MRI Based Cerebral Oxygenation Measures in Humans: Technical Development for Use Across Lifespan

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Abstract

MRI based cerebral oxygenation measures could enable brain-centered clinical care and improve understanding of brain energy use throughout human development. We made technical improvements to two MR oxygenation imaging techniques, and explored the concordance between various methods to quantify the cerebral metabolic rate of oxygen consumption (CMRO₂) and other aspects of cerebral metabolism in neonates with congenital heart disease (CHD).

Using a turbo gradient spin echo readout we reduced the scan time of the existing QUantitative Imaging of eXtraction of Oxygen and Tissue Consumption (QUIXOTIC) technique for measurements of global oxygen extraction fraction by eightfold while improving robustness to physiological noise and motion. We also corrected the effect of residual cerebrospinal fluid signal on transverse relaxation time quantification. The detected change in oxygen extraction fraction in the visual cortex during visual stimulation, demonstrated the technique’s suitability for absolute quantitative functional MRI experiments.

T2-Relaxation Under Spin Tagging (TRUST) has been used in several studies, including one with neonates, to quantify blood oxygen saturation. We investigated how involuntary subject motion affects quantification by incorporating volume navigators into TRUST to monitor motion during scans. We demonstrated that motion causes an upward bias in venous oxygen saturation quantification.

Finally, we used TRUST and phase contrast MRI to measure CMRO₂ in neonates with congenital heart disease (CHD) and explored the link between CMRO₂ and other aspects of metabolism examined by MR spectroscopic imaging. We found a relationship between cerebral oxygenation and lactate and glutathione concentrations in white matter. We also sought to extend the reach of absolute MRI-based quantification by comparing it to bedside near infrared spectroscopy-based measurements. We found good agreement between oxygenation measurements, but no agreement between cerebral blood flow measurements, suggesting that hemodynamics vary more rapidly than oxygenation.

Robust MR-based oxygenation imaging would improve clinical care and our understanding of how abnormal oxygen delivery affects brain development. Seeking a complete picture of cerebral metabolism throughout development, future work will perfect techniques to monitor cerebral hemodynamics, oxygenation and metabolism from midgestation to old age.

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Title: Professor, Department of Electrical Engineering and Computer Science
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Introduction

Methods are needed for quantitative cerebral oxygenation imaging across the human lifespan. There is clear necessity for non-invasive imaging to understand human development and to diagnose and inform treatment of the diseases that hamper it, but the use of MRI on neonates and fetuses lags far behind adult imaging. Even in adults, measurements of the cerebral oxygen consumption remain challenging. The most robust methods offer only global quantification of the cerebral metabolism rate of oxygen consumption (CMRO$_2$) and more specialized methods to take regional measurements suffer from long scan times. For neonatal imaging, smaller anatomy and involuntary motion challenge even the most robust adult methods. Imaging the unborn fetus requires even more drastic compromises to mitigate fetal motion and to spatially encode the large pregnant abdomen. There are significant challenges on all fronts, but MRI based cerebral oxygenation measures could break into the clinic if they were demonstrably robust and reliable.

Essential for the vast majority of cerebral energy production, techniques to measure the brain’s glucose and oxygen use reveal the location of abnormal metabolism in tumors, ischemia, or hypoxia, and can be used for functional brain imaging. The magnetic properties of hemoglobin, makes oxygen the target for MRI-based quantification of oxidative metabolism. Techniques have been developed to measure oxygen delivery to the brain, but none has demonstrated regional sensitivity, absolute quantification, and clinically feasible scan times without needing to be calibrated using inhaled gasses. We developed turbo QUIXOTIC (tQUIXOTIC) to fill this gap and demonstrated its utility for quantitative functional brain imaging.

MRI-based imaging of cerebral oxygenation is possible in adults, but technical barriers due to radically different scale, physiology and the motion during the scan have prevented using these protocols in the young. Oxidative metabolism is critical to sustain the energy demands of structural and functional development. Periods of oxygen deprivation and diseases that affect oxygen delivery to the brain during development can lead to cognitive impairment. We invented a technique for cerebral oxygenation imaging of the neonate that also monitors head motion by reworking and augmenting the techniques developed for adults. Mitigating how motion affects MRI-based measurements of cerebral oxygenation improves its utility for making brain centered treatment decisions and prognoses.
MRI-based oxygenation imaging gives only a snap-shot of the brain's oxygen use, but cerebral hemodynamics are dynamic—they change quickly. When it takes significant resources to move an infant severely ill with congenital heart disease from the intensive care unit to the imaging suite, the MRI scan must provide invaluable information or other modalities will be used to guide clinical management. We have sought to understand the value of MRI-based assessment of hemodynamics and oxygenation using two approaches. The first was to combine MR spectroscopy and MR oxygenation imaging to see if abnormal metabolite concentrations in the brain could be explained by abnormal hemodynamics and oxygenation. This would establish a definite link between the abnormal cardiovascular system and brain metabolism. In a cohort of patients with different types of severe cardiovascular abnormalities the large range of hemodynamics, blood oxygen saturations, and metabolite concentrations revealed some relationships between the two. The second was to look for correlations between bedside near infrared spectroscopy and MRI-based measurements of cerebral hemodynamics and oxygenation, with the goal of cross validating the relative regional blood flow and tissue oxygen saturation measurements from NIRS and the absolute global quantification of MRI.

These studies are the foundation of future work to perform cerebral oxygenation imaging on the fetus. Less constrained motion, smaller anatomy, and differences between adult and fetal blood circulation makes fetal imaging even more challenging. Currently, only coarse metrics of fetal brain oxygenation and hemodynamics are available, despite the clinical need to understand how brain development is affected by inadequate substrate delivery to the fetus in conditions like intrauterine growth restriction. After developing MR oxygenation imaging for adults, devising motion mitigation and applying quantitative MRI to neonates, further technical development for fetal imaging will permit tracking cerebral oxygen metabolism, and thus brain energy use, throughout development. Ultimately a detailed understanding of cerebral oxygen use and hemodynamics during development may point the way to critical markers of long term developmental outcomes.

The structure of this thesis is as follows:

Chapter 1 presents a brief introduction to brain energy use, metabolism and the various methods used to quantify oxygen and energy substrate consumption. Because hemoglobin is a remarkable endogenous contrast agent we describe some of its attributes and relate the effects of deoxygenated hemoglobin on magnetic fields to MRI signals. We review the current state of MRI based oxygenation measures paying particular attention to their suitability to neonatal and fetal imaging.
Chapter 2 introduces an eightfold faster version of QUIXOTIC, one of only a few methods for regional, absolute quantification of cerebral oxygenation. We demonstrate that turbo QUIXOTIC is well suited to block design functional MRI experiments and that baseline OEF imaging is possible in 3.5 minutes. We also describe how to eliminate the effect of diffusion attenuated cerebrospinal fluid on the QUIXOTIC signal, which improves the overall accuracy of the technique. This work was previously published as:

Chapter 3 explores how motion affects a robust, global cerebral oxygenation imaging technique called T2-Relaxation Under Spin Tagging (TRUST). TRUST has been used to measure cerebral oxygenation in neonates, but motion corrupted 20% of scans beyond usefulness, and more subtly affected many of the others. To monitor motion, we integrated a volume navigator into the TRUST sequence and discovered a coherent upward bias to the resulting blood T2 estimates. The bias is almost half of the observed group wise difference between healthy neonates and those with congenital heart disease. This work is previously published as:

Chapter 4 presents a hypothesis generating study in neonates with congenital heart disease looking for correlations between TRUST and phase contrast MRI derived measures of cerebral oxygenation (CBF, oxygen extraction fraction and CMRO2) and metabolite concentrations measured by MR spectroscopy. There are no known studies that consider the combination of cerebral oxygenation and MRS measures. Two hypotheses result from this work: 1) glutathione in white matter is upregulated in some neonates with congenital heart disease likely due to chronic mild oxidative stress, and 2) elevated lactate levels in
white matter negatively correlated with CMRO$_2$ suggest some subjects have inadequate cerebral oxygen supply.

Chapter 5 describes the observed correspondence between NIRS and MRI measurements of cerebral oxygenation in a subset of the neonates with congenital heart disease studied in Chapter 4. Though oxygen saturation and OEF were well correlated between the two modalities, measurements of CBF were not prompting the follow up studies described in Chapter 6. This work was previously presented as:


Chapter 1
Background and Significance

The brain is an energy hungry organ and most of the energy consumed by the brain comes from oxidative phosphorylation. Though glucose is the energy substrate most important to the brain, oxygen is easier to measure quantitatively via MRI given that the magnetic properties of hemoglobin change between its oxygenated and deoxygenated state—an endogenous contrast agent. Among the various ways to observe this contrast is by quantifying the transverse relaxation time ($T_2$) of blood. If venous blood can be isolated—much of the work presented in this thesis pertains to the process of isolating a blood signal and the challenges posed by squirmy neonates to the isolation paradigms—its $T_2$ is related to its oxygen saturation. Calculations of various oxygen consumption metrics are then possible. With the additional measurement of cerebral blood flow (CBF) the cerebral metabolic rate of oxygen consumption (CMRO$_2$) can be estimated. In adults, methods to assess CMRO$_2$ hold promise for the clinical evaluation of stroke, brain tumor, and neurodegenerative diseases. In children and fetuses, oxygen metabolism is part of the dynamics of brain growth and development. The details of human brain energy use during development remains a topic of investigation, and methods to quantify CMRO$_2$ via MRI would help these endeavors. Clinically, quantification of the effects of disease on brain oxygen use could promote brain-centered care of critically ill neonates. From the perspective of a researcher developing MRI-based methods to evaluate brain oxygen use, during development in utero oxygen transport is significantly more complex. Multiple blood pools, both maternal and fetal, need to be measured to understand the full picture of oxygen delivery through the placenta and to the fetal brain. In addition, the fetus is constantly moving. However, methods to measure placental oxygen transport and fetal CMRO$_2$ would be immediately useful in cases of intrauterine growth restriction and for assessing fetal viability after maternal injury.

1.1 Brain energy use and metabolism, the oxygen angle

1.1.1 ATP Production

In an adult, the brain is about 2% of the total body weight, but consumes 20% of the oxygen, and thus 20% of calories consumed by the body (1). In an infant, the brain weighs about 6% of the total body weight and consumes 40-65% of the body’s calories (2,3). The energy currency in the body is adenosine triphosphate (ATP). ATP is produced by the metabolism of various energy substrates, including glucose, ketone bodies, and fatty acids (4). Though the adult brain relies almost exclusively on glucose, and its
metabolism via oxidative phosphorylation, for its energy needs, the developing brain also relies on ketones and fatty acids (4) and may depend on glycolysis for some of its energy.

Though the details of cellular respiration can be found elsewhere (5), it is useful to outline the rudiments of this process to understand what aspect of metabolism is represented by the rate of oxygen consumption. When glucose enters the cell it is broken down into pyruvate via glycolysis and this produces two ATP. The same chemical pathway takes two names in the literature, anaerobic or aerobic glycolysis depending on whether it occurs in the presence of sufficient oxygen for oxidative phosphorylation to occur. From pyruvate, lactate is produced by lactate dehydrogenase or pyruvate can lead into the citric acid cycle and oxidative phosphorylation whereby significantly more ATP is produced, roughly 36, or more than 15x the rate of production via glycolysis. In summary, Glucose + 6 O₂ → 6 CO₂ + 6 H₂O + 38 ATP.

Measurement of the metabolic rate of oxygen consumption is directly related to the amount of oxidative phosphorylation occurring the tissue, but some energy is produced by glycolysis without the involvement of oxygen. Positron emission tomography (PET) experiments comparing the amount of glucose and oxygen metabolism occurring in different brain regions have shown that some glycolysis occurs even when there is sufficient oxygen to complete oxidative phosphorylation (2,6). This occurs because aerobic glycolysis also feeds the necessary precursors (carbon rich pyruvate and lactate) into the various pathways that synthesize biomass (7). For example, amino acid synthesis, axonal elongation, myelination and fast axonal transport all rely on aerobic glycolysis. The relative tissue intake of glucose, other energy substrates, and oxygen give insight into the processes of development.

1.1.2 Oxygen use in the developing and adult brain
Though this thesis focuses on oxygen, it is important to know that oxygen input is not always equivalent to energy output. By some estimates premature babies use 90% of the glucose entering the brain in aerobic glycolysis (not oxidative phosphorylation), versus only 10-12% in adulthood (7). If this is true, a considerable amount of the energy used by the developing brain in its early stages does not involve oxygen. However, a 9:1 ratio of glucose entering glycolysis to oxidative phosphorylation still means that the vast majority of the brain’s energy, 18:36 in terms of ATP produced, is being derived from oxidative phosphorylation. In other words, at no point can glycolysis alone sustain the brain’s energy demands. Oxidative metabolism is always essential (8).
The main use of energy in the brain is to maintain ionic and chemical gradients necessary for neuronal signaling. In the adult brain, the large majority of energy is devoted to the synaptic processes that maintain the balance of excitatory and inhibitory activity (1). In terms of the energy budget of neuronal signaling, 50% is used on postsynaptic glutamate receptors and 21% on action potentials, with the remainder being devoted to neurotransmitter recycling, pre-synaptic neurotransmitter release and other related cellular activities (9).

During development, there are additional processes that take considerable energy and biomass: cell differentiation, cell division, cell migration, axonal growth and maturation, myelination, dendritic arborization, and synaptogenesis. Though we do not have a clear picture of how the energy consumed by these processes ebbs and flows during human development, we can make inferences from comparisons to other mammals. Excellent reviews of brain development in terms of energy metabolism are found in (4,10). From data we do have, we know that premature babies (gestational age (GA) = 32-37 weeks) have a whole brain metabolic rate of glucose consumption of approximately 5.5 μmol/100g/min, which increases until about five years of age to 48 μmol/100g/min, which is about double the eventual adult rate (4). CMRO₂ follows a similar progression. Premature babies (GA = 26) have a CMRO₂ of about 2.6 μmol/100g.min (0.06 ml/100g/min, see section 1.3.1 on units) that rises until about 5 years of age to 230 μmol/100g/min (5.2 ml/100g/min) before falling to an adult level of roughly 150 μmol/100g/min (4).

The details of critical energy demands during normal brain development may help identify junctures were energy deprivation may lead to negative cognitive outcomes. Also identifying adaptations to oxygen or substrate deprivation during development could help clinicians better care for these patients. The problem is that techniques for monitoring cerebral oxygenation have been developed for use on adults and there are additional challenges posed by neonatal and fetal imaging.

1.2 The challenges of neonatal and fetal imaging

Non-invasive measurements of cerebral oxygenation follow a wider trend in MRI research: techniques are developed for use on adults. The first attempts using MRI to measure cerebral oxygenation in children (reviewed in (11)) or the fetus (12) were made using technology that is not tailored to their dramatically different anatomy and physiology.

The differences in Table 1-1 elucidate why neonatal and fetal scanning is particularly challenging. Neonates are not compliant subjects and though anesthesia and sedation can be used clinically when the
benefits of a motion free scan outweigh the risks, this is not possible in research scans that have to prove negligible risk. Fetal imaging is not performed with sedation or anesthesia due to risks to the fetus. Anesthesia also alters brain metabolism, and so cerebral oxygenation measurements during anesthesia may not be useful when trying to understand baseline physiology. This is not a concern for typical clinical studies of the brain’s structure.

A child’s brain and vasculature is considerably smaller than in adults. Smaller imaging voxels to capture smaller anatomy means lower signal to noise ratio (SNR) measurements. Also, given that adult hardware is used for the scans receive coils are further from the object, reducing SNR and the benefits of parallel imaging acceleration. In fetal imaging, a more complex field of view that contains maternal tissue means that a large volume still must be spatially encoded, so there are often compromises made in terms of resolution to avoid aliasing artefacts.

All these challenges make neonatal and fetal imaging considerably more difficult than adult imaging, but the fundamental problem is motion. Low SNR could be overcome by longer acquisition time, but motion limits this strategy. Motion is the critical challenge in fetal and neonatal imaging.

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>Neonate</th>
<th>Fetus (~36 weeks GA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean head circumference [cm]</td>
<td>57</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Potential motion (translation [mm]/rotation [degree])</td>
<td>1/1</td>
<td>5/10**</td>
<td>15/80**</td>
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<tr>
<td>Mean cerebral blood flow [ml/min]</td>
<td>730</td>
<td>100</td>
<td>426*</td>
</tr>
<tr>
<td>Cerebral O2 Sat (venous/arterial)</td>
<td>60/100</td>
<td>63/96</td>
<td>45/65*</td>
</tr>
</tbody>
</table>

Table 1-1: Comparison of anatomy, physiology, motion and imaging for adults, neonates and fetuses; * measured in superior vena cava since no studies to date have accurately measured SO2 in the fetal cerebral vasculature; ** pre-publication data. Sources: (13–22).

Researchers are still searching for a general solution to correct for motion during MRI scanning, but some strategies, reviewed in (15,23), have been proposed. Motion correction methods for adult imaging consist of three main categories optical, field and navigator based motion tracking. Motion correction can be applied during the acquisition of image data to correct slice positions in real time and this approach is called prospective correction. Retrospective correction is when the motion data is collected during image
acquisition but is then used afterward to improve data reconstruction or to reject data known to be corrupted by motion.

For neonatal motion correction, navigator based methods are the most appealing, though optical methods may be possible. Navigator based methods exploit the intrinsic imaging capability of the scanner. Fast acquisitions are made during pulse sequence “dead time” that previously was used exclusively for magnetization recovery (24). They can also be inserted in interleaved repetitions if the sequence does not have sufficient dead time for a navigator acquisition (25). These navigator images can then be used to track movements with no additional required hardware. Optical methods often rely on fiducial markers that require some degree of subject compliance (e.g. to retain a bite plate, or to leave a hat in place), so these are more challenging for neonates. Optical tracking methods may be possible in neonatal imaging if they rely on some noninvasive approach, such as face tracking or 3D imaging, and may be especially useful if high frequency tracking is need for prospective motion correction. Field based motion correction has not been tried for neonatal imaging, but relies on probes or active markers being affixed to the subject.

For fetal imaging, navigator methods are currently the only option since optical and field based approaches require invasive fiducial markers. Ultrasound can be used to track the fetus in real time to provide feedback to the scanner, but this approach has been met with only limited success (26).

Regardless of the approach to motion correction, spatial encoding still takes time, so techniques for accelerating image acquisition are also crucial for neonatal and fetal imaging. Parallel imaging acceleration that takes advantage of the non-uniform spatial sensitivity of receive coils in multi-coil arrays like GRAPA and SENSE are already routinely used. Though volume navigators have been used to do prospective motion correction in 3D sequences like MEMPRAGE (27), single slice acquisitions are often preferred because they minimize the time during which motion can corrupt the acquisition. Simultaneous multi-slice approaches are extremely promising for further accelerating single slice acquisitions so there is less time for motion to occur between all the slices in a volume (28).

1.3 State of the art quantification of cerebral oxygenation

1.3.1 Oxygen transport, CMRO$_2$ and units
Oxygen is not produced inside the body and must be continuously transported to tissues by blood. Oxygen enters the body through the lungs where it diffuses into blood where it is bound by hemoglobin
and dissolved in plasma. The oxygen carrying capacity of arterial blood is then \( \text{CaO}_2 = \text{CaO}_2,\text{plasma} + \text{CaO}_2,\text{heme} \). The percentage of bound oxygen molecules to available oxygen binding sites in hemoglobin (4 per hemoglobin molecule) is called oxygen saturation (SO2). The oxygen dissociation curve relates SO2 to the partial pressure of oxygen (pO2) in blood, and this relationship can be altered by blood pH, temperature, dissolved CO2 and 2,3-diphosphoglycerate concentration. The “Bohr effect” refers to the phenomena that higher pH and higher CO2 concentration shifts the dissociation curve to the right.

However, CaO2 is always directly related to SO2, regardless of the relationship between pO2 and SO2. This is important, because estimates of SO2 can be made by various means, and so calculating the amount of oxygen in blood only depends on the oxygen carrying capacity of hemoglobin, which is 1.39 ml O2/g Hb. For a given hemoglobin concentration in blood (\([\text{Hb}]\)) and a known oxygen saturation the carrying capacity of blood can be calculated: \( \text{CaO}_2 = (\text{pO}_2 \times 0.003 \text{ [ml O}_2/100 \text{ ml blood/mmHg]} \) + (\( \text{SaO}_2 \times [\text{Hb}] \times 1.39 \text{ [ml O}_2/g \text{ Hb]} \)). When a healthy adult breathes air the amount of oxygen dissolved in blood is much smaller than that bound to hemoglobin. (i.e. \( \text{CaO}_2,\text{plasma} \) is about 1% of \( \text{CaO}_2,\text{heme} \)) and so it is often ignored.

Oxygen amounts are expressed as milliliters of oxygen at standard temperature and pressure (STP) or in micromoles. Knowing that one mole of oxygen occupies 22.4359 L at 273.15K, and 25.4627 L at body temperature (310 K) at standard pressure, 1 ml of O2 at body temperature is equal to 39.27 \( \mu \text{mol} \) of O2.

Hemoglobin concentration in blood is expressed in \( \mu \text{mol/ml} \), but sometimes only hematocrit (Hct) is measured. Hematocrit is the fractional volume of red blood cells in a sample of blood (typically 0.3-0.5). Mean corpuscular hemoglobin concentration (MCHC) is needed to convert between Hct and [Hb]. Unless measured from a blood collection, typically MCHC of 0.34 g Hb/ml is assumed.

“Cerebral oxygenation” is used to refer to the related properties of arterial and venous SO2, the oxygen extraction fraction (OEF = (\( \text{SaO}_2 - \text{SvO}_2 \))/\( \text{SaO}_2 \)) and CMRO2. Fick’s principle can be used to relate cerebral blood flow, the metabolic rate of oxygen consumption and the concentration of oxygen in arterial and venous blood. That is, \( \text{CMRO}_2 = 1.39 \times \text{CBF} \times \text{OEF} \times \text{SaO}_2 \times [\text{Hb}] \), where CMRO2 is in units of ml O2/100g/min (and 1.39 [ml O2/g Hb] is the oxygen carrying capacity of Hb). In the same units, but in terms of Hct, \( \text{CMRO}_2 = 20.6 \times \text{CBF} \times \text{OEF} \times \text{SaO}_2 \times \text{Hct} \). If SaO2 is measured by a pulse oximeter or assumed to be 100% at sea level in healthy adults, [Hb] or Hct by a blood sample, and CBF by either phase-contrast or arterial spin labeling MRI, this means only SvO2 is needed to determine CMRO2. Luckily, the properties of hemoglobin mean estimates of SvO2 are possible via MRI.
1.3.2 Hemoglobin
In addition to its high affinity to bind oxygen, hemoglobin has remarkable properties that permit the quantification of blood oxygen saturation by both MRI and optics.

