THE REGIONAL CEREBRAL METABOLIC RATE OF OXYGEN
CONSUMPTION WITH PROTON DETECTED $^{17}$O MRI DURING
PRECISION $^{17}$O$_2$ INHALATION

Eric Albert Mellon

A DISSERTATION

in

Biochemistry and Molecular Biophysics

Presented to the Faculties of the University of Pennsylvania

in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

2008

____________________
Supervisor of Dissertation

____________________
Graduate Group Chairperson
ACKNOWLEDGEMENTS

It was 1997 when I was awarded my general education diploma after leaving high school. I would like to acknowledge everyone since then who told me that I wouldn’t, couldn’t, and shouldn’t. First, those who told me I wouldn’t go back to college. This is followed by others who told me I could never succeed as a pre-medical student with such little preparation. Then it was those undergraduate advisors who told me I shouldn’t do an MD/PhD program. “You can’t do both” I was constantly admonished, along with frequent other pieces of “advice”. My favorite was always the old adage that “the MD/PhD program does not award you a REAL PhD, but something more like a MS degree”. This thesis stands as my testament that they were wrong.

I thought once I began at Penn that I had heard the end of wouldn’t, couldn’t, and shouldn’t. Yet, somehow I found many lab doors closed to me. Some labs couldn’t take more graduate students. Others wouldn’t take MD/PhDs. Others I shouldn’t have joined, according to my advisors. Indeed, my graduate advisor at the time told me I could never succeed in an MRI lab. To him, I would be wasting my time without years of intense preparation in physics and math. I am glad I did not heed his words. It was instead Ravinder Reddy who told me “I once had a music major come into this lab and he was one of the best students I have ever had. If a
music major can do it, you can do it.” It is no wonder he became my advisor. For this, and many many other things, to him I am grateful and will always be so.

I must recognize my family for the direction (but not support) they have given me. After I was born, my mother had a psychotic break from which she periodically and incompletely returns. When I was eight, my father learned he was dying of alpha-1 antitrypsin deficiency induced cirrhosis, and our lives were forever changed yet a second time. As the only child of a truck driver and a store clerk, I feel like I have been fighting my whole life. Yet, I always had one advantage. My grandmother, an orphan of the great depression, somehow knew in the early 1980s that computers would become the great tools of the future. She put the TRS-80 Color Computer 2 in front of me when I was 3 years old, and a programmer was born.

Those who know me know that I take challenges head-on--with sincerity, honesty, and broader vision, and I acknowledge those who have cared to see me for that. I acknowledge Sridhar Charagundla and Dharmesh Tailor whose helpful discussions shaped both several portions of this thesis and my own career ambitions, Greg Kiska, and Ari Goldberg who are continuous sources of information and optimism, Mark Elliott and Tom Connick whose technical discussions have made much of this work possible and have enriched my
knowledge of MRI research. R Shashank Beesam and Santosh R Gaddam have been both my helpers and friends. There have been many others who have helped me along the way and I lament that I cannot name them all, but some deserve special mention for their frequent and exceptional support: Skip Brass, Maggie Krall, Marion Knaus, Letitia Cheatham, Kiarash Emami, and Allen Bonner. I thank you all.
Despite the importance of oxygen metabolism in health and disease, there are currently no clinically utilized techniques that directly measure tissue oxygen metabolism. Of the naturally occurring isotopes of oxygen, only oxygen-17 ($^{17}$O) is detectable by magnetic resonance imaging (MRI). Because $^{17}$O is non-radioactive and chemically identical to $^{16}$O, it is completely safe. Therefore, use of $^{17}$O MRI offers the unique possibility of clinically imaging human regional metabolism.

Yet there are two significant challenges for the use of $^{17}$O imaging. The first is the low sensitivity of detection of $^{17}$O. The second is the high cost of $^{17}$O enriched products. Both of these difficulties have been overcome in small animals. The first by the use of magnetic fields stronger, and radiofrequency coils smaller, than those used for clinical imaging. The second by the use of small animals that require very little oxygen in comparison to humans. Yet for human translation of
these techniques, these challenges must be readdressed. In the hope of realizing clinical utility for $^{17}$O MRI, this dissertation attempts to tackle these challenges in the swine, a large animal model, with commonly used MRI hardware.

For the study of metabolism, $^{17}$O$_2$ is inhaled by an organism, and the rate of water formation observed. In this work, metabolism is imaged before significant recirculation of $^{17}$O-labeled water produced from oxygen gas elsewhere in the body. This time is verified by arterial blood sampling and analysis to be under a minute after the start of inhalation. For such a brief course of inhalation, a fast pulse sequence is applied to the $T_1\rho$-based (“indirect”) detection of metabolically produced water. This is combined with a novel, precision-delivery breathing system to demonstrate H$_2^{17}$O detection in swine.

Additional modeling allows the measurement of cerebral hemispheric rate of oxygen consumption at 1.5 Tesla. This is correlated with 3 Tesla direct $^{17}$O spectroscopy. As a model of deranged metabolism, another series of swine are stimulated by the metabolic uncoupler 2,4-dinitrophenol. Measurements of $^{17}$O MRI oxidative metabolic stimulation correlate with whole body gas analysis. In total, this provides proof of feasibility for $^{17}$O metabolic imaging on clinical systems.
# TABLE OF CONTENTS

1. **CHAPTER 1: PURPOSE OF OXYGEN METABOLIC IMAGING AND INTRODUCTION TO OXYGEN-17 DETECTION** ........................................... 1

   1.1. Overview .......................................................................................................................... 1

   1.2. The use of molecular oxygen *in vivo* ........................................................................... 2

   1.3. Stroke: a motivation for studying cerebral oxidative metabolism ......................... 5

   1.4. The ischemic penumbra ................................................................................................. 12

   1.5. Clinical MRI Techniques for Imaging Penumbra ....................................................... 16

   1.6. Positron emission tomography techniques for imaging penumbra ......................... 19

   1.7. Other potentially applicable techniques for penumbra imaging ............................... 21

   1.8. MRI techniques other than $^{17}$O for the measurement of CMRO$_2$ ....................... 23

   1.9. Other potential applications for metabolic imaging ..................................................... 26

   1.10. Conclusion of purpose for metabolic imaging ............................................................ 27

   1.11. Overview of Oxygen-17 detection ............................................................................. 28

   1.12. Techniques for direct detection of $\text{H}_2^{17}$O *in vivo* ........................................... 29

      1.12.1. Basics of the $^{17}$O nucleus as applied to *in vivo* detection ... .................... 29

      1.12.2. Direct $^{17}$O *in vivo* uses in cerebral blood flow and metabolism .................... 34

      1.12.3. Demonstration of Direct $^{17}$O MRI/MRS in phantoms ..................................... 35

   1.13. Techniques for indirect detection of $\text{H}_2^{17}$O *in vivo* ........................................... 38
1.13.1. Principles of the $^{17}$O effect on $^1$H relaxation .................................................... 38
1.13.2. $T_1^\rho$ dispersion imaging .......................................................................................... 45
1.13.3. Basis for in vivo $^{17}$O detection in this work .......................................................... 48
1.13.4. Field strength comparison in indirect imaging ....................................................... 51
1.13.5. Indirect $^{17}$O in vivo uses in cerebral blood flow and metabolism .................... 54
1.14. Comparison of direct and indirect sensitivity and common issues ...................... 55
  1.14.1. Cost of $^{17}$O2 ........................................................................................................... 57
  1.14.2. Safety of $^{17}$O2 ....................................................................................................... 58
1.15. Conclusion and summary of technical challenges ............................................. 58
1.16. Specific aims of the thesis research ........................................................................ 59
  1.16.1. Aim 1: Improve the temporal resolution of $T_1^\rho H_2^{17}$O Imaging ..................... 60
  1.16.2. Aim 2: Create a precision-response, high-efficiency system for $^{17}$O2 delivery. .......................................................... 60
  1.16.3. Aim 3: Measure the regional rate of oxygen consumption in a large animal on a clinical scanner with $^{17}$O2 .......................................................... 61
  1.16.4. Aim 4: Repeat these CMRO$_2$ measurements in a model of deranged metabolism .................................................................................................................. 61

2.  CHAPTER 2: SINGLE SHOT $T_1^\rho$ IMAGING OF METABOLICALLY GENERATED WATER IN VIVO ................................................................. 63

  2.1. Introduction .................................................................................................................. 63
  2.2. A single shot $T_1^\rho$-weighted pulse sequence ............................................................ 65
  2.3. Methods of $T_1^\rho$ measurement in Oxygen-17 Water Phantoms ............................. 66
  2.4. Animal Imaging in this chapter ............................................................................... 69
  2.5. Oxygen-17 Phantom Single Shot Quantitative Imaging .......................................... 70
  2.6. Simulations of single shot imaging parameters ....................................................... 72
2.7. Signal changes in swine after two breaths of 40% $^{17}\text{O}_2$ delivery ................. 74
2.8. Delivery of $^{17}\text{O}_2$ as a 80% N$_2$ and 20% O$_2$ (70% enriched) mixture .......... 76
2.9. Chapter Discussion ................................................................................................. 79
2.10. Feasibility of $T_{1\rho}$ imaging of flowing solutions ............................................ 81

3. CHAPTER 3: DELIVERY OF $^{17}\text{O}_2$ IN BRIEF PULSES ....................... 86

3.1. Motivation ............................................................................................................ 86
3.2. The Kety-Schmidt Equation for CMRO$_2$ calculation ....................................... 87
3.3. Design considerations for the mechanical ventilator ........................................ 91
3.4. Design of the mechanical ventilator .................................................................... 92
3.5. Methods for ventilator validation ........................................................................ 97
3.6. Results: Step change in gas input and fast alveolar gas increases .................... 98
3.7. Chapter Discussion ............................................................................................. 101
3.8. A simple model of inhaled atomic fraction of $^{17}\text{O}$ during the first minute 102

4. CHAPTER 4: ESTIMATION OF REGIONAL CMRO$_2$ WITH PROTON DETECTED $^{17}\text{O}$ MRI DURING PRECISION $^{17}\text{O}_2$ INHALATION .......... 108

4.1. Introduction ......................................................................................................... 108
4.2. Materials and Methods ...................................................................................... 111

4.2.1. Animal Care .................................................................................................... 111
4.2.2. Determination of CMRO$_2$ after $^{17}$O$_2$ inhalation .............................................. 112
4.2.3. Magnetic Resonance Imaging .............................................................................. 115
4.2.4. Arterial Blood Sampling and $^{17}$O NMR spectroscopy of blood ...................... 116
4.2.5. Data Analysis .................................................................................................... 118
4.3. Arterial Blood Sampling ..................................................................................... 119
4.4. Direct $^{17}$O MRI and unlocalized MRS in a swine ........................................ 121
4.5. Correlation of observed indirect and direct signal ...................................... 123
4.6. CMRO$_2$ map and calculations for a series of swine ........................................ 125
4.7. Chapter Discussion .................................................................................... 128

5. CHAPTER 5: MEASUREMENT OF CEREBRAL OXIDATIVE
METABOLISM STIMULATION BY 2,4-DINITROPHENOL ..........135

5.1. Introduction ........................................................................................................ 135
5.2. Methods and Materials ..................................................................................... 136
  5.2.1. Animal Care .................................................................................................. 136
  5.2.2. Body $O_2$ consumption measured by exhaled gas analysis ....................... 137
  5.2.3. Dinitrophenol preparation ............................................................................. 138
  5.2.4. Direct and indirect imaging ........................................................................... 138
5.3. Whole body responses to DNP ......................................................................... 140
5.4. $^{17}$O$_2$ imaging of pigs before and after DNP stimulation ............................ 142
5.5. Chapter Discussion .................................................................................... 146
5.6. Detection of the low contrast H$_2^{17}$O effect in tissue ...................................... 148
6. CHAPTER 6: CONCLUSION ................................................................. 155

6.1. Future Directions .............................................................................. 155

6.2. Summary of dissertation ..................................................................... 157

REFERENCES ............................................................................................. 159
LIST OF TABLES

CHAPTER 1: PURPOSE OF OXYGEN METABOLIC IMAGING AND INTRODUCTION TO OXYGEN-17 DETECTION

Table 1.1. A comparison of relevant 1H and 17O nuclear properties ......................... 30
Table 1.2. A comparison of 1H and 17O observed NMR parameters. ......................... 31
Table 1.3. Comparison of indirect H₂¹⁷O detection sensitivity between 1.5 and 4.7T ................................................................. 54
Table 1.4. A comparison of indirect and direct ¹⁷O detection sensitivity at 4.7 Tesla ........................................................................................................ 56

CHAPTER 2: SINGLE SHOT T₁ρ IMAGING OF METABOLICALLY GENERATED WATER IN VIVO

Table 2.1. Steady-state and non-SS Calculations for bSSFP detecting a T₂ reducing agent such as H₂¹⁷O ................................................................. 72
Table 2.2. ¹⁷O contrast and SAR versus flip angle ............................................. 73

CHAPTER 3: DELIVERY OF ¹⁷O₂ IN BRIEF PULSES

Table 3.1. Parameters for simulation of ¹⁷O fractional enrichment absorbed during the first minute of inhalation .................................................. 105
CHAPTER 4: ESTIMATION OF REGIONAL CMRO$_2$ WITH PROTON DETECTED $^{17}$O MRI DURING PRECISION $^{17}$O$_2$ INHALATION

CHAPTER 5: MEASUREMENT OF CEREBRAL OXIDATIVE METABOLISM STIMULATION BY 2,4-DINITROPHENOL

Table 5.1. Summary of all DNP data. ................................................................. 146

CHAPTER 6: CONCLUSION ........................................................................ 155
LIST OF ILLUSTRATIONS

CHAPTER 1: PURPOSE OF OXYGEN METABOLIC IMAGING AND INTRODUCTION TO OXYGEN-17 DETECTION

Figure 1.1. An overview of Oxidative Phosphorylation................................. 3
Figure 1.2. A Computed Tomography image of acute intracerebral hemorrhagic stroke................................................................. 7
Figure 1.3. An example brain image showing a theoretical penumbra......... 14
Figure 1.4. The relationship between reduction in cerebral blood flow and time to tissue death in the ischemic penumbra................................. 15
Figure 1.5. Three Tesla $^{17}$O MRI/MRS demonstrations ........................... 37
Figure 1.6. Theoretical liquid water $^1$H spectrum with coupling to $^{17}$O in the absence of chemical exchange. ......................................................... 39
Figure 1.7. Simulated $^1$H spectra for two concentrations of H$_2^{17}$O with and without chemical exchange. .......................................................... 44

CHAPTER 2: SINGLE SHOT T$_{1p}$ IMAGING OF METABOLICALLY GENERATED WATER IN VIVO

Figure 2.1. The T$_{1p}$-prepared, center-out sampled bSSFP sequence.................. 65
Figure 2.2. Quantification of H$_2^{17}$O in T$_{1p}$-weighted images...................... 71
Figure 2.3. Time courses of three pig experiments with 40% enriched $^{17}$O$_2$. .... 75
Figure 2.5. Change in pig brain signal from increased O₂........................................ 77
Figure 2.6. Images and a signal time course generated by 70% ¹⁷O₂ in swine.... 79
Figure 2.7. Flowing blood simulation phantom. .......................................................84

CHAPTER 3: DELIVERY OF ¹⁷O₂ IN BRIEF PULSES
Figure 3.1. Schematic diagram of the system for mechanical ventilation...........93
Figure 3.2. Time response of the custom ventilator circuit.................................99
Figure 3.3. Helium concentration at the airway for two breaths of 100% helium
after equilibration with 100% oxygen.................................................................100

CHAPTER 4: ESTIMATION OF REGIONAL CMRO₂ WITH PROTON
DETECTED ¹⁷O MRI DURING PRECISION ¹⁷O₂ INHALATION
Figure 4.1. Serial arterial blood sampling with ¹⁷O₂ delivery. .............................120
Figure 4.2. Direct ¹⁷O MRI and unlocalized MRS in a swine. ..........................122
Figure 4.3. Correlation of observed signals. ........................................................124
Figure 4.4. Baseline swine metabolic map. .......................................................125
Figure 4.5. Hemispheric metabolic signal changes.............................................126
Table 4.1. Estimated.............................................................................................127

CHAPTER 5: MEASUREMENT OF CEREBRAL OXIDATIVE
METABOLISM STIMULATION BY 2,4-DINITROPHENOL
Figure 5.1. Whole body DNP oxygen stimulation time course......................140
CHAPTER 6: CONCLUSION
CHAPTER 1: PURPOSE OF OXYGEN METABOLIC IMAGING
AND INTRODUCTION TO OXYGEN-17 DETECTION

1.1. Overview

Oxygen metabolism is essential for tissue function and represents a useful biomarker in the setting of myriad diseases. While numerous techniques are in development, there are still no techniques used clinically to analyze the many diseases in which oxygen metabolism is altered either as a primary consequence of that disease or as a very important side effect. It is towards this goal that our nuclear magnetic resonance (NMR) research group has focused its expertise in magnetic resonance imaging (MRI) and spectroscopy (MRS) to the development of MRI techniques that exploit the only stable, NMR active nuclear isotope of oxygen, oxygen-17 or $^{17}$O, for the regional measurement of oxygen consumption. The work undertaken in this thesis represents important steps towards the clinical translation of technologies based on $^{17}$O MRI and MRS. But before delving into that data, several concepts must be reviewed in this introduction. First, a rationale for the study of in vivo oxygen metabolism will be established. Then the available techniques will be reviewed in order to discuss the status and limitations of the existing methods to measure oxygen metabolism.
1.2. The use of molecular oxygen in vivo

From basic biochemistry, we know that the metabolism of glucose and gaseous molecular oxygen is required for oxidative metabolism—the primary energy source for all aerobic organisms, including humans. The chemical equation for this reaction is the following.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 + 6 \text{H}_2\text{O} \rightarrow 6 \text{CO}_2 + 12 \text{H}_2\text{O}
\]  

Equation 1.1

Here we will focus on the oxygen to water conversion in this equation, as it is what is measured in this project. To very briefly summarize, in mammals oxygen gas is taken up through the lungs. The circulatory system transports that oxygen in blood, mostly while oxygen is bound to hemoglobin, to target organs. Once in the target organ, oxygen is driven to dissociate from hemoglobin by the decreased partial pressure of oxygen in metabolic organs. That oxygen then passively diffuses into the mitochondria of cells where it is converted to two water molecules by oxidative phosphorylation as shown below.
During oxidative phosphorylation, NADH and succinate produced in the citric acid cycle from the oxidation of glucose provide electrons to complex I or II, respectively, as shown above. These complexes pass these electrons down the electron transport chain through Coenzyme Q (Q) and Cytochrome C (cyt c) to complexes III and IV which use the energy to pump protons from the matrix to the intermembrane space of the mitochondrion. This establishes a proton gradient within the mitochondrion. ATP Synthase acts as a proton channel that allows protons back into the matrix along this proton gradient, while utilizing the energy that flow of protons down a gradient creates to attach phosphorus to an adenosine diphosphate (ADP) molecule. This process creates a total of 36-38 adenosine triphosphates (ATP) per glucose. If that same glucose molecule were
metabolized anaerobically only 2 ATP would be generated, which is insufficient for the functions of vital human organs such as brain and heart. The hydrolysis of that ATP to ADP will be the main power source for cells, and that ADP will then return to be rephosphorylated by this same process.

For later discussion it is important to note two features. First, molecular oxygen is the acceptor of the electrons pumped down this electron transport chain. During this process the oxygen is converted to water by Complex IV. The net stoichiometry of that conversion is the following.

$$4e^- + O_2 + 4 H^+ \rightarrow 2 H_2O$$  \hspace{1cm} \text{Equation 1.2}

It is this rate that is measured by techniques that measure oxygen consumption. One way to measure the rate of that reaction is to label the oxygen in the equation, as will be explained later. In the $^{17}$O labeled case, this turns into the following equation.

$$4e^- + ^{17}O_2 + 4 H^+ \rightarrow 2 H_2^{17}O$$  \hspace{1cm} \text{Equation 1.3}
Second, there are several drugs that act as mitochondrial uncouplers. The most common of these are 2,4-dinitrophenol and P-fluoromethoxyphenylhydrazone. These work as proton ionophores. That is, they cross the lipid membrane of the mitochondrion with or without protons. As such, these uncouplers shuttle protons down their gradient without harvesting the energy for ATP production. In response, the cell increases its oxygen metabolism to recreate the gradient necessary for ATP Synthase.

1.3. Stroke: a motivation for studying cerebral oxidative metabolism

The human body uses approximately its weight in ATP every day (1). As such, almost all organs in the human body rely on a constant supply of oxygen and fuel such as glucose to produce the ATP required for function by oxidative metabolism. If oxygen is restricted to a tissue, the state is known as hypoxia. The effects are rapidly seen in the brain, as unconsciousness occurs in seconds and permanent brain injury in as little as 15 minutes (2).

One widespread cause of hypoxia is cerebral ischemia, which often progresses to cerebral infarction. When occurring as an acute event, this is also known as a stroke. It is so common that cerebral infarction is the third most frequent cause of death in the western world (3). While many strokes are not fatal, affected
individuals often require hospitalization and extensive rehabilitation. The large
death toll and high rate of disability leads to an estimated $65.5 billion dollar cost
of cerebral infarctions for medical treatment and due to loss of productivity in the
United States (4).

Cerebral ischemia is caused when blood flow is reduced in an area of brain. The
mechanisms of that blood flow reduction are broadly categorized as ischemic and
hemorrhagic. An ischemic stroke occurs when a blood clot (thrombus) forms or
becomes lodged in an artery. This is commonly a result of a thrombus dislodged
from elsewhere in the body traveling through the blood (embolized) and
becoming lodged in an artery preventing downstream flow. Ischemic strokes
represent 83% of strokes (5).

The less common hemorrhagic strokes occur when a blood vessel ruptures,
spilling blood into surrounding brain. This reduces downstream flow and
increases pressure on the brain. One common way to distinguish the two types is
based on Computed Tomography (CT), which has a sensitivity for acute
hemorrhagic stroke of 89% but is almost completely unable to detect ischemic
stroke (6). As such, typically an absence of hemorrhage on CT indicates ischemic
stroke. An example of the effects of hemorrhage on CT images is shown below (Figure 1.2).

![Figure 1.2](image)

**Figure 1.2.** A Computed Tomography image of acute intracerebral hemorrhagic stroke. The white areas in the center of the brain are indicative of extravascular blood. Public domain image retrieved from http://en.wikipedia.org/wiki/Stroke.

Structural imaging and angiography are often able to locate a source, typically head trauma or aneurysm, which can be approached for surgical clipping or the minimally-invasive catheterization and placement of coils that block off the aneurysm.
The treatment of acute ischemic stroke is much more controversial. While medical management of the stroke, such as normalization of glucose levels and administration of aspirin and clopidogrel, are indicated, these primarily prevent a worsening or immediate repeat of the infarction elsewhere and do nothing to end the infarction itself. Instead, it may be possible to destroy the clot directly. Several enzymes activate plasminogen, which breaks down fibrin, a principal component of clots. These enzymes cause lysis of the clot and, therefore, have the potential to completely reverse a stroke. The most commonly used are urokinase, streptokinase, and the modern standard recombinant tissue plasminogen activator (rtPA, or alteplase).

