Investigating the myelin water fraction as a function of TR and the intra/extra cellular water geometric mean $T_2$ as a function of refocusing interval

by

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Abstract

In this thesis two studies were done using MRI. In chapter two, in vivo 3.0 T MRI data from white matter and grey matter in brain from 4 healthy volunteers was studied using a multi component $T_2$ relaxation analysis. The goal of this study was to find the dependence of myelin water fraction (MWF), the ratio of water in myelin bilayers to the total water component, with the repetition time of the MR sequence TR. Results showed that MWF increased with decreasing TR time. This behavior is believed to be influenced by the exchange of water between the myelin water and the intra/extracellular water pools. Several models were explored to explain this result, including a fast exchange model, a slow exchange model and a hybrid model in which myelin was proposed to contain regions of fast exchange and regions of slow exchange.

In chapter three, we addressed the questions: Does the intra/extracellular (IE) water geometric mean $T_2$ ($gmT_2$) of white and grey matter depend upon the refocusing interval? To answer these questions IE water $gmT_2$ times for different white and grey matter regions of interest were obtained from 5 healthy subjects. It was found that IE water $gmT_2$ times from both white matter and grey matter tissue decreased by approximately the same amount with refocusing interval prolongation from 10ms to 40 ms. Several mechanisms for this dependence were considered, including water exchange, existence of myelin, non-heme iron accumulation, or the effect of blood oxygenation. In this case, based on our simulations, exchange did not appear to play a role. Non-heme iron accumulation was related to $T_2$ time but not to the change in $T_2$ with echo spacing. Deoxygenation of blood results in the presence of paramagnetic deoxyhemoglobin (dHb), which increases the magnetic susceptibility, and thus the local magnetic field, which can shorten the transverse relaxation time; however, this mechanism would be expected to affect grey matter $T_2$’s more than white matter $T_2$s.
Preface

All of the data analysis and data interpretation presented in thesis was done by me. I was also involved in the experimental planning for chapter 3 along with Dr. Irene Vavasour and Dr. MacKay.

Research Ethics  The Ethics Approval Code from the UBC Clinical Research Ethics Board for the data presented in chapter two is H07-70237.
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Chapter 1

Introduction

Magnetic Resonance Imaging (MRI) is a medical imaging technique that produces high quality images of the human body using nuclear magnetic resonance (NMR) phenomena. NMR is based on interactions between the nuclear magnetic moment and the external magnetic field which is the basis for signal acquisition in MRI.

MRI deals predominately with the NMR signal detected from hydrogen for several reasons: hydrogen can be found in water which makes up approximately 60% of the human body, the most abundant isotope of hydrogen exhibits a nuclear spin, and nucleus of hydrogen gives a very high MR signal compared to other biologically relevant isotopes of carbon, sodium or phosphorous due to its large gyromagnetic ratio (~42.57 Mhz/T) [1].

1.1. Basics of NMR physics

Protons have randomly oriented spins (an intrinsic, quantum mechanical property) in the absence of an external magnetic field.

The nuclear spin is the sum of spins of all nucleons and since protons and neutrons have spin $\frac{1}{2}$, nuclei can have zero or $\frac{1}{2}$ or greater than $\frac{1}{2}$ spin. Nuclei with non-zero spin possess a magnetic moment. The magnetic moment is proportional to spin angular momentum, $J$, as
µ = γJ \quad (1.1)

where γ is the gyromagnetic ratio which is unique for each type of nucleus. In the nucleus, angular momentum is determined by the difference between number of protons and neutrons. Thus only isotopes with an odd number of protons or neutrons with a non-zero spin can generate an NMR signal. [2,3]

When protons placed in a magnetic field they start precessing. In addition they reorient themselves towards the direction of the magnetic field. This process of reorientation involves interaction with the surrounding. The result of this process is the minimum of potential energy for the ensemble of the spins.

\[ E = -\mu B \quad (1.2) \]