Hemoglobin is a metalloprotein made up of four monomers. The heme group at the center of each monomer has a high affinity to bind oxygen. In adults hemoglobin is made up of alpha and beta monomers, but in fetal hemoglobin alpha and gamma. The transition from fetal to adult hemoglobin starts before birth, but adult hemoglobin does not dominate until several weeks after birth. The binding affinity for oxygen is different between fetal and adult hemoglobin, with the fetal hemoglobin dissociation curve shifted to the left. This, along with the Bohr Effect, explains how oxygen moves across the placenta from maternal to fetal hemoglobin.

Hemoglobin exhibits different magnetic properties depending on whether or not it is bound with oxygen. When deoxygenated, the heme iron’s six highest energy electrons occupy the 3d orbitals leaving four unpaired electrons and giving Fe$^{2+}$ a spin $S = 2$. This means deoxygenated hemoglobin (dHb) is paramagnetic, with a magnetic susceptibility ($\chi > 0$). When oxygenated the electron configuration changes such that the six electrons pair up in the 3d orbitals and the spin is $S = 0$. This means oxygenated hemoglobin is diamagnetic, with $\chi < 0$. The magnetic susceptibility of blood scales linearly with dHb concentration, such that measurements of the magnetic susceptibility of blood can be converted into oxygen saturation. Additionally, this change in magnetic state affects the relaxation rates (transverse and longitudinal) of blood and the surrounding tissue and so mappings between relaxation times and oxygen saturation are another way to quantify blood oxygen saturation.

Hemoglobin’s optical properties also make it a target for the optical quantification of blood oxygen saturation. Between 650-1350 nm wavelength, near infrared light is predominantly scattered by tissue instead of absorbed. Because the absorption spectrum of the hemoglobin chromophore changes depending on whether it is oxygenated or deoxygenated, blood oxygen saturation can be quantified using near infrared spectroscopy. The pulse oximeter is the most common form of this technology, but more advanced systems enable the measurement of tissue oxygen saturation in the brains of newborns (29).

1.3.3 MRI based methods for quantifying cerebral oxygenation in neonates and fetuses
MRI-based methods for quantifying cerebral oxygenation can be divided into three broad categories, approaches based on quantitative BOLD, susceptibility and $T_2$, reviewed in (30,31). These reviews detail the applications and research directions pertaining to adult imaging. Neonatal imaging is more
challenging and only a few methods have been tried on neonates, as reviewed in (11). The following emphasizes each adult approach’s suitability for measuring cerebral oxygen in neonates and fetuses.

1.3.3.1 Calibrated BOLD

The blood oxygen level dependent (BOLD) signal was originally observed in rats in 1990 (32), and shortly thereafter in adults (33) using gradient echo based sequences sensitive to changes in $R_2^*$. Increased CMRO$_2$ during neuronal signaling in an activated brain region causes changes in cerebral blood volume, cerebral blood flow and oxygen metabolism that are combined in one extravascular $\Delta R_2^*$ signal. So it is a matter of careful modeling to back out the individual physiological changes that give rise to the observed BOLD signal. Careers have been spent refining models of BOLD contrast, and there are few better guides to physical and physiological basis of the BOLD signal and functional magnetic resonance imaging (fMRI) more generally than Richard Buxton (34,35).

At its most basic level, extracting $\Delta$CMRO$_2$ from observed changes in gradient echo based $R_2^*$ signal requires estimating the maximum achievable BOLD signal, the “M” parameter (36,37). This is achieved by inducing hypercapnia. CO$_2$ in the blood is a potent vasodilator that causes global CBF to rise without, presumably, affecting CMRO$_2$. Various methods have been proposed to induce hypercapnia for effective calibration including breathing CO$_2$ enriched air, breath hold and breathing a mixture of CO$_2$ and oxygen. However, calibration via hypercapnia only permits the estimation of the functional change in CMRO$_2$.

An additional calibration and measurement of CBF is necessary to permit the estimation of baseline OEF and CMRO$_2$ from the BOLD signal using a method called QUO2 (38–40). A dual echo pseudo-continuous ASL sequence is used to measure both CBF and BOLD signal changes. For calibration, a mask is fitted over the subject’s nose and mouth and then eighteen minutes of scanning is conducted while the subject breathes different gas mixtures to calibrate the model parameters, and acquire BOLD data during task activation. Gasses are administered by a physician in the magnet room who also ensures subject safety and comfort. With calibration QUO2 offers high frequency monitoring—compatible with block and event designed fMRI experiments—of a full range of absolute physiological parameters (CBF, CBV, OEF, CMRO$_2$) on a regional basis.

Although QUO2 is a major advance in quantitative oxygenation imaging, the challenges associated with using this technique for neonatal or fetal imaging are readily apparent. First, eighteen minutes of scan time is a long time for a neonate to remain still. Second, even if a neonatal subject were quiescent in the scanner, keeping the breathing mask on and monitoring subject wellbeing when they cannot describe how
they are feeling adds another level of complexity to a scan. Third, ASL scans to determine regional CBF in neonates are notoriously difficult because of motion, but also because of variation in arterial transit time in cerebral vasculature that is still developing (41).

There have been no proposals for how to obtain calibration measurements for fetal imaging.

1.3.3.2 Susceptibility

If \( \text{SaO}_2 \) is estimated from pulse oximetry and CBF from the MRI methods discussed below, absolute quantification of OEF and CMRO\(_2\) is possible with an estimate of \( \text{SvO}_2 \). Measurement of \( \text{SvO}_2 \) is the goal of both susceptibility and \( T_2 \) approaches to absolute cerebral oxygenation imaging.

When blood is deoxygenated in the capillary beds, its magnetic susceptibility changes, affecting the magnetic fields in and around the veins. The susceptibility of blood is directly proportional to OEF and hematocrit: 

\[
\Delta \chi_{\text{vein-water}} = \Delta \chi_{\text{do}} \cdot \text{Hct} \cdot \text{OEF},
\]

where \( \Delta \chi_{\text{do}} \) is the susceptibility shift between oxygenated and deoxygenated Hb (42). Susceptibility based techniques estimate blood oxygen saturation from the MRI phase signal.

Most uses of MRI rely only on the resulting magnitude image, where contrast originates from different rates of magnetic relaxation (\( T_1 \) and \( T_2 \)). However, contrast in the MRI phase signal originates from local magnetic field variation, the result of \( B_0 \) inhomogeneity, applied gradient fields, and tissue characteristics such as iron depositions, myelin and dHb concentration. To use MRI phase to estimate magnetic susceptibility the susceptibility effect must be separated from the others, and then modeled so as to relate magnetic field perturbations to changes in susceptibility. The relationship of signal phase to the underlying magnetic susceptibility is an ill-posed inversion problem.

One approach to the inversion is to model vessels as long cylinders parallel to \( B_0 \), and isolate the effect of dHb by comparing the phase measured within the vessel to the reference phase of nearby brain tissue or cerebral spinal fluid (42). Called MR susceptometry, this approach has been used in both adults (20,43,44) and neonates (45). Initially MR susceptometry required the visual identification of the vessel lumen such that a pure blood signal phase was used to calculate susceptibility, but later work explored using both magnitude and phase portions of the signal to correct for partial volume effects (46).

If the vessel of interest is not parallel to \( B_0 \), a more advanced approach can be used to determine vessel susceptibility from a regularized inversion. This technique is called quantitative susceptibility mapping.
Based on 3D encoded, dual-echo, flow compensated gradient echo volumes that can be acquired in about 10 minutes, QSM permits the reconstruction of susceptibility maps along the venous cerebral vasculature (48). However, this approach does not obviate the constraint that voxel size is smaller than the vessel lumen.

The advantages of magnetic susceptibility approaches to measuring OEF and CMRO₂, are that they are based on the standard gradient echo pulse sequence, which is widely available. The models used for the signal inversion to susceptibility require no calibration. QSM can be used to make regional estimates of OEF by examining small veins draining cortical regions (49), but it is not a truly regional technique in that the draining region must be inferred from the spatial location of the vein. There has been a proposal for how to match brain region with its draining veins (50).

Of particular importance to its application to neonatal imaging, the regional specificity is limited by the resolvable vessels at a given resolution. The straight sinus, superior sagittal sinus and jugular veins all would be suitable targets. Neonatal scanning to date has focused on the superior sagittal sinus (SSS) (45). The SSS is particularly convenient because in the supine position a portion of the large arcing vessel is almost always parallel to B₀. This permits quality estimates of blood susceptibility via MR susceptometry using single slice imaging. 3D imaging, requisite for QSM, requires longer acquisitions where motion would corrupt the entire volume being acquired.

There have been no attempts to use magnetic susceptibility to quantify the cerebral oxygenation of the fetus. It would be difficult because of the small vessel size and resolution constraints related to a large FOV. Furthermore, there is no constraint (like gravity holding the subject to the examination table in a supine position) that guarantees the vessel of interest would be parallel with B₀, and 3D encoded fetal imaging to permit QSM would require sophisticated motion correction that has not yet been developed.

1.3.3.3  T₂

T₂ is known as transverse or spin-spin relaxation. After an ideal 90-degree excitation the net magnetization of a voxel is entirely in the transverse plane. However, the individual spins that form this net magnetization experience slightly different magnetic fields depending on their spatial relationship to the other spins and microscopic field perturbers such as dHb. Furthermore these relationships are in constant change due to thermal motion such that each spin experiences fluctuating magnetic fields. These field fluctuations cause spins to precess at different rates, and the net result is decay of the transverse
magnetization. Technically, this phenomena is a dephasing of the isochromats within a voxel. $T_2$ relaxation is an intrinsic tissue property.

In actual experiments transverse magnetization decays more rapidly than predicted by microscopic tissue properties. This is because $B_0$ inhomogeneities, gradient imperfections and macroscopic field perturbations (e.g. air-tissue boundaries, metal implants, etc.) also cause field fluctuations leading to transverse magnetization decay. These extrinsic factors typically predominate. If all the causes of transverse magnetization decay are combined the resulting relaxation time constant is called $T_2^*$. In a single-voxel experiment, after an ideal 90-degree excitation, the resulting demodulated signal would be $S(t)=S_0\cdot\exp(-t/T_2^*)$, where $S_0$ depends on the proton density of the object.

The extrinsic and intrinsic relaxation effects can be separated by refocusing the transverse magnetization. First observed by E. Hahn the spin echo (SE) follows multiple RF-pulses (51). Most simply, if after a 90-degree excitation, a 180-degree refocusing pulse is played some time ($\tau$) later, and we observe the MR signal at $2\tau$, $S(t=2\tau)=S_0\cdot\exp(-2\tau/T_2)$. At $2\tau$ we would observe a local signal maximum, so we call this point in time the echo time (TE). To determine $T_2$, a series of refusing pulses could be applied at $2\tau$ (=TE) intervals after the first, and the observed maximum signals at multiples of the echo time fit to a decaying exponential of the form $S(t=n\cdot\text{TE})=S_0\cdot\exp(-t/T_2)$.

A different approach from SE to measure $T_2$ is called $T_2$-preparation. The advantage of $T_2$-preparation is that it is more robust to flow effects. If fresh spins with different spin histories flow into the imaging plane during an experiment (i.e. between the first and last TE) this can affect SE-derived estimates of $T_2$. A typical $T_2$ preparation module consists of a 90-degree excitation followed by a series of equally spaced refocusing pulses and then a -90-degree pulse. The net effect is that the longitudinal magnetization has been attenuated by some amount of transverse relaxation. Now that the transverse relaxation has been "stored" in longitudinal magnetization, an excitation followed by a readout at a consistent TE mitigates flow effects.

It is interesting to consider whether TE has any effect on the observed $T_2$. The relationship between $T_2$ and $T_2^*$ is often expressed as $\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'}$, where $T_2'$ is the relaxation due to extrinsic field inhomogeneities. This expression gives the impression that $T_2$ would be independent of the observed TE, because refocusing is eliminating dephasing due to extrinsic effects. The truth is that transverse relaxation is a very complex phenomena in biological tissues. Spins move in random walks around and within blood
vessels and erythrocytes, which means they are moving through the fluctuating magnetic fields produced by dHb. Spins also exchange between locations of high magnetic susceptibility, i.e. within erythrocytes to plasma or within blood vessels to interstitial space, which also means they are experiencing fluctuating magnetic fields. The net result is that what we call T2 depends on TE. The best way to think about this is that T2 is what remains after a certain amount of refocusing is achieved by producing a spin echo at a given TE. The longer the TE, the more irreversible dephasing takes place, which in the limit, without any refocusing, means that T2 has become T2* . This means that for T2-preparation based experiments, the amount of relaxation generated by module is related both to the spacing between the refocusing pulses as well as total duration of the module.

When the concentration of dHb increases in blood the magnetic fields within and around erythrocytes and blood vessels change which affects T2. Graham Wright first proposed a relationship between blood oxygen saturation and T2 in 1991 (52), and since then joint physical and empirical descriptions of the relationship have been produced (53). Though fast exchange of water between plasma and erythrocytes is the favored explanation for why T2 shortens with lower SO2, the diffusion of water around erythrocytes and small vessels also has some effect. Because the physical model is an incomplete representation of the complicated reality of transverse relaxation in a biological system, mapping T2 to SvO2 is often based on empirical results (54–56). These relationships are subtly different for fetal and adult blood and investigations into the root causes of these differences continue, whether due to the magnetic properties of fetal versus adult hemoglobin or fetal versus adult erythrocyte geometry.

Since brain parenchyma is only about 5% blood by volume (57), the challenge with using blood T2 to determine SO2 is to isolate the pure blood signal. Various methods have been proposed to do this. By visual inspection vessel lumens can be identified and blood voxels selected (58). Spin tagging can be used to isolate a blood in large vessels using a method called T2-relaxation under spin tagging (TRUST) (59), which will be discussed extensively in chapter 3. A more subtle time of flight effect—where inflowing spins provide fresh signal in the blood vessel but not in the surrounding tissue—is exploited by T2-TRIR (60). However, identifying SvO2 in large draining veins only provides information about the brain’s global oxygen use, so there has been great interest in techniques to isolate a blood signal in the microvasculature. Such a technique would be comparable to quantitative BOLD methods of measuring regional oxygen metabolism. Velocity-based venous blood isolation that takes advantage of blood flow velocity is called QUantitative Imaging of eXtraction of Oxygen and TIssue Consumption (QUIXOTIC) (61). Chapter 2 introduces an accelerated version of this technique. Recently, the pseudo-diffusion phenomena of blood in the microvasculature has also been exploited as an isolation strategy (62).
TRUST coupled with PC-MRI and T2-TRIR coupled with ASL have been used to quantify CMRO$_2$ in neonates (21,63). TRUST showed CMRO$_2$ rapidly increasing in the first weeks after birth in healthy neonates born around 35 weeks. T2-TRIR found a similar rise in CMRO$_2$ over the first weeks of life, and demonstrated OEF, but not CMRO$_2$, differences between premature neonates, premature neonates scanned at term equivalent age and neonates with hypoxic-ischemic encephalopathy. Both studies found that motion corrupted data to the point it could not be further analyzed in roughly 20% of scans, and that it severely impacted data quality over all. Motion robust techniques for neonatal cerebral oxygenation imaging are needed.

$T_2$ mapping—manually selecting voxels within the vessel lumen—remains the only method to date that has been used to determine oxygen saturation in fetal vessels (12). This study did not deploy any blood signal isolation techniques, instead focusing on large vessels where six voxels could be identified within the lumen. This strategy made total acquisition times very short (~25 seconds) which served to reduce motion artefacts in the scans. The drawback to this approach was that it focused on large vessels like the ascending aorta (a good estimate of SO$_2$ in the carotid arteries) and the superior vena cava (which receives blood return from the entire upper body above the diaphragm). Vessels to quantify cerebral oxygenation in isolation were too small to image. This study also underscores that a lack of access means blood properties essential for $T_2$ to SO$_2$ mapping, such as Hct, must be inferred or measured by some other means. One solution is to measure blood $T_1$ in addition to $T_2$ and then use both parameters to determine Hct and SO$_2$ simultaneously. Notably, one of the advantages of the $T_2$-TRIR technique is that blood $T_1$ estimates are generated simultaneously.

1.3.3.4 Cerebral blood flow
To calculate CMRO$_2$, CBF is needed along with OEF. CBF can be measured in two ways: velocity encoded phase contrast MRI (PC-MRI) of the major vessels feeding or draining the brain, and arterial spin labeling (ASL). PC-MRI is most often used with TRUST because both techniques give global measurements of CBF and OEF respectively. ASL has been paired with QSM or QUIXOTIC because all give regional measurements.

PC-MRI uses bi-polar gradients to encode blood velocity as phase. The maximum velocity that can be encoded before phase wraps from $\pi$ to $-\pi$ is known as VENC. Flow (Q) can then be calculated using the relationship $Q = A \sum v_i$ where $v_i$ are the velocity in each voxel of a hand drawn region or interest (ROI) around each vessel, and $A$ is the area of one voxel in the ROI.
Studies using PC-MRI have shown a dramatic increase in CBF in the first weeks of life (18,21), as well as abnormal CBF related to congenital heart disease (45,64). PC-MRI has also been used to assess fetal hemodynamics (65), identifying brain sparing physiology resulting from IUGR (66) and normal cerebral blood flow in congenital heart disease (12). Neonatal CBF measurement techniques are reviewed in (67).

ASL provides information about regional perfusion in absolute units. Standard ASL acquisition and quantification parameters have been proposed for adult imaging (68), but the technique is still being optimized for neonatal imaging (69–71). Various groups have explored the importance of blood hematocrit and T1 in order to accurately quantify CBF in neonates. However, the bigger problem is that ASL is extremely sensitive to motion since the relatively small perfusion signal is isolated by subtracting a control image and one where blood has been magnetically labeled. Any motion occurring between the two acquisitions corrupts perfusion quantification.

Nevertheless, ASL studies have found that whole brain CBF increases rapidly in the first months after birth (e.g. from 7 ml/100g/min at 31 weeks, to 29 ml/100g/min at 52 weeks post-menstrual age) and that the distribution of regional perfusion changes during this time (41,72,73). Studies of neonates with hypoxic ischemic encephalopathy showed that adverse outcomes were predicted by increased perfusion in the basal-ganglia and thalami (74–76).

Work is needed to address the inaccuracies in CBF measurements that arise from making measurements without synchronizing them to the neonatal or fetal heart rate. It is also important to recognize that though PC-MRI CBF measurements based on different combinations of vessels supplying (basilar artery, internal carotid arteries, and vertebral arteries) and draining (superior sagittal sinus, straight sinus, jugular veins) the brain show strong correlations (64,77) the underlying assumptions about flow distributions must be considered when comparing absolute CBF measurements between studies.

1.3.4 Other modalities

The ubiquity of MRI hardware in clinical and research settings plus the entirely non-invasive methods for ascertaining cerebral oxygenation make it a convenient and safe modality for absolute quantitative oxygenation imaging. However, it has its drawbacks too. From the proceeding discussion it is clear that MRI only makes indirect measurements of SvO2 and CBF and that quantification of these parameters involves a number of assumptions. PET on the other hand probes specific metabolic physiology using methods that are more directly quantifiable. Although there has been considerable interest in making MRI
scanners that are more compact and portable (78), MRI is not currently a bedside modality. NIRS or ultrasound on the other hand are well suited to bedside use. Other modalities offer advantages over MRI and studies that validate MRI quantification or use MRI as the reference standard to validate a bedside measurements will improve brain-focused clinical care.

PET remains the reference standard for regional quantification of oxygen metabolism. However, the experimental protocols for measuring CMRO₂ with PET are very complicated. Tracers that determine CBF, CBV and OEF are each different and are based on ¹⁵O which has a half-life of about 2 minutes. Tracer production thus necessitates an onsite cyclotron and radiochemistry lab, and careful coordination between the chemists and scan technicians. Nevertheless, reference standard measurements of CMRO₂ in neonates and adults have been made which makes PET studies important points of comparison to novel MRI methods. An important first step toward validating MRI versus PET methods for determine CMRO₂ is to compare ASL and PET-based CBF (79).

If the ultimate goal of cerebral oxygenation imaging is to monitor patients who are critically ill such that treatment decisions are brain health centered, then bedside monitoring will be necessary. NIRS enables regional measurements of cortical tissue oxygen saturation, and a variant of NIRS, diffuse correlation spectroscopy (DCS), enables quantification of cortical blood flow. Near infrared light is mostly scattered, rather than absorbed, by tissue and it penetrates several centimeters into the head. The thicker adult skull makes it more difficult to use NIRS on adults than on neonates. NIR absorption by hemoglobin is used to estimate tissue oxygen saturation. Local blood flow is quantified by modeling the scattering associated with microvascular flow. Cerebral blood flow quantified in this way is a local microvascular measurement with units akin to the coefficient of diffusion, cm²/s. Blood flow, oxygen saturation and CMRO₂ have been shown to correlate between simultaneous NIRS and MRI measurements (45,80), but the differences in the underlying physiological basis of the measurements remains an important topic of investigation.