While all of these compounds are capable of removing clots (7), their use also promotes hemorrhage in vivo. This was realized in perhaps the earliest study on thrombolysis, published in 1965 (8). That study used a combination of streptokinase and heparin administered within 4 days after the onset of stroke of undetermined cause (no patients were treated in the first several hours). Of 38 patients in the treatment group, 13 died, as compared to 4 deaths in the 33 member control group. This has since been attributed to the formation of hemorrhage by the combination therapy (9). A later study published in 1976 using urokinase administration within the first few days of ischemic stroke had 4
fatalities due to cerebral hemorrhage that were not seen on pre-administration angiography and no improvement in the treatment group (10).

In the early 1980s, a method for the production of rtPA was discovered (11) and it was hoped that it would be more efficacious or safe, as it is an analog to the tPA that the body typically uses to break down clots. This possibility was tested by Zivin et al by injecting many small clots into the carotid arteries of rabbits and observing how many rabbits died or showed obvious neurologic deficits with and without administration of rtPA (12). It turned out that the time of administration was crucial. The beneficial effects of rtPA were only realized when it was injected within 90 minutes of clot injection. This information combined with the earlier human trials of other thrombolytic agents determined that pharmacologic therapy could be beneficial, but time was of the essence!

That work in rabbits led to numerous trials released in the early 1990s of acute stroke of less than 90 minutes duration. A dose escalation study led to a dose that did not cause intracranial hemorrhage in 58 patients while causing major improvement in 46% of patients (13). This set the dose still used today. Other studies around the same time used varying doses and showed mixed benefits and intracranial hemorrhages due to rtPA (14,15). Another small study showed that
there may be benefits of rtPA up to 180 minutes after stroke onset, but just as many patients had cerebral hemorrhage as did patients who improved (16).

A large randomized, double-blinded, multicenter trial by the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group concluded soon thereafter (17). In both the 0-90 and 91-180 minute after stroke onset groups, despite increased hemorrhage with rtPA, a marked improvement in neurological outcome was found after 3 months in those treated with rtPA. Patients given rtPA were 30% more likely to have minimal or no disability than controls, and the benefits were clear even in the 91-180 minute time group. This represented the landmark trial that both propelled rtPA into clinical use and set the parameters for clinical utility.

It was from this study and from previous experience that the use of thrombolytic therapy has been restricted to 3 hours, which has led to the common term the “3 hour window” for stroke therapy. Yet, it is not clear that 3 hours is the correct amount of time. A pooled analysis of several trials of rtPA in stroke showed a clear benefit of rtPA to 4.5 hours (18). This led to renewed interest in determining the optimal time window. New trials are currently ongoing that may redefine the 3 hour window and extend it to later time points.
Still, the current time restrictions severely limit the use of rtPA. Due to poor public knowledge about stroke, delays in patient transport, and a limited number of capable centers (19), only about 20% of stroke patients arrive at a hospital within the 3 hour window and only 3-8% of eligible patients receive rtPA (20). An additional situation is one where the patient is not certain when their symptoms began due to confusion or symptoms upon waking from sleep. These patients are currently not considered eligible for therapy since it is not known when the stroke began. It is for all of these reasons that a very small percentage of stroke patients currently receive thrombolytic therapy.

Increasing the number of patients who receive rtPA beneficially is a goal of current research. Given that stroke is a common, debilitating, and often deadly disease and the one effective treatment is only sparingly used in the disease, many groups aim to increase the use of treatment by selection of those patients most likely to benefit from rtPA. One of the ways to select these patients is by using imaging modalities to detect which areas of brain are affected by the stroke, which areas are not affected, and most importantly, which areas of brain are threatened if the stroke is allowed to continue. This area of tissue that is threatened but potentially savable by therapy is called the ischemic penumbra. Some imaging studies have shown the penumbra to exist in select patients as long
as 17 hours after the start of the ischemic event (21). That may mean that in those select patients there is still a benefit from rtPA that outweighs the risks of tPA administration far into the future.

As we will discuss in the following sections, the penumbra has reduced oxidative metabolism compared to normal brain tissue. The penumbra also utilizes much more of the oxygen it receives from inflowing blood. Therefore, the long-term goal of our work is as follows. Techniques using $^{17}$O are being developed for clinical application for measurement of oxidative metabolism. This will provide a way to better delineate the penumbra of salvageable tissue in stroke. The clinician will then be able to select those patients who can benefit from thrombolytic therapy by selection of those individuals with recoverable tissue. This is likely to increase the number of patients who benefit from rtPA therapy during stroke.

1.4. The ischemic penumbra

During an acute stroke, the ischemic area can be divided into three regions. Depending on the measurement technique, the normal, unaffected, region of brain has a cerebral blood flow (CBF) rate in gray matter of about 1.05 ml/g/min and about .15ml/g/min in white matter (22). From this flow, the brain tissue takes a proportion of the oxygen in the blood, called the oxygen extraction
fraction (OEF), which is relatively constant in both gray and white matter of approximately 35% to 45% of the oxygen contained in the blood (23). By contrast, the ischemic core region downstream from a blockage in blood flow is tissue that receives very little blood or no blood flow. This tissue dies by cellular necrosis within minutes and is not thought to be salvageable due to the very limited time in which an intervention would have to be given.

The boundary region, termed the ischemic penumbra (Figure 1.3), has a reduced level of flow provided by collateral circulation that is not able to support normal function. The limited flow does prevent irreversible tissue damage for a period of hours depending on the amount of flow reduction.
Figure 1.3. An example brain image showing a theoretical penumbra. When a major blood vessel is obstructed, blood flow may be completely abolished to the core ischemia area shown in black. The ischemic penumbra is the area of potentially salvageable tissue shown in grey which has partial blood flow. Without intervention, the core could expand to fill the penumbra.

The rate at which the tissue dies depends, at least in part, on the amount of flow it receives. This survival time is determined by experiments in awake monkeys with middle cerebral artery occlusion (Figure 1.4) (24). The more flow there is to the tissue, the longer it can survive without permanent injury up to a threshold during which no injury will occur.
Figure 1.4. The relationship between reduction in cerebral blood flow and time to tissue death in the ischemic penumbra. The dashed line approximates the threshold at which tissue dies. When the local CBF falls below this line, injury is irreversible. The open circles represent measurements in normal tissue, open triangles in the infarct periphery, and closed squares represent infarcted tissue. The penumbra region is represented by the area of tissue that has reduced blood flow (in this case <17cc/100gm/min) at 0-3 hours, but has not yet progressed to infarct. Copyright by the American Association of Neurological Surgeons, reproduced with permission from Jones, et al. J Neurosurg 1981;54:773-782.
From an imaging perspective, many important parameters are changing in the penumbra. As shown above, the cerebral blood flow (CBF) is one parameter that changes. But many other measureable parameters change. For example, as flow is reduced, tissue extracts as much oxygen as it can from the arriving blood, which causes an increase in the oxygen extraction fraction of the affected tissue. The state of OEF enhancement in the face of diminished CBF is termed “misery perfusion”. If the tissue is unable to take enough oxygen from blood to sustain its normal rate of metabolism, the cerebral metabolic rate of oxygen consumption (CMRO\textsubscript{2}) declines. When CMRO\textsubscript{2} is insufficient to meet the tissue energetic demand, total energetic failure and necrosis occurs. Another imaging parameter that changes dramatically is the apparent diffusion coefficient (ADC) inside the ischemic core. There are myriad other changes occurring at the molecular level that are not currently measured by imaging (25), but are potential molecular imaging targets.

1.5. Clinical MRI Techniques for Imaging Penumbra

Several non-invasive clinical imaging modalities are able to give information about the core and penumbra; however, none are yet able to predict the extent of the penumbra region sufficiently and simply enough to guide care. The most commonly used imaging techniques for clinical study of the acute ischemic penumbra are perfusion and diffusion weighted MRI. A thorough treatment of
the MRI physics behind these techniques is beyond the scope of this review. However, it should be noted what perfusion and diffusion MRI tell us about the ischemic cerebral infarction.

Substantial decreases in diffusion (>50%) measured by diffusion weighted imaging (DWI) begins within minutes of ischemia in experimental models of stroke (26) and in humans (27). The DWI change is so consistent within the first 6 hours of stroke that 100% sensitivity has been reported (28). The fate of the region with restricted diffusion has been studied both in humans before and after rtPA therapy (29) and in rats with variable time course vessel occlusion (30). In summary, the change in ADC is a predictor of ischemic core and ischemic penumbra, but it is impossible to separate the two based on ADC alone. Complete recovery of ADC and function can be observed after treatment even when ADC is profoundly changed (29). When intervention is not pursued in the acute stroke of less than 6 hours duration, the area of restriction diffusion tends to increase in size over a period of days until at around 7 days when DWI and T2-weighted imaging show a similar sized area of permanent infarction. Overall, DWI is an early and very sensitive marker of acute ischemic injury, however it is not specific to core vs. penumbra and is not inclusive of all tissue that may proceed to infarction without treatment.
Perfusion weighted imaging (PWI) is another sensitive indicator of acute ischemic stroke. PWI is typically performed by looking at the kinetics of infused Gd-DTPA in its first pass through tissue (31), a relative measure of flow. It can also be performed by arterial spin labeling (ASL) techniques (32), which provide absolute information, but this is not yet common in the clinics. It has been observed that the area of diffusion abnormality expands over time to fill at least part of the area in the perfusion abnormality (33). One main drawback for Gd-DTPA based PWI currently is that there is no clinical consensus on which PWI parameters truly define the area of perfusion mismatch because each parameter gives different information (34). An even greater issue is that the area of perfusion abnormality is much larger than the area that will progress to permanent infarction (35). In one series of acute ischemic infarction patients, the average area of PWI abnormality was almost 3-fold larger than the area infarcted after 2 weeks (36).

Despite the drawbacks of the two imaging modalities, many groups have hypothesized that the penumbra can be defined by the “PWI-DWI mismatch”. The mismatch hypothesis suggests that the area of core infarction is given by DWI. That region is smaller than the area seen on PWI, which indicates reduced flow. That reduced flow region without DWI change delineates a threatened area that will progress to infarction (37). One multi-center trial has already
demonstrated that this definition of the penumbra can be used to guide therapy. Stroke patients with a DWI-PWI mismatch 3-6 hours after symptom onset had long-term symptom benefits with rtPA therapy when compared to those with a mismatch who did not receive therapy (38). Conversely, patients without a DWI-PWI mismatch were harmed by rtPA therapy when compared to those who did not receive therapy. Clearly, the DWI-PWI mismatch holds promise for selecting those patients who will benefit from rtPA therapy.

However, because DWI does not clearly separate core from penumbra and PWI is an uncertain measurement that overestimates the extent of threatened tissue, the extent and existence of the penumbra in the individual stroke patient is still not clear. For this reason, many groups are actively devising ways to better delineate the penumbra. Some of the other parameters, such as OEF and CMRO₂, may be investigated in patients by Positron Emission Tomography (PET). This is important for discussion because it is often claimed that these parameters better define the penumbra (39).

1.6. Positron emission tomography techniques for imaging penumbra

One way to study oxygen directly is to generate the short half-life isotope of oxygen, ¹⁵O. PET detects the positron emitted by the ¹⁵O nucleus when it decays.
For studies of metabolism, the typical products used are referred to as “triple oxygen” for the injection of labeled water, H$_2^{15}$O, for the measurement of CBF, labeled molecular oxygen, $^{15}$O$_2$, for the measurement of CMRO$_2$, and labeled carbon monoxide, C$^{15}$O, for the measurement of cerebral blood volume (CBV) (40,41). Another important parameter computed from these parameters is the OEF, which is easily derived from,

$$\text{CMRO}_2 = \text{CBF} \cdot \text{OEF} \cdot [O_2]_{\text{Art}}$$ \hspace{1cm} \text{Equation 1.4}$$

where CMRO$_2$ and CBF are measured and the arterial blood oxygen concentration, [O$_2$]$_{\text{Art}}$, is assumed based on normal hemoglobin concentrations or measured by blood sampling.

Soon after these techniques were developed in the 1970s and 1980s, groups found that certain parameters gave information about the evolving stroke. Several groups found that CMRO$_2$ was the best predictor of irreversible core ischemia in stroke patients (42,43). In a cat model of stroke where the evolution of the stroke was tracked, misery perfusion as an increase of OEF could be observed in the penumbra area along with reduced flow, followed by an eventual fall in OEF and CMRO$_2$ progressing to necrosis (44). Taken together, this indicates that $^{15}$O PET gives pertinent information about the ischemic penumbra.
Unfortunately, despite the fact that $^{15}$O is the longest lived radioactive nuclide of oxygen, its half-life is only just over 2 minutes. This limits its use to highly technical sites with an onsite cyclotron close to the scanner. Still, PET techniques show us that the direct imaging of oxygen gives us more specific information about the ischemic penumbra. Measurement of CMRO$_2$ by PET identifies the ischemic core as a hypometabolic region, as opposed to non-specific diffusion-weighted imaging which does not separate core and penumbra. Oxygen imaging also allows one to identify that tissue most at risk of energetic failure as that tissue with the highest oxygen extraction fraction. This gives a clear motivation for the use of other techniques for oxygen imaging that may be more clinically acceptable.

1.7. Other potentially applicable techniques for penumbra imaging

Perhaps other MRI techniques in development may provide a solution for a clinically acceptable technique for oxygen imaging. It has long been known that paramagnetic molecular oxygen, and more importantly, paramagnetic deoxyhemoglobin both have a small effect on T$_2$ and a large effect on T$_2^*$-weighted MRI signal. This is termed the Blood Oxygen Level Dependent (BOLD) effect (45). The T$_2^*$ (46), and T$_2'$ (47) effects have been used with mixed results as
indicators of the tissue OEF to identify tissue at risk during stroke in humans. Another very recent paper administered 100% oxygen to rats undergoing middle cerebral artery occlusion and showed penumbral differentiation based on the $T_2^*$ signal change from that additional inhaled oxygen (48). Several other approaches have been proposed and may have potential uses in stroke, such as proton (49) and phosphorus MR spectroscopy (50) and pH-weighted MRI (51).

An additional MRI technique that has shown some promise for giving stroke information is sodium MRI. The 100% naturally abundant sodium nucleus, $^{23}$Na, is relatively common in the body and has other properties that make it fairly easily detectable (albeit at lower resolution than $^1$H based MRI). The earliest study of $^{23}$Na in stroke showed that $^{23}$Na signal was increased in a cat measured 9 hours after an experimental cerebral infarction (52). Using techniques sensitive to the total sodium concentration in the brain, a study performed in 2 monkeys suggested that sodium increases during infarction progressively after the beginning of the infarct and progressing to a level indicating infarction (53). That same study suggested that infarcted human tissue could also be detected as having high sodium compared to the contralateral hemisphere, measured at about 12 hours after stroke and continuing for days after stroke. Additional work in rats indicated that the increase in sodium during infarct was linear over time, suggesting that the time of the start of the infarct can be estimated (54,55).
Clinical trials in humans would be necessary to make conclusions about the clinical usefulness of sodium imaging; but despite the early human work performed as far back as 1999, no such trial has yet been conducted. This could be because one large rat study indicated that sodium MRI was incapable of predicting experimentally induced ischemic penumbra. Its sensitivity was comparable DWI/PWI imaging at early time points (56).

1.8. MRI techniques other than $^{17}$O for the measurement of CMRO$_2$

There are four MRI techniques other than $^{17}$O for measuring CMRO$_2$, the parameter critical to differentiating between core and penumbra of infarction. One technique only once demonstrated in humans uses the turnover of $^{13}$C glutamate after $^{13}$C glucose infusion to measure citric acid cycle flux and therefore estimate CMRO$_2$ (57). Another high field approach also only once demonstrated in humans in an unlocalized fashion uses saturation of the inorganic phosphate resonance and measurement of the ATP resonance to measure ATP synthesis, thus measuring oxygen consumption (58). This assumes that ATP was generated by a constant fraction of oxygen to convert to number of oxygen molecules consumed, as opposed to an alternate, anaerobic energy source. Technical difficulties with both these techniques pose significant looming challenges to their clinical applicability. There are two more established and more finely resolved MRI techniques that provide CMRO$_2$ and OEF based in part on the
BOLD effect. The derivation of CMRO₂ is based on using multiple parameters to solve Equation 1.4.

The first method is a static, absolute method made by combining several measurements. Venous oxygen saturation, and then a calculated OEF, is measured locally by measuring R₂’ based on the BOLD effect (59). Both CBV, required to relate the R₂’ change to the OEF in the face of other field inhomogeneities, and CBF are measured by gadolinium perfusion measurements (60). Combining all of this information with an assumed or measured arterial oxygen content gives CMRO₂ (61). While it seems as if this technique holds much promise, it has been applied very sparingly to date. The same group measured CMRO₂ in a small series of acute stroke patients (62), but otherwise the techniques have not been applied in large clinical trials or trials of other diseases. Perhaps application is limited due to the numerous measurements and extensive processing required to make a single measurement. Another possibility that works against these techniques is the requirement for toxic gadolinium in a patient population which has undefined renal function in the acute setting.

The second method for MRI measurement of CMRO₂ is relative, dynamic, and is based on simultaneous imaging of CBF and T₂* after a single calibration (63).
Measurement of CBF is performed by Arterial Spin Labeling (ASL) (64). Because ASL is a subtraction technique based on a preparation cluster, one way of making simultaneous CBF and T₂* measurements is to look at signal in every other image (the ASL control images) with a fairly long echo time (TE) readout so that there is T₂*-weighting (65). This takes away from the maximum sensitivity of both techniques, and as such more sophisticated techniques interleave ASL and T₂* acquisitions (66) or use a multiecho readout to ASL (67). Because the T₂*-weighted signal is related to deoxyhemoglobin concentration by the BOLD effect, and specifically a quantitative exponential factor β, one has an indirect measure of OEF. CBV is estimated by relating it to CBF by an exponential constant, termed Grubb’s constant or α, taken from PET estimates (68).

A calibration must be performed to account for the change in signal due to each molecule of deoxyhemoglobin in the voxel because of tissue effects such as size and composition of blood vessels and partial volume effects with other tissues. This calibration is performed typically by having the subject inhale carbon dioxide, a pure vasodilator assumed not to have metabolic effects in a short time period (69), but has also been performed with 100% oxygen inhalation to increase [O₂]Art with negligible flow change (70). The change in signal during that inhalation is directly related to the change in deoxyhemoglobin by that increase in flow or oxygen, providing the calibration required for measuring relative
CMRO$_2$. This technique has since been used several times by numerous groups with fMRI paradigms (71-73), but since it only gives relative measurements based on multiple assumptions ($\alpha$, $\beta$, and baseline OEF), this technique is likely to not have utility in stroke where these couplings and assumptions likely do not hold.

### 1.9. Other potential applications for metabolic imaging

A robust technique for the clinical imaging of metabolism would not merely be useful for the imaging of the ischemic penumbra. There are myriad other diseases in which oxygen metabolism is involved either as a cause of the disease or as a diagnostically significant consequence. Another potential and significant application is the separation of benign and malignant tumors.

Tumor cells often undergo a switch to increased anaerobic metabolism as a part of their transition to malignancy (74). The increased glucose requirements for the more inefficient anaerobic glycolysis are thought to be directly associated with the increased uptake of $^{18}$F-fluorodeoxyglucose with Positron Emission Tomography (FDG-PET) (75). Therefore, a $^{17}$O or other MRI based technique for scanning metabolism could evaluate masses seen on MRI without use of radioactivity and during the same session. As whole body scanning with MRI becomes a clinical reality (76), this would become a powerful tool for the non-
radioactive detection of tumors. A mass with an associated increase in oxygen metabolism could be assumed to be a benign, while an absence of oxygen metabolism compared to normal tissue would be malignant. There is already evidence for this hypothesis from the PET literature, which compares $^{15}$O$_2$ measured oxygen metabolism with FDG-PET measured glucose metabolism (77,78).

1.10. Conclusion of purpose for metabolic imaging

As we have seen, the ischemic penumbra marks a target for stroke therapy with numerous metabolic parameters that can potentially be identified by imaging techniques. Existing $^{15}$O PET techniques give absolute, multiparametric information about the size of the penumbra that could be used for guiding stroke treatment, yet $^{15}$O is constrained by its very short half-life. Specifically, reduced CMRO$_2$ identifies tissue death, while increased OEF delineates the region of penumbra. The common MRI techniques of perfusion and diffusion imaging give useful but incomplete and imprecise information. Many other techniques are in development, but clinical utility has not yet been demonstrated.

Our group believes it is best to find other non-invasive imaging techniques, such as MRI, that measure OEF and CMRO$_2$, as these parameters have been shown to
be sensitive and specific to meaningful parameters in the setting of acute stroke by PET. Existing MRI techniques for this purpose are incomplete and indirect. In the next part of this chapter, we will discuss $^{17}$O MRI techniques. These are the only MRI techniques that directly measure oxygen consumption.

1.11. Overview of Oxygen-17 detection

Of the three stable isotopes of oxygen, only oxygen-17 ($^{17}$O) offers a net nuclear angular momentum required for NMR detection. For studies of oxygen metabolism with MRI, this makes $^{17}$O a logical choice. From this introduction, one can see the importance of measuring metabolism, yet there are some drawbacks to the use of $^{17}$O that have so far limited its widespread application. Still, $^{17}$O-enriched water ($\text{H}_2^{17}$O) and oxygen ($^{17}$O$_2$) have been utilized successfully by several groups in experimental animals, typically in small animals. This use is based on injection of $\text{H}_2^{17}$O acting as flow tracer for calculation of CBF and inhalation of $^{17}$O$_2$ acting as a metabolic tracer for calculation of CMRO$_2$.

The long-term goal of these techniques is to combine absolute CMRO$_2$ measurement by $^{17}$O$_2$ with perfusion imaging by $\text{H}_2^{17}$O, gadolinium, or arterial spin labeling in the setting of stroke. The CMRO$_2$ would then provide the information about the core of infarction, the CBF provides information about the
threatened region similar to PWI, and the OEF computed from Equation 1.4 defines the penumbra. This would provide a clinically acceptable technique for the measurement of the ischemic penumbra, and it is towards that goal that this thesis has been undertaken. But first, it is important to understand the theory and history of the cerebral metabolic use of these tracers so that one may put the current and future use of $^{17}$O into context.