The direction of B defines the longitudinal axis. The convention is that the magnetic field is applied in z-direction \( B = Bz \), and \( \mu_z = \gamma j_z \). Thus the proton energy would be

\[ E = -\mu_z B = -\gamma j_z B = -\gamma \hbar s_z B \quad (1.3) \]

Since \( s \) is \( \frac{1}{2} \) for protons there are two possible values for \( s_z \): \( \frac{1}{2} \) for spin up orientation and \( -\frac{1}{2} \) for spin down orientation. In the absence of magnetic field all spins are at the same energy level. Imposition of an external magnetic field \( B \) introduces splitting of the energy level in to a spin-up state and a spin-down state. This phenomenon is called the Zeeman effect. The spin-up state has a lower energy, \( E = -\gamma \hbar B/2 \), because in this state spins are oriented towards the magnetic field and the spin-down state has a higher energy, \( E = \gamma \hbar B/2 \), since the orientation of the spins is opposite to the magnetic field. In NMR or MRI a large number of protons are placed in a magnetic field. In this case the ratio of the population of spin-down (higher energy) protons to spin-up (lower energy protons) is defined as

\[ \frac{N_-}{N_+} = \frac{e^{-E_-/kT}}{e^{-E_+/kT}} = e^{-\Delta E/kT} = e^{-\gamma \hbar B/kT} \quad (1.4) \]
By applying a 1.5 T magnetic field at room temperature (T=300K) this ratio would be 1.0000102 which indicates that there are slightly more protons in the spin-up state than the spin-down state. This leads to the ensemble of spins gaining a total magnetic moment per unit volume called macroscopic magnetization given by

\[ M_0 = \mu(N_- - N_+) \]  \hspace{1cm} (1.5)

If the net magnetization vector of the protons is tilted away from the field direction it will be affected by a torque, \( \mathbf{N} \), and it will start to precess about the magnetic field direction.

\[ \mathbf{N} = \mu \times \mathbf{B} \]  \hspace{1cm} (1.6)

To describe the interaction of the protons spins with the magnetic field, Newton’s second law can be used:

\[ \mathbf{N} = \frac{\mathbf{d} \mathbf{j}}{\mathbf{d}t} \]  \hspace{1cm} (1.7)

which indicates that torque is the rate of change of angular momentum.

Substituting equations (1.1) and (1.6) in (1.7) leads to equation of motion for proton:

\[ \frac{\mathbf{d} \mu}{\mathbf{d}t} = \gamma \mu \times \mathbf{B} \]  \hspace{1cm} (1.8)

The solution of this equation of motion leads to what is known as the Larmor equation:

\[ \omega = -\gamma B \]  \hspace{1cm} (1.9)

where \( \omega \) is the angular frequency of the precession. Since the gyromagnetic ratio is unique for each type of nucleus, at a given field each nucleus will precess with a
characteristic Larmor frequency; this enables us to focus on MR signals from hydrogen and neglect MR signals from other nuclei in the body.

In order to generate a NMR signal, spin excitation is needed. Therefore an additional magnetic field, called $B_1$, which is rotating with Larmor frequency, is applied perpendicular to $B$ to tip the equilibrium magnetization from the $z$-axis. $B_1$ is applied in the form of short pulses and is called the radiofrequency or RF pulse.

Magnetization can be tipped by a specific angle $\alpha$ called the flip angle:

$$\alpha = \omega_1 \tau = \gamma B_1 \tau$$  \hspace{1cm} (1.10)

where $\tau$ is the length of the RF pulse. Of particular interest are 90 degree and 180 degree pulses. A 90-degree pulse tilts the magnetization vector to the transverse plane (xy plane) and a 180 degree pulse tilt the magnetization vector to the exact opposite direction of its equilibrium direction (from $z$ to $-z$).