Ultrasound is extremely useful for both structural imaging and high frequency quantitative hemodynamic measurements of the fetus at the bedside. Ultrasound quantifies blood by using the doppler effect to determine blood flow velocity. Indeed strong intermodality agreement between ultrasound and PC-MRI has been demonstrated (81). However, ultrasound measurements of blood flow velocity are strongly dependent on the angle between the vessel and the probe. Though a correction can be applied for misalignment, this dependence has meant that ratios of velocities (systolic to diastolic ratio, pulsatility index) that eliminate this dependence are most often used for diagnostic purposes (82).
1.3.5 MRS

CMRO$_2$ is a useful but limited metric of tissue metabolism. As previously discussed, though oxygen is an essential component of cellular respiration, the cell can produce some energy without oxygen. The quantification of other metabolic substrates or products drives research into magnetic resonance spectroscopic imaging (MRSI). MRSI relies on the frequency shifts experienced by protons in different chemical environments to quantify chemical concentrations. MRSI measurements are challenging in part because concentrations of the metabolites of interest are about 1000x lower than the concentration of water (~40 M), so the signals are very weak in comparison to the water signal used in structural imaging. Nevertheless n-acetylaspartate (NAA), choline (CHO), creatine (Cr), lactate (Lac), glutamate (Glu), glutamine (Gln), myoinsoitol (Ins) and glutathione (GSH), are each involved in different metabolic pathways and their concentrations provide insights into the cellular microenvironment. Interpreting the measurements is difficult, however, since the pathways the metabolites are involved in are often complex—reviewed in (83,84). Despite these difficulties some studies have examined brain metabolite concentrations during development and have found absolute differences in metabolite concentrations between health and disease (85,86), and also differences in how metabolite concentrations change during development (87).

1.4 Utility of oxygenation imaging

There are many overlapping use cases for oxygenation imaging. Robust and reliable techniques would be useful in the clinic and lab. Uses can also be divided between adults, neonates and fetuses on the basis of the diseases and research questions pertaining to these different populations. Given how little we understand of the human brain’s oxygen consumption during development the research and clinical uses overlap even more when we consider the fetus or neonate.

1.4.1 Adult clinical utility

Cerebral oxygenation would benefit adult patients with stroke, brain tumors and neurodegenerative disease. These clinical uses are well reviewed in (30,31). The recent advances in intravascular clot retrieval and stenting prompt the following elaboration on the clinical utility of oxygenation imaging in the context of stroke.

Stroke is a tragic reminder of the brain’s reliance on oxidative metabolism to maintain function. When perfusion is disrupted the time to reperfusion is essential for recovery, thus quick evaluation and
therapeutic intervention is extremely important. Indeed, “time is brain.” Stroke can be caused by ischemia or hemorrhage, and discriminating between these two forms is the first priority when a patient presents with symptoms. Computed tomography (CT) is the go to imaging modality for patients with acute stroke symptoms given that scans are fast and exclusion criteria for CT are minimal. If hemorrhage is ruled out, then contrast based CT angiography can be used to identify an occluded vessel for endovascular removal. Despite the fact that fast MR scan protocols have been developed that are of comparable length to CT protocols, patient screening and afterhours scanner availability are cited as reasons why MRI was not included in the clinical work up in three out of four recent endovascular clot removal clinical trials (88).

In the EXTEND trial of the effects of the thrombolytic drug, tissue plasminogen activator (tPA), in patients who had a stroke 4.5-9 hours prior to presentation the difference between door to needle times for patients receiving a CT versus a MRI was 78 minutes (88). There remain different opinions about the usefulness of MRI for clinical evaluation of acute stroke (89,90). MRI may still better select patients who would better benefit from thrombolytic therapy after the first three hours of stroke onset (90), but the best MRI based biomarker of brain tissue that remains viable after infarct (i.e. the stroke penumbra discerned by the perfusion-diffusion mismatch) is not as reliable indicator as PET measurements of elevated tissue OEF (91).

Though MRI may not be the go to modality for evaluating acute stroke, there is a role for MRI in evaluating stroke risk. Carotid stenosis affects millions of American and a half million carotid endarterectomies, where the plaque is removed in surgery, are performed each year. OEF is the best indicator of stroke risk in patients with carotid stenosis (92). A better technique for measuring hemispheric or regional OEF could potentially revolutionize the risk stratification of patients with carotid stenosis (93) guiding treatment decisions.

1.4.2 Adult research utility

Cognitive neuroscience has made extensive use of functional MRI (fMRI) to identify the neuroanatomical correlates to higher level brain processes. fMRI is a powerful tool, but explanations of the BOLD signal from first principles remain incomplete and the relative changes in BOLD signal used to identify activated brain regions are not quantitatively comparable between subjects. Questions of spatial selectivity (94), the relationship between vascular physiology and apparent BOLD signal (95), and the best models of the BOLD signal (96) remain active areas of investigation. Insofar as the BOLD signal is a combination of changes in blood volume, blood flow and oxygen content, a high frequency measurement of any one of these parameters would help researchers better understand the relationship between the BOLD signal change and underlying brain energy demands and might be a more effective metric of brain
activation. It is believed that OEF, and ultimately CMRO2, would better spatially resolve neuronal activation because these measures are more closely associated with cellular energy demands (97). Additionally, quantitative fMRI would in theory permit comparisons between the levels of response to a stimulus in different patients, perhaps opening the door to entirely new types of functional experiments.

1.4.3 Neonatal utility

Though babies also suffer from stroke and brain tumors, oxygenation imaging for neonates would also be useful in cases of congenital heart disease and hypoxic ischemic encephalopathy. Brain oxygenation imaging may also help us to understand the brain’s energy demands during development after birth.

Severe forms of congenital heart disease affect 3/1000 live births, and despite the development of life saving surgeries, neurocognitive impairment occurs in over 50% of these cases (98). Oxygenation imaging could help guide the timing of surgical intervention by monitoring brain development directly and intervening only when brain development begins to slow as a result of the cardiovascular disease. If metrics of brain oxygenation are linked to long term cognitive outcomes, then these would provide a surrogate endpoint for surgeons developing new repair techniques and guide perfusion protocols in the operating room (99). Oxygenation imaging may also help explain the cause of white matter injury which is extremely common in patients with CHD and likely causes cerebral palsy in these patients (100,101).

Studies using the currently available oxygenation imaging protocols reveal that CMRO2 in neonates with CHD is lower on average than healthy neonates (45). This finding is also supported by NIRS measurements (102). There is also some indication that different forms of CHD (i.e. different heart defects) have different implications for brain development (103). Studies to investigate how brain energy needs are met even when CHD affects cerebral perfusion would help personalize therapeutic decisions and having regional metrics of oxygen consumption might give insights into the brain regions most vulnerable to injury.

Hypoxic-ischemic encephalopathy (HIE) affects about 2/1000 term live births, and is the result of oxygen deprivation during delivery. Current treatment strategy is to monitor for HIE symptoms and then to cool affected infants to reduce the hyperperfusion and production of reactive oxygen species that results in reperfusion injury (104). Direct measurements of CMRO2 would provide an additional objective measurement of brain function to help clinicians decide when to use therapeutic hypothermia, determine cooling duration and guide post-cooling interventions depending on the severity of injury (105,106). An initial MRI-based study of premature neonates, and those with HIE found that OEF in babies with HIE
was about the same as that in premature babies and both were lower than premature babies at term equivalent age. Likely due to inaccuracies in ASL-based measurement of CBF the study did not find a difference in CMRO$_2$ between HIE and babies a term equivalent age (63).

Many studies have shown that CBF and CMRO$_2$ increase in the first weeks after birth (21,63,73,107). This is only the tip of iceberg for tracking development via quantitative oxygenation imaging. One study shows that the rate of CBF varies on a regional basis with the rate of increase in the frontal lobe being faster than the occipital between 32-46 week (73). There is much to learn about how the metabolic demands of the brain shift during development. Long term trends show the brain consuming an ever larger percentage of the body’s energy until about age 5 before slowly decreasing to adult levels after adolescence (10). Protracted human brain development has major implications for childhood nutrition.

1.4.4 Fetal utility
Much of our current understanding of human CMRO$_2$ during development comes from model organisms or premature babies, given the technical difficulties of taking fetal measurements in vivo. Fetal CMRO$_2$ is more difficult to measure because of small cerebral vessel caliber, arterial blood that is not fully saturated and the difficulty of accessing physiological signals for gating MRI acquisitions (Table 1-1). Some initial studies have yielded important discoveries.

Examining CHD, using a combination of PC-MRI and T$_2$ quantification one group as demonstrated that lower CMRO$_2$ in fetuses with CHD correlates with smaller brain volumes (12). However, these measurement of CMRO$_2$ are based on blood flows in the superior vena cava assuming that it mainly drains blood from the fetal brain, when in reality it drains blood from the entire upper body. Significant challenges remain if we wish to refine our estimates of in utero fetal CMRO$_2$. This finding is particularly interesting in the context of an MRSI study that found NAA concentration in the brains of healthy fetuses increased ever more rapidly than in the brains of fetuses with CHD. Fetuses with the severest forms CHD saw NAA level increase most slowly. In this context NAA was interpreted as a marker of neuroaxonal development that showed that fetuses with severe CHD faced cerebral oxygen-substrate supply inadequate to support normal development (87). These studies support the hypothesis that brain development is disrupted in utero in cases of CHD, which shapes expectations of what therapy can realistically achieve after birth.

Though there have been very few studies using oxygenation imaging to examine the effects on the brain of intrauterine growth restriction, initial investigations via MRI have demonstrated brain sparing
hemodynamics and lower fetal oxygen consumption than in healthy pregnancies (66). Recently an MRI-based diagnostic paradigm has been proposed for IUGR based on high frequency BOLD imaging of the placenta (108). The mother is asked to breathe oxygen enriched air and the bolus of oxygen can then be imaged as it washes through the placenta and transfers to the fetus. One study has focused attention on the time to signal plateau (TTP) after administering the oxygen, and in monozygotic twins found that the twin with poorer outcomes (i.e. based on observations of brain volume, liver volume, and birthweight) had a longer TPP (109). Though remarkable, the study examined monozygotic twins which elegantly controls for many confounding factors (i.e. differences in maternal vasculature, in genetics, in placenta location, in fetal programing, etc.) that currently prevent this metric being applied to diagnose singleton pregnancies. MR-based oxygenation imaging may help characterize baseline physiology sufficiently that TPP together with this additional data could be used to better diagnose IUGR.
Chapter 2

Functional oxygen extraction fraction (OEF) imaging with turbo gradient spin echo QUIXOTIC (turbo QUIXOTIC)

This chapter was previously published Stout JN, Adalsteinsson E, Rosen BR, Bolar DS. Functional oxygen extraction fraction (OEF) imaging with turbo gradient spin echo QUIXOTIC (Turbo QUIXOTIC). Magn. Reson. Med. [Internet] 2017; doi: 10.1002/mrm.26947, and appears here as the final submitted version (minor differences from the published version).

2.1 Abstract

Purpose: QUantitative Imaging of eXtraction of Oxygen and TIssue Consumption (QUIXOTIC) is a recent technique that measures voxel-wise oxygen extraction fraction (OEF), but suffers from long scan times limiting application. We implement multi-echo QUIXOTIC dubbed turbo QUIXOTIC (tQUIXOTIC) that reduces scan time by 8x and then apply it in functional MRI.

Methods: tQUIXOTIC utilizes a novel turbo gradient spin echo readout enabling measurement of venular blood transverse relaxation rate in a single tag-control acquisition. Using tQUIXOTIC we estimate cortical gray matter (GM) OEF, create voxel-by-voxel GM OEF maps, and quantify changes in visual cortex OEF during a blocked design flashing checkerboard visual stimulus. Contamination from CSF partial volume averaging is estimated and corrected.

Results: Average (N=8) cortical GM OEF was estimated as 0.38±0.06 using a 3.4 minute acquisition. Average OEF in the visual cortex was estimated as 0.43±0.04 at baseline and 0.35±0.05 during activation, with average %ΔOEF of -20%. These values are consistent with past studies.

Conclusion: tQUIXOTIC is introduced and shown to successfully estimate cortical GM OEF in clinical scan times and to detect changes in OEF during blocked design visual stimulation. tQUIXOTIC will be useful to monitor regional OEF clinically and in blocked design or event-related fMRI experiments.

2.2 Introduction:

Normal brain function depends on energy made available via cerebral oxygen metabolism. Oxygen extraction fraction (OEF) is directly related to the rate of cerebral oxygen metabolism (CMRO₂), and is a key quantitative indicator of brain function. As such, robust and reliable measurements of OEF have important clinical and basic neuroscience implications (30,110,111). Routine imaging of OEF, however, is
not commonly performed in clinical or research settings, in part due to long acquisition times and complex protocols of typical MRI and PET approaches (31,112).

Clinically, OEF is relevant as an indicator of stroke risk (110) and a potential indicator of stroke ischemic penumbra, that is tissue at risk, but still viable (111). From a neuroscience standpoint, OEF offers a more direct assessment of metabolic changes than the traditional BOLD response, which is the mainstay of functional MRI (35,113). OEF reflects the balance of oxygen delivery and oxygen metabolism and is related to CMRO$_2$ by Fick’s principle:

$$\text{OEF} = \frac{\text{CMRO}_2}{[\text{Hb}]-\text{CBF}\text{S}_\text{aO}_2}$$

where [Hb] is the blood concentration of hemoglobin, CBF is cerebral blood flow and S$_\text{aO}_2$ is the oxygen saturation of arterial blood (53,58). S$_\text{aO}_2$ can be measured using a pulse oximeter, or in healthy adults assumed to be fully saturated.

There are three main classes of MRI-based techniques for quantifying regional cerebral OEF: magnetic susceptibility, $T_2$ relaxometry, and quantitative BOLD, reviewed in (30,31,114). These all measure OEF by quantifying effects of paramagnetic deoxygenated hemoglobin (dHb) in venous blood. $T_2$-relaxometry approaches are particularly appealing, as they typically offer less sensitivity to non-dHb sources of magnetic field inhomogeneity by eliminating extravascular signal. QUantitative Imaging of eXtraction of Oxygen and TIssue Consumption (QUIXOTIC) is among these intravascular $T_2$-relaxometry based approaches, in which $T_2$ of post-capillary venular blood is estimated and calibrated to venous oxygen saturation (SvO$_2$) and OEF (52,115,116,54).

The key innovation of QUIXOTIC is its ability to isolate signal from post-capillary venular blood on a voxel-wise basis, by using specialized velocity selective pulses that exploit differential blood velocities through the circulation (61). A two-step data acquisition paradigm is used where control and tag acquisitions are interleaved. In the control acquisition, velocity selective module 1 (Figure 2-1) eliminates signal from all spins moving faster than a user-defined cutoff velocity ($V_{\text{CUTOFF}}$). If $V_{\text{CUTOFF}}$ is chosen correctly, the remaining signal will come from spins occupying the microvasculature. During the following “outflow” time (TO), a fraction of these remaining spins will accelerate into the draining venules, at which point imaging commences. In the tag acquisition, the experiment is repeated, but velocity selective module 2 is now applied at TO, and eliminates signal from the spins that have accelerated into the venules. Subtracting tag from control images generates an image in which the signal primarily originates from blood in the post-capillary venules.
A major limitation of QUIXOTIC is its long scan duration—only one echo time (TE) is acquired per repetition time (TR) and many repetitions are required to obtain enough SNR at the several echo times needed to determine $T_2$. The traditional QUIXOTIC approach therefore focused on acquiring one echo per scan, with each scan lasting several minutes. Standard single-run, blocked design functional MRI was not possible since $T_2$ (and thus OEF) could not be calculated every TR (117).

In this study, we have modified and improved the original QUIXOTIC approach (61) by implementing a turbo gradient spin echo (GRASE) (118) readout that acquires multiple echoes in a single TR, resulting in a several-fold decrease in imaging time, improved robustness to bulk motion and physiological noise, and providing the possibility to acquire a functional time series. We call this technique turbo QUIXOTIC (tQUIXOTIC). We demonstrate its effectiveness in estimating gray matter (GM) OEF in clinically feasible scan times, and then assess its ability to measure OEF changes in the visual cortex in response to a blocked design visual stimulus. We also estimate and correct for the effects of cerebrospinal fluid (CSF) diffusion attenuation on the tQUIXOTIC signal, a previously described confounder in the QUIXOTIC OEF measurement that is related to the application of velocity selective gradients intrinsic to the technique (61,119–121).

**2.3 Methods**

**2.3.1 Construction of turbo QUIXOTIC sequence using a turbo gradient spin echo (GRASE) readout**

To construct the tQUIXOTIC sequence, a custom GRASE readout utilizing adiabatic inversion pulses and spoiler gradients was added to the previously described QUIXOTIC pulse sequence (61) such that a complete set of control or tag images with different TEs is acquired in a single TR (Figure 2-1).

![Figure 2-1: Turbo QUIXOTIC Pulse Sequence. QUIXOTIC velocity selection followed by GRASE readout with one image acquired per TE.](image-url)
This implementation of GRASE differs from the original (122) in that multiple images are generated per TR. We used a RF-pulse train with spoiler gradients for accurate and robust $T_2$ mapping (123). The spoiling gradients alternate in sign and linearly decrease in magnitude with every $+/-$ pair. For improved robustness to B0 and B1 inhomogeneity, hyperbolic secant adiabatic pulses were implemented as the refocusing pulses. These pulses are nonselective, have a 3.142 ms duration, and are the same as those used in the velocity selective module (61,119). One complete echo planar imaging (EPI) readout is performed between each refocusing pulse and shortened to 12.6 ms using generalized autocalibrating partially parallel acquisitions (GRAPPA) parallel imaging technology (124), acceleration factor = 3. Notably, only even echoes from the GRASE readout were used for $T_E_1$, $T_E_2$, and $T_E_3$ (as seen in Figure 2-1), as the odd echoes are contaminated by non-linear phase accrual from a single adiabatic inversion pulse, which is reversed after a pair (125). The accuracy of $T_1$ and $T_2$ quantification using the standalone GRASE module was verified using doped agar phantoms (3% Agar, 0.03 mM gadolinium) with similar $T_1$ and $T_2$ measurements to GM (61,126). The tQUIXOTIC sequence parameters used in the following experiments were $V_{CUTOFF} = 2.3\text{cm/s}$, $T_{O} = 400$ ms, outflow time (TO) = 725 ms, with GRASE readout parameters voxel size = 3.9x3.9x8 mm$^3$, FOV = 250 mm, $T_E_1/T_E_2/T_E_3/TR$ =25.2/50.4/75.6/3000 ms, BW = 3256 Hz/pixel.

2.3.2 Subject Selection and Data Acquisition

All scans of adult subjects took place at the Athinoula A. Martinos Imaging Center at the McGovern Institute for Brain Research, MIT. Eight healthy volunteers (aged 19 to 30, 4 males and 4 females) were scanned with IRB approval on a Siemens Tim Trio 3T scanner (Siemens Healthineers, Erlangen, Germany) using the Siemens 32-channel head coil.

The slice position for tQUIXOTIC imaging was chosen to intersect an area of the visual cortex that demonstrated a strong BOLD response to a visual stimulation paradigm. The calcarine fissure was located by visually inspecting a 1 mm$^3$ isotropic resolution three dimensional $T_1$-weighted, gradient echo structural scan (FOV = 256x256x176 mm$^3$ $T_E/TR$ = 3.44 / 2530 ms, GRAPPA acceleration factor = 3, total acquisition time = 4.3 min). An axial 2D slice selective EPI spin-echo (EPI-SE) scan that covered the primary visual cortex with seven slices was then acquired during a blocked design visual stimulus (8 Hz flashing radial checkerboard with central fixation point projected onto a translucent screen and viewed through a mirror mounted to the head coil) with one-minute periods of a central visual fixation point interleaved with one-minute periods of a central fixation point plus a radial checkerboard, for a total of five minutes (Figure 2-2). The EPI-SE scan parameters were: voxel size = 3.9x3.9x8 mm$^3$, FOV = 250 mm, $T_E/TR$ = 60/3000 ms, $T_R$ delay = 2385 ms, BW = 3256 Hz/pixel, scan time = 5 min. These data were analyzed by fitting a linear signal model consisting of regressors representing the blocked design stimulus,
a linear and quadratic drift term, and a constant (DC) term. The slice showing the highest \( t \)-statistic values and largest volume of activation during the EPI-SE scan was selected for tQUIXOTIC imaging.

![EPI-SE Stimulus tQUIXOTIC](image)

Figure 2-2: Experimental Design. Visual stimulus paradigm of interleaved central visual fixation point (1 min) with flashing radial checkerboard (1 min), with total duration of 5 minutes for EPI-SE and 7 minutes for tQUIXOTIC. Example tQUIXOTIC tag or control image sets acquired for every TE in one TR = 3s.

tQUIXOTIC imaging then took place in four 7-minute runs (4 minutes total at baseline and 3 minutes total during exposure to visual stimulus). During each run the aforementioned visual stimulus paradigm was displayed (Figure 2-2). After each tQUIXOTIC run, a double inversion recovery (DIR) image for GM-only imaging was acquired using the same slice position and spatial resolution. DIR imaging parameters were, \( TE/\text{TR} = 13/4340 \) ms, inversion times = 3700 ms and 4280 ms to select for GM only, 2232 Hz/Px bandwidth, and scan time = 6 s.

2.3.3 Turbo QUIXOTIC Analysis for OEF measurement in cortical GM and during visual stimulation

Analyses were performed using custom scripts for NeuroLens (www.neurolens.org) and MATLAB (The Math Works, Natick, MA). Each tQUIXOTIC run was individually analyzed to determine OEF of cortical GM at baseline. Simple pairwise subtraction of tag and control images for corresponding TEs generated a tQUIXOTIC time series for each TE (\( TE_1, TE_2, \) and \( TE_3 \), Figure 2-2). Each series was smoothed with a 6
Figure 2-3: Representative data analysis for subject 7. A) t-statistic map \((T > 3.4)\) for the GLM fit of the \(T_E = 76.6\) ms venular blood weighted image series overlaid on the first tag image \((T_E = 25.2)\). SSS contaminated voxels determined from structural imaging are overlaid in purple and removed from further analysis. GLM regressors were stimulus convolved with hemodynamic response function \((EFFECT)\), DC offset \((DC)\) and linear drift. B) Corresponding plot of beta-coefficients from GLM \(\beta_{DC}\) and \((\beta_{DC} + \beta_{EFFECT})\) from within activation ROI (10 voxels) versus TE, with \(T_2\) fit lines.