To begin, one feature common to all techniques involving $^{17}$O is that inhaled $^{17}$O$_2$ gas is invisible to MRI. This is assumed to be due to its extremely fast relaxation (79). This is a strength compared to $^{15}$O, where $\beta$-decays from unmetabolized $^{15}$O$_2$ and metabolically produced H$_2^{15}$O are inseparable. In the $^{15}$O case, additional modeling is required to account for the decays by $^{15}$O$_2$ in the venous circulation or extracellular space local to the tissue of interest (80, 81). In the $^{17}$O case, the H$_2^{17}$O produced by metabolism (mH$_2^{17}$O) or injected can be detected by two distinct MR techniques termed direct and indirect.

1.12. Techniques for direct detection of H$_2^{17}$O in vivo

1.12.1. Basics of the $^{17}$O nucleus as applied to in vivo detection

Direct techniques for H$_2^{17}$O detection utilize MR spectroscopy or imaging at the Larmor frequency of $^{17}$O. The advantage to the direct detection of $^{17}$O is that the
physiologic signal change is specific to the $^{17}$O nucleus, and therefore to natural abundance $\text{H}_2^{17}$O or $\text{mH}_2^{17}$O. Though it is necessary when evaluating $^{17}$O techniques to remember the obvious—that this specificity does not extend to signal fluctuation due to noise, motion, or hardware instabilities. The main disadvantage to the direct detection of $^{17}$O is its insensitivity compared to other nuclei. MRI is most typically performed to detect $^1$H nuclei, and so in Table 1.1 some nuclear properties of $^{17}$O are compared to $^1$H to explain the low sensitivity of $^{17}$O (data from ref. (82)).

<table>
<thead>
<tr>
<th></th>
<th>$^1$H</th>
<th>$^{17}$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Abundance</td>
<td>99.98%</td>
<td>.038%</td>
</tr>
<tr>
<td>Gyromagnetic Ratio (Mhz/T)</td>
<td>42.58</td>
<td>-5.77</td>
</tr>
<tr>
<td>Spin</td>
<td>1/2</td>
<td>5/2</td>
</tr>
<tr>
<td>Relative Receptivity</td>
<td>1</td>
<td>$1.11 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Table 1.1. A comparison of relevant $^1$H and $^{17}$O nuclear properties.

These parameters underscore the difficulty of performing NMR or MRI with the $^{17}$O nucleus. The natural abundance of $^{17}$O strongly limits its detection in vivo. A smaller gyromagnetic ratio further reduces the detected signal. A combination of these terms with spin considerations to the net observed magnetization is defined
as receptivity as described in Ref. (82). This relationship is defined by Equation 1.5 below, where $D$ is the receptivity, $[N]$ refers to the natural abundance of the nucleus, $\gamma$ is the gyromagnetic ratio, and $I$ is the spin state.

$$D \propto [N] \{ | \gamma^3 | I(I+1) \}$$  

Equation 1.5

This equation indicates that for a volume of H$_2$O at a given field strength and temperature, one will receive about 90000-fold less initial magnetization from the $^{17}$O than from the $^1$H. Additional considerations influence the signal-to-noise (SNR) received when performing an in vivo experiment. These are summarized in Table 1.2 ($^{17}$O relaxation data from ref. (83), $^1$H from ref. (84)).

<table>
<thead>
<tr>
<th>Relative Magnetization</th>
<th>$^1$H</th>
<th>$^{17}$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7T rat brain tissue T1 (ms)</td>
<td>1600</td>
<td>4.47</td>
</tr>
<tr>
<td>4.7T rat brain tissue T2 (ms)</td>
<td>52</td>
<td>3.03</td>
</tr>
<tr>
<td>Relative SNR per unit of time</td>
<td>$1.97 \times 10^4$</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1.2. A comparison of $^1$H and $^{17}$O observed NMR parameters.
This adds information about relaxation and how that influences the experimentally observed SNR. First, the short T2 of 17O makes it difficult to detect by conventional MRI, and adds significantly to the observed point spread function (blurring the resolution that is achieved!). However, the short T1, allows one to average 17O more rapidly. This leads to the estimated decline in SNR of about 20,000-fold when doing a 17O experiment as compared to a 1H experiment at 4.7T. The proportional relationship of magnetization, field strength, T1, and T2 to the SNR per unit of time is shown in Equation 1.6 (85).

\[
\frac{SNR}{time} \propto D \cdot B_0^\beta \sqrt[2]{\frac{T_2}{T_1}}
\]  

Equation 1.6

The constant \(\beta\) reflects whether the noise in the experiment is coil dominated or sample dominated. In the coil dominated regime, \(\beta\) should theoretically be \(7/4\) (86), while in the sample dominated regime \(\beta\) is equal to 1. It has been previously shown that the proton noise contribution to SNR in the transition from .5T (1H-21.3Mhz) to 1T (1H-42.6Mhz) in a human head or abdomen is sample-dominated (87). In this regime, SNR increases linearly (\(\beta=1\)). Since the transition from coil-dominated to sample-dominated noise occurs because of sample size and frequency, it should be expected that direct 17O experiments performed on a human head would gain SNR linearly with field strength starting at 4T (17O - 23.1Mhz) and perhaps at even lower field.

Contrary to NMR theory, the relationship of field strength with SNR was empirically determined to be approximately quadratic for 17O, suggesting a \(\beta\) of
about 2 (85). It is unclear how that could have happened, but it should be noted that work did not account for coil Q and receiver losses. In any case, it was implied that $^{17}$O detection will benefit heavily from higher fields. However, this was only shown for a small coil and a small sample where the noise is likely to be coil-dominated. As noted in the previous paragraph, for a sample the size and conductivity of a human head, noise would likely be linear, allowing us to set $\beta=1$ for this comparison.

The dependence of SNR on $T_1$ reflects that the short $T_1$ of $^{17}$O allows for rapid averaging. Because the relaxation of $^{17}$O is predominately due to the quadrupole moment of $^{17}$O, the $T_1$ and $T_2$ do not change with field strength, as it does unfavorably for protons (i.e., $T_2$ decreases and $T_1$ increases). This further supports a SNR gain from direct $^{17}$O observation at higher fields. Nevertheless, one issue that has not been fully analyzed in the literature is the case of the specific absorption rate (SAR) limits for human high field $^{17}$O detection. This has been a significant issue for the high-field in vivo detection of the quadrupolar nucleus, $^{23}$Na (88) and will need to be analyzed for SAR-limited, human $^{17}$O experiments at 7 Tesla and beyond.
1.12.2. Direct $^{17}$O in vivo uses in cerebral blood flow and metabolism

The first study of metabolism using the direct technique showed the possibility of detecting $^{17}$O in vivo by doing spectroscopy of insects inhaling $^{17}$O$_2$ in a closed environment (89). This was then replicated in unlocalized spectroscopy of rats (90), however that group was unable to detect the small amount of $^{17}$O production in vivo during $^{17}$O$_2$ inhalation and they resorted to in vitro analysis of the rat brains. As will be discussed later, the more sensitive indirect techniques did demonstrate H$_2^{17}$O formation. Still, it was not long after that numerous other groups at higher field were successful at in vivo detection of the produced H$_2^{17}$O in brain ((91-93)). Another early application for H$_2^{17}$O was its injection and use as a tracer for flow. For example, one group demonstrated this for the measurement of the rate of fluid clearance in the eye (94). Several groups realized that $^{17}$O could be used to measure both flow and metabolism in the same study. Early unlocalized spectroscopy work demonstrated both flow and metabolism in cats (93). Localized measurements of flow and metabolism were soon after made by direct $^{17}$O imaging with a temporal resolution of 1 minute and nominal voxel size of .8mL by taking the change in water measured in the central voxel of a cat brain (92). In the setting of decreased metabolism due to hypothermia, direct $^{17}$O spectroscopy measured a change in metabolic rate in insects (95), and much more recently using higher resolution 3D spectroscopy on rats at 9.4T (96).
Future work with direct $^{17}$O imaging will likely take advantage of high field measurements, especially in humans, and looks to optimize $^{17}$O for human use. For example, one group recently showed the first images from clinical scanners of the natural abundance of $^{17}$O in human brains (97). However, a number of papers have been released recently using 3D chemical shift imaging (CSI) at high field by Wei Chen’s group (reviewed in (83)). For example, one abstract shows increased H$_2^{17}$O in the brains of humans inhaling $^{17}$O$_2$ gas. These investigators utilized CSI with 11 second temporal resolution and 3.5mL voxel size at 7 Tesla, though they were unable to make a metabolic measurement (98). Two other recent papers by that group mostly deal with the modeling of $^{17}$O inhalation and recirculation to be covered in a later section (99,100). The direct $^{17}$O imaging has relied entirely on gradient recalled echo or projection reconstruction type imaging. It has yet to be seen what effect fast imaging sequences, such as SPRITE (101) or TPI (102), used in the imaging of other quadrupolar nuclei, might have to improve $^{17}$O imaging.

1.12.3. Demonstration of Direct $^{17}$O MRI/MRS in phantoms

Towards direct $^{17}$O imaging at 3 Tesla, a 20 cm per loop $^{17}$O-tuned Helmholtz Pair was constructed by M. Rao and TJ Connick. This coil was placed around a phantom consisting of a 10 cm diameter jar of water to which six 15 mL conical tubes were taped on the outside. These phantoms contained 1X phosphate buffered saline and 6 concentrations of added H$_2^{17}$O (from 25% stock, Isotec,
Miamisburg, OH) increasingly linearly from 0 (natural abundance) to 25mM added. The same concentration phantoms will be used for indirect imaging in Figure 2.2. This arrangement was placed into the center of the scanner and the coil was connected to a custom-built $^{17}$O transmit/receive interface for the Siemens Trio clinical scanner.

A 3D spoiled gradient recalled echo (FLASH) sequence ran for 143 seconds with the following parameters: TR 10ms, TE 2.26ms (asymmetric echo), BW 260Hz/Px, FoV 400mm x 400mm, Matrix 64x64, slice thickness 20mm (4 slices). The signal from the brightest pixel from each of the phantoms was plotted against the added H$_2^{17}$O concentration and correlated ($r^2=.86$). For spectroscopy a 1 minute 2D chemical shift imaging sequence ran with the following parameters: TR 20ms, FoV 400x400mm, Matrix 32x32, slice thickness 20mm, 3 averages. These results are summarized in Figure 1.5.
Figure 1.5. Three Tesla $^{17}$O MRI/MRS demonstrations. Part a shows a $^{17}$O image of a water phantom surrounded by phosphate buffered saline phantoms with increasing amounts of $\text{H}_2^{17}\text{O}$ added from a final concentration of 20mM (natural abundance, top right) proceeding clockwise linearly to 45mM (left). The center (highest intensity) pixel signal value for each phantom was correlated with signal to produce
the plot labeled b. The spectra in c show two example $^{17}\text{O}$ spectra with applied line broadening. These are from the central pixels of chemical shift imaging of the phantoms shown in a taken with the parameters described in the text. The spectrum on the left is natural abundance and the right has 25mM $\text{H}_2^{17}\text{O}$ added. Note the y-axis for these figures (maximum y-axis value on left .6, maximum on right 2.0), which indicates higher $\text{H}_2^{17}\text{O}$ concentration with higher intensity.

1.13. Techniques for indirect detection of $\text{H}_2^{17}\text{O}$ in vivo

We have seen that direct detection of $^{17}\text{O}$ can only provide low resolution information about metabolism and flow due to its poor sensitivity, especially at clinical field strengths. This led many groups to investigate the possibility of detecting $^{17}\text{O}$ through its interactions with proton, a much more sensitive nucleus. The fact that the detection is performed by measuring protons leads to the term “indirect”. This is possible because in liquid water, $^{1}\text{H}$ and $^{17}\text{O}$ exhibit strong spin-spin coupling of 90Hz (103), reducing the bulk observed $T_2$ relaxation of water in proportion to the concentration of $^{17}\text{O}$.

1.13.1. Principles of the $^{17}\text{O}$ effect on $^{1}\text{H}$ relaxation

It is simplest conceptually to begin the discussion by showing the theoretical effects of the spin-spin coupling between $^{17}\text{O}$ and $^{1}\text{H}$ in water that is not
exchanging. Let us start by considering the scalar coupling Hamiltonian, where spin \( I_j \) is \(^1\)H and spin \( I_k \) is \(^{17}\)O. The interaction Hamiltonian is then given by

\[
H = 2\pi J_{jk} \left( \hat{I}_{jz} \cdot \hat{I}_{kz} \right)
\]

Equation 1.7

where \( J_{jk} \) is the coupling constant (see section 7.10, ref. (104)). The z-component of spin-angular momentum takes on six energy levels (-5/2, -3/2, -1/2, 1/2, 3/2, 5/2) for \(^{17}\)O because it is spin 5/2. This causes the proton resonance to split into 6 extra spectral peaks of equal intensity as shown in Figure 1.6.

![Figure 1.6. Theoretical liquid water \(^1\)H spectrum with coupling to \(^{17}\)O in the absence of chemical exchange. Each of the 6 spin states creates a peak separated by \( J = 90 \text{Hz} \). This figure is adapted from Ref. (105).](image-url)
Typically protons are exchanging between water molecules fairly rapidly and so extra steps have to be undertaken to experimentally observe figure 2.1. Mateescu et al. diluted H$_2^{17}$O in the non-exchanging solvent benzene and observed the scalar coupling multiplet (106). Protons also split the $^{17}$O resonance into two extra peaks by the +1/2 and -1/2 spin energy levels. This was observed by diluting H$_2^{17}$O in the non-exchanging solvent acetone (107).

Until now we discussed the case where water protons were not exchanging. In solutions of water and in vivo, they are exchanging, so the effect of $^{17}$O manifests itself in a different way. This spin-spin coupling effect of $^{17}$O on $^1$H in bulk liquid water was investigated thoroughly by Meiboom et al, who were seeking an explanation of the parabolic relationship of $T_2$ on pH (108). To understand what they found, one has to understand the effects of multiple site chemical exchange on spectra. A full discussion of this topic is beyond this document, however I recommend section 15.5 in Levit’s text on Spin Dynamics (104). To summarize qualitatively I quote Meiboom’s comprehensive work on this topic:

“The experimentally determined quantity is the increase in linewidth spectrum caused by these frequency changes. Qualitatively the changes observed in the NMR spectrum of a system as its exchange rate is increased gradually from zero to a high value are as follows: A gradual broadening of the resonance lines
involved in the exchange will be observed initially. This broadening will be increase until the individual lines overlap and a single broad line is observed. On continued increase of the exchange rate this line will narrow, and at very fast rates a single sharp line, at the center of gravity of the contributing lines is observed.” – S. Meiboom Ref. (109). 

In water, the exchange rate of protons between water molecules is sufficiently rapid to be on the order of the regime where there is a single narrow line, but one that is still broadened by the protons interacting with $^{17}\text{O}$. The exchange rate is increased by hydroxyl and hydronium ions that catalyze the following proton exchange reactions.

$$\text{H}_2\text{O} + \text{OH}^- \leftrightarrow \text{OH}^- + \text{H}_2\text{O} \quad \text{Equation 1.8}$$

$$\text{H}_2\text{O} + \text{H}_3\text{O}^+ \leftrightarrow \text{H}_3\text{O}^+ + \text{H}_2\text{O} \quad \text{Equation 1.9}$$

The minimum concentration of hydroxyl and hydronium ions occurs at neutral pH 7, and the amount of ions, and thus proton exchange rate, increases as one deviates from that pH. As the exchange of protons increases, the spectral line becomes increasingly narrow. Spectral linewidth is related to the $T_2$ by the following equation.
This qualitatively explains the $T_2$ dependence on pH. Very rapidly exchanging protons at the extremes of pH cause a narrow line with a higher observed $T_2$. An increasingly broad line at neutral pH due to slowed exchange of protons between $^{17}$O and $^{16}$O/$^{18}$O containing water molecules decrease the observed $T_2$.

Quantitatively, Meiboom explained the effect as follows. In the case where a continuous perpendicular magnetic field, $H_1$, is applied to a system, it is said to be “spin-locked” and the relaxation rate is termed $T_{1ρ}$. Using the Bloch equations (110) modified by McConnell for chemical exchange (111), this relaxation was derived to be,

$$\frac{1}{T_{1ρ}} = \frac{1}{T_{1ρ}^0} + \tau \sum_i \frac{P_i \delta_i^2}{1 + \tau^2 \left( \delta_i^2 + \omega_1^2 \right)}$$

Equation 1.11

where the $T_{1ρ}^0$ term refers to all the relaxation terms in the absence of $^{17}$O, $τ$ is the average time a proton is bound to an oxygen atom (~1.1ms at neutral pH), $P_i$ is the intensity of the $i$th line (6 lines for $^{17}$O as in Figure 1.6), $δ_i$ is the frequency difference between the $i$th line and the center line, and $ω_1$ is the frequency of the $H_1$ field in Equation 1.12.
\[ \omega_1 = \gamma H_1 \]

Equation 1.12

The \( \gamma \) term is the gyromagnetic ratio, in this case of the proton. In this work, spin locking is performed, but at the very low \( \omega \) of 100Hz. At that frequency, \(^{17}\text{O}\)-induced relaxation is almost identical to \( T_2 \) relaxation (112). As such, in the limiting case where \( \omega \) approximates 0, the relaxation is by \( T_2 \) and Equation 1.11 simplifies to Equation 1.13.

\[
\frac{1}{T_2} = \frac{1}{T_2^0} + \tau \sum_i \frac{P_i \delta_i^2}{1 + \tau^2 \delta_i^2} 
\]

Equation 1.13

The intensity of the \(^{17}\text{O}\) lines, \( P \) relates simply to the concentration of \( \text{H}_2^{^{17}\text{O}}, f \), as in Equation 1.14.

\[
P = \frac{f}{6} 
\]

Equation 1.14

From Equation 1.11 and Equation 1.13, one can see that if \( P \) is increased, \( T_1^{-1} \) and \( T_2^{-1} \) relaxation rates are enhanced. This is summarized pictorially by Figure 1.7.
Figure 1.7. Simulated ¹H spectra for two concentrations of H₂¹⁷O with and without chemical exchange. The spectrum shown in the top left corner is a conceptual rendition of natural abundance water in the absence of chemical exchange (see Figure 1.6). The H₂¹⁷O peaks are exaggerated for illustration purposes. When chemical exchange occurs, as is the normal condition in vivo, the outer lines are obscured and instead contribute to a broadening of the central line. If additional H₂¹⁷O is added, the amplitude of the H₂¹⁷O peaks is increased. In bulk liquid water, a broader central line is observed. The width of the spectrum is related to T₂ relaxation, and so relaxation can be related to the concentration of¹⁷O.
For \textit{in vivo} applications it is assumed that the rate of chemical exchange ($\tau$) does not change significantly within brain tissue. This is a good approximation even in the case of stroke where pH is known to change. This is because the pH change is not big enough to cause a significant change in proton exchange rate. One rat model of hyperacute stroke leading to complete infarction showed minimum overall tissue pH to be 6.6 (113). This is insignificant because the relaxation rate, $R_2$, induced by $^{17}$O only varies about 10% over the pH interval 6.2-7.8 according to Meiboom’s work (109).

\section*{1.13.2. $T_{1p}$ dispersion imaging}

Previous indirect $^{17}$O detection techniques relied upon $T_{1p}$ dispersion imaging. For a review of these techniques \textit{in vivo}, the works and theses of S. Charagundla and D. Tailor are recommended ((114,115)). To describe $T_{1p}$ dispersion imaging briefly, we follow the formalism of Tailor et al (116). This is based upon analysis of Equation 1.11, which states an increase in $\omega_1$ dampens the effect of $^{17}$O on relaxation, as well as feasibility demonstrations (112). This is referred to as spin-lock decoupling of the $^{17}$O effect. We begin by stating that for a $T_{1p}$ prepared, spin-echo readout based sequence the signal intensity is given by Equation 1.15,
\[ S_R = S \left[ 1 - e^{\frac{TR}{T_1}} \right] e^{\frac{TE}{T_2} e^{\frac{TSL}{T_{1,\rho}}}} \]  \hspace{1cm} \text{Equation 1.15} \\

where \( S \) is the signal in the absence of relaxation, \( S_R \) is the magnetization after relaxation, \( TR \) is the sequence repetition time, \( TE \) is the time from the first excitation to the center of the k-space, and \( TSL \) is the spin lock time.

Previously, it was shown that Equation 1.11 can be simplified to,

\[ \frac{1}{T_{1,\rho}(\omega_1)} = \frac{1}{T_{1,\rho}^0(\omega_1)} + R_{1,\rho}(\omega_1) \cdot f \]  \hspace{1cm} \text{Equation 1.16} \\

where \( T_{1,\rho}(\omega_1) \) is the observed relaxation of liquid water or tissue with \( \omega_1 \) spin-lock frequency, \( f \) is the concentration of H\textsubscript{2}\textsuperscript{17}O with a \( R_{1,\rho}(\omega_1) \) relaxivity, \( T_{1,\rho}^0(\omega_1) \) is the relaxation without H\textsubscript{2}\textsuperscript{17}O, and it is assumed the factors \( \tau \) and \( \delta \) are constant in Equation 1.11 (112). Substituting Equation 1.16 into Equation 1.15 and taking the ratio of signal intensities, \( S_l \) and \( S_h \), obtained at low and high spin lock, \( \omega_l \) and \( \omega_h \), one may state the following relationship.

\[ y = mf + b \text{ where} \]  \hspace{1cm} \text{Equation 1.17}
\[ y = 1/S_l, \]

\[ m = TSL \left[ R_{1\rho}(\omega_l) - R_{1\rho}(\omega_h) \right], \]

and

\[ b = \ln \left( 1/S_h \right) + TSL \left[ \frac{1}{T_{1\rho}^0(\omega_l)} - \frac{1}{T_{1\rho}^0(\omega_h)} \right] \]

Because of the spin-lock decoupling, \( R_{1\rho}(w_l) \), the effect of \( \text{H}_2^{17}\text{O} \) at low spin-lock, is greater than \( R_{1\rho}(w_h) \), the effect of \( \text{H}_2^{17}\text{O} \) at high spin-lock. This was verified in rats to obtain a master equation for in vivo \( T_{1\rho} \) dispersion imaging (116),

\[ f(t) = f_n \frac{\ln S_h - TSL \left[ \frac{1}{T_{1\rho}^0(\omega_l)} - \frac{1}{T_{1\rho}^0(\omega_h)} \right]}{\ln S_h - TSL \left[ \frac{1}{T_{1\rho}^0(\omega_l)} - \frac{1}{T_{1\rho}^0(\omega_h)} \right]} \]

Equation 1.18

where \( S_h \) is the signal from high amplitude spin-locking taken before the experiment, \( S_{l,t} \) is the signal at some time \( t \) to be converted to \( \text{H}_2^{17}\text{O} \) concentration, and \( S_{l,n} \) is the low spin-lock signal before adding \( \text{H}_2^{17}\text{O} \).
The $T_{1\rho}$ dispersion imaging technique is not implemented in this dissertation because adequate amplitude spin-locking (>1kHz) was not achievable because of RF power amplifier limitations in a head or body sized volume on our clinical 1.5T Siemens Sonata scanner. A second limit is tissue power deposition at higher fields such as 3T, which also does not permit sufficient amplitude and duration spin-locking for decoupling using human power deposition limitations.