The magnetization vector has three components in the laboratory reference frame, $M_z$, $M_x$, $M_y$. $M_z$ is parallel to the magnetic field, is called the longitudinal component, and is stationary in the laboratory frame. $M_x$ and $M_y$ are transverse to the magnetic field, are called transverse components, and precess about $B$.

$$\frac{dM}{dt} = \gamma M \times B$$  \hspace{1cm} (1.11)

$$\frac{dM_z}{dt} = 0 \hspace{1cm} \frac{dM_\perp}{dt} = \gamma M_\perp \times B$$

where $M_\perp$ is the transverse magnetization. When the RF pulse is turned off spins that are precessing tend to align with $B$ to minimize their energy. This process, which describes the time in which spins return to magnetization equilibrium, is known as relaxation.
1.2. Relaxation times

In relaxation, protons exchange energy with their surroundings, which can be either the lattice or other spins. The time constant describing transfer of energy from spins to the lattice, causing the magnetization to return to its equilibrium is called \( T_1 \) or the longitudinal relaxation time. In the transverse plane, spins lose phase coherence and as a result their transverse magnetization diminishes. This spin-spin interaction is called \( T_2 \) relaxation. In simple spin systems \( T_2 \leq T_1 \).

To describe the behavior of the magnetization in most MRI experiments, the Bloch equations [4] are used.

\[
\begin{align*}
\frac{dM_x}{dt} &= \omega_0 M_y - \frac{M_x}{T_2} \\
\frac{dM_y}{dt} &= -\omega_0 M_x - \frac{M_y}{T_2} \\
\frac{dM_z}{dt} &= \frac{M_0 - M_z}{T_1}
\end{align*}
\]

These are equations of motions for the magnetization in the case of interacting spins with the relaxation times \( T_1 \) and \( T_2 \).

When there is no \( T_1 \) relaxation \( \frac{dM_z}{dt} = 0 \) and in the presence of \( T_1 \) relaxation \( \frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1} \).

The solution of this modified equation is

\[
M_z(t) = M_z(0)e^{-t/T_1} + M_0(1 - e^{-t/T_1})
\]
which describes the process in which the longitudinal magnetization recovers to its equilibrium value $M_0$ which is along the z-axis. $T_1$ is the characteristic time of this process and is equal to the time needed to recover 63% of $M_0$. For $T_2$ relaxation:

$$M_{\perp}(t) = M_{\perp}(0)e^{-t/T_2}$$  \hspace{1cm} (1.16)$$

which indicates the diminishing of transverse magnetization over time. After time $T_2$ the magnetization is equal to 37% of $M_0$.

The solution of Bloch equation for each of magnetization components is

$$M_x(t) = e^{-t/T_2}(M_x(0) \cos \omega_0 t + M_y(0) \sin \omega_0 t)$$  \hspace{1cm} (1.17)$$

$$M_y(t) = e^{-t/T_2}(M_y(0) \cos \omega_0 t - M_x(0) \sin \omega_0 t)$$  \hspace{1cm} (1.18)$$

$$M_z(t) = M_z(0)e^{-t/T_1} + M_0(1 - e^{-t/T_1})$$  \hspace{1cm} (1.19)$$

Therefore after perturbation of the spins from the z axis, $M_x$ and $M_y$ precess about B and decay exponentially with $T_2$ while $M_z$ grows exponentially with $T_1$.

### 1.3. Central nervous system

The basic building block of the nervous system is a nerve cell known as a neuron. A neuron is a specialized cell, which transmits and receives nerve information impulses. Neurons are much like other body cells; they contain a nucleus, protected by its surrounding membrane and contain organelles.
One of the major parts of a neuron is the axon. In the nervous system axons are long projections, which are like electrical wires, and they transmit electrical signals around the body. Axons are usually covered by a fatty lipid-rich substance which acts as an insulating layer around it, called myelin sheath. Different axons have different numbers of layers of myelin sheaths. But generally, thicker axons have thicker myelin sheathes.