The acquisitions during visual fixation only (excluding the 12 seconds following each period of stimulation, to allow OEF to return to baseline) were averaged across time to get a single baseline tQUIXOTIC image per TE. The corresponding DIR-GM image was used as a mask to select GM voxels within the baseline tQUIXOTIC images and the average GM signal intensity was calculated. For one subject, DIR images were not acquired; in this case the GM mask was generated by segmenting the EPI-SE images using FSL tools \((BET and FAST)\) \((127)\). These resulting tQUIXOTIC GM signal intensities were plotted against echo time \((T_E = 76.6\) ms) and fit using the two-compartment signal model described below (Equation 3) using a non-linear least squares optimization method \((MATLAB)\) to determine \(T_2\) for venous blood in the cortical GM. \(T_2\) values were then calibrated to venular blood oxygen saturation \((SvO_2)\) based on empirical and biophysical models described in \((52,115,116,54)\). This mapping depends on the hematocrit value for each subject, which we assumed to be the average standard values for hematocrit 0.45 for males and 0.42 for females \((128)\), and on the inter-echo spacing of the GRASE train \((12.6\) ms). OEF for each state was then calculated as \((Sao_2-SvO_2)/Sao_2\), assuming fully saturated arterial blood in healthy adults \((Sao_2 = 1)\).
Representative baseline OEF maps were generated for each tQUIXOTIC run for a single subject. As before, the baseline tQUIXOTIC data was pairwise subtracted, smoothed (10 mm Gaussian kernel) (61) and averaged across time, similar to the original QUIXOTIC mapping approach. On a voxel-wise basis, the tQUIXOTIC signal in cortical GM was again fit using the two-compartment signal model described below (Equation 3) to determine T2 for venous blood in each voxel. SVo2 and OEF were determined as above.

To determine OEF of a functionally activating region during both baseline and activation, the four tQUIXOTIC runs were averaged together. The average 7 min tQUIXOTIC run was then pairwise subtracted and smoothed (6 mm Gaussian kernel). For each TE, a linear signal model was fit to the 7-minute time series of each voxel. The linear signal model consisted of regressors representing the blocked design stimulus, a linear drift term, and a constant (DC). Maps of activation t-statistics were generated for each TE (Figure 2-3a). The t-statistic map for TE3 was used to select a region of interest of the ten most significantly activating voxels (exceeding P < 6.5x10^4, corresponding to t-statistic > 3.35) excluding the region within 10 mm of the center of the superior sagittal sinus to avoid contamination from sinus venous blood. Beta-coefficients (βDC and βEFFECT) from the GLM fit were averaged over the region of interest. Fits of βDC (baseline) and βDC + βEFFECT (baseline plus effect size) for the three even echoes with the two-compartment signal model described below (Equations 2 and 3) determined T2 values at baseline and during activation (Figure 2-3b).

2.3.4 T2 Estimation and correction of CSF contamination to the tQUIXOTIC signal
Applying velocity selective gradients immediately before the imaging readout for the QUIXOTIC tag acquisition, but not during the control acquisition, results in a very small amount of diffusion weighting that is not eliminated upon control-tag subtraction (119). While this is negligible for most voxel constituents, a small component of CSF will persist and slightly contaminate the pure venular blood signal at longer echo times since T2,CSF is much longer than T2,blood (121). Assuming a two compartment model for blood and CSF in the voxel, the measured signal would be:

\[ S(TE) = k_{\text{blood}}e^{-\frac{TE}{T2,\text{blood}}} + k_{\text{CSF}}e^{-\frac{TE}{T2,\text{CSF}}} \]  \[2\]

Where, kCSF and kblood are the y-intercepts and T2,CSF and T2,blood are the transverse relaxation time constants for each compartment. Consequently, CSF signal contamination creates an upward-bias in the estimated T2,blood when a monoexponential fit is used, which results in a lower estimated OEF.

Corrected values for baseline T2,blood and S0 were determined by fitting a two compartment model, to the average tQUIXOTIC signal intensities, S(TE):
\[ S(TE) = S_0 \left[ (1 - X_{CSF}) e^{-\frac{TE}{T_2,\text{blood}}} + (X_{CSF}) e^{-\frac{TE}{T_2,\text{CSF}}} \right]. \]  

\( X_{CSF} \) is the magnetization fraction of diffusion attenuated CSF in a GM voxel acquired with the specific tQUIXOTIC protocol that we used. \( X_{CSF} \) and \( T_2,\text{CSF} \) were determined from calibration data from one subject through methods described in the appendix, and then used to correct the data for all other subjects. This assumes that the volume fraction of \( \text{CSF} \) within GM voxels is relatively constant across humans, which is supported by several studies (129,130).

Since there is increased cerebral blood flow during neuronal activation \( X_{CSF} \) (fundamentally the ratio of diffusion attenuated CSF signal to blood signal) will change during activation. To account for this change, corrected values for \( T_2,\text{blood} \) and \( k_{\text{blood}} \) during activation were determined by fitting Equation 2 to the average tQUIXOTIC signal intensities during stimulation, using \( k_{\text{CSF}} = X_{CSF} S_0 \) and \( T_2,\text{CSF} \) as determined from the corresponding baseline fitting. This approach reflects that CSF volume is assumed to remain constant during brain activation (129).

### 2.4 Results

Average cortical GM OEF at baseline for four runs from each subject are shown in Figure 2-4a, and values for \( T_2, \text{SvO}_2, \) and OEF are given in Table 2-1. The average \( T_2 \) for all subjects was 58±9 ms. Average \( \text{SvO}_2 \) was estimated as 62±6% and OEF as 0.38±0.06. Exponential fitting of the tQUIXOTIC signal averaged across GM voxels and over 3.4 minutes during baseline blocks, was high quality with \( R^2 > 0.99 \) for all fits. For OEF, the average coefficient of variation (COV) for all subjects across runs was 7%.
Representative OEF maps at baseline in GM are shown in Figure 2-5 for Subject 1. Average voxel-by-voxel OEF was 0.35±0.01 across the four maps, compared to 0.36±0.01 for the GM mask analysis.

During visual stimulation, significant changes in $T_2, SVO_2$, and OEF were observed in the visual cortex. Results corrected for CSF contamination are given in Table 2-2. Figure 2-4b presents average cortical GM OEF at baseline, and OEF in the visual cortex at baseline and activation for each subject. Average baseline $T_2$ was 50±6 ms and activation $T_2$ was 64±8 ms. OEF changed (paired $t$-test, $P = 0.00001$) from 0.43±0.04 at baseline to 0.35±0.05 during activation with an average relative change of -20%. Exponential fitting of the tQUIXOTIC signal at baseline and activation averaged across ten voxels in the visual cortex was high quality with $R^2 > 0.99$ for all fits.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$T_2$ baseline [ms]</th>
<th>$T_2$ activation [ms]</th>
<th>$\Delta T_2$ [ms]</th>
<th>$SVO_2$ baseline [%]</th>
<th>$SVO_2$ activation [%]</th>
<th>OEF baseline</th>
<th>OEF activation</th>
<th>%ΔOEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>71</td>
<td>18</td>
<td>60</td>
<td>70</td>
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<td>0.30</td>
<td>-26</td>
</tr>
<tr>
<td>2</td>
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<td>75</td>
<td>16</td>
<td>64</td>
<td>72</td>
<td>0.36</td>
<td>0.28</td>
<td>-23</td>
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<tr>
<td>3</td>
<td>53</td>
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<tr>
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<td>55</td>
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<td>61</td>
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<td>54</td>
<td>63</td>
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<td>8</td>
<td>45</td>
<td>62</td>
<td>17</td>
<td>54</td>
<td>66</td>
<td>0.46</td>
<td>0.34</td>
<td>-25</td>
</tr>
<tr>
<td>Mean±STD</td>
<td>50±6</td>
<td>64±8</td>
<td>14±4</td>
<td>57±4</td>
<td>65±5</td>
<td>0.43±0.04</td>
<td>0.35±0.05</td>
<td>-20±5</td>
</tr>
</tbody>
</table>

Table 2-2: Baseline and activation $T_2$, $SVO_2$, and OEF values from the visual cortex showing a significant decrease in OEF with activation ($P = 0.00001$).

Data above is reported after correction for CSF contamination using $X_{CSF} = 0.112$, determined from calibration scans in cortical GM at 3.9x3.9x8 mm$^3$ resolution. Fit parameters for determining $X_{CSF}$ as described in the appendix are given in Table 2-3. CSF correction did not alter the statistical significance of any reported comparisons. In cortical GM at baseline, uncorrected $T_2$ was on average 16±0.4 ms higher than corrected. Average uncorrected OEF was 0.29±0.05, or 0.09±0.01 lower than corrected OEF. $X_{CSF}$ during activation—recalculated from $k_{CSF}$ and $k_{blood}$ after fitting—was 0.083±0.01 across all subjects.

<table>
<thead>
<tr>
<th>Model</th>
<th>Residual sum of squares</th>
<th>$x_1$</th>
<th>$x_2$</th>
<th>$x_3$</th>
<th>$x_4$</th>
<th>$x_5$</th>
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<tbody>
<tr>
<td>$(x_1(\Delta TE) + x_2)e^{-TE/x_3} + (x_4(\Delta TE) + x_5)e^{-TE/1.2}$</td>
<td>0.0511</td>
<td>-57.1</td>
<td>6.85</td>
<td>0.061</td>
<td>-4.88</td>
<td>0.835</td>
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Table 2-3: Fit parameters for model given in Equation A3, with $T_{2,CSF} = 1.2$ s.
2.5 Discussion

We have introduced a GRASE implementation of QUIXOTIC that permits estimation of regional cerebral oxygenation in a fraction of the original imaging time, making the technique feasible for clinical and functional imaging. The average estimate of baseline cortical GM OEF falls in the expected physiological range and is comparable to results from other MRI (38,58,131) and PET studies (57,132,133). We were able to demonstrate measurement of OEF in GM in 3.4 minutes, making tQUIXOTIC one of the fastest approaches for determining baseline OEF (31). It permits GM OEF mapping and was validated by performing a blocked design functional activation experiment using a flashing visual stimulus. Our results demonstrate a significant decrease in OEF during full field visual stimulation (average %ΔOEF = -20%, \( P = 0.00001 \)) in agreement with previously reported values for OEF in visual activation paradigms (Table 2-4). To our knowledge, these results are the first MRI based measurements of regional OEF change with brain activation, based on a single functional time course, and without the use of external gases like oxygen or carbon dioxide. This becomes possible since tQUIXOTIC permits estimation of \( T_2 \) every two TR, since all echoes are acquired per TR.
tQUIXOTIC results without CSF correction are similar to the previously published QUIXOTIC results (see values in Table 2-4). Though QUIXOTIC scans are lengthy, baseline QUIXOTIC and tQUIXOTIC measurements of T2 and OEF in cortical GM from one subject were acquired as a sanity check. Both scans were acquired from the same slice location, and GM was selected using a DIR scan as a mask. QUIXOTIC sequence parameters were as described in (61). For QUIXOTIC GM T2 and OEF were 83±19 ms (mean ± standard error of the estimated parameter) and 0.25, respectively, and for tQUIXOTIC 77±3 ms and 0.28. These values are indistinguishable considering the standard errors, and fall within one standard deviation of the mean values for all subjects scanned with each technique.

The standard errors given for both T2 estimates underscore that though raw SNR over an imaging run is 2x lower using the GRASE readout in tQUIXOTIC, we observe improved fit quality in tQUIXOTIC (R²>0.99) versus QUIXOTIC (R²>0.95), which we attribute to the lower physiological noise and less bulk motion captured with tQUIXOTIC. tQUIXOTIC 8x faster overall, meaning that in terms of SNR per unit time tQUIXOTIC outperforms QUIXOTIC by 4x, which reduces the physiological noise during acquisition. Furthermore, by
acquiring images from all TEs in every control and tag repetition there are far fewer periods were bulk motion can disrupt the tag-control difference images, and any motion should affect one repetition's images similarly.

Numerous PET and MRI studies find baseline OEF around 0.4 (57,134–136). Table 2-4 summarizes the OEF findings from other studies and supports the following comparisons. Without correcting for CSF contamination our estimates of cortical GM OEF values are in the lower MRI range (38,49,58), however after correction they are better agreement with both PET and MRI estimates (57,112,134,135,137,138), suggesting the CSF correction improves QUIXOTIC accuracy. Our standard deviation across subjects for cortical GM OEF of 0.06 is similar to the values reported by PET and MRI studies. Since tQUIXOTIC scans to determine OEF in GM at baseline required just a few minutes of data, we were able to perform multiple OEF measurements for each subject to ascertain intrasubject, same session, variability (Figure 2-4 and Table 2-1). The average same session COV, a metric of measurement noise, was 5.6% (discounting the large variation for subject 5 caused by a motion degraded run), which is slightly higher than the 3.2% reported for the whole-brain T$_2$-relaxation under spin tagging (TRUST) technique (136), even though TRUST benefits from high SNR in comparison to tQUIXOTIC. The intersubject COV, which in addition to measurement noise reflects the variance due to different physiology between subjects, was 15%. This is similar to that reported for other MRI techniques and corroborates the idea that baseline OEF is quite variable between subjects (139).

tQUIXOTIC is a voxel-by-voxel technique capable of producing cortical OEF maps in 3.4 minutes, as demonstrated in Figure 2-5. We observe that variation between maps from different runs is small, and that these maps appear qualitatively similar to those generated by qBOLD (134), and T$_2$ calibrated BOLD (135). It is reassuring that average OEF calculated for the voxel-by-voxel analysis and whole GM analysis were
in excellent agreement. However, fits were generally not possible in white matter due to low SNR. Combining tQUIXOTIC runs was attempted to increase SNR, but suspected out-of-plane motion between runs and physiological noise (140) (over the course of 28 minutes of total scanning) diminished SNR gains despite the use of 2D-motion correction, and OEF mapping of white matter was not improved. Since tQUIXOTIC, as implemented, is a single slice technique with fairly low in-plane resolution (64x64 voxels in plane), out-of-plane motions are not well addressed by standard motion correction approaches. Future approaches will consider prospective motion correction strategies to improve SNR with longer acquisitions (15).

A robust response in the visual cortex to a flashing radial checkerboard stimulus makes it a popular paradigm for characterizing changes in cerebral oxygenation upon stimulation (38,58,115,138,141). We examined changes in OEF in the visual cortex due to visual stimulation to further validate tQUIXOTIC GM results and demonstrate the feasibility of tQUIXOTIC for detecting functional changes in brain state. The GRASE implementation is an important advance for functional imaging, as the required echoes for \( T_2 \) fitting and SvO\(_2\) or OEF estimation are acquired in single tag-control acquisitions, giving a time point every two repetitions, which is similar to standard arterial spin labeling approaches that have been commonly used for functional challenges (113). This advance permits use of standard single-run, blocked and event-related functional paradigms, not possible with the original implementation. Whereas functional imaging with BOLD depends on blood volume, blood flow and tissue metabolism (35), and arterial spin labeling is only a marker of vascular changes (113), OEF-based functional imaging is related to the underlying balance between tissue metabolic demand and oxygen delivery. Ultimately, changes in CMRO\(_2\), which could be estimated by combining tQUIXOTIC derived OEF with a measure of local CBF (61), would permit direct quantitative assessment of cerebral metabolic demand. Further, given that baseline SvO\(_2\) or OEF has been shown to modulate BOLD and CBF signal changes (137,139), fast approaches to OEF mapping may also provide useful calibration in multi-subject fMRI studies.

Our results for changes in OEF with visual stimulation are comparable to two previous calibrated BOLD studies at 3T (38) and (138), see Table 2-4. Our value for \%\(AOEF\) is in excellent agreement with (38), -20% versus -21%, which used a similar visual stimulus (off 1 min, on 3 min, off 2 min). Notably our result for absolute OEF of 0.43 at baseline in the visual cortex is higher than that found in (38) of 0.29. Calibrated BOLD uses the information provided by hyperoxia and hypercapnia calibration experiments to determine baseline properties of the BOLD signal, which are then used to estimate absolute OEF and CMRO\(_2\). In contrast, tQUIXOTIC is used to directly measure the \( T_2 \) from the venular blood compartment, which is empirically and theoretically related to SvO\(_2\) (54). Thus, despite different modeling and dominant signal
source (intravascular versus extravascular) the agreement between (38) and tQUIXOTIC for assessing relative change in OEF during activation in the visual cortex suggests both methods are sensitive to the same physiological change with stimulation when a similar stimulation paradigm is used. However, the different absolute OEF may be attributable to these underlying differences in methodology. Interestingly, the combined hyperoxia-hypercapnia calibrated BOLD technique found a higher baseline OEF of 0.435 at 7T (137), nearly identical to our value.

The other calibrated BOLD study, (138), found an absolute OEF at baseline in the visual cortex of 0.48, which is even higher than our own, but a much smaller %ΔOEF of -6.25% than observed in (38) or our own study. The authors of (138) attribute the small %ΔOEF to a decline in stimulus induced CMRO₂ over the long 20 min stimulation paradigm, which was necessary to present a graded gas challenge to calibrate the BOLD signal model used to calculate OEF and CMRO₂. These differences draw attention to the fact that the metabolic response to visual stimuli is still being investigated (142) and variations in the stimulus presentation such as visual field coverage, presentation duration and subject attention may also influence the OEF response.

We compared OEF in the visual cortex and in cortical GM for eight subjects (Figure 2-4b). In 6 subjects visual cortex OEF is higher than GM OEF (Figure 2-4b), which is a trend supported by MRI and PET studies (112,138). In two of the subjects the trend is reversed which is similar to that found in (38,39). Differences in the apparent distribution of OEF across the cortex could be explored with an upgraded tQUIXOTIC with improved SNR and multi-slice acquisition as we discuss below, while a greater number of subjects would be needed to address the overall tendencies in OEF within the primary visual areas.

Our correction for signal contamination due to diffusion-attenuated CSF resulted in a greater change in $T_{2,\text{blood}}$ (-15.5 ms) than a previous study (-10.2 ms) that explored the contamination in the QUIXOTIC technique (121). Differences in sequence implementation that affect the degree of diffusion attenuation or the volume of the selected blood pool would directly impact the magnetization fraction, $X_{\text{CSF}}$, which could explain these different changes in $T_2$. The approach of biexponential modeling to determine the signal contamination of CSF has been used previously (143). The signal contamination we determined is small given that the CSF volume fraction in a typical voxel (10.7% at baseline) is about double the blood volume (129). Most of the CSF signal is being eliminated during control-tag subtraction. In the healthy young adults who were subjects in our study, we are confident that our approach to CSF correction requires calibration scans only once per voxel size and QUIXOTIC parameter set, and the relevant correction factor can be applied to all similar scans. Differences in the CSF volume fractions between subjects has only a small
effect on corrected OEF estimates (COV = ±3.4% and no bias, based on a Monte Carlo simulation using the 13% intersubject variation in CSF volume fraction reported in (130) as the expected variation in $X_{CSF}$) in comparison to the average same session variation we found (COV = 5.6%).

Many central nervous system pathologies that cause focal or regional oxygenation changes will not have significant fluctuations in CSF content, particularly in their early stages (e.g. early ischemic stroke). When detecting changes in OEF, non-CSF corrected tQUIXOTIC will certainly be useful in some cases, as the lesional OEF values can be compared to OEF in regions of normal brain. However, several central nervous system pathologies (e.g. Alzheimer’s disease, certain brain tumors) and old age, can lead to different CSF to tissue volume ratios, in which case $X_{CSF}$ might be a source of error in corrected OEF estimates. The proposed CSF correction addresses the volume fraction effect arising from single voxels (0.12 ml) deemed GM based on DIR scans. It is unclear how an overall change in CSF volume and brain volume affects the volume fraction of CSF in a nominally GM voxel of the size we use, since most studies investigating CSF volume fraction changes have been undertaken to correct the metabolite concentrations obtained from much larger (~8 ml) MR spectroscopy voxels, with one study suggesting no detectable change (144) while another shows larger intersubject variation that accompanies disease (145). Though higher resolution may minimize these fluctuations in CSF volume fraction, in specific settings like Alzheimer’s disease, brain tumor, or studies of aging, we suggest that measuring $X_{CSF}$ is important to ensure that estimates of OEF are not biased.

A two compartment exchange model was used to perform the $T_2$ to $SvO_2$ calibration (59,54), which depends on the subject’s hematocrit and inter-echo spacing of the $T_2$ preparation module, with model coefficients empirically derived from $T_2$-relaxation under spin tagging (TRUST) data (54). TRUST varies TE based on pre-readout $T_2$ preparation, which is inherently insensitive to flow, in contrast to our GRASE approach, which will be slightly sensitive to flow effects. However, we assume that flow effects are minimal between the short 12.6 ms echoes, particularly given that most of the venular blood signal within the cortex is originating from low-velocity microvasculature. We acknowledge a small bias may exist since later echoes will have their corresponding signal intensity biased upward due to increased signal accumulation in venules. Experiments varying the inter-echo spacing will further characterize the magnitude of this error, although preliminary work in (61) suggests that this is a small source of error.

Additional work is also needed to characterize the tQUIXOTIC signal originating from larger vessels, such as the superior sagittal sinus, which limited the choice of activated visual cortex voxels in this particular study. Theoretically, though tQUIXOTIC velocity selection should eliminate signal in large veins, turbulence and changes in flow direction when vessels emerge from the cortex and combine with larger
veins may mean that some apparently slowly moving blood arrives in the superior sagittal sinus during TO. Similarly, signal appearing in the superior sagittal sinus in velocity selective arterial spin labeling experiments has been attributed to the nonmonotonic velocity of blood as it drains from the microvasculature into large vessels (146).

Future work on tQUIXOTIC will focus on improving the SNR of the technique and implementing a multi-slice readout. One approach to improve SNR will be to optimize the radio frequency pulse train in the GRASE module to reduce the signal intensity difference between odd and even echoes (147,148). Optimization will also reduce specific absorption ratio (SAR). Currently we operate tQUIXOTIC scans with the SAR limit set to IEC Level 1, and we were SAR limited to acquire only 6 echoes for the 7 minute tQUIXOTIC runs. We could further minimize SAR by empirically determining the lowest power necessary to meet the adiabatic threshold for the inversion pulses. tQUIXOTIC was implemented as a single slice technique such that one complete image could be acquired per TE and 6 second temporal resolution could be achieved overall. However, a multi-slice acquisition would significantly increase the value of tQUIXOTIC, in both clinical and functional settings, where regions of interest may not be known a priori and more regional measurements are desired. Specifically, improved volume coverage would permit exploration of how OEF is tied to neural activity, perhaps using retinotopic mapping to examine the specificity of BOLD in comparison to tQUIXOTIC based OEF. When a lower image-to-image temporal resolution is acceptable (for example in clinical and non-functional MRI applications), but multiple slice coverage is desired, the GRASE module could be substituted with a 3D segmented readout (e.g. 3D spiral or 3D GRASE) (149,150). For fMRI applications that still require high image-to-image temporal resolution, simultaneous multi-slice acquisition technology will allow acquisition of several slices per TR (28).