Thus in the following section a $T_2$-based formalism is followed because the basics for $T_2$-based detection have been derived in the literature and are analogous to those to be shown for low amplitude spin-locking. This is true fundamentally because low amplitude spin-locking approximates $T_2$ contrast, but is powerful enough to clear effects due to macroscopic background field inhomogeneities. Conceptually, the work in the following section can be considered to be analogous to the system in this section without high amplitude spin-locking, taking the low amplitude spin-lock signal at the beginning of $^{17}$O$_2$ delivery to be the natural abundance signal.

1.13.3. *Basis for in vivo $^{17}$O detection in this work*

Let us begin by simplifying Equation 1.13 to Equation 1.19 similarly to the derivation of Equation 1.16 by removing the pH dependence on chemical
exchange and fixing the other constants to the relevant values for $^{17}$O-$^1$H spin-spin coupling. That was performed by Stolpen, Reddy, and Leigh for \textit{in vivo} detection and the framework here will continue to follow theirs (105).

$$\frac{1}{T_2} = \frac{1}{T_2^0} + R_2 f$$

\textbf{Equation 1.19}

All of the relaxation terms are lumped into $R_2$, the transverse relaxivity due to $^{17}$O in a tissue, for a tissue with a given exchange time. For a 5% gelatin phantom, that group measured $R_2=3.28$ per at. % per second with almost no change in $R_1$ (.01 per at. % per second). From theoretical calculations, that corresponds to an exchange time of $\tau=0.5$ms. They also show that for physiologically relevant concentrations of $^{17}$O, $R_2$ remains a linear factor.

Since we wish to determine the concentration \textit{in vivo} of $H_2^{17}$O, Stolpen et al went on to derive the equations for the calculation of $f$. I present a slightly modified but analogous derivation for solving for $H_2^{17}$O by taking the uncoupled $T_2$ at the beginning of the experiment to reflect the baseline $^{17}$O. Beginning with the signal obtained after $T_2$ relaxation of tissue, where $S$ is the signal in the absence of relaxation and $S_R$ is the signal with relaxation, added relaxation due to $H_2^{17}$O is reflected in Equation 1.20.
We further deviate from the literature derivation because no decoupling occurs in these experiments. Instead of using $T_2^0$ in the absence of $^{17}\text{O}$, we introduce a variable $T_2^i$, the $T_2$ at the beginning of an experiment including the existing amount of $^{17}\text{O}$. The atomic fraction $^{17}\text{O}$ added is termed $\Delta f$. This yields a pair of equations for the signal before the addition of $^{17}\text{O}$ ($S_i$) and after the addition of $^{17}\text{O}$ ($S_o$) as follows.

\[
S_i = S \cdot e^{-\frac{-\text{TE}}{T_2^i}} 
\]

Equation 1.21

\[
S_o = S \cdot e^{-\frac{-\text{TE}}{T_2^0} + R_2 \Delta f} 
\]

Equation 1.22

The fractional change in signal due to the addition of water $^{17}\text{O}$ becomes Equation 1.23.

\[
\frac{S_i - S_o}{S_o} = \exp\left(\text{TE} R_2 \Delta f\right) - 1
\]

Equation 1.23

Within the limit $(\text{TE} R_2 \Delta f) \ll 1$, Equation 1.23 simplifies as follows.
Solving Equation 1.21 and Equation 1.22 for $\Delta f$ with this simplification yields the final equation used for determining metabolism in this thesis.

$$\Delta f = \frac{\ln\left(\frac{S_i}{S_o}\right)}{TE \cdot R_2}$$  \hspace{1cm} \text{Equation 1.25}

In this thesis the $R_2$ set by Stolpen is used to measure the $^{17}$O concentrations in tissues. Because this has only been validated in semi-solid phantoms, it may be imprecise. A more precise value for $R_2$ \textit{in vivo} has not been determined because of the difficulties in measuring this for actual brain tissue. One must assume measured $^{17}$O concentrations in this work are somewhat imprecise in the absence of a proper way to measure in vivo $R_2$. Based on Stolpen’s simulations for proton exchange time modulation of $R_2$, the maximum theoretical $R_2$ is 18% higher based on an exchange lifetime of 0.9 ms, providing evidence the $R_2$ used is within 20% of the correct value.

\textbf{1.13.4. Field strength comparison in indirect imaging}

As discussed for Equation 1.6, when ignoring the SAR concerns at higher field, the sensitivity of direct detection increases with field. The improvement in water $^{17}$O sensitivity with
field strength for indirect techniques is more complicated but is considered in the following discussion culminating in Table 1.3. For indirect proton-based detection of samples the size of human body parts at 0.5T or more, the noise is always sample-dominated (87). Hence only a linear increase in SNR due to field strength is expected. This must be taken into consideration in combination with the fact that proton relaxation at lower field strength has shorter T₁ and longer T₂. So faster averaging can be performed, and there is more time to sample the transverse magnetization. Thus, the expected SNR gain for a pulse acquire experiment at 4.7T over 1.5T is only about 2-fold from Equation 1.6 (using the 1.5T data from (117) in Table 1.3).

The contrast due to ¹⁷O is dependent on chemical exchange, which Meiboom found to be unrelated to main field strength (B₀) (108), and the spin-spin coupling strength is also unrelated to B₀ (thus R₂ is unaffected B₀). Thus for comparison purposes, the TE for generation of ¹⁷O contrast was chosen to be equal to one tissue T₂ in the case of proton. This approximates the optimum echo time for contrast to noise (CNR) derived for small amounts of added ¹⁷O (105). The contrast due to 1 at % in Table 1.3 is then calculated by taking the ratio of Equation 1.22 over Equation 1.21 (natural abundance H₂¹⁷O) assuming the R₂=3.28 per at. % per second (105) and setting the brain T2s equal to those in
Table 1.2. One finds based on this that a longer TE allows for more change in signal (contrast) due to added $^{17}$O, which means that a longer starting $T_2$ at lower fields increases $^{17}$O contrast. Meanwhile, the proportional signal decay at an echo time equal to baseline tissue $T_2$ remains constant ($e^{-1}$). Thus to obtain a ratio of the CNRs for the two field strengths, the contrast due to $1$ at % were multiplied by their relative SNR per unit time.

All of these steps are summarized below, leading to the conclusion that field strength has little influence on indirect $H_2^{17}$O sensitivity in the common, clinically-approved range (1.5-4T).

<table>
<thead>
<tr>
<th>$^1$H Field Strength</th>
<th>1.5T</th>
<th>4.7T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Magnetization</td>
<td>1</td>
<td>3.13</td>
</tr>
<tr>
<td>Brain tissue $T_1$ (ms)</td>
<td>998</td>
<td>1600</td>
</tr>
<tr>
<td>Brain tissue $T_2$ (ms)</td>
<td>91</td>
<td>52</td>
</tr>
<tr>
<td>Relative SNR per unit of time</td>
<td>1</td>
<td>1.87</td>
</tr>
<tr>
<td>Signal Change (Contrast) due to 1 at</td>
<td>.26</td>
<td>.16</td>
</tr>
<tr>
<td>Contrast to Noise Ratio</td>
<td>.26</td>
<td>.30</td>
</tr>
<tr>
<td>Estimated Relative $^{17}$O Sensitivity</td>
<td>1</td>
<td>1.15</td>
</tr>
</tbody>
</table>
Table 1.3. Comparison of indirect H$_2^{17}$O detection sensitivity between 1.5 and 4.7T.

A lower field strength is also beneficial to mitigate other the competing effects of signal change. For example, paramagnetic deoxyhemoglobin, a contributor to the BOLD effect, exerts less of an influence at lower field strengths (118). Also, field inhomogeneity ($\Delta B_0$) artifacts in T$_{1p}$ imaging (demonstrated in (119)) are reduced at lower fields.

1.13.5. Indirect $^{17}$O in vivo uses in cerebral blood flow and metabolism

The early manuscripts on the indirect detection of H$_2^{17}$O mainly showed the potential for in vivo detection. One early paper showed the feasibility of detecting H$_2^{17}$O with a steady state sequence after injection in mouse brain (120). The sequence they used was an early forerunner to the one used to detect $^{17}$O in Chapter 2, but due to gradient limitations in 1988 it took six seconds to acquire k-space as opposed to the one second or faster acquisition on modern scanners. A collaboration involving the same group went on to use a T$_2$ spin-echo based sequence to measure CBF in the mouse brain after injection using the Equation 1.25 presented here and an additional equation for calculation of CBF (121). As a feasibility experiment, another group used similar techniques to detect H$_2^{17}$O after injection of artificial oxygen carrier in the dog (122).
A few years later, spin-locking techniques (described in Section 1.13.2) showed promise for the decoupling of H$_2^{17}$O from relaxation (112). This would be later shown for off-resonance spin locking as well (123,124). These techniques were later used to measure flow (116) and metabolism (125) in rats. Another method for decoupling involves the use of irradiation at the $^{17}$O frequency during the relaxation period (126). This was also utilized to image metabolism (127) and flow (128) in rats. The $^{17}$O decoupling technique requires double-tuned hardware and a second transmit channel often found on small animal scanners but rarely found with clinical scanners. High power is required with all of these techniques, which makes them difficult to implement without violating tissue power absorption limits and the duty cycles of MRI radiofrequency power amplifiers.

1.14. Comparison of direct and indirect sensitivity and common issues

To truly appreciate the sensitivity advantage of indirect $^{17}$O techniques, we present

Table 1.4 for experiments at 4.7T.
Relative SNR per unit of time

<table>
<thead>
<tr>
<th></th>
<th>$^1$H (Indirect)</th>
<th>$^{17}$O (Direct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remaining SNR after TE</td>
<td>$1.97 \times 10^4$</td>
<td>1</td>
</tr>
<tr>
<td>Signal Change (Contrast) due to 1 at %</td>
<td>.16</td>
<td>26</td>
</tr>
<tr>
<td>Contrast to Noise Ratio</td>
<td>1160</td>
<td>26</td>
</tr>
<tr>
<td>4.7T Estimated Relative $^{17}$O Sensitivity</td>
<td>45</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1.4. A comparison of indirect and direct $^{17}$O detection sensitivity at 4.7 Tesla.

Relative SNR was derived in Table 1.2.

Equation 1.6, which accounts for $T_2$ in SNR, was originally derived for pulse acquire spectra, so it adapts directly for $^{17}$O chemical shift imaging, which assumes a very short acquisition delay. The echo time delay for a spin echo sequence is approximated as a shift in the acquisition window to start at the echo time, with a signal loss due to exponential decay (Equation 1.21). There is SNR loss due to waiting for the echo time to generate contrast, but this allows for the highest CNR due to the added $^{17}$O. In the direct case, adding more $^{17}$O to the system increases the SNR and CNR linearly, where 1 atom (at) % provides a 26-fold increase over natural abundance signal. Combined, this shows a 45-fold improvement in sensitivity for indirect detected $^{17}$O over direct detection at this
relatively high *in vivo* field strength. At 1.5T, the most common clinical field strength, the estimated indirect sensitivity is estimated to be well over 100-fold greater than direct measurements. This is because the $^{17}$O SNR scales down at least linearly with field strength but the $^1$H-detected sensitivity barely changes.

### 1.14.1. Cost of $^{17}$O$_2$

There are two issues common to all use of $^{17}$O-enriched products. The first is the cost of $^{17}$O enrichment. Current pricing for 70% enriched O$_2$ is $2,200 per liter (at sea level pressure and room temperature). This cost is a minor issue for small animal experiments since they breathe a tiny amount of gas. For example, a rate of 6mL/min in rats makes them convenient for low cost $^{17}$O$_2$ experiments (125). But future applications in humans require consideration of the human respiratory system. A typical human at rest breathes approximately 500mL per breath, 12 times per minute (129), or 6L of total air. Use of gas injudiciously would be tremendously wasteful in this setting, and even proper application is costly. This expansion of size presents an obstacle to large animal implementation of these techniques, one that is addressed in this work to bring the estimated $^{17}$O$_2$ cost for human measurement of CMRO$_2$ to only $2,640 per experiment (1.2L) with today’s prices. Personal communication with the manufacturers of $^{17}$O-enriched gas suggests that increased usage will provide an economy of scale, where future bulk use would reduce cost per liter of gas.
1.14.2. Safety of $^{17}$O$_2$

A second issue is that there is no Food and Drug Administration approval for the delivery for $^{17}$O$_2$ to humans. Still, within the past few years at least one Institutional Review Board has given permission for limited studies using inhaled $^{17}$O$_2$ (98). This is because recently a commercial provider of $^{17}$O products has become certified for current good manufacturing practices (Nukem Gmbh, Aschaffenburg Germany). As a result, it is expected an investigational new drug approval will provide later clearance for human experiments. It is almost certain that $^{17}$O is completely safe. The best evidence comes from the safety of Oxygen-18 containing compounds such as H$_2^{18}$O and $^{18}$O$_2$ (reviewed in ref. (130)), which have been tested extensively in animals and are routinely used in human experiments. In the meantime, we have chosen swine as a large animal model, thanks to their similar breathing volumes and circulatory systems.

1.15. Conclusion and summary of technical challenges

In summary, I have presented the basics for in vivo detection of H$_2^{17}$O and the past uses of H$_2^{17}$O and O$_2$ in cerebral blood flow and metabolism. Because the vast majority of the work on $^{17}$O was and continues to be performed on small animals, the goal of this thesis is the detection of H$_2^{17}$O formation after the
inhalation of $O_2$ for the calculation of CMRO$_2$ on clinical scanners \textit{in vivo}. This “scaling up” of the techniques presents significant challenges. Only indirect detection of $^{17}O$ is feasible for localization on clinical scanners, as direct detection is just too insensitive at today’s field strengths on large coils. In addition, clinical scanners are not capable of $^{17}O$ scanning. Decoupling becomes an almost insurmountable challenge due to SAR concerns and required amplifier duty cycles. Gas cost and requirements must be considered carefully. Additionally, modeling of $O_2$ delivery and $H_2^{17}O$ recirculation in large animals are two additional critical parameters required for metabolic measurement. Yet, despite all of these challenges, our group has made significant advances in $^{17}O$ delivery and imaging to be presented in the following chapters.

1.16. \textbf{Specific aims of the thesis research}

The inadequacies of existing metabolic imaging techniques have rendered them unable to fill an important clinical role. In this chapter, we have seen an overview of one potential technique, $^{17}O$ imaging, for the measurement of oxygen metabolism and blood flow. It is towards the goal of clinical implementation of $^{17}O$ techniques that I present the following aims.
1.16.1. **Aim 1: Improve the temporal resolution of $T_{1\rho}H_2^{17}O$ Imaging**

The goal of improving the temporal resolution of $H_2^{17}O$ measurements provides motivation to explore fast indirect imaging techniques for measurement of $^{17}O$. Previous measurements using these techniques for this purpose have been taken on the order of 10 seconds per measurement (125), which is unsuitable for experiments that limit the usage of costly $^{17}O_2$ (1-2 minutes of delivery). After this work, presented in Chapter 2, measurements will be made every 3-3.6 seconds. These data also serve to demonstrate the “single shot” quantification of $H_2^{17}O$ in phantoms and the large animal in vivo.

1.16.2. **Aim 2: Create a precision-response, high-efficiency system for $^{17}O_2$ delivery.**

In Chapter 3, I will present a method of $^{17}O_2$ delivery to humans or large animals and then model the change in $^{17}O$ atomic fraction in the lungs. This work was developed in collaboration with Dr. James Baumgardner. The circuit for this delivery is novel in being able to provide a step change function of gas at the mouth through 40 feet of tubing from a system outside the scanner room. Before this work there was no animal ventilator that allowed low-loss, single breath changes of delivered gas, limiting our ability to deliver $^{17}O_2$ over short time courses. This allows us to minimize use and waste of the expensive $^{17}O_2$ gas as
well as make time measurements of delivery start and stop with precision. This same system can be used in future, human experiments.

1.16.3. Aim 3: Measure the regional rate of oxygen consumption in a large animal on a clinical scanner with $^{17}$O$_2$

The combinations of Aim 1 and 2 allow for high quality indirect imaging of metabolically produced H$_2^{17}$O imaging in the swine. A modeling of CMRO$_2$ estimation is presented in Chapter 4 and arterial blood sampling is performed to validate the time period of CMRO$_2$ measurement in this model. This verifies the ability of these methods to measure CMRO$_2$ with clinical equipment on an animal almost the size of a human.

1.16.4. Aim 4: Repeat these CMRO$_2$ measurements in a model of deranged metabolism

Finally, I will show the ability of these methods to detect deranged metabolism in the swine in the setting of metabolic stimulation by the stimulant 2,4-dinitrophenol. This work in Chapter 5 serves to further solidify the feasibility and reproducibility of the current techniques. It is hoped that future clinical translation of this work will yield a clinically useful and widespread technique.
2.1. Introduction

As discussed in Section 1.13, indirect techniques hold a great deal of promise at today’s clinically useful field strengths (typically 1.5 Tesla). The goal of this work is to improve upon the pulse sequence groundwork for measurements of CMRO$_2$ in large animals and humans on clinical scanners.

One challenge is that the relaxation enhancement due to $^{17}$O is subtle. This leads to long echo times in conventional imaging sequences to develop contrast and resultant $T_2^*$ effects in gradient echo-based sequences that lead to unstable artifacts that often change throughout the study. The approach used here attempts low amplitude spin locking, where field inhomogeneities are eliminated but contrast based on $\text{H}_2^{17}\text{O}$ remains virtually the same. The sequence development keeps in mind that with high amplitude spin locking $T_1\rho$ dispersion imaging is possible with this same sequence (112).
The pulse sequence of choice for $T_{1\rho}$-based $H_2^{17}O$ studies has been $T_{1\rho}$ preparation of turbo spin echo. Increasing turbo factors to reduce time per image has led to unacceptable k-space blurring \textit{in vivo} and as such it has taken multiple repetitions in order to fill all of k-space. These long times per image on the order of 10s/image have proven unacceptable when trying to measure the kinetics of $^{17}O_2$ metabolism. This is because we propose to measure CMRO$_2$ based on the initial dip in signal before the metabolically generated water has had time to recirculate, a time on the order of 60 seconds.

Many complexities have appeared in the attempt to reduce this time per image. For example, implied in each $T_{1\rho}$-based indirect model of $H_2^{17}O$ measurement is the assumption that $T_2$ does not change while images are being generated and, therefore, the contrast used to calculate $H_2^{17}O$ is entirely based on the pre-encoding cluster. This assumption produces slight errors for sequences with short TE since the $T_2$ and $T_{1\rho}$ both change as $H_2^{17}O$ is added. However, most standard single shot sequences use linear phase encoding, acquiring several lines of k-space before the center line of k-space, where most contrast information is acquired. Minimum TE starts at 20ms for Echo Planar Imaging (EPI) (131) and increases for other single shot sequences, creating increasing amounts of error. Centric encoding of k-space, provided with balanced steady state free precession
readouts (132), with the associated short effective TE greatly diminishes this problem.

Here we report single shot T$_{1p}$ imaging with readout based on T$_{1p}$-prepared balanced Steady State Free Precession (bSSFP). We use pigs as a model for their similar lung capacities and circulations to humans. While the detection of H$_2^{17}$O with a steady state sequence has been reported (120), we show theoretically H$_2^{17}$O sensitivity is improved by a sequence with a T$_{1p}$ or T$_2$ pre-encoding cluster followed by signal acquisition without the steady state directly after pre-encoding. The efficacy is demonstrated by phantom experiments and in vivo.

2.2. A single shot T$_{1p}$-weighted pulse sequence

![Diagram of the pulse sequence](image)

Figure 2.1. The T$_{1p}$-prepared, center-out sampled bSSFP sequence.

The single shot pulse sequence is shown in Figure 2.1. A spin-lock cluster generates T$_{1p}$ contrast. The preparation cluster includes a 90 degree pulse to put
magnetization in the transverse plane followed by spin lock (SL) pulses of spin lock time (TSL) duration and with the provided phase alternation and finally a 90 degree pulse to place the magnetization back in the longitudinal plane. After this cluster, a crusher removes unwanted magnetization in the transverse plane. The bSSFP readout uses an $\alpha/2$ RF pulse and a train of +/-$\alpha$ pulses with fully balanced gradients. K-space sampling begins at the center to maximize the weighting by the preparation cluster. The image is taken in a single shot and a delay before the next repetition allows for $T_1$ recovery.

2.3. Methods of $T_1\rho$ measurement in Oxygen-17 Water Phantoms

All images were acquired with a Siemens 1.5T Sonata MR scanner. Simulations were performed with custom-written software in Matlab.

The ability of the sequence to detect changes in $\text{H}_2^{17}\text{O}$ concentration was measured by imaging six 15mL conical tubes filled with 1X Phosphate Buffered Saline doped with $\text{H}_2^{17}\text{O}$ (Isotec, Miamisberg, OH) in steps of 5mM from 20mM (natural abundance) to 45mM. These tubes were sealed and placed into a 9cm jar filled with water. A receive only loop coil was placed around the jar and used in combination with the body transmit coil. Cross-sectional images of the tubes were acquired with a spin lock amplitude of 100Hz and spin lock times of
200,400,600,800, and 1000msec. Imaging parameters are: FoV 90mm², ST 5mm, TR/TE 10.4/5.2ms, Matrix 128², BW 130Hz/Px, α=180°.