Myelin is a lipid-protein [5] (the dry weight of myelin composed of 80% lipid, 20% protein) lamellar membranous structure which is produced by oligodendrocytes in a process called myelination. The function of the myelin sheath is to protect the axon and to accelerate signal propagation. Myelin has a significant role in CNS since it is critical to healthy functioning of the central nervous system. Myelin is one of the most lipid rich structures hence it is an electrical insulator for neurons. It also accelerates nerve signal transmission by a process called saltatory conduction [20]. Damage to the myelin sheath and myelin loss leads to decreased brain functionality and is an aspect of neurodegenerative disorders like Multiple Sclerosis (MS), Leukodystrophies, and Schizophrenia [6].

The nervous system is divided into the central nervous system and the peripheral nervous system. The central nervous system (CNS) is composed of the brain and the spinal cord. The brain and spine contain white matter and grey matter. Grey matter consists of neuronal cell bodies (area of the neuron which contains nucleus), non-myelinated axons, and capillaries. The grey color of grey matter is due to capillaries and neuronal cell bodies. Hence, blood volume is significantly greater in grey matter than white matter. On the other hand, white matter is composed mostly of myelinated axons, which are covered by a white layer of myelin known as the myelin sheath. The water trapped in between myelin sheath bilayers is called myelin water. White matter structures have more myelin and myelin water than gray matter structures [7,8].

Other water reservoirs in CNS are intra/extra cellular water and cerebrospinal fluid (CSF); they can be distinguished through their different T2 values. Fischer et al showed that T2 times for all the water in grey and white matter are greater than 10 ms and hence are accessible to MRI [9].
1.4. $T_2$ relaxation analysis of brain water pools

The most common MRI technique used to measure the $T_2$ relaxation time of a given sample is a pulse sequence developed by Carr, Purcell, Meiboom, and Gill known as the (CPMG) sequence [10,11]. CPMG is a multi spin echo sequence consisting of a 90 degree excitation RF pulse about the x-axis, followed by a train of equally spaced 180 degree refocusing pulses along the y axis. Each of the 180 degree pulses will generate an additional echo. Due to $T_2$ dephasing, the peak signal amplitude of each echo is reduced over time leading to a smoothly decaying curve known as the $T_2$ decay curve. For homogenous environments the $T_2$ decay curve can be fitted by a single exponential function. However, for non-homogenous environments when there are different compartments with unique $T_2$ relaxation times, the $T_2$ decay curve should be fitted by a multi exponential function. Different brain structures exhibit different $T_2$ decay curves [12].

For clinical application of spin-spin relaxation a quantitative technique for tissue decay curve measurement, $T_2$ analysis and interpretation is needed. The most common analysis approach is to use the Non-Negative Least Squares (NNLS) method to analyze multi component $T_2$ decay curves. The equation representing $T_2$ decay curve is

$$y(t_i) = y_i = \int_a^b s(T_2) e^{-t_i/T_2} \, dT_2 \quad i = 1,2,\ldots,N \quad (1.20)$$

where the N data points $y_i$ are measured at times $t_i$. $s(T_2)$ is the amplitude of the spectral component and $e^{-t_i/T_2}$ represents the amount that a signal has decayed in time $t_i$. Discrete $T_2$ distributions consist of delta functions each with $s_j$ amplitude corresponding to a distinct $T_2$ time. Discretized version of equation (1.20) will be

$$y_i = \sum_{j=1}^{M} s_j e^{-t_i/T_{2j}} \quad i = 1,2,\ldots,N \quad (1.21)$$
A general form of equation (1.21) replacing the exponential terms with a single variable, $s$, can be written as

$$y_i = \sum_{j=1}^{M} A_{ij} s_j \quad i = 1, 2, \ldots, N$$  \hspace{1cm} (1.22)