2.6 Conclusion

Turbo QUIXOTIC marks a 8x decrease in acquisition time over the original approach, enabling clinical and functional OEF imaging including cortical GM OEF mapping in 3.4 minutes and generation of an OEF time course for functional MRI. Future experiments will use tQUIXOTIC to quantify OEF changes during different challenges (e.g. gas inhalation and non-visual functional tasks), demonstrate CMRO$_2$ quantification (with additional cerebral blood flow measurement), and evaluate OEF and CMRO$_2$ in pathology such as stroke and brain tumor.
2.7 Appendix

In the original QUIXOTIC technique (61), $T_2$ of blood is determined by fitting a monoexponential decay to the venular-blood weighted signal:

$$S(TE) = k e^{-\frac{TE}{T_2}}.$$  \[A1\]

However, due to diffusion weighting from the velocity selective module applied only for tag acquisitions, some CSF signal remains after control-tag subtraction (61,119). Thus, the $T_2$ of blood is overestimated by monoexponential fitting since the signal includes some portion of CSF with its much longer $T_2$.

One proposal for how to empirically correct this CSF signal contamination in QUIXOTIC (121), assumes that any QUIXOTIC signal remaining after a long TE (~400 ms) originates from CSF alone, since the $T_2$ of blood is considerably shorter than the $T_2$ of CSF and will have decayed into the noise. This CSF signal can then be subtracted from the QUIXOTIC signal to compensate for the CSF contamination at short TEs where the blood signal predominates:

$$S_{\text{corrected}}(TE) = S_{\text{uncorrected}}(TE) - k_{CSF} e^{-\frac{TE}{T_{2,CSF}}}$$  \[A2\]

Since $k_{CSF}$ is determined empirically by measuring the signal intensity from a second long TE image, this provides a simple empirical method to correct for CSF contamination in QUIXOTIC.

For tQUIXOTIC, we first attempted the approach described in (121), but increasing the number of refocusing pulses (from 6 to 32) at the nominal delta TE of 12.6 ms to reach TE = 400 ms was not possible due to SAR limits. We thus increased the ATE to acquire data at longer TEs, which utilized less refocusing pulses and was less SAR intensive.

We acquired calibration data from one subject to determine the appropriate CSF correction for a typical grey matter voxel and then applied this result to all our data acquired with the same resolution and acquisition parameters. The calibration data consisted of tQUIXOTIC acquisitions with different ATE values from one subject. ATE values were 12.6, 50, 60, 70, 80, 90 and 100 ms. Five echoes were acquired with each ATE, except for the standard three echoes for $\Delta TE = 12.6$. The total scan duration was 317s per acquisition. A DIR image for GM-only imaging was collected after each acquisition using the parameters described previously.

Since the signal from CSF is purposefully suppressed by subtraction, we first determined the value of $T_{2,CSF}$ within GM using the pre-subtraction images. Images were smoothed and averaged over all grey matter. Each acquisition with different $\Delta$TE was analyzed separately. Those acquisitions with two or more average
signal intensities with TE ≥ 500 ms were then fit assuming monoexponential decay (Equation A1), since all signal remaining at these long TEs should only be from CSF. These fits resulted in average $T_{2,CSF} = 1.2$ s, which is similar to that computed in (121).

The calibration data was then analyzed using the same methods described for the tQUIXOTIC experiments. In short, the data was pairwise subtracted, smoothed (6 mm Gaussian kernel), and averaged across time. The corresponding DIR-GM image for each acquisition was used as a mask to select GM voxels and the average GM signal intensity was calculated.

We fit Equation 2, with $T_{2,CSF} = 1.2$ s, to the average GM signal intensity and observed a large residual due to signal discontinuities when similar TEs were acquired with different ΔTE (Figure 2-6), suggesting that $k_{CSF}$ and $k_{blood}$ had some dependence on the inter-echo spacing (ΔTE) of the GRASE readout. We altered the Equation 2 model for the average signal intensity measured at each echo time ($S(TE)$) by introducing a linear dependence on ΔTE for both y-intercept terms:

$$S(TE, ΔTE) = (x_1(ΔTE) + x_2)e^{-TE/x_3} + (x_4(ΔTE) + x_5)e^{-TE/1.2}. \quad [A3]$$

This data-driven alteration of the model dramatically improved fitting with an $F$-statistic = 133, $P < 5 \times 10^{-15}$. We suspect the variation in y-intercept with ΔTE is biophysical and may be due to exchange of water between voxel compartments or diffusion around high susceptibility veins in the CSF (53,151).

We then determined $X_{CSF}$, the magnetization fraction of diffusion attenuated CSF within a GM voxel, which is specific to the tQUIXOTIC parameters used in our experiments. Specifically, for $ΔTE = 12.6$ ms, $X_{CSF} = k_{CSF}/(k_{blood}+k_{CSF}) = 0.112$, where $k_{CSF} = x_4(0.0126)+x_5$ and $k_{blood} = x_1(0.0126)+x_2$ with $x_i$ given in Table 2-3. The magnetization fraction ($X_{CSF}$) is valid for $ΔTE = 12.6 - 100$ ms, but $X_{CSF}$ for $ΔTE > 0.5(1/T_{2,blood})$ are likely not relevant for tQUIXOTIC in practice, as the blood signal will have significantly decayed at these longer echo times. Variation in $X_{CSF}$ for different ΔTE is due to the same biophysical properties.
hypothesized to affect the y-intercepts in equation A3. To be clear, $X_{CSF}$ is not the CSF volume fraction. It is the fraction of diffusion attenuated CSF that is not eliminated by control-tag subtraction in comparison to the venular-blood weighted signal resulting from QUIXOTIC velocity selection (i.e. the magnetization fraction). As long as the parameters of the velocity selective modules (i.e. the gradient, gradient pulse separation, and gradient pulse duration) are the same as what we used here, we would expect the same relative contribution of CSF contamination so this result could be applied to other studies. However, the approach of (121) is subject specific when used to correct QUIXOTIC acquisitions.

Corrected $T_{2,blood}$ values were then determined as described above, by fitting tQUIXOTIC data with the two-compartment model given in Equations 2 and 3.
Chapter 3

Assessing the effects of subject motion on T2 relaxation under spin tagging (TRUST) cerebral oxygenation measurements using volume navigators

tQUIXOTIC permits whole brain OEF measurements in 3.4 minutes, and high temporal resolution regional measurements when data can be averaged over 28 minutes of acquisition time, but these durations are too long for neonatal imaging. Table 1-1 gives average neonatal head size and the magnitude of typical neonatal head movements during scanning. Both factors make tQUIXOTIC imaging of neonates extremely difficult. tQUIXOTIC is limited by SNR and neonatal imaging currently takes place using MRI receive coils designed for adult imaging. With receive coils further from the neonatal head SNR is reduced as are the advantages of parallel imaging acceleration. In addition, the assumption of only small involuntary head movements is invalid. A prospective motion correction strategy would be needed to permit tQUIXOTIC imaging of neonates. However, there are other MRI based techniques for measuring global OEF, and the TRUST technique has been used previously to measure global CMRO2 in healthy neonates (21). This chapter examines the effects of motion on TRUST quantification and was previously published as Stout JN, Tisdall MD, McDaniel P, Gagoski B, Bolar DS, Grant PE, Adalsteinsson E. Assessing the effects of subject motion on T2 relaxation under spin tagging (TRUST) cerebral oxygenation measurements using volume navigators. Magn. Reson. Med. [Internet] 2017;78:2283–2289. doi: 10.1002/mrm.26616. It appears here as the final submitted version (minor differences from the published version).

3.1 Abstract

Purpose: Subject motion may cause errors in estimates of blood T2 when using the T2-relaxation under spin tagging (TRUST) technique on non-compliant subjects like neonates. By incorporating three-dimensional volume navigators (vNavs) into the TRUST pulse sequence, independent measurements of motion during scanning permit evaluation of these errors.

Methods: The effects of integrated vNavs on TRUST-based T2 estimates were evaluated using simulations and in vivo subject data. Two subjects were scanned with the TRUST+vNav sequence during...
prescribed movements. Mean motion scores were derived from vNavs and TRUST images, along with a metric of exponential fit quality. Regression analysis was performed between $T_2$ estimates and mean motion scores. Also, motion scores were determined from independent neonatal scans.

**Results:** vNavs negligibly affected venous blood $T_2$ estimates and better detected subject motion than fit quality metrics. Regression analysis showed that $T_2$ is biased upwards by 4.1 ms per 1 mm of mean motion score. During neonatal scans, mean motion scores of 0.6-2.0 mm were detected.

**Conclusion:** Motion during TRUST causes an overestimate of $T_2$. Predicted errors in $T_2$ estimates by as much as 7.4 ms in neonatal scans urges caution when comparing TRUST results between non-compliant cohorts.

### 3.2 Introduction

A variety of quantitative measures derived from MRI have been found to demonstrate significant biases due to even small amounts of subject motion (152–156), and motion is known to cause high failure rates for MRI studies in non-compliant subjects (21,24,157). $T_2$ relaxation under spin tagging (TRUST) is a quantitative technique used to measure cerebral oxygenation (59,158), which is of particular interest in neonates (11,45,12,159,160), but subject motion is prevalent in this cohort. 17% of scans in a previous study of neonates using TRUST were unusable due to motion artifacts (21), and this rate has been as high as 30% in our own investigations (161). Biases due to motion may confound comparisons of cerebral oxygenation measurements between studies.

Similar to many quantitative measures derived from MRI, TRUST relies on comparing changes between image volumes acquired in different experimental conditions. In particular, TRUST uses spin tagging to isolate the venous blood signal in large draining veins of the brain via label and control image subtraction (59). This blood signal is then imaged with varying amounts of $T_2$ weighting and then fitted with an exponential function to estimate the $T_2$ relaxation rate of blood. The $T_2$ of blood is empirically related to its oxygen saturation (52,54,55,162). Motion occurring between these two images would presumably affect the venous blood $T_2$ estimation.

Previous work to assess motion confounds of the TRUST technique was limited to small motions that occurred in otherwise compliant subjects (59,163). This level of evaluation makes sense for compliant subjects considering that two dimensional (2D) registration during data processing would only account for in-plane movements (59,163,164). In relatively still subjects, a correlation between more subject motion and worse exponential fit quality was confirmed, but no statistically significant effect of motion on $T_2$ or
venous oxygen saturation (SvO₂) was reported (59,163). These conclusions cannot be extrapolated to cases where subject motion is large or frequent.

Volume navigators (vNavs) were developed as a means to detect low-frequency subject motion during a scan by using the three-dimensional (3D) imaging capability of the MRI scanner itself (27,165). vNavs are 3D-encoded echo planar images with low resolution, typically 8x8x8 mm³, that can be acquired in under 300 ms. vNavs are inserted into a parent sequence during dead-time where magnetization recovery or flow is taking place. Despite the low spatial resolution, sub-millimeter rigid body motions in three dimensions are quantifiable (27,166).

Though vNavs were developed for prospective motion correction, here we used them as a retrospective tool to track subject motion during the TRUST sequence and we evaluated the effects of motion on the estimates of blood T₂. It is extremely difficult to evaluate the effects of motion in the neonatal and infant cohort itself. Anesthesia or sedation that creates a quiescent state may also affect baseline physiology (167-170), and limited scan time for these subjects makes it unlikely that matched baseline no motion and motion corrupted scans can be acquired in the same session. For these reasons, we attempted an initial characterization of the effects of motion on TRUST using healthy adult volunteers. We demonstrated the negligible impact of vNavs on the TRUST-based quantification of T₂ in still subjects, before quantifying the effect of subject motion on T₂ estimates. Finally, we used motion tracks gathered during structural brain scans of neonates to approximate the impact of neonatal motion on TRUST results.

3.3 Methods

3.3.1 TRUST Sequence Modification
We implemented the TRUST sequence (Figure 3-1A) following the published pulse sequence description (59). Two changes were made: we used adiabatic refocusing pulses in the T₂-preparation module, and we inserted vNav modules before the T₂-preparation module.
The T₂ preparation module consisted of a +90-degree pulse, pairs of hyperbolic secant modulated adiabatic refocusing pulses, and a -90-degree pulse. Inter-echo spacing was 10 ms, such that effective echo times (\(T_{\text{EEFFECIVE}}\)) of 0, 18, 36, 72 and 144 ms could be generated. The method for determining the correction applied to \(T_{\text{EEFFECIVE}}\) due to the duration of the adiabatic pulses and the performance of these pulses has been reported previously (61,126).

vNav acquisition modules (27), consisting of a low resolution 3D-EPI volume acquisition, were inserted into the TRUST sequence. The module was placed directly before the T₂-preparation module, and thus ends at \(T_I - T_{\text{EEFFECIVE}}\) after the inversion pulse. It was inserted here to be close to the TRUST readout, and so that consistent contrast in the vNav was maintained within \(T_{\text{EEFFECIVE}}\). vNav imaging parameters were: \(T_E/T_R = 5.2/11\) ms, flip angle = 3°, resolution = 8 x 8 x 8 mm³, field of view = 256 mm, 6/8 partial Fourier, bandwidth = 4464 Hz/Px, EPI factor = 32, total acquisition time = 300 ms. To test our hypothesis that vNav modules would have negligible effects on the TRUST image magnetization in the absence of subject motion, Bloch simulations of the vNav module were performed (171).

3.3.2 Experiments
All scans of adult subjects took place at the Athinoula A. Martinos Imaging Center at the McGovern Institute for Brain Research, MA, USA. Four adults (2 male, 2 female, mean age=21) were scanned with
IRB approval on a Siemens Tim Trio 3T scanner (Siemens Healthineers, Erlangen, Germany) using the Siemens 32-channel head coil. The TRUST image was positioned by visual inspection of a 1 mm³ isotropic gradient echo structural scan, 25 mm above the confluence of the sinuses perpendicular to the superior sagittal sinus (SSS). The matrix size was selected to be the same as that used in neonatal imaging so that the number of voxels across the SSS would be similar, but the field of view was 240 mm (160 mm, for neonates). TRUST imaging parameters were: TE/TR = 12/5000 ms, TE_{EFFECTIVE} = 0, 18, 36, 72, 144 ms, resolution = 3.4 x 3.4 x 5 mm³, inversion time = 1200 ms, tagging width = 100 mm, tagging gap = 25 mm, 3 sets for averaging, total acquisition = 2:30.

TRUST can be used to determine SvO₂ by relating T₂ to saturation via an empirical relationship (54,55,162,172,173). We quantified the effects motion on T₂ estimates, since this relationship is complicated and depends on blood hematocrit and possibly the types of hemoglobin—adult or fetal—present in the blood (172,173).

Each TRUST trial was analyzed using custom Matlab (MathWorks, Natick, MA) routines. The three brightest voxels in a manually drawn ROI of approximately 25 voxels near the SSS were averaged to give a signal intensity (S(TE_{EFFECTIVE})) value for one post-subtraction image. Fitting to 
\[ S(TE_{EFFECTIVE})=S_0\exp(TE_{EFFECTIVE} \times C) \] was performed by taking ln(S(TE_{EFFECTIVE})) and performing a linear least squares fit. The T₂ of blood was then determined as 
\[ T_2,\text{blood}=1/(R_1,\text{blood}+C), \ R_1,\text{blood}=0.62 \] (59). Goodness of fit was measured by the standard deviation of the residuals (SDR) of the linear fit.

3.3.2.1 Empirical test of vNav module’s effects
Alternating TRUST and TRUST+vNav trials were gathered from two subjects who were instructed to remain still during the scan, to test the effects of vNav modules on T₂ quantification.

3.3.2.2 Evaluating the effects of motion on TRUST
To evaluate the effects of motion, two compliant adult subjects were instructed to move their head in a prescribed manner or remain still for alternating TRUST+vNav trials. Motion was rehearsed before entering the scanner for the types, magnitudes and timings of movement given in Table 3-1. These different motion descriptions were to guarantee a variety of movements, not to validate the accuracy of vNavs nor specify every possible movement. The movement magnitudes were roughly calibrated using the nose bridge on the 32-channel head coil as a guide by asking the subject to move their nose to different positions relative to the nose bridge from the center line (see descriptions in Table 3-1). Large,
medium and small motions equated to a nose movement of approximately 35 mm, 17 and 10 mm respectively with the exact size depending on the facial geometry of the subject. The timing of movements was left up the subjects and so was unknown with respect to sequence timing, but instructions were given to move continuously or re-position with rests between movements.

<table>
<thead>
<tr>
<th>Type of motion</th>
<th>Magnitude of motion</th>
<th>Timing of motion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;yes&quot; nod (rotation about a right-left axis)</td>
<td>Large (move the nose from touching one side of the nose bridge to the other, or similar perceived motion)</td>
<td>Continuously (&quot;slowly move without stopping&quot;)</td>
</tr>
<tr>
<td>&quot;no&quot; shake (rotation about an inferior-superior axis)</td>
<td>Medium (move the nose from center to touching on one side nose bridge, or similar perceived motion)</td>
<td>Re-position with rests (&quot;move to a new position and hold it for a couple of breaths, then repeat&quot;)</td>
</tr>
<tr>
<td>random (combinations of nodding and rotation as well as translations)</td>
<td>Small (from center to the side without touching the nose bridge, or similar perceived motion)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-1: Prescription for motion, one attribute from each column was selected for each motion trial.

To accommodate the spin history shadow from the label pulse (Figure 3-1A), offline registration of the vNav images was performed using the FSL FLIRT tool (174) and custom Matlab scripts. We adapted the methods proposed in (16) to co-register volumes from a region of interest excluding the spin history shadow. Rigid, six degree of freedom, affine translations between each vNav and the first vNav \( (T_{1,N}) \) were generated. Translations between label and control image pairs were calculated using \( T_{L,C} = T_{1,C} (T_{1,L})^{-1} \).

We defined a motion score to reflect movement in the volume near the SSS, reasoning that this motion is what adversely affects blood signal isolation via spin tagging. We adapted the root mean square deviation framework proposed by Jenkinson (175), by setting the volume of interest (VOI) to be a sphere centered on SSS with a 16 mm radius. Thus the motion score for each label and control image pair was:

\[
E_{RMS} = \frac{1}{\sqrt{5}} R^2 \text{Trace}(A^T A) + (t + A x_c)^T (t + A x_c), \quad \text{where} \quad M = I - T_{L,C} = \begin{bmatrix} A & t \\ 0 & 0 & 0 \end{bmatrix},
\]

\[ R = 16 \text{ mm}, \quad \text{and} \quad x_c = \text{center voxel in the SSS}. \] 

Mean \( E_{RMS} \) were calculated for each trial.
For comparison, two dimensional (2D) rigid registrations were performed between label and control
TRUST images using FLIRT with the mutual information cost function.

3.3.2.3 Neonatal motion estimation

To estimate the potential effect of neonatal motion on T₂ estimation, without facing the previously
discussed obstacles to performing experiments on neonates, we calculated motion trajectories from vNav
data obtained from structural neonatal brain scans gathered as part of another study that took place with
IRB approval at Boston Children’s Hospital. vNavs from five prospective motion corrected MEMPRAGE
scans (Siemens WIP 711), acquired with a Siemens Tim Trio 3T scanner using a 32-channel head coil,
were examined to select 2.5 minute periods of motion that had occurred during scanning. The E₉MS for
movements between TRs was determined using the same methods described previously for our adult
study.

3.4 Results

3.4.1 TRUST Sequence Modification and Empirical test of vNav module’s effects

Bloch simulations of the non-selective, 3° flip angle vNav excitations produced very little attenuation in
the difference signal, resulting in a negligible (0.003%) relative decrease in estimated T₂ value of blood.
The empirical ratio of TRUST+vNav post-subtraction signal to the TRUST signal was 95.6% (Figure
3-2). There were no statistically significant differences in T₂ estimates from the two sequences (Table
3-2).

<table>
<thead>
<tr>
<th>Subject #</th>
<th>T₂ [ms] (avg±std)</th>
<th>SDR (avg±std)</th>
<th>N, trials each sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRUST</td>
<td>TRUST+vNav</td>
<td>TRUST</td>
</tr>
<tr>
<td>1</td>
<td>64.2±3.5</td>
<td>66.5±0.6</td>
<td>0.11±0.021</td>
</tr>
<tr>
<td>2</td>
<td>64.6±1.1</td>
<td>65.0±1.3</td>
<td>0.073±0.016</td>
</tr>
</tbody>
</table>

Table 3-2: T₂ estimation and fit quality changes due to the vNav module.

3.4.2 Evaluating the effects of motion on TRUST

Label to control relative motion trajectories for a voxel near the SSS during one moving TRUST scan as
measured by the vNav modules (3D) and from TRUST images (2D) are shown in Figure 3-3.
For 32 TRUST trials, from subjects 3 and 4, where the subject was asked to remain still or to move, mean \( E_{\text{RMS}} \) and SDR are compared as motion classifiers based on maximum a posteriori probability. The error probability identifying motion trials given mean \( E_{\text{RMS}} \) was 0.06, and with SDR was 0.17.

Figure 3-2: Empirical signal attenuation due to vNav. The average blood signal intensity (mean value of the three brightest voxels in the neighborhood of the SSS after Label-Control image subtraction) for three trials each of TRUST+vNav and TRUST. Each trial consisted of three acquisitions for each of five \( T_{EFFECTIVE} \).

The effect of motion on \( T_2 \) estimation is shown in Figure 3-4 and Table 3-3. Table 3-3 gives the descriptive statistics for \( T_2 \), SDR and mean motion score between label and control images for alternating motion/still trials. In Figure 3-4, the \( T_2 \) error (difference of \( T_2 \) and mean \( T_2 \) for still trials) is plotted so that data from two subjects can be combined even though they have different baseline \( T_2 \). \( T_2 \) error is
significantly correlated with $E_{\text{RMS}}$ ($R^2 = 0.33$, $P = 0.0005$).