The concentrations of the phantoms were determined experimentally according to the following. In Equation 1.25, the H₂¹⁷O change for a voxel over time was calculated. If however, that individual voxel is not changing during the experiment, but is instead static as would be a series of phantoms, a slightly modified equation is necessary. This is because the coil sensitivity varies across multiple measured phantoms in space, and so S, the signal in the absence of relaxation, is not the same for all phantoms as it would be for a tissue that is changing in ¹⁷O content but not moving. So let us begin with Equation 1.22 though using the nomenclature in this chapter, where ST₁ρ is the signal observed in a T₁ρ weighted acquisition, S is the signal in the absence of relaxation, TSL is the spin locking time (analogous to TE), T₁ρ is the relaxation time at natural abundance H₂¹⁷O, R₁ρ (=1/ T₁ρ) is relaxation rate at 100Hz (a value almost the same as R₂) imparted by ¹⁷O, and Δf is the isotopic fraction of ¹⁷O in excess of natural abundance.

\[
S_{T_{1\rho}} = S \times e^{-\frac{TSL}{T_{1\rho}} \left( \frac{1}{T_{1\rho}} + R_{1\rho} \Delta f \right)}
\]

Equation 2.1
To account for coil sensitivity, we apply Equation 2.1 over a proton density weighted image ($S_{PD}$). In this case, the same sequence is used that is shown in Figure 2.1, but with a preparation cluster that only consists of two 90° pulses placing the magnetization along the transverse plane and then immediately recalling it to the longitudinal axis. Substituting $S_{PD}$ for $S$ yields,

$$\frac{S_{T_{1,\rho}}}{S_{PD}} = e^{-TSL\left(\frac{1}{T_{1,\rho}} + R_{1,\rho}\Delta f\right)}$$

Equation 2.2

and then we rewrite as in Equation 2.3.

$$\ln\frac{S_{T_{1,\rho}}}{S_{PD}} = -TSL\left(\frac{1}{T_{1,\rho}} + R_{1,\rho}\Delta f\right)$$

Equation 2.3

The constant $T_{1,\rho}$ will be the same between phantoms, and so to cancel this term, we should let the equation take the following form, where ‘,NA’ is added to the natural abundance phantom terms and ‘,\textsuperscript{17}O’ is added to the \textsuperscript{17}O phantom terms.

$$\Delta f \cdot R_{1,\rho} \cdot TSL = \ln\left(\frac{S_{T_{1,\rho},\text{NA}}}{S_{PD,\text{NA}}}\right) - \ln\left(\frac{S_{T_{1,\rho},\text{\textsuperscript{17}O}}}{S_{PD,\text{\textsuperscript{17}O}}}\right)$$

Equation 2.4
If we rearrange Equation 2.4 to solve for the total atomic fraction of $^{17}$O in a phantom, $f(p)$, and add an $f_n$ term to account for natural abundance, the final form in Equation 2.5 for finding the concentration of $^{17}$O in a phantom is reached.

$$f(p) = f_n + \left[ \ln\left( \frac{S_{T_{1p},NA}}{S_{PD,NA}} \right) - \ln\left( \frac{S_{T_{1p},^{17}O}}{S_{PD,^{17}O}} \right) \right] \frac{1}{R_{1p} \cdot TSL} \tag{Equation 2.5}$$

### 2.4. Animal Imaging in this chapter

The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all animal experiments. A live pig was placed supine on a vendor supplied surface coil. Once placed inside, the intubation tube was connected to a custom precision delivery breathing circuit (Chapter 3) that drove the pig’s breathing at 6 breaths/min at a tidal volume of 18mL times the weight in kg. A standard $T_1$-weighted localizer sequence was run to find a suitable coronal image of the middle portion of the pig brain that included cortex, brainstem, and ventricle. A $T_1$-weighted IR-prepared GRE sequence (MP-RAGE) was performed to obtain anatomical images of the pig brain and the desired slice. Sequence parameters for the $T_{1p}$-prepared bSSFP are as follows: TR/TE 9.7/4.8ms, ST 5 or 10mm, FoV 200mm$^2$, 128$^2$ matrix, BW 130Hz/Px, $\alpha=180^\circ$, SL Amp 100Hz, fat
saturation on, 1.6 second readout time, 2 second delay between acquisition, time per image 3.6/sec.

2.5. Oxygen-17 Phantom Single Shot Quantitative Imaging

A demonstration of the $T_1\rho$-prepared bSSFP sequence shows that measurements of $H_2^{17}O$ concentrations can be made with single shot imaging very rapidly (Figure 2.2). Once $R_1\rho$ for the phantoms was determined, the quantification was performed with only two repetitions of the pulse sequence, once with $T_1\rho$ preparation and once without. The relaxivity measured with the single shot sequence was only 10% different than the relaxivity measured with a much longer spin echo pulse sequence. This indicates that the single shot sequence may lose a small amount of sensitivity to $^{17}O$ over a non-single shot technique. Still, the spin echo technique represents the highest possible contrast readout. The readings from the first 15mM of added $H_2^{17}O$ were almost identical, and a 10% $R_1\rho$ performance is excellent considering the gain in temporal resolution. For rough comparison, in Figure 1.5 the direct quantification used a voxel volume of 7.8cm$^3$, while the volume in Figure 2.2 for indirect quantification was .12cm$^3$. 
Figure 2.2. Quantification of $\text{H}_2^{17}\text{O}$ in $T_{1\rho}$-weighted images. Part a shows cross-sectional images of 15mL conical tubes filled with phosphate buffered saline and doped with increasing amounts of $\text{H}_2^{17}\text{O}$ to concentrations from 20mM (natural abundance) to 45mM (25mM added). The middle image, b, is a proton density image using only two 90 degree pulses and no spin locking ($T_{SL}=0$) as a preparation cluster. The right image, c, shows the contrast developed with 100Hz spin locking and the single shot readout. In part d, two $T_{1\rho}$ maps were taken. The first, shown as green squares, are the $T_{1\rho}$ values for the single shot sequence. The second, shown in the pink diamonds corresponds to a $T_{1\rho}$-prepared spin echo sequence with the same TR but required 6 minutes per image. The slope of the line is the relaxivity ($R_{1\rho}$) of these phantoms with the single-shot sequence. This is then is applied to Equation 2.5 along with the signal intensities from the above images to calculate $\text{H}_2^{17}\text{O}$ concentrations. The calculated concentrations agree very well with the actual concentrations ($R^2>.99$). Vertical error bars for each point are the size of or smaller than the size of the diamond representing the value.
2.6. Simulations of single shot imaging parameters

Shown in

Table 2.1 is a comparison of bSSFP for detecting a $T_2$ reducing agent without a preparation cluster in the steady state or with a preparation cluster (not in steady state).

<table>
<thead>
<tr>
<th>Steady State</th>
<th>Non-Steady State</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation</strong></td>
<td>$M_{SS} = M_0 \frac{\sin(\alpha)}{1+\cos(\alpha)+(1-\cos(\alpha))(T_r/T_2)}$</td>
</tr>
<tr>
<td><strong>Contrast</strong></td>
<td>$C_{SS} = M_{SS}(T_{2,1}) - M_{SS}(T_{2,2})$</td>
</tr>
<tr>
<td><strong>Optimal (\alpha)</strong></td>
<td>$60^\circ$</td>
</tr>
<tr>
<td><strong>Contrast at optimal (\alpha)</strong></td>
<td>$.0011M_0$</td>
</tr>
<tr>
<td><strong>Scan Time</strong></td>
<td>1.28s</td>
</tr>
<tr>
<td><strong>SNR Efficiency</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>CNR Efficiency</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2.1. Steady-state and non-SS Calculations for bSSFP detecting a $T_2$ reducing agent such as $H_2^{17}O$.

To show that high SNR and contrast are maintained by using a sequence with a pre-encoding cluster versus detecting pure $T_2$ changes in the steady state, Bloch
equation simulations were performed to show that detecting in the steady state gives less contrast and CNR than non-steady state methods. These values were obtained by using the $T_1$ and $T_2$ given for 1.5T in Table 1.3, assuming a drop in $T_2$ of 1ms. In essence, by filling the center of k-space first with a high flip angle, the contrast achieved is much higher than when obtaining $T_2$ contrast in the steady state. The simulated differences are summarized in Table 2.1. Despite the possibility of generating images faster, steady state acquisition offers less SNR and CNR efficiency.

<table>
<thead>
<tr>
<th>Flip Angle ($\alpha$)</th>
<th>Contrast vs. 180°</th>
<th>CNR Efficiency vs. Steady State</th>
<th>SAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>180°</td>
<td>100%</td>
<td>660%</td>
<td>100%</td>
</tr>
<tr>
<td>150°</td>
<td>83%</td>
<td>486%</td>
<td>69%</td>
</tr>
<tr>
<td>120°</td>
<td>66%</td>
<td>309%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Table 2.2. 17O contrast and SAR versus flip angle.

Because SAR is a concern in fast imaging sequences, we show in
Table 2.2 how contrast and SAR reduce with decreases in flip angle. CNR Efficiency vs. Steady State compares the CNR from a non-steady state acquisition with the given flip angle against CNR from steady state acquisition with optimum flip angle (~60°) for CNR in the steady state.

2.7. Signal changes in swine after two breaths of 40% $^{17}$O$_2$ delivery

To demonstrate detection of H$_2^{17}$O in vivo, $^{17}$O$_2$ gas was delivered to a pig during imaging. At first we began by delivering 2 breaths of $^{17}$O$_2$ at 40% enrichment. Signal decreases shown in Figure 2.3 demonstrate the detection of $^{17}$O$_2$ conversion to H$_2^{17}$O. The 40% $^{17}$O$_2$ was delivered to three pigs. The signal traces over time from whole brain regions of interest in those three animals are averaged together to create Figure 2.3, and all three individual signal traces take that general form. This represents the shortest time course delivery of $^{17}$O$_2$ gas with H$_2^{17}$O detection in the literature.
Figure 2.3. Time courses of three pig experiments with 40% enriched $^{17}\text{O}_2$. Their results are pooled. These were performed with TSL=200ms to develop a higher percent signal change, but with SNR about 40:1 as opposed to 150:1 at TSL=75ms in Figure 3. The whole brain signal trace is an average of these three experiments, though ROIs from different regions show similar kinetics. Two pigs were imaged with a protocol of 5 minutes of 100% $\text{O}_2$, 2 breaths of 40% $^{17}\text{O}$ enriched $\text{O}_2$, then 5 minutes of 100% $\text{O}_2$. The third pig was imaged with 5 minutes of room air then 2 breaths of 20% $\text{O}_2$ (40% enriched $^{17}\text{O}_2$) mixed with 80% $\text{N}_2$. The start of $^{17}\text{O}_2$ is indicated by the arrow. Because the normalized signal trace from the third experiment is so similar, it has been pooled. Signal changes are statistically significant when compared to baseline (see Figure 2.4).

Figure 2.4 demonstrates that the changes due to $^{17}\text{O}$ results are statistically significant. Still, this is not a measurement of metabolism, as the subtraction occurred over three minutes after $^{17}\text{O}_2$ delivery. As will be discussed later, significant contamination of metabolic information with flow information by recirculating H$_2^{17}$O has occurred.
Figure 2.4. The signal change in one experiment is shown to be statistically significant. The second pooled dataset was singled out and region of interest analyses from 62 images pre-$^{17}$O$_2$ and 62 images post-$^{17}$O$_2$ were analyzed in order to demonstrate that signal changes are due to $^{17}$O$_2$. Statistical significance has been shown in every dataset in this chapter.

2.8. Delivery of $^{17}$O$_2$ as a 80% N$_2$ and 20% O$_2$ (70% enriched) mixture

Delivery of 100% O$_2$ enriched with $^{17}$O has two undesired effects. First, 100% O$_2$ has to be delivered over a period of minutes to reach a steady state in blood plasma. While hemoglobin has been 100% saturated after about 16% air O$_2$ concentration, a small amount of oxygen will dissolve in the blood, and this leads to signal changes from the delivery of 100% O$_2$ alone (133). This is demonstrated in Figure 2.5.
Figure 2.5. Change in pig brain signal from increased O₂. Using the same sequence, one swine inhaled increasing amounts of natural abundance oxygen for 2 minutes (pink boxes) and then that oxygen was turned off for 2 minutes (room air, ~20% O₂). The signal increases with oxygen inhalation then undershoots its initial baseline. This is a clear motivation to maintain arterial oxygen saturation and not to deliver ¹⁷O₂ as 100% oxygen.

Thus, if one delivers 100% O₂ enriched in ¹⁷O as a bolus after room air delivery, the two signal change effects compete destructively (¹⁷O decreases signal while increased dissolved oxygen increases signal). So five minutes of 100% O₂ equilibration was applied when necessary in Figure 2.3.
Additional consideration of the most efficient means to deliver the costly 70% $^{17}\text{O}_2$ led to Figure 2.6. As previously noted, hemoglobin becomes completely saturated when exposed to about 16% O$_2$. Room air contains about 21% O$_2$. So delivering 100% enriched gas is tremendously wasteful when one considers that most will not be taken up by the body. This means that about 80% of the product will be exhaled unused! In Figure 2.6 then, as with all the additional experiments in this thesis (except two later experiments as noted at 40% enrichment), 1.2 to 2.4 breaths 70% enriched $^{17}\text{O}_2$ was diluted with nitrogen to simulate room air and delivered over one to two minutes at 6-8 breaths/minute. The same amount of oxygen is used by the body in this case, more $^{17}\text{O}_2$ is used as less is exhaled to waste, and from that, more H$_2^{17}\text{O}$ is generated.
Figure 2.6. Images and a signal time course generated by 70% $^{17}$O$_2$ in swine. In this experiment two minutes of 20% $^{17}$O$_2$ (70% enriched) was delivered mixed with 80% nitrogen. In a, a T1 weighted image of the imaging slice is presented. Then b shows an example T1$\rho$-prepared bSSFP image. A series of 376 of these images were obtained (3.6 sec/image). After 10 minutes imaging with room air, 2 minutes of 70% enriched $^{17}$O$_2$ (NUKEM Group, Germany) was delivered (again as an O$_2$/N$_2$ mixture) and room air resumed. To obtain the difference map in part c, 32 images after $^{17}$O$_2$ delivery (150 sec - 262 sec) were subtracted from 32 baseline images. The color bar describes the signal decrease due to H$_2^{17}$O. Part d shows the difference map overlaid on the image from part a. Moving average signal traces in part e are plotted over time from the beginning of $^{17}$O$_2$ delivery (time 0).

2.9. Chapter Discussion
In conclusion, it is hoped that this chapter provides many of the technical details of and proof of concept for improvements in indirect detection of $\text{H}_2^{17}\text{O}$ by $T_{1p}$ on clinical scanners. Due to the preparation cluster, this sequence can also be used on research equipment and can be used for $^{17}\text{O}$ decoupling experiments. Still, the ultimate goal of this thesis is the clinical *in vivo* imaging of metabolism. To go from detection of $\text{H}_2^{17}\text{O}$ to computation of $\text{CMRO}_2$ is not a straightforward task and requires many additional considerations. These considerations will be the focus of following chapters. The following provides a brief discussion of how this sequence can be used for one of these later considerations.

In order to compute the $\text{CMRO}_2$, several parameters require modeling or measurement. First, the delivery rate and uptake of $^{17}\text{O}_2$ must be known. With the precision-delivery breathing circuit a step change of gas concentration is provided to the airway, and the rate of uptake through the lungs and tissue transport can be modeled. The rate of production of $\text{H}_2^{17}\text{O}$ is related to signal change. However, a parameter requiring consideration is the arterial input function (AIF) from recirculation of produced $\text{H}_2^{17}\text{O}$.

There are two methods of measuring the AIF. The first method assumes that it will take some time for $\text{H}_2^{17}\text{O}$ to recirculate through the body after production in
the mitochondria. For example, at least 60 seconds elapse before $\text{H}_2^{17}\text{O}$ “wash-in” after the start of $^{17}\text{O}_2$ delivery in a cat (92). That gives a window during which to compute $\text{CMRO}_2$ while ignoring recirculation. In the three experiments that were used to prepare Figure 2.3, a small hump was observed always at around 90 seconds. It could be speculated that after this point wash-in due to recirculation begins, however, this effect is not seen in every experiment.

2.10. Feasibility of $T_{1p}$ imaging of flowing solutions

A second method involves the imaging of an artery during $\text{H}_2^{17}\text{O}$ delivery. To this end, Zhang et al. utilized an implantable coil placed around the carotid artery for small animals using direct $^{17}\text{O}$ measurements (99). A small implanted coil with a localized RF field is required because direct imaging is unable to resolve something the size of an artery. That group argued with this invasive technique that there was little to no time for recirculation in that model; however, the recirculation time for a very small animal like a rat may be much shorter than for a large animal like a pig. But along these lines, a possible future direction for this work is imaging with this bright blood, fast imaging sequence to address the imaging method for measuring AIF in vivo. It could be suggested that in a large animal the carotid arteries can be imaged with a surface coil over time to look at changes in $\text{H}_2^{17}\text{O}$ concentration.
The pulse sequence described in this chapter is uniquely equipped to perform these measurements. A spin lock pulse applied by a body coil can be considered to apply to all the spins within a fairly large slab, as it is non-selective. As such, one can imagine all the spins from the heart to the carotid artery to be labeled by a spin-lock pulse. The readout pulse is slice selective, and we wish to readout downstream of the heart in the carotid artery. So if $\alpha$, the readout flip angle, is set to less than 180° in this sequence, and spins are flowing perpendicular to the slice plane (as in an artery), one would obtain fresh, spin-lock prepared magnetization with each readout pulse, assuming the readout is less time in duration than one cardiac cycle. This is the basis behind a magnetization prepared “bright blood” sequence such as that found here (134).

As a suggestion for future work and as a demonstration of the feasibility of this approach, a solution of 50% glycerol/50% water was prepared to simulate the arterial blood $T_1$ and $T_2$ at 1.5T (135) and mixed with .01% sodium azide to prevent spoilage. This was flowed through Cole-Parmer L/S 15 tubing with an inner diameter of 4.8mm, very close to the swine carotid artery diameter of 5mm (136), by a rate-adjustable continuous flow pump (Fisher Scientific 13-876-4). The pump remained outside the room, while long tubing carried the solution from a reservoir close to the center of the magnetic field, through a beaker of
water with a static phantom, and back continuously. $T_{1p}$ maps of the flowing solutions and a static phantom were taken with this sequence with the solution flowing at various rates. The resulting values were all within 10% of the static values, independent of flow rate, as shown in Figure 2.7. Incorporating this work \textit{in vivo} with cardiac gating may lead to another method of determining the $^{17}$O arterial input function over long time periods.
Figure 2.7. Flowing blood simulation phantom. Details are described in the text. The small areas of signal within a dark ring is the flowing simulation fluid within the tubing, while the larger tube is a 50mL conical tube containing the same fluid. In part a is an image with 50ms of 100Hz spin locking and readout $\alpha$ of 90° with 10m/s flow through the tubing. In part b, an image is shown with a $T_1$-prepared HASTE sequence (a “dark blood” spin echo based sequence) with the same flow rate. No signal from the flowing solution is observed. Phase contrast images in vivo revealed the flow in the swine carotid arteries to be about 30cm/s. This corresponded nicely with the maximum flow rate of the pump. In part c, 100Hz spin-locking was
performed at 50, 100, 150, 200, and 250Hz and repeated at 10cm/s (low flow),
20cm/s (medium flow), and 30cm/s (high flow) flow rates. Flow rates through the
tubing were verified by standard scanner phase contrast imaging and processing.
The names at the bottom (static and flow 1-4) correspond to small regions of
interest within each tube. An example T₁ρ map is shown in part d, here with medium
flow.
CHAPTER 3: DELIVERY OF $^{17}$O$_2$ IN BRIEF PULSES

3.1. Motivation

Prior studies have delivered gaseous $^{17}$O$_2$ by inhalation for a period ranging from 2 minutes (96,98) to 40 minutes (125). Given the cost of $^{17}$O$_2$ gas for large animal research, a goal of $^{17}$O research is to make measurements from as little gas as possible. But this leaves an open question--how little is little enough? A potential target is to deliver just enough $^{17}$O$_2$ to make measurements of the produced metabolic H$_2^{17}$O (mH$_2^{17}$O) before the mH$_2^{17}$O has had time to recirculate to other organs and complicate the modeling for CMRO$_2$. This time is on the order of one minute and has been shown by arterial blood sampling in a cat for $^{17}$O$_2$ (92) and in humans for $^{15}$O$_2$ (137). As such, delivery of $^{17}$O$_2$ in very brief (1 minute) pulses could, in theory, provide significant advantages for imaging of CMRO$_2$.

However, mechanical ventilators for large animals and humans are not typically designed to provide rapid step changes in gas concentration at the airway. Modern, servocontrolled, open-circuit ventilators that are commonly used in the intensive care unit can provide a change in inspired gas concentration with a time constant of a few seconds (138,139). The use of an open-circuit ventilator, however, would lead to tremendous waste of $^{17}$O$_2$ gas. Closed-circuit and low
flow, semi-closed systems for mechanical ventilation are designed to conserve administered gases such as inhaled anesthetics. These systems, however, have time constants for changes in gas concentrations at the airway that are on the order of a few minutes (140).

We designed and tested a mechanical ventilator specifically developed to produce rapid gas concentration changes at the airway through long runs of tubing between the ventilator mechanical parts outside an MRI scanner and a subject inside the scanner (141). The ventilator was then used in all of the animal experiments later described in the text and is the basis for the as-yet unpublished alveolar diffusion simulations. For these reasons we reproduce relevant portions of that work here.

3.2. The Kety-Schmidt Equation for CMRO₂ calculation

Because the ¹⁷O incorporated into water produces an MR signal, but gaseous ¹⁷O₂ does not, MR imaging with ¹⁷O provides a way to image metabolically produced water without any need to account for concurrent changes in gas. Ideally, CMRO₂ could be calculated from a local signal that is a simple function of the water produced locally in the mitochondria. Some of this mH₂¹⁷O, however, leaves the region of interest (ROI) by diffusing to the venous circulation. Additionally, water
produced outside the ROI also diffuses to its venous circulation, and re-circulates into the ROI in the arterial blood. The changes in H$_2^{17}$O in the ROI, therefore, are related not only to CMRO$_2$, but also to CBF, and to the arterial input function for H$_2^{17}$O. Fiat and Kang first presented a comprehensive model of the relationship between local concentration of H$_2^{17}$O and CMRO$_2$ (142), based on the mass balance principles developed by Kety and Schmidt (143). This is summarized as,

$$\frac{d}{dt}C_b(t) = \frac{2\alpha(t)}{f_1}(\text{CMRO}_2) + \frac{Q}{f_2}[C_a(t) - C_v(t)]$$  \hspace{1cm} \text{Equation 3.1}$$

where $C_b(t)$ is the local concentration of H$_2^{17}$O in excess of natural abundance, $\alpha$ is the $^{17}$O enrichment fraction of the inhaled $^{17}$O$_2$ gas (treated as a constant for long administration times), Q is cerebral blood flow (CBF), $C_a(t)$ and $C_v(t)$ are the concentrations of H$_2^{17}$O in excess of natural abundance for arterial and venous blood, and $f_1$ and $f_2$ are unit conversion factors.

Delivery of a very brief pulse of $^{17}$O$_2$ could potentially simplify the relationship between locally measured H$_2^{17}$O and CMRO$_2$. Immediately after a breath of $^{17}$O$_2$, the water that is produced outside the ROI should be delayed in its entry into the ROI, as it must diffuse to the local venous circulation and transit through the heart and lungs before entering the arterial circulation. The venous-arterial convection delay for an adult human is typically on the order of 10-15 seconds.
(144). For a brief period immediately after beginning inhalation of $^{17}$O$_2$, therefore, the arterial input function of H$_2^{17}$O should be zero, which would eliminate the C$_a$(t) term in Equation 3.1. In contrast, the local production of H$_2^{17}$O should commence almost immediately, as the expected diffusion lag for $^{17}$O$_2$ can be estimated to be less than one second. This diffusion time lag is characterized by the time constant $\delta^2/D$, the average diffusing distance squared divided by the diffusivity of oxygen in tissue (145). For an average capillary spacing in brain of 50 microns (146), each capillary can be estimated to supply a cylinder of radius 25 microns. The spatially averaged diffusing distance, assuming uniform distribution of mitochondria around the capillary, is then estimated at 17 microns. For a simple one-dimensional diffusion model into a finite slab, solution of the transient diffusion equation with an oxygen diffusivity in tissue of 2.4 x 10$^{-5}$ cm$^2$/sec (147) estimates that the average 0-90% rise time in mitochondrial concentration after a step change in arterial concentration would be approximately 130 msec. This diffusion delay in mitochondrial $^{17}$O$_2$ utilization can therefore be neglected for all but the shortest arterial pulses.