The non-negative least squares method inverts a relaxation decay curve to a relaxation time distribution, which is a plot of signal intensity versus relaxation time. Using NNLS, decay curves can be decomposed into an arbitrary number of exponential components. The term to be minimized is

$$\chi^2 + \mu \sum_{j=1}^{m} s (T_{2j})^2$$  \hspace{1cm} (1.23)

where $\mu$ is the smoothing parameter which smooths out the resulting $T_2$ distribution; large $\mu$ leads to a smoother $T_2$ distribution while a small $\mu$ causes spikier $T_2$ distributions. In other words $\mu$ determines the range of regularization. Setting $\mu$ to zero gives the least squares ($\chi^2_{\min}$) solution. $\chi^2$ is the misfit which indicates the quality of the fit.

$$\chi^2 = \frac{\sum_{i=1}^{m} [Y_i - \sum_{j=1}^{m} s_j \exp(-t_i/T_{2j})]^2}{\sigma^2}$$  \hspace{1cm} (1.24)

The NNLS algorithm fits the relaxation decay curve by minimizing the factor $\chi^2$. Often $\chi^2$ must be in a predefined range to produce robust $T_2$ distribution plots [13]. When there is no regularization the resultant $T_2$ distribution consists of a few discrete peaks [14]. Previous in vivo work at 1.5T [15,16,17] showed that water components in healthy human brain can be separated into three components. The short $T_2$ time ($T_2 \sim 15$ms) arises from water trapped between myelin bilayers and is known as myelin water MW, the intermediate $T_2$ time ($T_2 \sim 70$ms) arise from water in intra/extra cellular space called intra/extra cellular water IEW, and the longest $T_2$ time ($T_2 \sim 3$s) is from cerebrospinal fluid [18].
In general, two water pools contribute to the $T_2$ distribution of white matter; Intra/extracellular water and myelin water. An example of a $T_2$ distribution of white matter is shown below.

Figure 1. A shows the $T_2$ decay curve and B shows the $T_2$ distribution of myelin water, which is trapped in between myelin bilayers, and intra/extracellular water.

The area under each peak is proportional to the number of water protons contributing to that water reservoir.

Since all of the water in brain contributes to the MR signal [17], the sum of all $T_2$ component amplitudes is proportional to the total water content. The fraction of water trapped between myelin bilayers called the myelin water fraction (MWF) is the ratio of the myelin water signal (short $T_2$ component) to the total signal [15].
In the $T_2$ distribution the intra/extra cellular water peak can be characterized by its geometric mean $T_2$ ($\text{gm}T_2$). The $\text{gm}T_2$ which is the mean on a logarithmic scale, and can be calculated using

$$
\text{gm}T_2 = \exp\left[\frac{\sum_{T_2 \text{ max}}^{T_2 \text{ min}} S(T_2) \log T_2}{\sum_{T_2 \text{ max}}^{T_2 \text{ min}} S(T_2)}\right]
$$

(1.25)

where $S(T_2)$ are the $T_2$ distribution intensities.

$T_2$ decay curve measurements demonstrate that individual brain structures have markedly different MWFs and different geometric mean $T_2$’s for the intra/extracellular $T_2$ peak [19]. At 1.5T, the geometric mean $T_2$ times for the intra/extracellular water pool were found to vary between 70 and 90 ms with no discernable separation between grey and white matter structures. Water content is larger for grey matter than for white matter and myelin water fraction varies by more than a factor of 2 between different brain white matter structures and is quite low in all grey matter areas. These variations between different brain regions may arise from differing tissue microstructures [12]. Water molecules which interact with proteins, lipids and other nonaqueous molecules undergo faster relaxation and water molecules which are in free solution undergo slower relaxation; this is why white matter has shorter $T_1$ and $T_2$, and grey matter has longer $T_1$, and $T_2$. 
Chapter 2