<table>
<thead>
<tr>
<th>Subject</th>
<th>No Motion</th>
<th>Motion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$, trials</td>
<td>$T_2$ [ms]</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>60.6 ± 3.9</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>51.0 ± 3.0</td>
</tr>
<tr>
<td>All</td>
<td>16</td>
<td>55.8 ± 6.0</td>
</tr>
</tbody>
</table>

Table 3-3: Mean +/- standard deviation for $T_2$, SDR and mean motion score for all trials (* indicates $P > 0.05$ for the unpaired T-test between no-motion and motion states)

3.4.2.1 Neonatal motion estimation

The range of mean $E_{\text{RMS}}$ motion scores during three periods of motion in neonatal scans was 0.6-2.0 mm. The range of $T_2$ overestimate predicted from the linear fit was 1.7-7.4 ms. Assuming an actual $T_2$ of 60 ms, a 7.4 ms overestimation of $T_2$ would result in an absolute 3.9% bias in $\text{SvO}_2$ ($\text{Hct} = 0.4$, tCPMG = 10 ms) (54,176). Overestimation of $\text{SvO}_2$ with all other factors equal would lead to an underestimation in oxygen extraction fraction and cerebral metabolic rate of oxygen consumption.
3.5 Discussion

vNav modules were used to quantify head motion in young adults during TRUST scans. Our main finding is that motion leads to a positive bias in $T_2$ estimates (4.1 ms per mm of mean $E_{\text{RMS}}$), as well as increased variance. This bias could be as large as half of the physiological change some studies have tried to detect. In addition, we found that motion detection using vNavs was a more accurate classifier than relying on quality of $T_2$ fit. These findings are important because when the TRUST technique is used in studies with poor patient compliance to instructions to remain still (21), or in cohorts with different propensities to motion, due to disease or anesthesia (161), the bias introduced by motion should be considered when drawing conclusions about brain state in these different groups. A conundrum results, because obvious methods to mitigate motion, like sedation, anesthesia or restraint may all alter the baseline CMRO$_2$ being quantified (167–170) and it is generally impossible to sedate or anesthetize healthy controls for still comparison between brain states.
There are a few caveats pertaining to the approximate T2 bias of 1.7-7.4 ms due to neonatal motion. vNavs were acquired every 2.52 seconds in the MEMPRAGE scans so the motion tracks provide a lower bound on the TRUST mean ERMS (TR = 5 seconds) since subjects would have more time to move between the label and control images in the TRUST sequence. Also, though we tried to mimic the partial volume effects expected in neonates by matching image matrix sizes between adult and neonatal scans, there is a fundamental difference in signal to noise ratio in the different voxel sizes. We expect this difference to affect the variance rather than the bias of the T2 estimate. Lastly, neonates may move differently than the adult motions we prescribed.

Despite these caveats, a 7.4 ms bias in T2 is important since it exceeds the largest same subject standard deviation (SD) in T2 observed in this study (3.9 ms, Table 3-3) and the mean same subject SD given in a study investigating the test-retest characteristics of TRUST (4.5 ms, backed out from the Svo2 results in (136) by assuming a hematocrit of 0.4, Svo2 of 62% (136), and a $\tau_{CPMG}$ of 10 ms). Potential bias due to motion alone (3.9% absolute Svo2) is greater than half the observed group difference in Svo2 in some studies of neonates with congenital heart disease (7.5% absolute Svo2) (102).

Motion leads to increased measurement variance in addition to a positive bias as seen in Table 3-3 and Figure 3-4. Our observed average intrasession (same subject measurements from one session) SD for motion trials (8.4 ms) shows that motion adds significant measurement uncertainty—of a comparable magnitude to the observed intersubject SD for still trials (6.0 ms)—to the T2 estimate in addition to the
upward bias. This is further evidence that motion may confound intersubject comparisons. The intrasession and intersubject SD (Table 3-3) for still trials are similar to the values previously reported by the TRUST developers (59,136). Still trial intrasession variance is due to measurement and physiological noise. The additional variance with motion not explained by mean $E_{RMS}$ could be due to motion affecting only some label-control pairs, or motion could be sufficiently severe to affect the volume of the cortex that is labeled by the inversion affecting the overall signal to noise ratio of the technique.

To explore how motion may affect the underlying TRUST signal, the equations in (59) can be modified to include a change ($\Delta$) in blood volume fraction in a voxel ($X_b$) between the control and label acquisitions, where $0 \leq X_b \leq 1$ and $X_{b-1} \leq \Delta \leq X_b$.

$$\Delta S = X_b S_{blood, control} - \Delta S_{tissue} - (X_b - \Delta) S_{blood, label}$$

Lumping terms that do not depend on TE_{EFFECTIVE} (subscript b, blood, and t for tissue):

$$\Delta S = S_1(X_b - \Delta)e^{TE_{EFF}(\frac{1}{T_{1,b}} + \frac{1}{T_{2,b}})} + S_2(\Delta)e^{-TE_{EFF}/T_{2,b}} - S_3(\Delta)e^{TE_{EFF}/T_{2,t}} + S_4(\Delta)e^{TE_{EFF}(\frac{1}{T_{1,t}} + \frac{1}{T_{2,t}})}$$

Considering that for moving subjects data is gathered with different $\Delta$ values for each TE_{EFFECTIVE}, and that this will lead to different weighting of the exponentials, it is unsurprising that much of the final variance in $T_2$ is unexplained by mean $E_{RMS}$.

The derivation of Equation 3 assumes no motion during the readout and no velocity effects causing additional signal modulation. Higher frequency motion tracking, perhaps obtained via optical methods (15), could be used to explore these effects. However, given our observations, further work on motion correction for TRUST should adopt a detect and reject strategy using volume navigators, or perform prospective motion correction for all label, saturation, preparation and readout pulses using a sequence-independent motion tracking system.

To assess and correct for motion in TRUST, researchers have relied on 2D registration of label and control images, visual inspection of the post-subtraction images, and/or some quality of exponential fit metric (21,59,136,163,177). All these methods rely on the intrinsic information in the 2D TRUST images. This information is incomplete since out of plane motions go undetected as is demonstrated by Figure 3-3. Figure 3-4 shows that 3D motion tracks describe more of the variance in $T_2$ error than do 2D motion tracks ($R^2 = 0.27$ versus 0.33). Against the vNav benchmark, Figure 3-4 shows that the 2D mean $E_{RMS}$ metric actually captures the majority of the variance in $T_2$ error. This might be due to the basically perpendicular orientation of the SSS with respect to the imaging plane, and suggests motion tracks
derived from previously acquired data sets could be used to inform intersubject or group-wise comparisons of $SvO_2$.

We did not implement the suggested pulse sequence upgrades in (164), but we do not expect either improvement to affect our conclusions. Areas of the brain may be in a different metabolic states between motion and still trials, but previous studies suggest this is negligible for global measurements (178,179).

In conclusion, vNavs used to monitor 3D motion during TRUST show that motion causes an overestimate of $T_2$, and this bias may affect $SvO_2$ estimates in non-compliant subjects such as infants or neonates.
Chapter 4
Quantifying hemodynamics and metabolism in neonates with congenital heart disease using MRI

4.1 Introduction

Severe congenital heart disease (CHD) affects 3/1000 live births, and even after corrective heart surgery leads to high rates of neurological impairment (98,180). The etiology of this impairment is unknown, but studies of infants with CHD have revealed abnormal cerebral hemodynamics and metabolism (45,85,160), and other studies have found similar abnormalities in fetuses (12,159). There is evidence that brain injuries occur before surgery, but how baseline physiology predisposes or protects against perioperative insults is not understood. Clinical care decisions could be optimized to improve long term cognitive outcomes if reliable metrics of brain health were available (101).

Work continues to develop techniques to measure brain hemodynamics and metabolism. MRI, doppler ultrasound and near infrared spectroscopy (NIRS) each offer unique advantages, but MRI permits the absolute quantification of cerebral blood flow (CBF), oxygenation extraction fraction (OEF), and the cerebral metabolic rate of oxygen consumption (CMRO₂) in neonates (reviewed in (11)) while also measuring the concentrations of metabolites essential to brain function, via MR spectroscopic imaging (MRSI) (84).

Cerebral blood flow and blood oxygen saturation are related by Fick’s principle, such that CMRO₂ can be estimated from measurements of CBF and OEF: CMRO₂=OEF·SaO₂·[Hb]·CBF, where SaO₂ is arterial oxygen saturation measured by pulse oximeter and [Hb] is hemoglobin concentration in blood measured from a blood draw. CBF can be measured by phase contrast MRI (PC-MRI) (18), and OEF by T₂-relaxation-under-spin-tagging (TRUST) (21,59). A previous MRI study has documented lower CMRO₂ in neonates with CHD, but scans took place just before surgery after anesthesia had begun (45). Anesthesia makes infants motionless in the scanner, but it also may disrupt baseline hemodynamics and metabolism (170). There have also been studies of CMRO₂ based on NIRS that show lower CMRO₂ in neonates with CHD (102,181).
Studies measuring brain metabolite concentrations in neonates with CHD have focused on n-acetylaspartate (NAA) and lactate (Lac) concentrations as general markers of neuronal health and hypoxia (85,182–185). NAA levels increase and lactate levels decrease during development (159,186). NAA levels in neonates with CHD have been consistently lower than in healthy controls (187). NAA concentrations are about 20 mM in the adult brain and so provides a high signal-to-noise (SNR) target for MRSI studies. NAA is generally considered a marker of neuronal health since it is primarily synthesized in neurons (84). Reported lactate levels in neonates with CHD have been variable (187,188). The lactate doublet overlaps with lipid signals at low echo-times (TE), but inverts and becomes clearer around $TE = 135$ ms (189). Lactate is synthesized from pyruvate via the lactate dehydrogenase enzyme. Lactate can be quickly produced and catabolized due to the energetics of the reaction. In cells with high aerobic demands, the mass action ratio of the reaction shift to favor pyruvate, but when the coupling between glycolysis and the Krebs cycle is disrupted, as in cases of acute hypoxia, lactate concentrations quickly rise (84).

Abnormal blood flow and blood oxygenation resulting from CHD likely disrupts normal brain development even before birth. A lower cerebral resistivity index assessed by Doppler ultrasound is thought to be a sign of brain sparing physiology whereby a maximal amount of blood is diverted to the cerebrovasculature, and these fetuses have a smaller head circumference (190). MRI measurements of blood flow and oxygen delivery in utero confirm that low cerebral oxygen delivery corresponds to a reduction in fetal brain volume (12). Though NAA/Cho is expected to rise throughout gestation, fetuses with CHD show a slower progression, which suggests a failure of normal brain development beginning in the third trimester (159).

After birth, periventricular white matter injury (PWMI) is the most common brain injury among CHD patients (191,192). PWMI constitutes of spectrum of disease ranging from focal cystic necrotic lesions (periventricular leukomalacia appreciable via structural MRI imaging) to diffuse disturbances of myelination (193). PWMI pathogenesis is a result of three main factors, the incomplete development of the brain’s vascular system supplying white matter, the immaturity of cerebrovascular autoregulation, and the vulnerability to oxidative stress of certain oligodendrocyte precursor cells (194). Past studies of PWMI in CHD using diffusion weighted MRI and MRSI have documented a pattern of injury similar to that of prematurity (85,182,185), but the relationships between PWMI detectable on structural imaging and measurements of WM microstructure are ambiguous. One study showed agreement between diffusion MRI and brain injury score (185), while another failed to find concordance (184). Though the
pathophysiology of PWMI is well characterized, how the spectrum of PWMI is manifested on quantitative imaging is not.

While developing reliable metrics of brain health for neonates with CHD, their abnormal cardiovascular physiology provides a range of hemodynamic states unobserved in healthy neonates or adults. Thus, we hypothesized that links between cerebral hemodynamics and metabolism would be revealed in these patients. The pathways involving each metabolite quantifiable by MRSI are numerous and complex, so few MRS signals can be interpreted in a vacuum. CMRO₂ measurements may provide the context to interpret them correctly. Though previous studies have combined diffusion MRI and MRSI to understand the injury to white matter resulting from CHD (85,184,185), no previous study has combined CBF and cerebral oxygenation imaging and MRSI to understand how hemodynamics affected by CHD relates to metabolite concentrations in the developing brain. We undertook a prospective study in unanesthetized neonates with CHD, to generate hypotheses relating hemodynamic effects to metabolic changes in their brains. Does CBF and cerebral oxygenation combined with metabolite concentrations yield additional information relevant to clinical management and the prognosis of neonates with CHD?

4.2 Methods

4.2.1 Subjects

25 neonates with CHD were enrolled in a prospective observational study between June 2014 and August 2016 at Boston Children’s Hospital. Written consent approved by the institutional review board at Boston Children’s Hospital was obtained from parents or guardians. Inclusion criteria were neonates greater than 35 weeks gestational age (GA) with CHD who underwent surgery within the first 30 days after birth. Exclusion criteria were birthweight less than 2.5 kg, recognizable phenotypic congenital syndrome, known chromosomal abnormalities and known intracranial abnormalities. Table 4-1 gives the demographic data, diagnostic groups for the subjects. Diagnostic groups were assigned by a cardiologist based on the patient’s physiology and type of surgery required for correction. The single ventricle (SiV) patients required a stage-1 Norwood procedure. The transposition of the great arteries (TGA) patients required an arterial switch operation. The bi-ventricle (BiV) patients were a mixed group with aorta-related defects (coarctation of the aorta, interrupted aortic arch, aortic stenosis and truncus arteriosus) and also Tetralogy of Fallot. All patients were stable at the time of transport to the MRI scanner.

To ensure subject safety a cardiologist, nurse and MRI technician were present at all scans. Neonates were fed, wrapped in a blanket and positioned on a vacuum immobilizer. If subjects became agitated or
their vital signs deteriorated during a scan, as assessed by the cardiologist, scanning was halted. Scanning would resume only with doctor approval and when the subject became quiescent.

<table>
<thead>
<tr>
<th></th>
<th>CHD patients</th>
<th>TGA</th>
<th>SiV</th>
<th>BiV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>25</td>
<td>7</td>
<td>11</td>
<td>7</td>
</tr>
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<td>4</td>
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<tr>
<td>Gestational age [wk]</td>
<td>38.5±1.3</td>
<td>38.4±1.5</td>
<td>38.5±1.3</td>
<td>38.5±1.3</td>
</tr>
<tr>
<td>Age at scan [days]</td>
<td>4.2±2.4</td>
<td>4.0±1.3</td>
<td>3.3±1.2</td>
<td>5.8±3.7</td>
</tr>
<tr>
<td>Postmenstrual age at scan [weeks]</td>
<td>39.1±1.2</td>
<td>39.0±1.4</td>
<td>39.0±1.3</td>
<td>39.3±1.0</td>
</tr>
</tbody>
</table>

Table 4-1: Patient demographic data.

4.2.2 MRI Protocol

The following MRI protocol was used for scanning on a Siemens Tim Trio 3T scanner (Siemens Healthineers, Erlangen, Germany) using the Siemens 32-channel head coil:

1. **T<sub>1</sub>-weighted structural imaging using volume navigated MEMPRAGE** (195). (Field of view (FOV) = 180 x 180 x 128 mm<sup>3</sup>, resolution = 1 mm isotropic, repetition time (TR) = 2520 ms, echo times (TE<sub>1</sub>/TE<sub>2</sub>/TE<sub>3</sub>/TE<sub>4</sub>) = 1.75/3.6/5.45/7.3 ms, inversion time (TI) = 1450 ms, flip angle (FA) = 7 degrees, GRAPPA acceleration factor = 2, total acquisition time (TA) = 5:15.)

2. **T<sub>2</sub>-weighted structural imaging using turbo spin echo imaging**, axial or sagittal oriented acquisition. (FOV = 180 x 144 mm<sup>2</sup>, resolution = 0.5 x 0.4 x 2 mm<sup>3</sup>, number of slices = 55, TR = 11000 ms, TE = 110 ms, FA = 120 degrees, GRAPPA acceleration factor = 2, TA = 3:09.)

3. **Time of flight angiogram (MRA)** positioned to include the Circle of Willis and the neck. (FOV = 192 x 176 mm<sup>2</sup>, resolution = 0.8 x 0.8 x 2 mm<sup>3</sup>, number of slices = 50, TR = 20 ms, TE = 4.83 ms, FA = 60 degrees, GRAPPA acceleration factor = 2, TA = 2:16.)

4. **Velocity encoded phase contrast flow imaging (PC-MRI)** positioned manually based on the MRA perpendicular to the basilar artery (BA) and interior carotid arteries (ICA), sometimes acquired in two scans if BA and ICAs were not parallel (18). (FOV = 140 x 127 mm<sup>2</sup>, resolution = 0.5 x 0.5 x 4 mm<sup>3</sup>, TR = 16.65 ms, TE = 4.67 ms, FA = 10 degrees, velocity encoding (VENC) = 100 cm/s, number of averages = 20, TA = 1:19.)

5. **T<sub>2</sub>-relaxation under spin tagging (TRUST)** (11,59) positioned 15 mm above the confluence of the sinuses perpendicular to the superior sagittal sinus. (FOV = 160 x 160 mm<sup>2</sup>, resolution = 2.3 x 2.3 x 5 mm<sup>3</sup>, TR = 5000 ms, effective echo times (TE<sub>EFFECTIVE</sub>) generated by T<sub>2</sub> preparation = 0, 18,
36, 72, 144 ms, inversion time = 1025 ms, tagging width = 50 mm, tagging gap = 15 mm, number of averages = 3, TA = 3:05.)

6. 3D MR spectroscopic imaging (MRSI) using a custom spiral chemical shift imaging readout, with a LASER excitation box positioned completely inside the brain covering the basal ganglia.

(chemical shift imaging matrix size = 16 x 16 x 8, voxel size = 12.3 x 12.3 x 12.6 mm³ (2 ml), excitation volume = 70 x 70 x 40 mm³, TR = 1800 ms, TE = 30 and 135 ms, averages = 3, TA = 3:00.)

4.2.3 OEF Analysis

Estimates of the T₂ of deoxygenated blood in the SSS were determined using custom Matlab routines, generally following the procedure previously described in (21,196). The three brightest voxels in a manually drawn ROI of approximately 25 voxels near the SSS were averaged to give a signal intensity (S(TEFFECTIVE)) value for one post-subtraction image. The post subtraction images were evaluated for motion occurring between label and control image acquisition and only S(TEFFECTIVE) from low or no motion acquisitions were fit to estimate T₂. Motion was determined to have occurred between control and label acquisitions when the standard deviation of pixel intensity values or the mean of the absolute value of the pixel intensities of the difference image exceeded preset thresholds (standard deviation > 20 or the mean > 10). This strategy was based on the observation that motion creates errors in the subtraction of non-moving tissue, and was adopted in lieu of active motion tracking afforded by volume navigators (24,196). Fitting to \( S(TEFFECTIVE) = S₀exp(TEFFECTIVE \times C) \) was performed by non-linear least squares fitting. The T₂ of blood was then determined as \( T₂,\text{blood}=1/(R₁,\text{blood}-C) \), \( R₁,\text{blood}=0.62 \) (59). Goodness of fit, ΔR₂, was measured by the 95% confidence interval found for \( R₂ (=1/T₂) \) following (21). Venous SO₂ (SvO₂) was determined from T₂ using a published calibration (172) based on umbilical cord blood. Hct was measured from a blood sample sent to hospital's lab. Arterial SO₂ (SaO₂) was determined from the average of before and after MRI scan readings from a pulse oximeter used for patient monitoring in the intensive care unit. OEF was calculated using \( \text{OEF} = (S_aO₂-S_vO₂)/S_aO₂ \).

4.2.4 CBF Analysis

Estimates of total blood inflow to the brain were generated from reconstructing and analyzing the raw MRI data using custom Matlab routines. Raw data was exported from the scanner, and complex coil sensitives were estimated by fitting with 4th-order polynomials to the ratio of individual coil images to the sum squared combined image. Phase-difference and magnitude images were then generated via SENSE reconstruction (197). Blood flow velocity was estimated for the right and left ICA and BA from the
phase-difference image by averaging the phase (\(\phi\)) from all the voxels within a region of interest (ROI) for the vessel. The ROI was selected semi-automatically using the threshold method from (81). Briefly, a seed voxel was selected at the vessel center and a vessel ROI was grown such that all voxels had signal intensity values greater than a threshold calculated from an adjacent, background region. In our study the threshold was set at 50% of the difference between the average magnitude signal of the background region and the seed. Average phase-difference (\(\phi\)) was then converted to average blood flow velocity (\(v\)), using \(v = \text{VENC} \cdot \phi / \pi\). VENC was 100 cm/s in this study. Blood flow through the vessel was determined using \(BF = v \cdot A\), where \(A\) is the cross-sectional area of the vessel determined from the vessel ROI. Total blood inflow was the sum of the flows through each vessel.

To calculate CBF, total blood inflow was divided by the total brain mass determined by an automatic segmentation of the T1-weighted structural imaging using Freesurfer (Massachusetts General Hospital, Boston, MA). The total volume of the cerebrum and cerebellum was multiplied by brain density (1.06 g/ml (21)) to determine brain mass in grams.

The CMRO2 was determined using:

\[
CMRO_2 = 1.39 \cdot CBF(\text{OEF} \cdot S_a O_2)[Hb].
\]

Where \([Hb]\) is the concentration of hemoglobin as determined from the measured Hct using (61):

\[
[Hb] = \frac{Hct}{3.0(\text{ml/g}) \cdot 0.016125(\text{g/umol})}.
\]

4.2.5 MRS Analysis

MRSI data was analyzed using TARQUIN (198) and custom Matlab routines. Spectra for single voxels were fit using default TARQUIN parameters, except reference signals were NAA, Cr, Cho, and pulse sequence was LASER. For TE = 30 ms spectra, concentration ratios to total choline concentration (i.e. Cho + GPC) (TCho) were determined for total NAA (i.e. NAA +NAAG) (TNAA), creatine (Cr), glutathione (GSH), and glutamate plus glutamine (Glx). Lactate to TCho ratios were calculated from the TE = 135 ms data. Metabolite levels are expressed as ratios to a metabolite concentration that remains relatively constant over time and between subjects in order to avoid an additional scan to determine the water peak height for absolute quantification. For adult MRSI this is often creatine (Cr), but Cr concentrations are known to increase with gestational age whereas Choline (Cho) remains relatively constant over the first weeks after birth (199). Some studies have examined the ratio of Lac/NAA in CHD (86,186), but this measurement will be directly affected by changing NAA level over time.
Bilateral gray matter voxels in the thalamus, extending out to the basal ganglia, and bilateral white matter voxels in the posterior white matter, and for two subjects in the anterior white matter, were selected manually for all subjects (Figure 4-1). Visualization was via FreeView (Massachusetts General Hospital, Boston, MA) with voxels selected based on T2-weighted structural images. Spectra from these voxels gathered with 30 ms TE, exceeding a signal to noise threshold of 14.5 for gray matter and 10.5 for white matter, and for 135 ms TE exceeding a signal to noise threshold of 10.5 for GM and 8 for WM were included in the subsequent analysis.