Additionally, because water movement through lipid bilayers tends to be restricted (148), locally produced water may be delayed in its egress to the venous circulation as it passes through the mitochondrial, the plasma, and the capillary endothelial membranes. A diffusional delay in non-cerebral tissues would further
extend the period of a negligible arterial input function. Additionally, substantially restricted diffusion of water from mitochondria to the venous circulation within the ROI would minimize the $C_v(t)$ term in Equation 3.1. For a brief period after initiating a pulse of $^{17}$O$_2$ delivery, then, the relationship between $\mathrm{H}_2^{^{17}}\mathrm{O}$ concentration and CMRO$_2$ should approximately obey the simplified relationship,

$$\text{Equation 3.2}$$

$$CMRO_2 = \frac{1}{2} \frac{dC_b(t)}{dt} \frac{f_1}{\alpha(t)}$$

where $\alpha(t)$ is the time dependent arterial $^{17}$O atomic fraction. The constant $\alpha$ of Equation 3.1 has been replaced by $\alpha(t)$ because the kinetics of alveolar gas dilution cannot be neglected for brief pulses of $^{17}$O$_2$ (see Figure 3.4).

Prior studies in small animals have used a simplified equation, similar to Equation 3.2 with no arterial input and no venous washout terms, to successfully estimate CMRO$_2$ after inhaling $^{17}$O$_2$ (96,100,125,149). We wish to verify the time period over which this simplified equation is valid and deliver $^{17}$O$_2$ within that time period. This provides the motivation for precision $^{17}$O$_2$ delivery.
3.3. Design considerations for the mechanical ventilator

The main design consideration for the ventilator was the goal to produce sharp step changes in gas concentration at the airway. This dictated avoidance of any sudden transitions in diameter of the gas flow pathway (such as conventional one-way valves), which could slur a sharp gas concentration front. This also dictated that the inspiratory limb tubing be of the smallest diameter feasible, reducing transit time from the gas source to the airway and minimizing diffusive mixing between sharp concentration boundaries.

Some design constraints were imposed by the goal ultimately to apply this ventilator to functional imaging studies, where it is desirable to avoid stimulatory cues. For this reason, use of gas switching valves near the subject in the scanner was restricted, which in turn means that the gas switching valves and all mechanical devices would ideally be outside of the scanner. This in turn required atypically long tubing runs, placing an even greater premium on narrow-bore tubing.

Some design constraints were imposed by the expense of \( ^{17}\text{O}_2 \) gas. Delivery of the gas in very brief pulses has a natural advantage of minimal gas use per CMRO\(_2\) measurement. From a financial perspective, it was considered desirable for the
ventilator to be capable of pressurizing $^{17}$O$_2$ mixtures from un-pressurized sample bags rather than pressurized gas cylinders, thereby avoiding costly mistakes in gas handling that might occur in the early developmental stages.

Finally, in the interests of versatility, it was decided that the ventilator should be capable of either comfortable, supported spontaneous ventilation in awake subjects or controlled mechanical ventilation in anesthetized subjects.

### 3.4. Design of the mechanical ventilator

The system for mechanical ventilation appears schematically in Figure 3.1.
Gases are pumped to and from the subject by two large peristaltic pumps (Cole-Parmer, Chicago IL; Masterflex I/P digital drive with dual standard pump heads and silicone I/P 73 tubing), one for assisting or controlling inhalation (“PPI”), and one for assisting or controlling exhalation (“PPE”). The pumps outside of the MRI scanner are connected to the subject inside the scanner by 25 feet of 0.25 inch ID Tygon tubing (Cole-Parmer L/S 17). At the maximum flow provided by
the pumps of 16L/min, the Reynolds number for pure oxygen is 3800, i.e. the flow is in the turbulent range at the maximal volumetric flow rate. At this maximal flow rate, the transit time for 25 feet of the 0.25 inch ID tubing is 0.90 seconds. The pressure drop across the transit tubing for maximal flow is 12 cm H₂O, which is over 100 fold less than the maximal operating range of the peristaltic pump. Pressure at the subject’s airway (“Paw”) is transmitted by 0.125 inch ID nylon tubing to a pressure transducer (Freescale Semiconductor, Chandler, AZ, MPX2010DP) with signal amplification by a custom opamp circuit. Information on airway pressure is transmitted via a multifunction DAQ device (National Instruments, Austin TX, USB-6008) to a computer that uses feedback control to adjust the pump speeds for both pumps during assisted spontaneous ventilation and for the exhalation pump during volume-controlled mechanical ventilation. Inhaled gases can be chosen via a computer controlled stream select valve (“inh”) (VICI® Valco Instruments, Houston, TX, Model C45) to select air, regular 100% O₂, oxygen with enriched ¹⁷O₂ or other test gases. The oxygen and test gases are sealed in Tedlar® gas sampling bags (Cole-Parmer®). Exhaled air can be directed, via a second stream select valve (“exh”), to either waste (“W”), or to recovery of the partially enriched ¹⁷O₂. All data acquisition and control software was written in Labview 7.1 (National Instruments™, Austin TX).
The long tubing connections between the mechanical components and the subject required a significant departure from prior approaches to mechanical ventilation. Although a wide variety of ventilator modes are available from conventional ventilators, exhalation is almost universally passive, i.e. the exhalation pressure at the ventilator is set to a prescribed value and the lungs exhale passively against that exhalation pressure. Ventilator exhalation tubing is usually sized in a large enough diameter to make expiratory flow resistance negligible, thus keeping the pressure at the airway equivalent to the set exhalation pressure in the ventilator. For the system constructed here, large bore exhalation tubing would greatly increase the circuit priming volume, and would also tend to slur any sharp concentration fronts in the exhalation limb, making clean recovery of partially enriched $^{17}\text{O}_2$ more difficult. Passive exhalation, therefore, leads to design requirements for the exhalation tubing that are fundamentally at odds with the desire for efficient use and recovery of the expensive $^{17}\text{O}_2$ gas. The system depicted approaches these problems by actively assisting exhalation as well as inhalation. Pressure at the airway is monitored and fed back to the control system, which then adjusts the exhalation pump speed. The target exhalation pressure at the airway can then be specified at any given level, and for the subject it feels as though he or she is exhaling passively at that pressure, even though the pressure required at the end of the exhalation tubing varies markedly according to the expiratory flow rate.
At the inspiratory side, mechanical ventilators typically have either a gas bellows, or a piston and cylinder, both of which can slur rapid step changes in gas concentration (150), and both of which increase the circuit priming volume. We used an additional roller pump here which eliminates the reservoir volume and also avoids any large sudden changes in tubing diameter, helping to preserve step changes in gas concentration.

Control of the pumps was implemented with a sequential state machine, with a single inspiration state and a single expiration state for each breath. Within each state, the opposing pump was stopped and the appropriate pump for the state (for example the inhalation pump for the inspiration state) was controlled according to the airway pressure with simple proportional control. For transitioning between states, awake subjects were instructed to initiate inspiration by transiently (5 ms) reducing airway pressure below a critical value (-33 cm water) and then to inhale as normally as possible. Transition to the expiration state was signaled by a transient (5 ms) increase of airway pressure above a critical value (33 cm water). For safety, the awake subjects manually held a breathing mask to their faces for a tight seal and were instructed to remove the mask if breathing became uncomfortable.
For use in large anesthetized animals, we also implemented a much simpler algorithm for volume controlled ventilation. Transitions between the states were strictly determined by the inspiratory and expiratory times, as determined by the desired respiratory rate and inspiratory/expiratory ratio. For example, a respiratory rate of 10 breaths/min and an I:E ratio of 1:2 entered by the user would translate to an inspiratory time of 2 seconds and an expiratory time of 4 seconds. Inhalation pump speed was accelerated to a constant value, determined by the desired tidal volume that was maintained throughout the inspiratory time. Exhalation pump speed during the expiratory state was again controlled according to airway pressure.

3.5. Methods for ventilator validation

With Institutional Review Board approval, the system for mechanical ventilation was tested in a sitting human volunteer in spontaneous ventilation mode, with use of helium as a surrogate for $^{17}$O$_2$, and with monitoring of gas concentrations at the airway with a micropore membrane inlet respiratory mass spectrometer (151). The time response of the mass spectrometer was determined by placing the sample port in a stream of flowing gas, directly downstream of a switching valve (Valco Instruments Model C45), and switching the gas source from 0% to 100%
helium. The subject breathed in assisted spontaneous ventilation mode through the circuit, where both inspiratory and expiratory pump flows were controlled by the pressures generated at the airway by the subject. After switching to 100% O₂ and denitrogenation, the inhalation valve was switched to a source gas of 100% helium and the change in gas concentration at the airway was recorded by the mass spectrometer. After two inhaled breaths of helium, the source valve was switched back to 100% oxygen. Exhaled gas was directed, by the exhalation valve, to the recovered ¹⁷O₂ gas sampling bag for these two breaths of helium and for the following breath. Helium concentration in the recovered ¹⁷O₂ bag was then measured with the respiratory mass spectrometer.

3.6. Results: Step change in gas input and fast alveolar gas increases

Acceleration to full speed from a full stop for the pumps of Figure 3.1 was well described by a mono-exponential time constant of 0.88 sec. Similarly, deceleration was well described by mono-exponential decay with a time constant of 1.46 seconds. The inhalation and exhalation pump flow changes in response to changes in airway pressure were adequate to provide subjectively comfortable spontaneous breathing in the normal human subject. The 0-90% response time of the respiratory mass spectrometer was 2.2 seconds. The step change in gas concentration at the airway provided by the ventilator circuit was fast enough to
approach the temporal resolution of the mass spectrometer, with a measured 0-90% response time of 4.0 seconds Figure 3.2.

After correction for the mass spectrometer time response by de-convolution, the estimated 0-90% response time at the airway was 2.4 seconds.

The time course of helium concentration changes at the airway during two breaths of helium are shown in Figure 3.3, with labeling of the phases of the respiratory cycle.
The measured end-tidal helium concentration after a single deep breath was 41%. After further practice by the subject breathing as deeply as possible with the ventilator system, the end-tidal helium after a single deep breath was 48%. For the idealized scenario of a perfect step change in gas concentration in a healthy individual breathing through an apparatus of negligible resistance, the maximum end tidal helium concentration predicted from standard pulmonary function testing (PFT) nomograms (152,153) is 68%, with a conservative estimate (based on +/- one STDV for a coefficient of variation of 20%) of the range of normal values around this mean extending from 54% to 82%. Thus, achieving an end-tidal concentration of up to 48% in one individual after a single breath of helium indicates that the ventilator circuit is able to provide a change in gas concentration in the lung that is approaching the theoretical maximum, despite providing this breath of helium through 25 feet of narrow bore tubing. The

Figure 3.3. Helium concentration at the airway for two breaths of 100% helium after equilibration with 100% oxygen.
measured concentration of helium in the recovered \textsuperscript{17}O\textsubscript{2} gas sampling bag was 47\%, which compares favorably with the maximum possible recovered concentration of 49\%.

3.7. Chapter Discussion

The ventilator we designed and tested is capable of delivering step changes in gas concentration at the airway through long runs of narrow bore tubing between the mechanical pumps of the ventilator and a subject in the MR scanner. After this step change at the airway, the kinetics of \textsuperscript{17}O\textsubscript{2} uptake into arterial blood should be predicted by mathematical models. A crucial remaining issue for the use of very brief pulses of \textsuperscript{17}O\textsubscript{2} for CMRO\textsubscript{2} measurement is the length of the time window for which Equation 3.2 applies. This topic will be address later in this work.

The limited experimental data that has been reported previously on this topic suggests that the arterial input function may be delayed beyond the expected convection delay. Zhu and coworkers in a study presented in abstract form in 2002 and discussed in their 2005 review (83), showed in rats that cerebral washout of H\textsubscript{2}\textsuperscript{17}O after a bolus arterial injection was faster than washout after cessation of \textsuperscript{17}O\textsubscript{2} breathing, implying at least qualitatively that diffusion from the mitochondria to the venous circulation is restricted in the brain. This is in
addition to the studies cited in Section 3.1. The availability of the new mechanical ventilator reported here facilitates additional experimental studies on tissue using $^{17}$O$_2$ to investigate CMRO$_2$.

3.8. A simple model of inhaled atomic fraction of $^{17}$O during the first minute

It is necessary to know or model the atomic percentage of $^{17}$O in the oxygen metabolized by cells because while the mH$_2^{17}$O rate of formation is measureable by MRI, the fractional enrichment of the $^{17}$O$_2$ generating that water at the cellular level during that time is not known. This is not as much of a problem for small animals that breathe very rapidly and equilibrate their lung gas with the inhaled gas rapidly. Instead, for the large animal that breathes relatively deeply and slowly, we generated a simple step-wise model for estimating the fractional enrichment, $f_a(i)$, of $^{17}$O in the lungs for each breath. Each step (breath) is modeled as an instantaneous inhalation, mixing of $^{17}$O$_2$ in the lungs, and exhalation via a simple dilution model with the addition of O$_2$ uptake into the body from the lungs.
Let us begin with an inspired volume of $^{17}$O, $V_{i}^{17}$O. That volume is given by the tidal volume (TV), the fraction of oxygen in that gas ($f_{i}O_{2}$), and the $^{17}$O enrichment of that gas ($\alpha$).

$$V_{i}^{17}O = TV \cdot f_{i}O_{2} \cdot \alpha$$ \hspace{1cm} \text{Equation 3.3}

This volume adds to the $^{17}$O left in the lungs at the end of the previous exhalation, $V_{e}^{17}$O. That volume is determined by the functional residual capacity (FRC), the fraction of oxygen in that exhaled gas ($f_{e}O_{2}$), and has the $^{17}$O enrichment of the breath before it, given by $f_{a}(i-1)$.

$$V_{e}^{17}O = FRC \cdot f_{e}O_{2} \cdot f_{a}(i - 1)$$ \hspace{1cm} \text{Equation 3.4}

To calculate $f_{e}O_{2}$, one must approximate the amount of oxygen consumed during the breath. For simplicity, it is assumed that the inhalation, mixing, and exhalation happen instantly to produce a step change and the oxygen consumption occurs on the post-exhaled gas. This is given on a per breath basis by,

$$f_{c}O_{2} = \frac{VO_{2}}{RR \cdot FRC}$$ \hspace{1cm} \text{Equation 3.5}

where $f_{c}O_{2}$ is the fraction of $O_{2}$ consumed from the exhaled lung volume during each breath, $VO_{2}$ is the per minute volume of $O_{2}$ consumed, and RR is the
respiratory rate in breaths/min. Hence, to take into account the O₂ consumption in the “exhaled” gas set FeO₂ as in Equation 3.6.

\[
feO₂ = fiO₂ - \frac{\dot{V}O₂}{RR} \frac{1}{FRC}
\]

Equation 3.6

The incoming tidal volume of O₂ mixes with the lung end exhalation volume to create the fmO₂ (the mixed fraction of O₂).

\[
fmO₂ = \frac{(TV \cdot fiO₂) + (FRC \cdot feO₂)}{FRC + TV}
\]

Equation 3.7

The combination of all these factors leads to Equation 3.8 where \(fa(i)\) is the estimated alveolar \(^{17}O\) enrichment at that breath.

\[
f_a (i) = \frac{A}{TV \cdot fiO₂ \cdot \alpha + FRC \cdot feO₂ \cdot f_a (i-1)} \cdot \frac{B}{(TV + FRC) \cdot fmO₂}
\]

Equation 3.8

The numerator in the equation specifies the volume of \(^{17}O\) while the denominator specifies the overall volume of oxygen. To convert fmO₂ to the overall volume of O₂ in the denominator, it must be multiplied by the total volume TV+FRC. The portion of the equation labeled \(A\) represents an incoming breath (Equation 3.1), the portion labeled \(B\) accounts for the uptake of oxygen into the body from the
lungs (Equation 3.4 and Equation 3.6 to take consumption into account). The portion labeled $C$ normalizes the equation to units of fractional enrichment.

Using this model, the first minute of $^{17}$O$_2$ consumption was simulated according to the parameters shown in Table 3.1.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value (Ref)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV</td>
<td>18</td>
<td>mL/kg</td>
</tr>
<tr>
<td>$\text{fiO}_2$</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>FRC</td>
<td>50 (*)</td>
<td>mL/kg</td>
</tr>
<tr>
<td>$\text{VO}_2$</td>
<td>5 (**)</td>
<td>mL/kg/min</td>
</tr>
<tr>
<td>RR</td>
<td>6</td>
<td>breaths/min</td>
</tr>
</tbody>
</table>

References * (154) ** (155,156)

Table 3.1. Parameters for simulation of $^{17}$O fractional enrichment absorbed during the first minute of inhalation.
The results of this simulation, the fractional enrichment of the $^{17}$O in the brain tissue after each breath, are shown in Figure 3.4. This simulation estimates that there is a 10 second delay from the inhalation start time until the inhaled oxygen-$^{17}$ reaches the brain. There is a 2.4 second delay from the pump to the airway (Figure 3.2). From there, the lung to brain transit time is estimated at 7 seconds (157). This is very close to 10 seconds.

![Figure 3.4](image)

**Figure 3.4.** Simulation of $^{17}$O tissue enrichment during inhalation. Plotted is a step function simulation of brain tissue $^{17}$O fractional enrichment in oxygen gas when 15 breaths of 20% oxygen at 70% $^{17}$O enrichment is given to a pig with the parameters in table 1. The time of delivery for the CMRO2 estimates in this paper is 6 breaths (1 minute), but data out to 15 breaths is shown here to demonstrate the time to asymptote at the delivered enrichment. Delivery of gas begins at time 0, but a 10
second delay is given for breathing circuit delay, mixing time, and uptake and transport time of oxygen to the brain.

From the average of points at 10 seconds through 59 seconds, these simulations estimate a mean of 58.81% of the delivered gas concentration being metabolized during the CMRO₂ calculation time (10-59sec). This is a significant correction that must be considered when computing CMRO₂ in the next chapter.
CHAPTER 4: ESTIMATION OF REGIONAL CMRO₂ WITH PROTON DETECTED $^{17}$O MRI DURING PRECISION $^{17}$O₂ INHALATION

4.1. Introduction

Since the vast majority of $^{17}$O studies have been performed on small animals, the objective of this chapter is the measurement of CMRO₂ with clinical hardware with the goal of human studies using $^{17}$O₂. As such, adolescent swine that are the size of an adolescent human are used with similar cardiovascular and respiratory systems to an adult human in a 1.5 Tesla clinical scanner. The scaling of metabolic measurement by $^{17}$O to large animals and humans presents some unique challenges. First, $^{17}$O₂ gas is expensive, and the body sizes of large animals dictate much more tracer must be used than in small animals. Therefore, short time course inhalation is used here for large animals, the shortest yet reported for $^{17}$O₂, and the uptake is modeled for slowly breathing, large lung volume, large circulation volume animals. A low loss, precision breathing circuit is employed for this delivery. With such a short time course of inhalation, fast sequences must be used with sufficient contrast-to-noise, signal-to-noise, and spatial resolution to make measurements (Chapter 2). This must be used without decoupling due to radiofrequency power amplifier limitations on our clinical scanner that are
common to most clinical scanners. Still, decoupling can be used with these same techniques if a high spin lock amplitude or second transmit channel is achievable, but with magnet stability and without subject motion decoupling may not be necessary as control images may be taken immediately before the delivery of $^{17}$O$_2$ gas.

Similar scaling issues hinder the human application of direct $^{17}$O techniques at high field as well. For example, while $^{17}$O in H$_2$-$^{17}$O has a short T$_1$ which allows for very rapid acquisition and averaging in small animals, specific absorption rate limits applied to humans partially hinders this advantage. It is well established that the low sensitivity of the nucleus presents SNR limitations for detection. Unfortunately, the dramatic signal to noise gain granted by using very small radiofrequency coils on small animal heads is abolished unless very superficial H$_2$-$^{17}$O measurements are made in humans with small coils. Further, at least as much $^{17}$O gas, scaled for body weight, will be required and the efficiency and precision of delivery should be considered in that case as well.

One additional consideration is required. To arrive at the CMRO$_2$, the arterial input function must be known for the recirculation of metabolic H$_2$-$^{17}$O ($m$H$_2$-$^{17}$O) produced elsewhere in the body. This recirculation must be either modeled
(100,125), measured (99), or otherwise accounted for in order to obtain CMRO₂. In large animals there will be a time delay between the start of mH₂¹⁷O generation in parenchyma and the start of incoming metabolically generated water washed in from other tissues after ¹⁷O₂ delivery. During this time delay, PET studies have estimated CMRO₂ in normal subjects (158). While an analogous recirculation delay for ¹⁷O is expected and has been suggested (92), no group has combined ¹⁷O delivery in a large animal model with fast imaging techniques to further investigate the possibility of measuring CMRO₂ from the pre-recirculation time.

Therefore, the three purposes of this chapter are: (i) to demonstrate the combined use of low loss precision breathing delivery circuit to minimize ¹⁷O gas use combined with fast imaging on clinical scanners to measure the kinetics of metabolically produced H₂¹⁷O in large animals. (ii) Determine the time delay before significant wash-in of H₂¹⁷O occurs from other regions. (iii) Show that CMRO₂ can be estimated in large animals by measuring the formation of mH₂¹⁷O in brain during the recirculation delay with single-shot low amplitude T₁ρ imaging. In total, this work provides a basis for future ¹⁷O studies in humans.
4.2. Materials and Methods

4.2.1. Animal Care

All animal experiments were approved by the Institutional Animal Care and Use Committee and the guidelines of the “Principles of laboratory animal care” were followed. Pigs weighing 19kg to 41kg were used. Induction of anesthesia was performed by an initial IM injection of ketamine 22mg/kg, xylazine .025mg/kg, and atropine .05mg/kg and maintained with ketamine 60mg/kg/hr and diazepam 2mg/kg/hr. Fluid balance was maintained with normal saline at 2ml/kg/hr. Animal heart rate and oxygen saturation were monitored with a standard infrared pulse-oximeter and full oxygen saturation (98-100%) without fluctuation was required for the experiments to proceed. All experiments begin with T₁-weighted sequence for localization and anatomical imaging of the brain.