Variation of myelin water fraction as a function of TR

2.1. Introduction

In white matter, most axons of neurons are covered by multiple lipid rich bilayers known as the myelin sheath. Myelin, which consists of membranes with high lipid content, is an electrical insulator that accelerates nerve signals conducted along the axons. These nerve impulses are known as the action potential. The action potential is the voltage change across the cell membrane which propagates along axons and transfers information throughout the nervous system. If axons were covered by one continuous myelin sheath the signal would be dissipated. In order to boost the signal there are some gaps between myelin sheath known as Nodes of Ranvier (where the axonal membrane interface with its surroundings). This propagation of action potential is called saltatory conduction [20]. Myelin can be found in both the central and the peripheral nervous system of vertebrates. Damage to the myelin sheath causes reduction in signal conduction velocities, which occurs in diseases such as multiple sclerosis [21, 22, 23] schizophrenia [24] and Phenylketonuria (PKU) [25, 26]. Hence it is important to be able to measure in vivo myelin content in order to diagnose and manage myelin related disorders.

The water trapped between the myelin bilayers is called myelin water. The ratio of myelin water to the total water content is called the myelin water fraction (MWF). MWF is particularly important since it has been proposed as a marker for myelination in vivo [15,19] and also validated as a myelin content marker by histological staining in guinea pig brain [27], sciatic nerve [28], and human brain [29, 30].
Measuring MWF in a clinical time frame may require the use of shorter TR times. Reducing TR time leads to $T_1$ weighting and acquired $T_2$ decay curve becomes $T_1$ weighted. Hence it is important to understand $T_1$ relaxation in brain. The nature of $T_1$ in white matter is not well understood. One phenomenon that can influence $T_1$ in brain is the time scale of magnetization exchange between myelin water and intra/extracellular water.

Dula et al. [31] showed that in ex vivo rat spinal cord, water exchange between myelin water and intra/extracellular water affected transverse relaxation and that exchange rate varied as a function of microanatomy; in regions with similar myelin content, water exchange occurred at a faster rate in axons with smaller diameters and thinner myelin sheaths. Decreased exchange would result in increased MWF [32]. If the exchange occurs slowly on the brain $T_1$ timescale (~1s), $T_1$ could have two components. In this case amplitude of each $T_1$ component should reflect the population of each water reservoir. However if the exchange happens at a time close to the $T_2$ time scale, then the amplitudes of the peaks in the $T_2$ distribution will be shifted towards less short $T_2$ component signal and more intermediate $T_2$ component water signal, leading to a MWF which underestimates myelin content.

In typical MR imaging, an inversion pulse lasts for few milliseconds while non-aqueous magnetization decays to nearly zero in about 100 microseconds, which makes the signal from non-aqueous tissue in brain undetectable directly by MR. While MRI inversion pulses flip the magnetization of protons in water by 180 degrees, due to their limited bandwidth they have little effect on the magnetization of non aqueous protons (lipid, protein, etc.). Hence, after an inversion pulse non-aqueous protons and water protons are not in equilibrium with each other which leads to magnetization exchange between the two spin species. Studies by Gochberg et al. [33,34] Pranter et al. [35] showed that in white matter the re-equilibration of magnetization between water protons and non-aqueous protons resulted in water signal changes that could be mistaken for $T_1$ relaxation.

While most of the literature reports single component $T_1$ relaxation in white matter, Labadie et al. suggested that white matter has two $T_1$ components [36]. The Labadie in vivo study was done at 4T in human white matter and two $T_1$ components were measured
(~120 ms and 1050 ms) with similar amplitudes to the T\textsubscript{2} components assigned to myelin water and IE water. This would suggest that in white matter magnetization exchange is slow on the T\textsubscript{1} timescale.

In this study, in vivo MRI data collected from white matter and grey matter regions of 4 healthy subjects at 3.0 T. MWF was measured at various effective repetition times (TR\textsubscript{eff}) in different brain structures using multi component T\textsubscript{2} relaxation imaging. TR\textsubscript{eff} is the time from the last 180 degree refocusing pulse to the beginning of the next sequence. In multi spin echo sequences each 180 degree pulse inverts the z magnetization, preventing the signal from recovering along the z direction by T\textsubscript{1} relaxation. Since reducing scan time is an important goal in making myelin water imaging a clinical technique, it is important to understand the influence of reducing TR on measured MWF.