Comparisons of metabolite concentration ratios were performed using Student’s t-test. Metabolite ratios from right- and left-sided voxels were compared, and subsequently left and right sided ratios, separately in GM and WM, were averaged for each subject if both voxels had sufficient SNR. Comparisons were made across the three diagnosis groups (i.e. bi-ventricle, TGA and single-ventricle) and between the top and bottom quartiles of OEF. Correlation analysis using Spearman’s rank order correlation was performed between OEF, CBF, CMRO2 and the metabolite ratios.

4.3 Results

4.3.1 Motion and data quality

We did not anesthetize or sedate the neonates. During more than half the scans the child started crying and was thus repositioned at least once to calm them. Motion during scanning meant that we successfully collected the entire MRI protocol in only 12 cases. TRUST data was successfully collected for 20 subjects.
Figure 4-2: SvO₂, CBF and CMRO₂ results compared between our study and two previous studies. TEA stands for healthy premature neonates scanned at term equivalent age. Point indicates mean, whiskers indicate standard deviation and * indicates t-statistic with P < 0.05.

(20% failure rate). PC-MRI data was collected for all 25 subjects, but the wrong phase contrast sequence was used for the first eight scans preventing CBF estimation. Thus, estimates for CMRO₂ were possible for only 12 subjects. Spectroscopy data could not be collected at all due to motion in one case, and was collected at both echo times for 20 subjects (20% failure rate). There were 22 estimates of metabolite ratios in GM and 20 in WM from TE = 30 ms scans with sufficient SNR (>14.5 GM and > 10.5 WM). Different numbers of subjects were included for CBF, oxygenation and metabolite concentration correlations depending on the available data meeting the given quality thresholds.

4.3.2 OEF Results

For the twenty TRUST scans without severe motion, \( T_2 = 53 \pm 15 \) (mean ± standard deviation), \( S_o_2 = 53 \pm 9 \), OEF = 0.41 ± 0.10. Quality of fits, assessed by the 95% confidence interval for \( R^2 \), varied markedly between trials with \( \Delta R_2 \) values from 2.4 – 408 s⁻¹, though all trials except two had \( \Delta R_2 \) less than 30. There was no correlation between fit quality and control/tag pairs rejected from the analysis of a scan due to motion. There were no significant comparisons between diagnostic groups (\( N_{BI} = 8 \), \( N_{SI} = 7 \), \( N_{TGA} = 5 \)).

For the twelve subjects for which TRUST and PC-MRI was successfully acquired, \( T_2 = 49 \pm 14 \), \( S_o_2 = 51 \pm 10 \), OEF = 0.45 ± 0.09, and all fits had \( \Delta R_2 < 30 \). Figure 4-2a shows a comparison between these results and literature values.
4.3.3 CBF Results, including CMRO\textsubscript{2}

For all fourteen subjects with analyzable PC-MRI data, CBF = 10 ± 2 ml/100g/min and brain mass = 420 ± 40 g. There were no significant differences between diagnostic groups.

For the twelve subjects for which TRUST and PC-MRI was successfully acquired, CBF = 10 ± 2 ml/100g/min and CMRO\textsubscript{2} = 38 ± 12 µmol/min/100g. Figure 4-2b-c shows a comparison between these results and literature values. CMRO\textsubscript{2} trended lower in the SiV (29 ± 4 µmol/min/100g, N = 3) compared with the BiV group (47 ± 13 µmol/min/100g, N = 5, P = 0.06) and the combined BiV and TGA group (42 ± 12 µmol/min/100g, N = 9, P = 0.1).

4.3.4 MRS Results

Table 4-2 gives the average metabolite concentration ratios for gray matter and white matter.

4.3.4.1 Comparisons between right and left sides
The only significant difference in metabolite ratios on different sides of the brain were found in WM, where the left sided GSH ratio was 0.70 ± 0.23 and the right sided ratio was 0.47 ± 0.14 (paired sample T-test, P = 0.009).

4.3.4.2 Comparisons between diagnostic groups (TE = 30 ms data)
We found no statistically significant differences in metabolite ratios between diagnostic groups.

4.3.4.3 Correlation analysis of MRS data (TE = 30 ms) and OEF data
In WM, GSH was negatively correlated with OEF for the 16 subjects with both MRS and OEF measurements (Figure 4-3) (Spearman’s rank correlation coefficient = -0.56, P = 0.03, N = 16), this was the only significant correlation between blood flow and oxygenation parameters and metabolite ratios. Additionally, when comparing mean concentration ratios for the subjects in the upper and lower quartiles of OEF (mean
OEF first quartile = 0.27, fourth quartile = 0.49, in WM TNAA was higher in the first quartile \((P = 0.04)\) and GSH higher in the first quartile \((P = 0.01)\).

4.3.4.4 Lactate \((TE = 135 \text{ ms data})\)

Figure 4-4 shows the average ratio for all three groups. For \(TE = 135 \text{ ms data}\) sufficient SNR was defined as >10.5 GM and >8 WM. In WM, Lactate/TCho was significantly lower in the BiV group \((0.18 \pm 0.07, N = 5)\) in comparison to the TGA group \((0.27 \pm 0.06, N = 5)\) \((P = 0.04)\). In GM, Lactate/TCho trended lower in the SiV \((0.11 \pm 0.02, N = 6)\) than the TGA and BiV group \((0.14 \pm 0.04, N = 11)\) combined \((P = 0.07)\). Figure 4-5 shows plots of Lac/TCho versus OEF and CMRO2 for both GM and WM, with a significant negative linear correlation between Lac/TCho and CMRO2 in white matter \((R^2 = 0.475, P = 0.04)\), though the Spearman rank correlation was not significant.

4.4 Discussion

This work combines two MRI techniques to more fully examine cerebral metabolism in a mixed cohort of neonates with CHD. Quantifying CMRO2 gives an instantaneous big picture view of the brain metabolic state, but it does not characterize past oxygen demand or future development potential. Metabolite concentrations derived from MRS give a granular view of the brain’s ongoing biochemical reactions, but in itself cannot resolve the specific cause of an abnormal concentration due to the complexity of the implicated pathways. Both modalities probe metabolism, and by combining them we have found evidence that oxygen supply to the brain is inadequate in some cases, and has been inadequate for some time prior to the scan. Specifically, observing that Lac/TCho is negatively correlated with CMRO2 in WM suggests that oxygen supply is not meeting demand for oxidative metabolism in some subjects and finding that GSH/TCho is negatively correlated to OEF in WM suggests an adaptation to a precarious balance between oxygen and supply and demand in the brain.
4.4.1 CBF, OEF and CMRO$_2$

When compared with literature, the CMRO$_2$ for our cohort is not significantly different from that reported for healthy neonates (21) (Figure 4-2). This apparent discrepancy with the literature is discussed in Chapter 5, and is likely attributable to the different average PMA between the two cohorts.

Figure 4-5: Lac/TCho versus OEF and CMRO$_2$.  

\[ \text{CMRO}_2 \text{ (mol/min/100g)} \]
The CMRO₂ for our cohort is significantly higher ($P < 0.05$) by about 50% than that reported for anesthetized neonates with CHD (45). A similar pattern was found with NIRS. Comparing the results of Dehaes et al. (102) to Jain et al. (45), CMRO₂ is more than double in unanaesthetized neonates. The effects of anesthesia using fentanyl on CMRO₂ are still being explored. In animal models there is mixed evidence about the drug's effect, with a dose dependent effect on CBF and CMRO₂ in rats (167) and no effect in dogs (168). In humans, preliminary reports support the notion that anesthesia does lower CMRO₂ (170). Baseline CBF, OEF and CMRO₂ in neonates with CHD are most likely not as abnormal as measurements of anesthetized neonates may suggest.

Due to the small sample size and large intersubject variation we do not find statistically significant difference in OEF, CBF or CMRO₂ between diagnostic groups. The average CMRO₂ in the SiV group trended lower, which makes sense given a single ventricle heart delivering lower SaO₂ to organs with precariously balanced vascular resistance has less ability to accommodate the rapidly increasing oxygen demands of the brain. Nevertheless there is considerable variation in CMRO₂ between subjects in each diagnostic group. The coefficient of variation for the SiV, BiV and TGA groups are 13%, 28% and 28%, respectively. This variation is much larger than the 6% test-retest variation reported for neonatal CMRO₂ measurements using TRUST and PC-MRI (21) suggestive of the different hemodynamics caused by the unique heart anatomy of each subject. To better understand the group wise trends in CBF, OEF and CMRO₂ more subjects are needed within each diagnostic group.
4.4.2 Lactate

Our MRS results are similar to the metabolite ratios (TNAA/TCho, Lac/TCho and Lac/NAA) from past studies of healthy (182,200,201), premature (186) and CHD neonates (85,182) (see Table 4-2). There are known issues when comparing metabolite ratios between studies employing different acquisition (single voxel versus MRSI (202)) and analysis techniques (proprietary vendor based analysis versus offline spectrum fitting (198)) so comparisons must be made cautiously, but the basic agreements between reported values builds confidence in the methods that we have employed. The average TNAA/TCho in both the thalamus and posterior white matter for our cohort is lower than that in studies of healthy control neonates and Lac/TCho and Lac/NAA are higher. This trend matches other MRS studies of neonates with CHD (85,182), and it makes sense in neonates with CHD that neuronal health might be poorer as reflected by NAA and anaerobic metabolism more common as indicated by lactate.

Despite the small number of samples in each diagnostic group, finding that Lac/TCho in WM was higher among neonates diagnosed as TGA versus BiV suggests that Lac/TCho could differentiate neonates with different metabolic status (Figure 4-4). With more subjects it may be that these three conditions would stratify into three different Lac/TCho levels, or, like the trend in GM, differentiate one group (SiV) from the other two (BiV + TGA). Though it is commonly thought that lactate is undetectable in the brains of healthy neonates, data from both (85) and (182) show that some lactate is always detectable, and this makes sense considering that lactate is important to normal brain metabolism. Notably, elevated Lac/TCho is often not a statistically significant finding when comparing healthy controls and neonates with different types of CHD (85,184). That we found significant difference in Lac/TCho between neonates with different forms of CHD suggests that the makeup of a mixed cohort would affect the average magnitude of Lac/TCho, and could obscure the fact that some subjects have significantly higher Lac/TCho reflecting their individual physiology. In common with other studies of healthy (186) and CHD neonates (182), Lac ratios were higher in the posterior WM than thalamic GM (Figure 4-4).

We hypothesized that lactate level would be related to abnormal cerebral hemodynamics in this cohort. The only relationship that we observed was a significant linear correlation between Lac/TCho and CMRO₂ in WM, though this was driven by two data points with high CMRO₂ and negligible lactate. Outcomes data (i.e. time in the intensive care unit after surgery (99)) could be used to verify these two subjects presumed healthy pattern of higher CMRO₂ and lower Lac. Though elevated lactate is commonly interpreted as a general marker of hypoxia, it is really a marker of acute injury since the concentration of lactate in the brain depends on pyruvate synthesis, cellular redox potential and lactate diffusion and transport (84). Higher lactate concentrations have been related to more severe brain injury in cases of HIE...
and in shaken baby syndrome (203). Our data does not show a conclusive link between hemodynamics and lactate levels, but further work is needed to determine why lactate levels are variable in patients with CHD. Specifically, sufficient data to explore lactate level coupling to CBF, OEF and CMRO$_2$ in the different diagnostic groups could reveal the effects of group-specific physiology, and may show which patients have experienced glucose limited brain growth versus acute oxygen deprivation (204).

4.4.3 Glutathione

Findings in WM, but not GM, underscore the known vulnerability of WM in patients with CHD. It is possible that the GSH/TCho correlation with OEF (Figure 4-3) reflects the precarious hemodynamic status of CHD neonates after birth. GSH is a tripeptide that is found in relatively high concentrations in the brain (2-3 mM). It is synthesized from glutamate, cysteine and glycine in reactions requiring ATP. Synthesis is cystine limited since glutamate and glycine occur at relatively high intracellular concentrations (205). In adults [GSH] is lower in WM than in GM. Glutathione depletion results in mitochondrial dysfunction and decreases NAA (83,206). Studies of how oxygenation affects GSH levels in tissues and cells do not give a clear picture since both hypoxia and hyperoxia are known to create an imbalance between antioxidants and reactive oxygen species leading to oxidative stress (205,207). There is, however a consistent finding that mild stress upregulates GSH levels through various pathways (205).

We found that GSH/TCho is negatively correlated to OEF in white matter, but we do not observe a significant correlation with CBF or CMRO$_2$. Two possible explanations are that GSH is being depleted at higher OEF, or GSH is being upregulated at lower OEF. GSH depletion is a sign of acute oxidative stress brought on by either hypoxia or hyperoxia (205,207). Treating Lac/TCho as a marker of hypoxia, high Lac/TCho should correlate with a low GSH/TCho if GSH depletion were a result of hypoxia, but we do not find a significant correlation. If GSH depletion were a result of hyperoxia, we would expect a higher tissue pO$_2$, a higher SvO$_2$ and a lower OEF, but this effect would be opposite our observation. Thus, we suggest that GSH levels are upregulated as a result of persistent mild oxidative stress at low OEF. Upregulation of GSH has been previously observed in an in vitro model of ischemia/reperfusion beginning within 24-hours of the hypoxic preconditioning event (208). GSH levels are higher in some cancer cells along with GSH-related enzymes and transporters, which is thought to be a major factor in chemoresistance of some tumors. In addition, GSH appears to play a role in the pathogenesis of Parkinson’s disease and possibly Alzheimer’s disease as well (209). OEF, on the other hand, is not a reliable marker of brain health (105), but low OEF has been associated with brain injury. Low OEF due to acute hypoxic injury (HIE) is attributed to neuronal loss and immature autoregulation (63). In longitudinal studies, OEF has been shown to increase with age (63,210), mostly due to the transition from fetal to
adult hemoglobin, so a lower OEF could also indicate immature brain development. The relationship between OEF and GSH level may provide more information than considering each individually.

Elevated GSH levels are likely related to WMI. GSH levels increase in pathologies where astrocytes are activated (i.e. they undergo morphological and functional changes in response to injury) (211), and in diffuse WMI the primary pathological feature appears to be diffuse astrogliosis (i.e. an increase in the number of astrocytes and their processes as reaction to injury) (211,212). Furthermore, higher GSH levels translate to a more reducing environment promoting cellular division as opposed to cellular differentiation. In particular, the redox status of oligodendrocyte/type-2 astrocyte progenitor cells has been shown to affect the fate of the cells with a more reducing environment promoting cell division (205). In astrogliotic regions of diffuse WMI, there is evidence that there are large populations of pre-oligodendrocytes that fail to normally generate oligodendrocytes (212). Based on this evidence, we hypothesize that GSH could be associated with WMI, but we did not find a significant difference in GSH/TCho between subjects with appreciable WMI and those without. Previous studies have not found a consistent link between WMI appreciable on structural imaging and quantitative measurements of the WMI based on diffusion MRI or MRSI (184,185), which suggests that the acute lesions detectable on structural imaging give only a partial picture of PWMI and that quantitative MR may better resolve the spectrum of disease. With the goal of associating the diagnosis of WMI in CHD to long term outcomes, work to clarify the relationship between microstructural abnormalities, metabolite imbalances, and appreciable lesions is clearly needed.

4.4.4 Limitations

4.4.4.1 CBF, OEF and CMRO\textsubscript{2} Quantification

All MRI scans of unanesthetized neonates are affected by subject movement and we observed a 20% failure rate due to motion, which is typical (21). We have previously investigated the effects of subject motion on TRUST scans, and plan to use a TRUST pulse sequence with additional volume navigators for motion rejection in future studies (196). This should improve the T\textsubscript{2} fit quality, which was poor for some trials.

We measure oxygen delivery because hemoglobin is an ideal target for MRI. However, the delivery of metabolic substrates may also be disrupted by a malfunctioning cardiovascular system. Glucose delivery to the brain may also be abnormal, but we can only measure the metabolic rate of glucose consumption with PET. Any abnormal finding in oxygen delivery is only one dimension of brain metabolism.
4.4.4.2 MRS

There is no standard way to analyze and assess the quality of MRSI data. Here we elected to use a threshold on SNR to select quality data for analysis, so that all metabolite concentrations from selected voxels were analyzed regardless of individual metabolite Cramer-Rao lower bounds. Our selection criteria for MRS spectra is actually quite high in comparison to previously published studies where SNR of approximately 5 is the normal cutoff, with FWHM < 0.15 (213,214). Tarquin's output includes its own fit quality metric, Q (198). Q equal to one is extremely high data quality. TE = 30 ms data had a Q less than 2.4, and TE = 135 ms data less than 2.8. A published study of brain tumors, used data with Q less than 2.5 (214).

The spectrum of GSH overlaps with gamma-aminobutyric acid, Glu and Gln, making it impossible to resolve visually and challenging to fit (83,84). However, the default Tarquin basis set includes GSH and fit quality, Q, was adequate. More work is needed to validate the accuracy of GSH quantification at 3T using Tarquin. This could include tests of Tarquin's fit quality on simulated signals matching physiological spectra with various GSH and noise levels, as in (198), and tests on phantoms that mimic physiological metabolite concentrations with different levels of GSH. If resolving GSH at 3T is demonstrably harder than our preliminary data here suggest, future scans at 7T would have higher SNR and measurement precision, and spectral editing techniques could be used (215).
Chapter 5

Optimizing unanesthetized cerebral oxygen consumption measures: comparison of MRI and near-infrared spectroscopy (NIRS) approaches in neonates with congenital heart disease

5.1 Abstract
Concern for cerebral perfusion in neonates with congenital heart disease (CHD) has driven investigations into cerebral hemodynamics. MRI in combination with bedside NIRS has the potential to provide complementary measures of hemodynamics to guide surgical timing and assess response to surgery. We compare MRI and NIRS measures in neonates with a variety of specific heart defects, expecting to observe a wide range of natural variation in cerebral oxygenation and hemodynamics. Modality results compare well to literature studies, but intermodality correlation is limited. Before combining modalities additional studies are needed to better understand why cerebral blood flow and CMRO₂ measures in MRI and NIRS differ.

5.2 Introduction
Moderate to severe CHD affects 6/1000 live births, with severe CHD resulting in adverse neurodevelopmental outcomes in over 50% (98,180). The precise etiology of neurodevelopmental disorders is unknown but evaluation of the hemodynamic state of CHD infants pre- and post-surgically has become a focus with CMRO₂ identified as a key parameter for clinical evaluation (45,12,159,160). MR measurements of cerebral blood flow and oxygenation are quantitative in absolute units and can be both global and regional. Additionally, while the neonate is in the MRI scanner a complete clinical protocol can also be acquired. However, a pre-surgical MRI of neonates with CHD is not standard of care. It remains unclear what this additional information about the brain can add to clinical management of the life threatening heart condition.

Near infrared spectroscopy, on the other hand permits continuous bedside measurement of CMRO₂, but in relative units and only in particular brain regions. Because near-infrared light is scattered by tissue it does not penetrate far into the head and thus the measurement is biased to the cortex. These drawbacks could
be overcome if NIRS and MRI measurements of CMRO$_2$ could be combined such that the absolute, global information provided by MRI were linked to the regional, relative information provided by NIRS. In essence, using MRI to calibrate the NIRS measurements on a per subject basis could make this multi-modal approach equivalent to continuous monitoring of CMRO$_2$, in absolute units, at the bedside, providing a marker of brain-health in the critical period of early development.

This synergy has been previously recognized. Jain, et al. (45) demonstrated correlations between MRI and NIRS measures of CMRO$_2$ pre-surgically when measured simultaneously in anesthetized neonates with CHD. They demonstrated good correlation between the two measurements. Alderliesten, et al., compared NIRS and MRI measurements of regional blood oxygen saturation in neonates, both healthy and with a variety of conditions, and found good agreement as well (62). We wished to confirm these results and demonstrate inter-modality correlation on unanesthetized infants with CHD, since anesthesia may disrupt baseline hemodynamics and metabolism (170).

To characterize baseline physiology we measured hemodynamics and oxygenation in unanesthetized neonates with a variety of heart defects. We expected to observe a wide range of hemodynamic and oxygenation states maximizing the likelihood of finding good agreement between MRI and NIRS measurements of OEF, CBF and CMRO$_2$.

5.3 Methods

Subjects for this study were the same as those described in Chapter 4, with a subset of nine subjects selected for comparison between MRI and NIRS (GA = 38.8±0.9 weeks, age at scan = 3.6±1.3 days, 8 male, 1 female). Data was selected if the NIRS and MRI scans occurred within 12 hours before to 7 hours after the MRI scan and before the neonate went to surgery. Only data with sufficient quality was compared. For MRI data this meant that the 95% confidence interval in the estimation of $R^2 < 30$. For NIRS data quality was evaluated according to the algorithm described in (29). All studies were performed at Boston Children’s Hospital with IRB approval and parental consent.

The MRI protocol and analysis was the same as that described in Chapter 4 and included:

(1) T1 structural imaging (volume navigated MEMPRAGE)
(2) time of flight angiogram (MRA) positioned to include the circle of Willis and the neck
(3) velocity encoded phase contrast image positioned manually based on the MRA perpendicular to the basilar artery and interior carotid arteries (TE/TR = 4.67/16.65 ms, resolution = 0.5 x 0.5 x 4.0 mm, velocity encoding = 100 cm/s, $T_{aeq} = 1:19$)
(4) T2-relaxation under spin tagging (TRUST) (21) positioned 15 mm above the confluence of the sinuses perpendicular to the superior sagittal sinus (TE/TR = 15/5000 ms, resolution = 2.3 x 2.3 x 5 mm, inversion time = 1025 ms, tagging width = 50 mm, tagging gap = 15 mm, $T_{aq} = 1:19$). Post-processing to estimate cerebral blood flow and venous T2 was performed in MATLAB.