The breathing circuit described in Chapter 3 was utilized with a respiratory rate set to 6 breaths/min and a tidal volume of 18 times the weight in kg. In these experiments, 1.2 times the tidal volume of enriched $^{17}\text{O}_2$ was diluted with nitrogen to a final concentration of 20% enriched $^{17}\text{O}_2$ and 80% N₂ and delivered over 6 breaths. Delivery begins at time 0 in all plots.
4.2.2. Determination of CMRO₂ after \(^{17}\text{O}_2\) inhalation

Beginning with Equation 3.1, for the calculations here we make a number of assumptions. First, there is a natural abundance of H\(_2^{17}\text{O}\) in naturally occurring water (20.35µmol/g). But, it is assumed that this labeling is constant throughout the time of the experiment and that all change is due to metabolic production. The first part of the equation represents locally produced mH\(_2^{17}\text{O}\) while the second part represents outflow and inflow of mH\(_2^{17}\text{O}\). In the short time course of \(^{17}\text{O}_2\) measurement where there is not significant recirculation of mH\(_2^{17}\text{O}\), \(C_a(t) - C_v(t)\) approximates 0 during the measurement time. This is a common assumption in the \(^{17}\text{O}\) and \(^{15}\text{O}\) PET literature. Then integration of Equation 3.1 with this simplification yields,

\[
\Delta C_b = \int_{t_0}^{t} \frac{2\alpha(t)}{f_1}(\text{CMRO}_2)
\]

Equation 4.1

where \(\Delta C_b\) is the change in mH\(_2^{17}\text{O}\) concentration over the time of \(t_0\) to \(t\), the time of the CMRO₂ calculation (previously derived in (149)). Equation 4.1 can then be reduced as follows.

\[
\text{CMRO}_2 = \frac{\Delta C_b f_1}{2f'}
\]

Equation 4.2
As in section 3.8, $\alpha(t)$ cannot simply be approximated by $\alpha$, and instead is replaced by the average enrichment over the time of CMRO$_2$ calculation, $f$. The amount of water generated per minute can be solved from the signal change by the equation for finding the concentration of a T$_2$ contrast agent, Equation 1.25. That equation is from Stolpen’s work (105), but it is restated here in the case of brain detection where $\Delta C_b$ is analogous to $\Delta f$ and T$_1$ detection where $R_2$ approximates $R_{1p}$ at 100Hz as described in Section 1.13.1.

$$\Delta C_b = \frac{\ln \left( S_i / S_o \right)}{TSL \cdot R_{1p}}$$  \hspace{1cm} \text{Equation 4.3}

Instead of using the echo time, TE, the constant TSL is the sum of the T$_{1p}$ preparation time and the time from the first excitation to the first (center line of k-space) echo time. Combining Equation 4.2 and Equation 4.3 yields the final equation used to calculate CMRO$_2$ for indirect imaging in Equation 4.4.

$$\text{CMRO}_2 = \frac{\ln (S_i / S_o(t)) f_1}{TSL \cdot R_{1p} \cdot 2f}$$  \hspace{1cm} \text{Equation 4.4}

Until now we described how to solve for a change in H$_2^{17}$O water concentration without units of time. For the purposes of measurement during an experiment, the term of $S_i$ represents the initial signal while the $S_o(t)$ represents the signal change due to H$_2^{17}$O per unit time. In this case signal change is measured over
about 50 seconds, but the final measurement is based on the linear fit of change over that time, then extended to one minute to produce signal change per minute.

For direct $^{17}$O spectroscopy, the change in signal is directly proportional to the increase in concentration of mH$_2^{17}$O, leading to Equation 4.5,

$$\text{CMRO}_2 = C_b(0) \frac{(S_o(t)/S_i)}{2f^1}$$

where $C_b(0)$ is the concentration of H$_2^{17}$O at the start of the experiment (in units of $\mu$mol H$_2^{17}$O/g water), $S_i$ is the signal of $^{17}$O at the start of the experiment, $S_o(t)$ is the final concentration after the measurement period of metabolism from a linear fit of the rising signal over the calculation time, and $f_1$ is set to .77 (159).

The cerebral metabolic rate of oxygen consumption is calculated from the first 50 seconds of oxygen inhalation, after a 10 second delay. This delay is due to the delay in signal change, as expected between the start of inhalation and cellular conversion to water due to breathing circuit delay, mixing time, and uptake and transport time of oxygen.
4.2.3. Magnetic Resonance Imaging

Direct imaging was performed on a broadband-enabled 3T Siemens Trio scanner. Proton images were taken with the body coil and then without moving the pig, a custom built interface was connected to the scanner and interfaced with a home built 9cm surface coil tuned to 16.71MHz placed on top of the pig’s head. Calibration of the rectangular pulse used was performed by taking a number of gradient recalled echo images and maximizing signal-to-noise over the brain region. One $^{17}$O image was taken in each plane. Parameters were as follows: repetition time (TR) 100ms, TE 1.8ms, field of view (FoV) 40x40cm, 64x64 matrix, bandwidth 200Hz/Pixel, asymmetric echo. Following that, a series of pulse acquire spectra were taken with the same hard pulse over 15 minutes. Pulse acquire parameters were: TR 100ms, 256 points, two step phase cycling, 40kHz bandwidth, repeated 9000 times over 15 minutes.

Indirect imaging was performed on separate occasions on a 1.5T Siemens Sonata scanner. Images were taken with a 15cm vendor supplied surface coil placed on the head of the pig. Serial images during room air and $^{17}$O$_2$ delivery were taken with a $T_{1p}$-prepared single-shot, high flip angle, centrically encoded, fully-balanced gradient echo sequence reported shown in Figure 3. A more thorough treatment of this sequence and its use to detect mH$_2^{17}$O in vivo are presented in a separate manuscript (160). Parameters were: TR 9.7ms, TE 4.7ms, slice
thickness 6mm, FoV 200mm², 128x128 matrix, bandwidth 130Hz/Pixel, flip angle 180° (opening pulse 90°), spin locking amplitude 100Hz, spin locking time 75 or 200ms, fat saturation on, time per image 1.6 seconds, 2 second delay for T₁ recovery.

4.2.4. Arterial Blood Sampling and $^{17}$O NMR spectroscopy of blood

An arterial catheter was placed under ultrasound guidance into the femoral artery in the large animal fluoroscopy suite. During imaging, approximately 2cc of blood was collected over 3-5 seconds into each Vacutainer Serum Separator Tube (Becton-Dickinson, #367983) at a rate of one sample each 10 seconds for the first 12 samples and then 60 seconds each for six more samples. Four control samples were taken before the experiment began. The tubes used are designed to reseal immediately after needle puncture. Their ability to remain sealed was tested by taking several tubes at random, creating a strong negative pressure inside the tube with a vacuum pump attached to a needle, and then repeated puncture with larger diameter needles than used for blood sampling. The pressure inside the tubes was checked after over a dozen punctures and had changed less than 10%. Storage of a sampling of tubes over a period of a week showed no repressurization over time.
A concern is that water $^{17}\text{O}$ will exchange with ambient $\text{CO}_2$ through bicarbonate ion, a very fast reaction when catalyzed by carbonic anhydrase as in whole blood (161,162). To minimize losses to the air at the time of analysis, serum was separated by centrifugation soon after the experiment to remove the carbonic anhydrase from analyzed fluid. Centrifugation places a gel between the serum and red blood cells in these tubes to fully separate the two. Tubes were then frozen at -20C and stored sealed. This procedure serves to protect the samples in two ways. First, because the tube is sealed, blood can mix only with the small amount of $\text{CO}_2$ (~450 parts per million) in the small amount of air (<1mL) drawn into the tube, a trivial source of mixing. A small amount of mixing can occur with the blood bicarbonate, but this reaction is extremely fast and occurs in the body regardless. As blood bicarbonate is <30mM (163), this represents a trivial loss of $^{17}\text{O}$. Second, carbonic anhydrase has almost no activity in serum (164). So when the serum is re-exposed to air, the reaction catalyst is missing, and the NMR measurement completed within 10 minutes suggests that the loss of $^{17}\text{O}$ is less than 5% during that time (165).

An 11.7T Bruker DMX400 Avance Spectrometer equipped with a 1H/X nucleus decoupler probe tuned to $^{17}\text{O}$ was used to measure 1mL of each sample loaded into 5mm ID NMR tubes just before analysis. Spectra were recorded without lock.
at room temp. The parameters for $^{17}\text{O}$ spectroscopy were: TR 41ms, 4096 points, bandwidth 50kHz, 4000 averages, flip angle 90°.

4.2.5. **Data Analysis**

For the direct measurement of mH$_2^{17}\text{O}$ production, the integrals of the pulse acquire spectra from 4 averages per data point (400ms each) were normalized to the natural abundance. The signal trace during $^{17}\text{O}_2$ delivery was fit linearly over 50 seconds beginning 10 seconds after the start of the $^{17}\text{O}_2$ pulse to generate the mH$_2^{17}\text{O}$ production in Table 4.1. The slope and standard error of the linear fit was used in Equation 4.5 as $S_o$. The plots of the direct signal change were temporally smoothed over 16 seconds before and after to show the trend in signal change.

For indirect measurement of CMRO$_2$, region of interest analyses were performed by segmenting each half of the pig brain without including ventricles based on anatomical contrast images and a pig brain anatomy atlas. The time course of each ROI in the indirect imaging was temporally smoothed over 16.2 seconds (4 points) before and after to filter high frequency noise. A linear fit was performed over 50 seconds approximately 10 seconds after the start of the $^{17}\text{O}_2$ pulse, and the slope of that line was used to estimate the signal change and fitting error per minute as in Equation 4.4.
To compute the statistical significance of the slope of signal change due to mH$_2^{17}$O production evaluated against no change, a standard formula was applied. The t-score (t) is found according to (166),

\[ t = \frac{b_1}{SE} \]

where $b_1$ is the slope and $SE$ is the standard error of the slope. With 15 points per measurement, the degrees of freedom is 13, which requires the slope to be 2.16 times greater than the standard error for $p<.05$ (two-tailed). All hemispheric measurements made in this chapter fulfill this requirement except for one.

### 4.3. Arterial Blood Sampling

Blood sampling was performed during scanning on two separate occasions. The delay between the start of $^{17}$O$_2$ delivery and the start of wash-in is defined as the time until there is a data point greater than the standard deviation of the natural abundance measurement. This is shown in Figure 4.1 to be at least 60 seconds in 40.8kg and 19.8kg pigs. This verifies that recirculation of mH$_2^{17}$O is minimal until at least 60 seconds after the start of $^{17}$O$_2$ delivery, and thus verifies the validity of Equation 4.1 for this purpose.
Figure 4.1. Serial arterial blood sampling with $^{17}$O$_2$ delivery. Shown are the results of a serial arterial blood sampling experiment during and after one minute of $^{17}$O$_2$ delivery. Plot a is for a 40.8kg pig and plot b is for a 19.8kg pig. Delivery of $^{17}$O$_2$ as 80% N$_2$/20% O$_2$ (70% enriched) begins at time 0 seconds and ends at 60 seconds as in all the following figures in this chapter. Serum concentration of H$_2^{17}$O was calculated by integrating the H$_2^{17}$O peak obtained by $^{17}$O spectroscopy of the blood
samples taken at the given time points. The solid and dashed lines are 2 point moving averages of the data points. The first data point is an average of 5 samples taken before the start of $^{17}$O$_2$ delivery, set at 20mM H$_2^{17}$O. The error bars are the standard deviation of those initial 5 points of 0.17 mM (.8%) in part a and .19 mM (.9%) in part b. In both pigs there is no point one standard deviation over the baseline (>30% the 180 second point) until at least 60 seconds after inhalation begins. Sustained recirculation (over 2-3 minutes) is on the order of .6mM added H$_2^{17}$O. Plots up to 5 minutes remain stable at those levels. The two points at 80 and 90 seconds in part b are uncharacteristically and unexplainably high. Removal of those two points makes part a and part b appear very similar.

4.4. Direct $^{17}$O MRI and unlocalized MRS in a swine

Figure 4.2a-c shows direct $^{17}$O images taken at 3 Tesla with a surface coil placed on the head of the pig in each plane to demonstrate the area examined by the time series spectra in Figure 4.2d. This is overlaid on $^1$H images taken with the body coil. Figure 4.2d shows a time series plot derived from the integrals of $^{17}$O spectra taken with the same coil configuration and excitation pulse.
Figure 4.2. Direct $^{17}$O MRI and unlocalized MRS in a swine. a.-c. Three color scaled $^{17}$O images taken before $^{17}$O$_2$ inhalation (natural abundance) are overlaid over grayscale proton images from the same locations (Tra-Transverse, Sag-Sagittal, Cor-Coronal). A color bar on the right shows the linear relative $^{17}$O signal. The differences in signal are mostly due to the surface coil sensitivity profile and not due to any differences in $^{17}$O content except in large difference areas such as the air spaces. This is presented to show the area that H$_2^{17}$O is averaged over in the measurements for pig 1, also in Fig 6a. d. The same excitation pulse as in a-c was
used to take serial pulse acquire spectra and the H$_{2}$O peak integrals are plotted over time to indicate increase in µmol H$_{2}$O/g water.

4.5. Correlation of observed indirect and direct signal

The correlation of indirect and direct imaging is shown in Figure 4.3a. Figure 4.3b shows the time series plot of blood sampling in Figure 4.1a combined with direct imaging shown in Figure 4.2d.
Figure 4.3. Correlation of observed signals. a. The metabolic water change measured by separate experiments using direct $^{17}$O spectroscopy and proton-detected $\text{H}_2^{17}$O is presented to show the similarity in vivo. The direct $^{17}$O spectroscopy plot is used to derive the CMRO$_2$ for pig 1 ($1.19 \pm 0.43$). The indirect $^{17}$O trace is the result of whole brain ROI analysis (excluding ventricles) from pig 2 ($1.17 \pm 0.21$). Excellent agreement between the indirect and direct signals verifies the
specificity of the current methods. The indirect signal is converted to concentration of mH$_2^{17}$O by Equation 1.25. b. Demonstrated is the rise in metabolic water from brain without a concurrent rise in recirculated metabolic water. The difference between the two lines indicates the CMRO$_2$. Since the difference between them is so great, the recirculation can be effectively ignored.

4.6. CMRO$_2$ map and calculations for a series of swine

Figure 4.4 shows an example image and metabolic map.

![Image of baseline swine metabolic map]

Figure 4.4. Baseline swine metabolic map. a. An example image taken during the time series of pig 5. Areas of enhancement correspond to regions at least partially filled with cerebral spinal fluid. b. A pixel-by-pixel linear fit for pig 5 during the delivery of $^{17}$O$_2$ converted to CMRO$_2$ per pixel, cropped to fit the brain. The smoothing function used is a 1 pixel Gaussian, leading to a final nominal resolution.
of $3.6 \times 3.6 \times 6\text{mm}$ at full width half maximum. The largest change is seen around the edge of the brain representing brain cortex and in the brainstem, both areas expected to have the highest metabolism. Some noise is seen, however very low metabolism is seen in high fluid regions such as in the ventricles.

Figure 4.5 demonstrates the changes in $\text{mH}_2^{17}\text{O}$ post $^{17}\text{O}_2$ delivery during the first 60 seconds and shows the linear fit to the data points used to calculate CMRO$_2$. The slope of the signal change in half of the brain in one central slice of brain gives the hemispheric $\text{mH}_2^{17}\text{O}$ production and that is calculated for each hemisphere in Table 4.1 and converted to CMRO$_2$ measurements.

Figure 4.5. Hemispheric metabolic signal changes. The hemispheric changes (Right and Left) in water production used to generate CMRO$_2$ estimates in the swine listed in Table 4.1. The signal from each ROI at each time point was entered into Equation
6 to convert to units of µmol mH$_2^{17}$O/g water. The straight lines show the linear fits to the individual points, the slope of which is the slope referred to in Table 4.1. The small amount of deviation over that time represents the standard error of the measurement.

Table 4.1. Estimated cerebral metabolic rates of oxygen consumption (CMRO2)

<table>
<thead>
<tr>
<th></th>
<th>Direct $^{17}$O</th>
<th>TSL</th>
<th>CMRO$_2$</th>
<th>CMRO$_2$ error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f$</td>
<td>$f_i$</td>
<td>µmol/g/min</td>
<td></td>
</tr>
<tr>
<td>Pig 1 Whole Brain</td>
<td>.41</td>
<td>.77</td>
<td>1.19</td>
<td>0.43</td>
</tr>
<tr>
<td>Pig 2 Left Hemisphere</td>
<td>.24</td>
<td>205</td>
<td>1.03</td>
<td>0.33</td>
</tr>
<tr>
<td>Pig 2 Right Hemisphere</td>
<td>.24</td>
<td>205</td>
<td>1.30</td>
<td>0.26</td>
</tr>
<tr>
<td>Pig 3 Left Hemisphere</td>
<td>.24</td>
<td>205</td>
<td>1.49</td>
<td>0.39</td>
</tr>
<tr>
<td>Pig 3 Right Hemisphere</td>
<td>.24</td>
<td>205</td>
<td>1.63</td>
<td>0.62</td>
</tr>
<tr>
<td>Pig 4 Left Hemisphere</td>
<td>.41</td>
<td>80</td>
<td>0.88</td>
<td>0.52</td>
</tr>
<tr>
<td>Pig 4 Right Hemisphere</td>
<td>.41</td>
<td>80</td>
<td>1.46</td>
<td>0.7</td>
</tr>
<tr>
<td>Pig 5 Left Hemisphere</td>
<td>.41</td>
<td>80</td>
<td>1.34</td>
<td>0.33</td>
</tr>
<tr>
<td>Pig 5 Right Hemisphere</td>
<td>.41</td>
<td>80</td>
<td>1.54</td>
<td>0.59</td>
</tr>
<tr>
<td>Indirect Average</td>
<td></td>
<td></td>
<td>1.33 ± 0.26</td>
<td></td>
</tr>
</tbody>
</table>

127
The $f$ values correspond to the fractional enrichment of $^{17}$O delivered where .41 corresponds to delivery of 70% $^{17}$O$_2$ and .24 corresponds to delivery of 40% $^{17}$O$_2$ (58.81% of the isotopic enrichment from Figure 3.4). Taken together, these demonstrate that CMRO$_2$ can be calculated by the signal change of 1 minute of inhalation by the indirect technique at 1.5 Tesla.

4.7. Chapter Discussion

The CMRO$_2$ of pigs of comparable size using a similar continuous infusion ketamine and benzodiazepine anesthesia regimen has been estimated to be $1.63 \pm .19 \ \mu$mol/g/min by $^{15}$O PET (167). The spatial resolution of those measurements presented here is the same as those $^{15}$O PET images without the need for an onsite cyclotron or radioactivity. Still, differences in pig CMRO$_2$ found in the literature vary significantly depending on the technique, anesthesia regimen, and size of pig used. One study averages $1.20 \pm .29 \ \mu$mol/g/min and $1.15 \pm .46 \ \mu$mol/g/min by radioactive and fluorescent microspheres respectively (168). Another radioactive microsphere study in pigs of different ages found CMRO$_2$ ranging from $1.30$ to $1.75 \ \mu$mol/g/min (169). The measurements made here compare favorably to these values.
The major technical limitation of $^1$H detected $^{17}$O imaging is the relatively small effect of physiologic levels of $^{17}$O on $^1$H relaxation. We are confident that this problem can be overcome in human applications. The human brain is so much larger than pig brain that region of interest analyses should be more easily obtainable and more meaningful. The $^{15}$O PET literature lends evidence for this claim. The CMRO$_2$ maps taken from humans (170) are more impressive than those taken from swine (171), and quantification can then be made regionally instead of hemispherically. As humans are far more cooperative than pigs and do not require anesthesia, human studies should be easier to perform. Further, as units of $^{17}$O will cost less as more is produced, it is hoped that the cost of these techniques will be improved by increased interest and usage by the research community.

It is unclear if these techniques will be able to measure CMRO$_2$ in functional MRI due to the limited sensitivity of the technique and the competition from the flow and blood oxygen level dependent effects. Electrical stimulation of the paw in rats for example imparts a 19% increase in CMRO$_2$ (172) which is a small and very spatially localized change. Future improvements in these techniques may make this possible. Nevertheless, the primary intent of our group is to develop these techniques for use in major disturbances in metabolism, such as ischemia, tumor, and neurodegeneration, whose metabolism cannot be studied by existing
functional MRI techniques. Studies are underway to demonstrate this possibility, and future studies in humans should become easier due to the reasons listed above.

It must be noted that the equations used to quantify CMRO$_2$ have sources of potential error for absolute measurements. The value of the relaxation enhancement due to $^{17}$O on $^1$H, R$_2$, for example is difficult to measure in vivo or with ex vivo tissues, and as such it may be imprecise. As well, R$_2$ may vary based on different tissue parameters such as grey or white matter or cerebral blood volume per voxel. The value of $f$, the tissue fractional enrichment of $^{17}$O, chosen is based from a very simple, approximate model of inhalation appropriate for averaging over several breaths; however more sophisticated modeling could potentially be used. This factor has not been considered in the estimation of CMRO$_2$ with $^{17}$O$_2$ previously because small animals have high respiratory rates and the slurring of the $^{17}$O fractional enrichment may be neglected because gas mixing in the lungs occurs much more quickly. Also, it becomes more reasonable to neglect $f$ with longer time periods of inhalation where the fractional enrichment has had sufficient time for equilibration with the enriched $^{17}$O gas. Despite these considerations, relative CMRO$_2$ in the same subject or across subjects can be estimated using the current techniques.
This study was performed with a body coil transmitter and surface receiver, which is suggested for the high homogeneity of the body coil for spin-locking. Decoupling of the $^1$H-$^{17}$O interaction can be performed with high spin lock amplitudes as well. While decoupling is not performed in this study due to implementation difficulties and specific absorption rate concerns on clinical scanners, decoupling experiments similar to those previously conducted (112,173) can be performed using the sequence and delivery techniques presented here. Also, it may be possible that the same estimates could be made with fast $T_2$ weighted sequences such as spin echo echo planar imaging or single shot turbo spin echo methods. In particular, spin echo echo planar imaging presents the possibility of obtaining 3D metabolic images. However, caution must be exercised using sequences, as any possible distortion or artifact may mask the small proton signal change generated by physiologic short-term $mH_2^{17}$O production. $T_{1p}$ imaging, as shown here, enables us to measure the small changes due to $mH_2^{17}$O that may not be seen with other sequences due to artifacts that may obscure small signal changes in long $T_2$-weighted images or due to artifacts that cause spatial distortions from echo planar imaging.