The purpose of this study was twofold: 1) to investigating the dependence of MWF on TR\textsubscript{eff} in white matter. 2) Explore the MW/IEW exchange regime in white matter. If MW/IEW exchange occurs in a fast regime, MWF should be independent of TR\textsubscript{eff}. But in a slow MW/IEW exchange regime MWF should increase with TR\textsubscript{eff} decrease. In other words, If both myelin water and IE water have the same T\textsubscript{1}, MWF is independent of TR, If MWF has a shorter T\textsubscript{1}, then MWF increases as TR decreases.

2.2. Methods

Four healthy volunteers underwent MR scan on a 3.0T whole body MR scanner (Achieva 3.0T, Philips Medical Systems, Best, The Netherlands) using an eight-element phased-array head coil. All subjects provided signed, informed consent prior to participation and all examinations were performed with approval from the University of British Columbia Clinical Research Ethics Board.

A CPMG sequence with 32 spin echoes was repeated at five TR times: 1100, 1200, 1300, 1500, 1600 ms. Due to specific absorption rate (SAR) limitations, a 32 echo sequence
with 10ms echo spacing could not be used. In order to measure the myelin water fraction, we used a sequence with 16 pulses at 10ms echo spacing followed by 16 pulses with 50ms echo spacing.

The last 180° refocusing RF pulse was 935ms later than the initial 90 pulse, hence $\text{TR}_{\text{eff}} = \text{conventional TR} - 935\text{ms}$. $\text{TR}_{\text{eff}}$ is the time from the last 180° refocusing pulse to the beginning of the next sequence and it is the time that the system is given to recover back to its equilibrium. We measured MWF at effective $\text{TR}_{\text{eff}}$ times of 165, 265, 365, 565, and 665 ms. Imaging parameters were: number of slices = 7, slice thickness = 5 mm, FOV = 24 cm, matrix size = 256 x128.

2.3. Data analysis

Each subject's images were registered to the longest TR image (TR = 1600 ms) using FLIRT (FMRIB's Linear Image Registration Tool). Regions of interest (ROI) were drawn for five white matter structures; minor forceps (MN), major forceps (MJ), genu (GU) and splenium (SP) of the corpus callosum and posterior internal capsules (IC) and three grey matter structures; putamen (PU), thalamus (TH) and caudate nucleus (CA). The mean MWF from all pixels within each ROI was extracted.

2.4. Results

Myelin water fraction (MWF) at various $\text{TR}_{\text{eff}}$ times from genu (GU), posterior internal capsules (IC), major forceps (MJ), minor forceps (MN), splenium (SP) of the corpus callosum, caudate nucleus (CA), Putamen (PU), thalamus (TH) are shown in the table below.
Table 1. MWF for the selected white and grey matter structures at five TR\textsubscript{eff} times. The standard error associated with each measurement is shown inside the parentheses.