The NIRS protocol included frequency-domain near-infrared spectroscopy (FD-NIRS) and diffuse correlation spectroscopy (DCS) (216) measurements and analysis previously described in (102). FD-NIRS provides regional measurements of oxygenated and deoxygenated hemoglobin, which was used to compute cerebral tissue oxygen saturation (StO2). For comparison to MRI, $SvO_2$, OEF and CMRO2 were calculated assuming that the ratio of venous blood to total blood volume ($\beta$) was 0.85, such that $StO_2 = 0.15 \cdot SaO_2 + 0.85 \cdot SvO_2$ (217). To be consistent with the other NIRS studies when comparing CMRO2, $\beta = 0.75$. DCS provides a measure of microvascular perfusion by quantifying intensity fluctuations of multiply scattered light due to the movement of red blood cells (RBC) inside the sampled tissue. Regional CBF, measured as blood flow index (CBFi), has been previously validated (218,219).

5.4 Results

Table 5-1 gives the descriptive statistics for the MRI and NIRS studies. Figure 5-1 shows the correlation between SvO2, OEF, CBF and CMRO2 measured by MRI and NIRS. Figure 5-2 shows the comparisons between our results and the literature (similar to Figure 4-2, there with a larger number of subjects).
Figure 5-1: MRI-based versus NIRS-based measurements of SvO₂, OEF, CBF and CMRO₂ (P = 0.85). Significant correlations between MRI and NIRS measurements of SvO₂ and OEF in the 9 subject sample.

5.5 Discussion

The large range of venous oxygen saturation and OEF measured in this cohort demonstrated strong correlation between MRI and NIRS measurements, however CBF and CMRO₂ did not correlate. This suggests that either changes in CBF occur quickly and that the delay of several hours between MRI and NIRS based measurement of CBF meant that the underlying physiology had changed state, or that one of the modalities was inaccurate. Though we consider the latter possibility, we believe same modality comparisons between our results and the literature suggest that both techniques were accurate. Further work is needed to explore the underlying causes of the disagreement in CBF measurements between MRI and NIRS.
Results for a larger cohort are provided in Chapter 4, but here we focus on the nine subjects for which we acquired both NIRS and MRI data. When compared with literature, the CMRO$\textsubscript{2}$ measured by MRI is not significantly different from that reported for healthy neonates (21) (Figure 5-2). This finding contrasts with what has been reported from NIRS studies, that CMRO$\textsubscript{2}$ is lower in neonates with CHD (45,102) (Figure 5-2). We know, however, that cerebral blood flow increases rapidly over the first weeks of life (220) and is correlated to PMA (73,221), also that CMRO$\textsubscript{2}$ increases with PMA (21). Thus the comparison to the average CMRO$\textsubscript{2}$ reported for healthy neonates by Liu et al. (21) must be corrected for their cohort’s lower average PMA of 37.4 weeks compared to our subjects PMA of 38.8 weeks. Extrapolating from their data, and assuming that post-natal age does not significantly alter CMRO$\textsubscript{2}$ in neonates, an average CMRO$\textsubscript{2}$ of 49.6 $\mu$mol/min/100g would be expected for healthy neonates with PMA equal to our own (21). Interestingly, this suggests that our CHD cohort has a 33% lower CMRO$\textsubscript{2}$ than the healthy controls and this is in good agreement with the 27% reduction determined using NIRS (102).
The mean CMRO₂ for this cohort of unanesthetized neonates with CHD is significantly higher ($P = 0.01$) by about 43% than that reported for anesthetized neonates with CHD (45). A similar pattern was found with NIRS. Comparing the results of Dehaes et al. (102) to Jain et al. (45), CMRO₂ is more than double in unanaesthetized neonates. The effects of anesthesia using fentanyl on CMRO₂ are still being explored. In animal models there is mixed evidence about the drug's effect, with a dose dependent effect on CBF and CMRO2 in rats (167) and no effect in dogs (168). In humans, preliminary reports support the notion that anesthesia does lower CMRO₂ (170). Baseline CBF, OEF and CMRO₂ in neonates with CHD are likely not as abnormal as measurements of anesthetized neonates may suggest.

Examining correlations between modalities, SvO₂ and OEF are in good agreement suggesting that SO₂ measures capture similar information and/or are relatively stable in this cohort (Figure 5-1). The NIRS measurement of StO₂ is sensitive to the deoxygenated and total hemoglobin concentrations in the tissue and is not a measurement of the venous oxygen saturation exclusively (217). Thus SvO₂ is calculated from StO₂ and SaO₂ scaled by the relevant volume fractions. The correlation shown in Figure 5-1 is for $\beta = 0.85$ (217), though the two prior studies assume $\beta = 0.75$ for neonates (45,102). This is one example of how absolute measurements via MRI could be used to improve the accuracy of NIRS based measurements.

There was no significant correlation between CBF and CMRO₂ (Figure 5-1). There are three possibilities for the discrepancies between NIRS and MRI measurements of CBF and CMRO₂: (1) Measurements were not performed simultaneously and subjects with CHD are unstable. (2) Sample size was limited as neonates needed to be stable and asleep to have a successful MRI scan. (3) MRI and NIRS rely on different models and assumptions that could lead to different measurement biases.

Neonatal physiology even in healthy subjects can change rapidly. CBF is known to increase by about 70% from 32-40 weeks gestational age (222), and has been shown to increase by 50% in the first two weeks after birth (220). It is unclear how neonates are influenced by circadian rhythms, but in adults variation in CBF of 20% has been documented depending only on the time of day (223). Also, internal temperature, pCO₂ and pO₂ of blood, hematocrit, mechanical ventilation, pressors, sedation and anesthesia have all been shown to affect CBF (170,224,225). Simultaneous measurements of CBF via NIRS and MRI as performed by (45), eliminates these possible confounding effects and this alone would explain why they found good correlation between MRI and NIRS measurements of both CBF and CMRO₂ and we did not.
We studied stable preoperative neonates without anesthesia and although the sample size was small as was discussed previously the results compare well to other same modality measures. As was previously discussed in Chapter 4, a cohort with a variety of congenital heart defects may obscure comparisons between healthy subjects and those with CHD, but this variety should only further reveal correlations between measurement techniques given the large physiological range that is observable. However, more longitudinal observations are needed to understand how cerebral physiology in neonates with different heart conditions changes during the first weeks after birth.

MRI CBF measurements are based on large cerebral artery blood volume inflow, whereas NIRS measurements are based on microvascular RBC flow and these differences may also explain the lack of correlation we observed. We know that CBF increases at different rates in different brain regions during early post-natal development (73). Though the MRI measurement of flow is a whole brain average, the NIRS flow measurement is a measurement wherever the probe is positioned.

Finally, it could be that either NIRS or MRI measurements were biased due to some methodological limitation. There are several ways that PC-MRI could be improved for subsequent studies. Though the supplying arteries targeted for CBF measurement in this study were the internal carotid and basilar arteries, these have been shown to be well correlated with measurements from the internal carotid and vertebral arteries (64). In either case, maintaining sufficient resolution to ensure that more than 3 voxels span the vessel lumen (81,226) and that VENC is sufficient to prevent phase wrapping being silently incorporated into the averaged dicom image is important. Also, gating PC-MRI to the neonatal heart rate should improve accuracy by about 10% (17).

5.6 Conclusion

MRI and NIRS provide complementary methods for quantification of cerebral hemodynamics that if cross-validated would increase our confidence in both modalities and lead to more comprehensive clinical monitoring. However, before data between these two modalities can be compared or combined, additional studies are needed to better understand how CBF varies through time in early development and the relationship between large vessel bulk flow and microvascular red blood cell flow. SvO2 measurements in our study are significantly correlated between modalities, but CBF, and therefore CMRO2, are not in good agreement either due to differences in physiology or biases in these measurements.
Chapter 6
Future Work

6.1 QUIXOTIC SNR

By introducing a turbo gradient and spin echo readout module into the QUIXOTIC technique we were able to reduce the acquisition time required for whole brain OEF measurements by eightfold. This dramatic acceleration was largely due to reductions in physiological noise and motion achieved with a shorter scan. However when making OEF maps, voxels in WM regions gave unreliable exponential fits due to suspected low SNR, and voxels in GM where not as uniform as expected in comparison to PET studies possibly due to macrovascular contamination. Low SNR in WM makes sense since WM CBF is approximately 71% lower and CBV 50% lower than in GM (227). It is not unreasonable that a 50% reduction in velocity selected blood volume for QUIXOTIC imaging would result in low SNR for some voxels, however this is unverified. Macrovascular contamination has been observed in VSASL (146) and in our results near the SSS (Figure 2-5). Signal in veins with flow velocities much higher than should be obtainable during TO, is hypothesized to originate from apparently slow moving blood actually moving quickly through tortuous vessel geometry or in turbulent regions. Confirming this hypothesis may lead to techniques for eliminating this signal.

6.1.1 Impact

- Optimizing tQUIXOTIC SNR to create whole brain, not GM only, OEF maps would enhance the clinical utility of the sequence, for instance to identify strokes in watershed regions.
- Establishing the relationship between velocity selective module parameters and specific blood compartments would permit an optimization of desired vascular compartment and SNR.
- SNR and vascular compartment selection are fundamental to the QUIXOTIC technique and may motivate further sequence based optimizations such as multiple direction velocity encoding (228).

6.1.2 Proposed Approach

- When targeting venous blood using QUIXOTIC, $V_{CUTOFF}$ for VS1 and VS2 (Figure 2-1) are the same. So the parameter space for $V_{CUTOFF} = 1$, 1.5, 2, 2.5, 3 cm/s with $TO = 500$, 600, 700, 800, 900 ms could be mapped in in less than 90 minutes of scan time with the 3.5 minute acquisition we used for baseline scanning. SNR could be quantified as a ratio of the average QUIXOTIC venular blood signal in a voxel to the standard deviation of the venular blood signal on a per TE basis. Fit quality for $T_2$ could also be used as a metric for parameter optimization.
• A physiological flow phantom of the brain would be ideal for performing a more extensive parameter space mapping, but we are not currently aware of any such phantoms with physiological vascular properties. Parameter space mapping could be attempted on a perfused placenta model. Though it does not seem that the ex vivo perfused placenta would be a suitable analogue for cerebral perfusion, interest in placental imaging in its own right would motivate trying QUIXOTIC on the ex vivo placenta (229). Velocity selective ASL imaging has been applied to the placenta (230), but information about the selected blood compartment and the effects of motion on perfusion quantification would be extremely valuable.

• Numerical simulations using multiscale vascular models would assist in relating module parameters to vascular compartments (231,232) to clarify the origin of the macrovascular signal.

6.1.3 Metrics of success

• A determination of the maximum achievable SNR in the prescribed $V_{CUTOFF}$ and TO parameter space during a 3.5 minute acquisition and reliable whole brain OEF maps.

• Using a numerical multiscale vascular model, vessel calibers and blood volumes selected by each $V_{CUTOFF}$ and TO parameter set should confirm the empirical parameter optimization.

• Motion free estimates of ex vivo placental perfusion that match within 5% the expected perfusion given the external pump flow rate and placental volume. Some error might be expected due to vessel leakage or inhomogeneous perfusion.

6.1.4 Potential pitfalls and alternative strategies

• If the maximum achievable SNR is not sufficient for reliable estimation of OEF in WM, a longer acquisition could also improve SNR. However, motion correction is needed to enable longer scans. For baseline acquisitions without the temporal resolution necessary for functional imaging, a 3D segmented readout (e.g. 3D spiral or 3D GRASE) (149,150) could be used to acquire volumes amenable to registration even during out of plane motion.

6.2 Neonatal simultaneous flow measurements

Chapter 5 describes uncorrelated measurements of CBF when several hours separated the NIRS and MRI scans. This lack of correlation meant that CMRO$_2$ measurements did not agree either. Given that previous groups have demonstrated good agreement between the two modalities, this was surprising. It is either a result of inaccurate measurements, changing physiology or a differences between macrovascular and microvascular blood flow. We hypothesize that this lack of agreement is due primarily to a variety of factors described in chapter 5 that lead to physiological variation in CBF. If CBF is sufficiently variable in a single subject throughout the course of a day that we failed to find inter-modality agreement, this
would support previous findings (105) that indicate CBF is a more important marker of brain function than oxygenation alone. It also suggests that the magnitude or frequency of variation might be an important marker of brain health.

6.2.1 Impact
- Simultaneous NIRS and MRI measurements of oxygenation and blood flow before surgery would establish whether measurement error or physiological CBF variation explains the findings in Chapter 5.
- Simultaneous measurements could be used to calibrate the hemodynamic history provided by NIRS to create a metric of CBF variation through time.
- Calibrated CBF flow variation through time could be compared with measurements of Lac and GSH to test hypotheses detailed in Section 6.3.

6.2.2 Proposed Approach
- Healthy neonates and those with CHD would be scanned frequently leading up to surgery, before a simultaneous MRI/NIRS scan takes place.
- An MRI compatible DCS flow probe would be used to quantify CBFi in the frontal lobe during the MRI acquisition of CBF. The MRI CBF protocol would consist of cardiac gated and ungated PC-MRI acquisitions of the ICAs and BA.
- Correlation analysis would be performed between CBFi and CBF with and without cardiac gating. A metric of CBF variation would be standard deviation of CBFi divided by the ratio of mean CBFi to absolute CBF.

6.2.3 Metric of success
- Good correlation between simultaneous CBFi and CBF measurements would demonstrate that the results in Chapter 5 are due to physiological variability in CBF.
- The creation of a reliable CBF variation metric for use in the experiments described in section 6.3.
- A difference between CBF variation in healthy neonates and those with CHD. We might expect healthy neonates to have more stable CBF.

6.2.4 Potential pitfalls and alternative strategies
- If simultaneous measurements are not correlated:
  - This would suggest a measurement inaccuracy. PC-MRI is a well-researched method and we would wish to try a high resolution, cardiac gated acquisition with a vendor supplied protocol.
○ This could be due to the difference between microvascular flow, measured by NIRS, and larger artery flow, measured by MRI. We could attempt ASL or velocity selective ASL such that a regional microvascular measurement of CBF could be compared to the NIRS measurement (73).

○ We could use two DCS probes or move the one probe to the parietal or occipital lobe and repeat the simultaneous measurement.

6.3 GSH and Lactate quantification with frequent NIRS monitoring

Monitoring neonates with CHD in the ICU, during and after surgery with a focus on improving long term neurological outcomes is a challenging area of research. Further development improvement of monitoring technology coupled with multimodal analyses may reveal surrogate markers for long term developmental outcomes. Preliminary data presented in Chapter 4 raises two testable hypotheses:

1) Elevated GSH is a result of precarious, perhaps inadequate, oxygen supply leading to persistent mild oxidative stress in the time before the MRI scan.

2) Lactate levels generally decrease after birth, so elevated lactate in neonates with CHD is either a sign of cerebral dysmaturity or hypoxia.

6.3.1 Impact

- Confirming the hypothesis that GSH is higher in the presence of mild oxidative stress would create a new marker of oxygen supply inadequacy.

- Connecting Lac levels to dysmaturity or hypoxia would explain the biological mechanism behind a common radiological finding in this cohort.

6.3.2 Proposed Approach

- The presence of mild oxidative stress or ischemic episodes would be confirmed by performing continuous NIRS monitoring for tissue oxygen saturation and cerebral blood flow in the period between birth and surgery. Repeated drops in CBF or spikes in OEF would indicate inadequate oxygen supply. Comparison to continuous monitoring data from healthy controls may be necessary to classify theses drops.

- Scanning neonates with CHD before surgery, we would use the MRS protocol described in Chapter 4 for Lac quantification, and a MEGA-PRESS protocol for GSH quantification (233). This approach would also permit comparison of GSH quantification as performed in Chapter 4 to the validated MEGA-PRESS protocol.

- Acquire simultaneous MRI/NIRS CMRO₂ measurements to facilitate subject-wise comparisons of the NIRS data.
6.3.3 Metrics of success

- Demonstration that MRS measurements of GSH and Lac are markers for stress occurring over a period of time before the measurement, by showing larger variation in CMRO$_2$ and CBF between birth and the MRI scan correlates with measured metabolite concentration ratios.
- Confirm or invalidate the approach to GSH quantification described in Chapter 4 by comparing the two methods of GSH quantification on the same subjects.

6.3.4 Potential pitfalls and alternative strategies

- Though lactate levels rise in the seconds after an hypoxic insult, it is not the best maker of hypoxia severity, which is more linearly related to alanine or glycerol-3-phosphate concentrations (84). Alanine was difficult to resolve in our data, so we could not attempt an analysis, but with more SNR at 7T alanine may be the better marker of hypoxia.
- Transition may be the largest insult neonates with CHD face from conception to surgery. If continuous NIRS measurements do not show repeated mild drops in blood flow and oxygen delivery, pre and post-natal CMRO$_2$ measurements using MRI may help us understand how transition affects oxygen delivery to the brain. Pre-birth CMRO$_2$ measures that are comparable to neonatal measures would need to be developed.

6.4 MRF for fetal organs and placenta

As a step toward quantifying fetal cerebral oxygenation, we propose exploring novel methods for quantitative T$_1$ and T$_2$ mapping of the fetus and placenta. Quantitative T$_1$ and T$_2$ may improve diagnosis and monitoring of placental and fetal disease. Relative placental $\Delta R_2^*$ mapping during maternal hyperoxia correlates with placental pathology and birth weights in discordant twins (109). T$_1$ and T$_2$ are also sensitive to placental physiology. T$_2$ correlates with placental fibrin density and T$_1$ and T$_2$ relate to blood oxygen saturation and hematocrit (234,235). Additionally, T$_1$ is sensitive to changes in brain water content and the progression of normal myelination (236,237). Even the methods for T$_1$ and T$_2$ quantification in cardiac imaging (238) are suboptimal for use during pregnancy given the longer relaxation times of fetal tissues, unpredictable and substantial fetal motion, and $B_1^+$ inhomogeneities. We aim to use magnetic resonance fingerprinting (MRF) (239) to estimate regional T$_1$ and T$_3$ of the placenta, fetal liver and brain. We would build on initial findings (240) by accelerating MRF acquisition using sliding-window reconstruction (241) during a single breath hold, validate quantification using an anthropomorphic 22-week fetal phantom and correct for $B_1^+$ inhomogeneity (242). MRF offers fast absolute parameter mapping making it potentially well suited to placental and fetal imaging.
6.4.1 Impact

- This study will establish reference values for $T_1/T_2$ of the human fetus liver, brain and placenta at 3T. No studies of this sort have been published for the liver or brain, and only one study of the placenta at 3T.
- Quantitative $T_1/T_2$ would be closely related to blood oxygen saturation and hematocrit in the placenta which is mostly (>60%) blood. This would permit characterization of its baseline oxygenation state, which directly affects the baseline $R_2^*$ signal. Accurate baseline characterization may enable comparison between $R_2^*$ time courses in singleton pregnancies.
- With sufficient resolution maternal and fetal blood compartments of the placenta may be resolved based on their $T_1/T_2$ parameters.
- Large FOV MRF needs optimal $B_1^+$ and slice profile corrections, which we will develop for imaging pregnancy, but can applied to abdominal imaging generally. Preliminary results (Figure 6-1) show promising improvements to $T_1$ accuracy from $B_1^+$ correction.

![Figure 6-1: Results of ROI analysis of fetal phantom. Mean ROI $T_2$ versus $T_1$ with error bars representing standard deviation of values within the ROI. (+ = reference methods, o = MRF, □ = MRF w/B1+ correction)](image)

6.4.2 Proposed Approach

- Using a 22-wk fetal phantom we will determine the optimal $B_1^+$ and slice profile correction strategy by attempting to match MRF and reference method $T_1/T_2$ in five compartments. Fully sampled MRF data (a 10 ms tanh-modulated adiabatic inversion pulse for homogenous inversion across the FOV, repetition times (TRs) = 12 to 12.81 ms (Perlin noise pattern), flip angles (FA) = sinusoidally varied 3-78°, echo time (TE) = 2.7 ms, data acquisition was by a constant density spiral trajectory of 30 interleaves with one interleave used per acquisition, resolution = 3x3x4 mm³, field of view (FOV) = 390 mm, 720 TRs acquired in 30 x 9 = 270 sec) will be used to try different correction approaches based on the previously published methods (243,244).
• We will then collect $T_1/T_2$ maps of the placenta, fetal brain and fetal liver, at baseline and during maternal hyperoxygenation. Preliminary results (Figure 6-2) indicate that this is achievable with a breath hold during the short 9 second scan.

![Parameter maps from a scan during pregnancy (27\+ weeks), with breath hold and B1+ correction. Solid line liver, dotted line placenta, dashed line brain, and arrow descending aorta.](image)

Figure 6-2: Parameter maps from a scan during pregnancy (27\+ weeks), with breath hold and B1+ correction. Solid line liver, dotted line placenta, dashed line brain, and arrow descending aorta.

6.4.3 Metrics of success

• 5% error in $T_1$ and $T_2$ estimates would be tolerable after B1+ and slice profile correction. Error of this magnitude would permit baseline characterization, detection of pathology in the placenta (234), and estimation of fetal or maternal blood $SO_2$ and Hct (245).

• Detect change in placental $T_1/T_2$ during maternal hyperoxygenation.

• Improved predictive value of time to plateau measurements in singleton pregnancies after correcting for baseline placental physiology ($T_1/T_2$).

6.4.4 Potential pitfalls and alternative strategies

• Though the accuracy of MRF is thought to be fairly good (246), with the constraints of fetal motion and large FOV imaging MRF may not provide the 5% accuracy we believe is necessary for quantitative $T_1/T_2$ to be clinically useful. In this case we will need to explore alternative approaches to quantitative relaxometry. These could include machine learning based approaches to MRF reconstruction (247) or the abandonment of the MRF approach in favor of fast single-slice acquisitions like multi-inversion EPI for $T_1$ mapping (248) and $T_2$ preparation and bSSFP readout for $T_2$ mapping (12).

• Fetal motion is unpredictable and maternal breathold during hyperoxia challenge is suboptimal. Motion correction for MRF during pregnancy is a harder case than that previously explored for cardiac imaging (249), but the magnetization preparation approach may permit first order motion correction by rejecting those TRs during which motion has occurred from fingerprint matching. This approach may fail given that the spin history will be different for out of plane tissue that may
move into the imaging plane during the acquisition, so a more complex approach where a motion model is included in the reconstruction itself may be necessary (248).
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