While it is recognized that the anesthesia regimen may change the vascular recirculation of $mH_2^{17}$O as opposed to awake humans, multiple $^{15}$O PET studies have modeled the time course of recirculation for metabolic water using similar
time courses with a single breath hold in humans and these have also made CMRO$_2$ measurements claiming recirculation during the first minute to be negligible (158,174). Indeed, the measurements made here and the modeling used for CMRO$_2$ measurement is very similar to $^{15}$O experiments performed in humans (137). In that study, 40 seconds were used to measure CMRO$_2$ after a 20 second delay after start of inhalation. Recirculation of H$_2$^{15}O was measured and found to be minimal during that time. The error in CMRO$_2$ calculation ignoring wash-in was simulated to be less than 15% for very critically low values of oxygen extraction fraction (<.15), and less than 5% for typical oxygen extraction fractions (>.3). With the current data and the PET data taken together, it seems the recirculation delay is comparable in humans, and as such, anesthesia in pigs is not thought to be a significant factor for the kinetics of mH$_2$^{17}O recirculation. If anything, these experiments should become easier to perform in humans who have larger brains, higher resting brain metabolic rates, and do not require anesthesia for imaging.

Another difference between the current study and many short time course PET studies is that for delivery PET studies frequently use a single tidal volume inhalation of tracer with a breath hold. We do not employ this strategy here for several reasons. First, any fluctuation in blood oxygen content results in a change in MRI signal (70,175). A single bolus of 100% oxygen after breathing room air
will cause an increase in MR signal that will interfere with the measurement (suggested by above references and demonstrated in Section 2.8). A large single breath of $^{17}\text{O}_2$ could potentially be held longer to decrease the amount of exhaled $^{17}\text{O}$, however this risks even small amounts of hemoglobin desaturation which will artificially enhance the $^{17}\text{O}$ effect by $T_2^*/T_2/T_1\rho$ reduction by paramagnetic venous deoxyhemoglobin. Still, with careful bolusing and hemoglobin saturation accounting, a single breath MRI method could be obtained that would use approximately the same amount of $^{17}\text{O}_2$ used here.

In conclusion, we have demonstrated the feasibility of measuring CMRO$_2$ in large animals on clinical scanners using a single tidal volume of $^{17}\text{O}_2$ gas delivered with a precision delivery circuit. By minimizing gas required for CMRO$_2$ measurements, employing a large animal model, and utilizing clinical hardware, this represents a crucial first step towards the translation of small animal $^{17}\text{O}_2$ studies to humans. A simple model of lung mixing and delivery to tissues is presented. Arterial blood sampling and analysis of mH$_2^{17}\text{O}$ content shows the time when recirculation begins to be 60-80 seconds--ample time for fast imaging techniques to obtain numerous images for CMRO$_2$ estimation. High temporal resolution indirect and direct imaging is correlated to show similar results for the estimation of CMRO$_2$ non-invasively during those 60 seconds. Work is already underway to detect experimental derangements of metabolism. It is hoped that
future studies will detect metabolic derangements in humans using these
techniques. For indirect imaging only a clinical 1.5T scanner with standard
hardware and an easily programmable pulse sequence is necessary.
5.1. Introduction

There are some drawbacks to using large animals for the purpose of studying metabolism. In the setting of cerebral ischemia, large animal models require invasive surgery for production of focal ischemia (176). In swine this typically involves one of a transorbital approach with direct vessel obstruction (177) or cutting a hole in the skull and applying direct pressure to the brain itself to produce ischemia (178). These measures are required because swine, like other large animals, have a well collateralized cerebral circulation (179,180). One less invasive way of causing global metabolic change is by induction of hypothermia. This was shown recently in rats with 17O MRS (96) and hypothermia has also been shown to reduce the metabolism of swine (168). Still, the lack of uncertainty about core body temperature and instability in body temperature led us to seek a more stable model for metabolic derangement.

From this we settled upon a different approach for the derangement of oxidative metabolism for study with 17O techniques: the administration of 2,4-dinitrophenol (DNP) to stimulate metabolism. In brief, DNP acts a proton shuttle
that disrupts the mitochondrial proton gradient used by ATP synthase. This disruption stimulates increased metabolism by cells to reestablish the proton gradient, the net effect of which is an increased conversion of \( \text{O}_2 \) to \( \text{H}_2\text{O} \) by complex IV. While DNP has been used in humans and many animals, there are no previous studies outlining the drug’s effects in swine (181,182).

The goal of this manuscript is to characterize the effects of large animal stimulation with DNP and assess its suitability for studies of metabolism. Changes in metabolism are then addressed by indirect \(^{17}\text{O} \) MRI and direct MRS techniques for the first time in large animals. This will provide additional evidence of the potential utility of indirect \(^{17}\text{O} \) MRI in humans.

5.2. Methods and Materials

5.2.1. Animal Care

All experiments were approved by our Institutional Animal Care and Use Committee. Swine of 25-40kg were anesthetized with ketamine 25mg/kg, medetomadine 0.1mg/kg, and glycopyrrolate 0.02mg/kg intramuscularly. Anesthesia was maintained with ketamine 20-80mg/kg/hr by continuous IV infusion. DNP was initially found to decrease blood glucose to levels undetectably low by glucose strip analysis. All imaging experiments had blood glucose levels
maintained at a normal level by the addition of continuous infusion of .045% saline, 5% dextrose, 20mEq/L potassium chloride solution. Swine were ventilated through an endotracheal tube with the custom precision delivery breathing circuit that maintained a respiratory rate of 8 breaths per minute and produced a tidal volume of approximately 25mL/kg. This stabilized arterial blood saturation measured by pulse oximetry at ~100% at all times. After the experiment, all animals were euthanized with pentobarbital while under anesthesia.

5.2.2. **Body O₂ consumption measured by exhaled gas analysis**

Whole body metabolism was measured by capture of exhaled gas over 3 breaths in a Tedlar gas sampling bag (Jensen Inert Products, Coral Springs, Florida) periodically during the experiment and the concentration of oxygen, and carbon dioxide was measured with a respiratory monitor (Gemini Analyzer, CWE Inc., Ardmore, PA). Measurements were converted to whole body oxygen consumption, VO₂ (mL/min), by Equation 5.1 (183),

\[
VO₂ = \left\{ \left[ \left( \frac{1 - FEO₂ - FECO₂}{1 - FIO₂} \right) FIO₂ \right] - FEO₂ \right\} VE \cdot RR \quad \text{Equation 5.1}
\]

where FEO₂ is the expired fraction of O₂, FECO₂ is the expired fraction of CO₂, FIO₂ is the inspired fraction of O₂ (room air, .208), VE is the volume of expired
per breath (tidal volume), and RR is the respiratory rate. Values are reported as oxygen consumption per weight by dividing over the weight of the animal (kg).

5.2.3. Dinitrophenol preparation

The DNP was acquired in the highest commercially available grade (>99.5% by certificate of analysis; Sigma-Aldrich D198501). As suggested previously (184), solutions were prepared within 1 day of use by adding 2.5mg/mL of DNP and 5mg/mL sodium bicarbonate (Sigma-Aldrich 13433) to .09% saline. This was then brought to a near boil with constant stirring to dissolve the bright yellow powder, returned to room temperature, and filtered through a .1 micron filter (Millipore SCVPU02RE).

5.2.4. Direct and indirect imaging

Imaging was performed as described in Chapter 4. In summary, single shot, low amplitude $T_{1p}$ prepared images were acquired in a 15 minute series at 3 seconds per image. After 5 minutes of room air inhalation, a mixture of 80% N$_2$/20% O$_2$ (70% $^{17}$O enriched) was inhaled for one minute. A center weighted average of 9 images measured just before the start of one minute of $^{17}$O$_2$ inhalation was used as a control for the signal dip that began at 9 seconds after the start of inhalation.
The signal drop over the following 51 seconds was fit linearly and converted by Equation 4.4.

This was performed twice, once before DNP administration and once 30 minutes after DNP administration. Image parameters were as follows: TR=3s, TE=5ms, BW=130Hz/Px, FoV=256x256, Matrix=128x128, slice thickness=5mm, flip angle=180°.

Direct measurements were made by placing a 9cm surface coil over the head of the animal at the 17O frequency. A series of free induction decays were acquired once before DNP administration and once after 30 minutes after DNP administration. Image parameters were as follows: TR=100ms, 256 points, BW=40kHz, two step phase cycling, repeated 9000 times over 15 minutes. Data were binned into 30 averages per point (3 seconds) and processed according to Equation 4.5.

The estimated average enrichment of 17O2 reaching tissue over the measurement time, f=.55, was based on the model presented in Figure 3.4 with a tidal volume of 25 mL/kg, inhaled oxygen fraction of .2, 17O enrichment of gas of .7, functional
residual capacity of 50, VO$_2$ of 5mL/kg/min (measured in this study as 4.9 +/- 1.19, n=7), and an RR of 8 breaths/min.

5.3. Whole body responses to DNP

DNP served as an excellent experimental model of oxygen metabolism stimulation. A concern in the use of DNP is animal temperature increase; however, we observed temperature changes after DNP administration of 1°C or less in all but one pig. Whole body metabolic stimulation was very stable owing to the long half-life of DNP as shown for two pigs in Figure 5.1.

![Figure 5.1. Whole body DNP oxygen stimulation time course. Two animals were administered 6mg/kg of DNP twice at the times indicated by the red arrows (data](image-url)
points at 0 and 30 minutes were taken just before). The points at time 0 represent an aggregate of 3 control measurements, and the two standard deviations are .02 and .17 mL O₂/kg/min. The animals were followed for 3 hours to check for the stability of the metabolic stimulation. The very minor variations in metabolism on the time scale of the 15 minute imaging experiment indicates that DNP provides a stable metabolic boost for metabolism experiments.

DNP stimulated porcine whole body oxygen consumption by 11.9% +/- 2.5% per mg/kg animal weight of DNP administered intravenously (7 pigs, total of 16 doses). The pooled data are summarized in Figure 5.2.
Figure 5.2. Body O₂ consumption increase versus DNP dose. Plotted is the stimulation in whole body oxygen consumption for a series of animals given DNP. For each point 3 baseline measurements were compared to 3 measurements after administration of the DNP. The Y-axis shows the increase in O₂ consumption measured by whole body exhaled gas analysis. The individual points are shown for each of 7 pigs and a pooled regression was performed. All pigs receiving DNP showed an expected and consistent increase in metabolism.

5.4. $^{17}$O₂ imaging of pigs before and after DNP stimulation

Five pigs were given a bolus of $^{17}$O₂ before and after DNP administration during indirect imaging. Hemispheric CMRO₂ was computed from the initial dip of signal change. A pixel by pixel map of this signal change converted to units of
CMRO$_2$ is shown in Figure 5.3. ROIs were chosen from each midline to the outside of the slice, not including lateral ventricle for each animal. From the ROI signal change over time, the mean change per hemisphere was derived by the decrease in signal over the first minute after inhalation. The data for all 4 pigs are shown in Figure 5.4 and compared to the whole body VO$_2$ change in Table 5.1. Overall, CMRO$_2$ tended to increase slightly more than whole body metabolism (Increase=17%, SD=10%, p=.08). A sixth pig underwent direct, unlocalized $^{17}$O spectroscopy at 3T to verify the indirect measurements and those results are included in Table 5.1.
Figure 5.3. Metabolic map of CMRO\textsubscript{2} pre- and post-DNP. (A) The image shows the corresponding coronal (magnet axial) T1-weighted image slice of the pig brain. (B) The map displays cerebral metabolism based on the signal decline after \textsuperscript{17}O\textsubscript{2} administration. (C) 9mg/kg DNP was administered to the pig and a 30 minute delay was introduced for stabilization of the DNP effect. The imaging with \textsuperscript{17}O\textsubscript{2} bolus was repeated and an increase in metabolism observed.
Figure 5.4. Summary of DNP with indirect $\nu$O$_2$ data. A series of 5 pigs were administered $\nu$O$_2$ as a metabolic tracer during imaging twice before (Pre) and about 30 minutes after (Post) DNP administration. The dip in signal from the generated H$_2$$\nu$O was converted into hemispheric (L-Left and R-Right) measurement of metabolism using the techniques described in the Methods.
Table 5.1. Summary of all DNP data. The estimated CMRO\(_2\) is compared to the increase in whole body metabolic measurements of metabolism by respiratory gas analysis. The metabolic change by \(^{17}\text{O}_2\) inhalation MRI is comparable to and slightly greater than the whole body O\(_2\) increase. The CMRO\(_2\) measured by unlocalized \(^{17}\text{O}\) direct spectroscopy is comparable to the CMRO\(_2\) obtained by hemispheric indirect measurements. The unlocalized spectroscopy includes surrounding skin and temporalis muscle surrounding the brain.

5.5. Chapter Discussion

Here we have measured the metabolic change due to DNP administered in a series of pigs to establish DNP as a model for oxidative metabolic change in the pig. The action of the drug directly stimulates oxygen metabolism by increasing the required proton flux within mitochondria to generate ATP. As such, DNP
directly manipulates the oxygen metabolism that is studied by $^{17}$O. We show this change to be stable over the course of an imaging experiment. The whole body metabolic stimulation is then compared to the brain metabolic stimulation by the indirect and direct $^{17}$O techniques to demonstrate the ability of these techniques to measure metabolism.

It is important to note that these studies have been performed using a large animal model while human approval for $^{17}$O$_2$ use is awaited, but these techniques are easily transferable to humans. The indirect measurements were performed using clinical equipment, and all of the imaging techniques can be directly translated to human use.

The metabolic map generated here is comparable in quality to the metabolic maps obtained by $^{15}$O PET (167), which also reports metabolism reported hemispherically, yet the $^{17}$O measurements are much easier to obtain as an onsite cyclotron to generate the three minute half-life $^{15}$O isotope is not required. Still, the results of the $^{15}$O experiments shed some light about the potential of $^{17}$O imaging. The brain size of humans is much greater than that of pigs. As such, while $^{15}$O measurements on pig brain are hemispheric, more recent $^{15}$O measurements on the much larger human brains can be more localized (185). We
anticipate $^{17}$O measurements in humans will be similarly more regionally or locally defined.

In conclusion, we have demonstrated the ability of indirect $^{17}$O techniques using about a single breath of $^{17}$O$_2$ to detect direct stimulation of cerebral metabolism in swine. This is performed with clinical hardware and techniques that would be easily adaptable to humans. We hope that future indirect $^{17}$O imaging will provide a measurement of oxidative metabolism for disease detection in humans.

5.6. Detection of the low contrast H$_2^{17}$O effect in tissue

A central concern to reviewers of this work has been that the raw signal change imparted by one minute of generated H$_2^{17}$O is small and that this may obscure the metabolic measurement. To investigate the sensitivity of indirect imaging to detect CMRO$_2$, a set of simulations were performed based on experimentally derived parameters.

First, let us consider the noise in a single scan. Hemispheric ROI signal values were acquired every 3 seconds over 5 minutes at the end of a 15 minute scan using the parameters in Section 5.2.4. It is assumed that the lack of systematic
variation in signal seen here indicates that it is primarily physiologic and thermal noise. The spatial SNR was estimated from an ROI of the entire brain divided by an ROI outside the brain from an image within this series. This yielded a spatial SNR of 425:1 with a voxel size of 2\times 2\times 5\text{mm}. Perhaps more important is the temporal SNR, as CMRO$_2$ is measured from the slope of the signal change over time. The temporal SNR was then determined for one hemisphere the size of a typical measurement in Chapter 4 and Chapter 5. This was measured to be .24\% from the standard deviation of the normalized hemispheric ROI signal over time. This signal over time is plotted in Figure 5.5.

![Figure 5.5](image.png)

Figure 5.5. A plot of observed noise over time. Specifically this is the 5 minute end of the pre-DNP scan for pig #2, left hemisphere.
As a further test of the possibility of improving temporal SNR by increasing the voxel size, we recorded noise from a second swine. In this swine, FoV was increased to 400x400mm to make a voxel size of 3.125x3.125x5mm, and signal was simply recorded over time without $^{17}$O$_2$ administration. Bandwidth, slice thickness, number of averages, and all other imaging parameters were kept the same. This yielded a measured spatial SNR based on a whole brain and a noise ROI of 1380:1. These points are plotted over time in Figure 5.6. The temporal noise standard deviation is almost identical. This suggests that the dominant contributor to the noise in these swine is physiologic fluctuation or from some hardware source that cannot be reduced by simply changing imaging parameters.
Figure 5.6. A plot of observed temporal noise with a larger voxel size. In this case the voxel size was 2.4-fold larger, yet the observed temporal noise was approximately the same.

To examine the ability of these techniques to measure CMRO2 during the first minute of delivery, the effect produced by $\text{H}_2^{17}\text{O}$ due to metabolism. An example of this without noise is seen in Figure 5.7 was simulated.
Figure 5.7. Simulated signal change due to metabolism without noise. Assuming that metabolism of $^{17}$O$_2$ begins at 9 seconds, is metabolized linearly, and progresses to 60 seconds (the period of the measurement), a metabolic rate of 1.33 µmol/g/min produces the signal change seen in the red squares. The stimulated metabolism due to DNP at 2.66 µmol/g/min produces the signal change seen in the blue diamonds.

Now let us add the empirically determined noise, .24%, to the calculated signal decline during metabolism as plotted in Figure 5.8. The simulated noise is based on a Gaussian distribution with mean of 0 and standard deviation of 0.24%. By taking measurements from a 9 point centered moving average as in the previous chapters, the random noise was smoothed over time to make more consistent
measurements. This line is the one that then received a linear fit to produce the metabolic measurement.

![Graph](image)

**Figure 5.8.** Simulated metabolic signal change with noise. The red squares show the signal change due to DNP-stimulated metabolism. The green squares show the signal change with the physiologic level of noise added. The blue line shows the 9 point moving average line of the green squares and that is fit to make the CMRO$_2$ measurement.

The simulation in Figure 5.8 was repeated to evaluate the effect of randomly generated noise. That is, random Gaussian noise was added to the metabolic signal change 20 times for each of the pre-DNP and post-DNP signal changes. The actual simulated pre-DNP CMRO$_2$ mean was 1.33 µmol/g/min, and the
simulated mean with noise was 1.44 µmol/g/min +/- 44% (n=20). The actual simulated post-DNP mean was 2.66 µmol/g/min, while the simulated mean with noise was 2.81 µmol/g/min +/- 37% (n=20). It is clear that this technique is at the limit of detection, but that it is still possible to make measurements. Improvements to the measured precision could be made if measurements were made faster with higher signal to noise, if the observed noise could be reduced, the gas delivery and measurement time for metabolism was longer, more ^17O could be added to the system in the metabolic measurement time, or if multiple measurements were made in the same individual.
CHAPTER 6: CONCLUSION

6.1. Future Directions

Several areas for future development were suggested in the text, and these will be reviewed and supplemented with additional considerations.

The lack of human studies is a significant current limitation of $^{17}$O measurements. Progress in the field requires one of two things to happen. The first possibility is that the FDA will provide an investigational new drug (IND) approval for $^{17}$O$_2$ and/or H$_2^{17}$O. The application process for this is a significant undertaking, and so far, as we know, no institution or company has begun this process. The other possibility is that significant progress will be made in another country in which the regulations are not as strict for the use of stable, chemically identical isotopes. According to personal communications with collaborators, these are already underway in India.

Still, it would be beneficial to combine CMRO$_2$ with CBF measurements to compute OEF, as that is the parameter that seems most useful for delineating the penumbra in $^{15}$O PET studies. This seems straightforward theoretically, but
nobody has done this study yet. A combination of these techniques in a large animal model of stroke would be especially interesting to the scientific community. As discussed in Section 5.1, this could be accomplished by surgical or perhaps other techniques.

The pulse sequence suggested in Chapter 2 could be easily implemented for H$_2^{17}$O decoupling either by simultaneous $^{17}$O transmission (127), interleaved $^{17}$O transmission (186), or T$_{1\rho}$ decoupling (125). For the current clinical hardware at our institution any of these paths would require hardware upgrades. It seems especially advantageous to implement this at 1.5T, as the SAR concerns, which increase with field strength, will give much more leeway to decouple with a short TR. Many of the possibilities for detection improvement discussed at the end of Section 5.6 could potentially be applied to the pulse sequence.

It is still possible that the pulse sequence could measure the H$_2^{17}$O in blood over time. Then in the Kety-Schmidt equation (Equation 3.1) the simplification for recirculation time would not be necessary and averaging of the rate of metabolism could take place over a longer period than one minute. Still, due to the subtleties of this experiment, it is recommended that a pulsatile flow simulator be used to extend the work shown in Section 2.10. Once the optimal
bandwidth and systolic delay are determined, this could be applied to the neck of a large animal.

6.2. Summary of dissertation

In Chapter 1, I discussed cerebral infarction as a motivation for the in vivo imaging of metabolism. Stroke presents a common and debilitating target for an uncertain therapy that could be improved by imaging to determine who would benefit from therapy. One metabolic imaging technique that would shed light on cerebral infarction is 17O imaging, and the principles and history of 17O techniques were reviewed. Our group chose indirect, proton-detected, 17O imaging because it is available on clinical scanners and provides suitable sensitivity that makes it the most likely method to be clinically translated. To image metabolism, 17O₂ was inhaled by swine, and the rate of H₂17O formation was recorded.

Several challenges to the implementation of 17O imaging were tackled in this dissertation. First, the cost of 17O₂ gas dictated assessment of the most efficient methods of 17O₂ gas delivery. This limited both the amount of time over which 17O₂ could be delivered, and mandated a rethinking of the supply method of the 17O₂. This short gas delivery time led to a consideration of the time required for
image acquisition. The consideration of all of these challenges was provided in Chapter 2 and Chapter 3, with an emphasis on imaging in the former and a novel circuit for gas delivery in the latter.

With this new framework for precision delivery and fast measurement of $^{17}\text{O}_2$, in Chapter 4, it was shown how CMRO$_2$ can be measured in the adolescent swine on a clinical scanner. This was performed with modeling and verification of the recirculation delay by arterial blood sampling. This was followed by the conclusion in Chapter 5 where metabolism in the swine was purposely altered by administration of a metabolic stimulant, and CMRO$_2$ was correlated with whole body metabolic change.

In conclusion, we anticipate that this work will lead to future studies of metabolism in humans and in disease. The studies performed here serve to bridge the gap from small animal research to human research. Techniques based on $^{17}\text{O}_2$ have strong potential for future use as a metabolic tracer, and it is hoped that this clinical translation will be the focus of future work.
REFERENCES


34. Kane I, Carpenter T, Chappell F, Rivers C, Armitage P, Sandercock P, Wardlaw J. Comparison of 10 different magnetic resonance perfusion imaging processing methods in acute ischemic stroke: effect on lesion size,


81. Lammertsma AA, Wise RJ, Heather JD, Gibbs JM, Leenders KL, Frackowiak RS, Rhodes CG, Jones T. Correction for the presence of


114. Charagundla SR. Indirect $^{17}$O detection with proton magnetic resonance imaging; 2000. xiv, 135 p. p.

115. Tailor DR. Indirect $^{17}$O-magnetic resonance imaging of cerebral blood flow and oxidative metabolism; 2002. xv, 121 p. p.


182. Clark WG, Clark YL. Changes in body temperature after administration of antipyretics, LSD, delta 9-THC, CNS depressants and stimulants,


