poster presentation

September 2012 – August 2013, *CIHR strategic training program in CaRTT*  
$26700 total funding per year towards PhD program

May 2012 – April 2013, *OGS*  
$15000 towards PhD program

May 2011 – April 2012, *OGS*  
$15000 towards PhD program

September 2009 – present, *Schulich Graduate Scholarship*  
$6,938 for school tuition per year

September 2009 – August 2010, *NSERC CGS-M*  
$17500 towards Masters program

*McMaster University*

May 2008 – August 2008, *NSERC USRA*  
$4500 towards research (declined)

September 2006 – April 2007, *Senate scholarship*  
$800 for academic excellence

September 2008 – April 2009, *Hooker’s award*  
$1500 for academic excellence

September 2005 – April 2009, *Queen Elizabeth II scholarship*  
$14000 for academic excellence

September 2005 – April 2007, *Nortel Networks Entrance Scholarship*  
$6000 for academic excellence

September 2005 – April 2007, *McMaster President’s Award*  
$6000 for academic excellence

*Teaching Experience*

January 2012 – April 2013, *Teaching Assistant, Western University, London*  
- Medical Biophysics 3507, Oxygen transportation in blood
- Taught analytical diffusion problems and implementation in Matlab
- Led the question and answer session and marked assignments
**September 2007 – April 2009, Teaching Assistant, McMaster University, Hamilton**

- Engineering 1DO4, basic algorithms with the aid of C sharp
- Led classroom of 40-50 students as a tutorial teaching assistant
- Served as a question and answer session teaching assistant

**Additional Research Experience**

**August 2009 – April 2013, Research Assistant, Robarts Research Institute, London**

- Design and construction of $^1$H, $^3$He, $^{129}$Xe coils for low field (0.075T) and 3T MRI
- Repair and maintenance of multinuclear MRI hardware

**May 2007 – August 2007, Research and Development, Evertz Microsystems, Burlington**

- Employed as summer CO-OP Design Engineer
- C++ programming for menu structures of LCD displays

**May 2006 – Aug 2006, Research and Development, Evertz Microsystems, Burlington**

- Employed as summer CO-OP Design Engineer
- Emphasis on C++ and VHDL programming

**Volunteer Leadership Experience**

**September 2009 – April 2011, Co-President, Western Punjabi Association, Western Ontario, London**

- Oversaw the operations and provided direction to committee members
- Organized and led fundraising events

**September 2007 – April 2009, Vice President Social, BEAMS, McMaster University, Hamilton**

- BEAMS - Biomedical Engineering at McMaster Society
- Organized and collaborated social events
- Managed volunteers for smooth operation during the social events

**May 2008 – April 2009, Treasurer, Engineer World Health, McMaster Chapter, McMaster University, Hamilton**

- Managed allocation of financial resources
- Organized and helped in fundraising events

**September 2006 – November 2008, Captain, Outdoor Soccer team, Intramural sports, McMaster University, Hamilton**

- Led the intramural soccer team for 3 years
- Made it to the semi-finals each season