<table>
<thead>
<tr>
<th>Structure</th>
<th>165 ms</th>
<th>265 ms</th>
<th>365 ms</th>
<th>565 ms</th>
<th>665 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU</td>
<td>0.134 (0.023)</td>
<td>0.147 (0.022)</td>
<td>0.136 (0.023)</td>
<td>0.140 (0.021)</td>
<td>0.133 (0.024)</td>
</tr>
<tr>
<td>IC</td>
<td>0.234 (0.030)</td>
<td>0.212 (0.017)</td>
<td>0.193 (0.033)</td>
<td>0.182 (0.027)</td>
<td>0.215 (0.012)</td>
</tr>
<tr>
<td>MJ</td>
<td>0.177 (0.019)</td>
<td>0.152 (0.017)</td>
<td>0.142 (0.023)</td>
<td>0.126 (0.020)</td>
<td>0.124 (0.014)</td>
</tr>
<tr>
<td>MN</td>
<td>0.094 (0.021)</td>
<td>0.089 (0.009)</td>
<td>0.081 (0.009)</td>
<td>0.082 (0.008)</td>
<td>0.072 (0.007)</td>
</tr>
<tr>
<td>SP</td>
<td>0.208 (0.016)</td>
<td>0.180 (0.024)</td>
<td>0.153 (0.020)</td>
<td>0.141 (0.024)</td>
<td>0.162 (0.020)</td>
</tr>
<tr>
<td>CA</td>
<td>0.062 (0.016)</td>
<td>0.036 (0.011)</td>
<td>0.042 (0.015)</td>
<td>0.030 (0.008)</td>
<td>0.040 (0.014)</td>
</tr>
<tr>
<td>PU</td>
<td>0.058 (0.010)</td>
<td>0.033 (0.004)</td>
<td>0.050 (0.015)</td>
<td>0.031 (0.010)</td>
<td>0.047 (0.014)</td>
</tr>
<tr>
<td>TH</td>
<td>0.046 (0.007)</td>
<td>0.020 (0.014)</td>
<td>0.026 (0.006)</td>
<td>0.015 (0.011)</td>
<td>0.033 (0.011)</td>
</tr>
</tbody>
</table>
Figure 2. MWF values from different white and grey matter brain structures at five $T_{Reff}$ times. Standard errors are shown as error bars.

To better distinguish the difference between white matter and grey matter we summed all the MWF values for all white matter and all grey matter regions for each $T_{Reff}$.

<table>
<thead>
<tr>
<th>TR</th>
<th>165ms</th>
<th>265ms</th>
<th>365ms</th>
<th>565ms</th>
<th>665ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter</td>
<td>0.169(0.025)</td>
<td>0.156(0.020)</td>
<td>0.141(0.018)</td>
<td>0.129(0.016)</td>
<td>0.141(0.023)</td>
</tr>
<tr>
<td>Grey matter</td>
<td>0.055(0.004)</td>
<td>0.029(0.004)</td>
<td>0.039(0.007)</td>
<td>0.025(0.005)</td>
<td>0.040(0.004)</td>
</tr>
</tbody>
</table>

Table 2. Averages of MWF of white matter and grey matter regions for five $T_{Reff}$ times. The standard error associated with each measurement is shown inside parentheses.
These results showed that the measured myelin water fraction (MWF) from white matter structures decreased with increasing TR$_{\text{eff}}$. On the other hand, for grey matter structures MWF remained approximately constant across the range of effective TR times.

2.5. Discussion

The main finding of this study is that for white matter structures at short TR$_{\text{eff}}$ times (shorter than about 400 ms) the measured myelin water fraction increased as TR$_{\text{eff}}$ decreased. To find out the reason for this increase we investigate three models for magnetization exchange between myelin water and intra/extracellular water.
Most of the literature reports a single T$_1$ component in white matter regions. If there was a fast exchange between MW and IEW on the T$_1$ timescale, both water pools should have the same T$_1$ and undergo the same T$_1$ weighting at all TR$_{eff}$ times and the plot of MWF vs. TR$_{eff}$ would be a straight line for all the structures. According to Figure.4 This model is not consistent with our results for white matter.

![Graph showing MWF vs. TR$_{eff}$](image)

**Figure 4.** Synthetically generated fit showing MWF vs. TR$_{eff}$ assuming both MW and IEW pools have the same T$_1$ = 400 ms.

It should be taken into account that after a MRI pulse, which affects largely water, there is a re-equilibration between non-aqueous and water protons at short times which looks like a short T2 component.

If there was a slow exchange or no exchange between MW and IEW on the T$_1$ timescale, then each water pool should have distinct T$_1$. To investigate this scenario we tried to fit our white matter data to a two component exponential in which T$_{1MW}$=200ms and T$_{1IEW}$=1200ms.
Figure 5. Synthetically generated MWF fit assuming that the myelin water $T_1=200\text{ms}$ the IE water $T_1=1200\text{ms}$ plotted along with the experimental MWF data vs. $T_{\text{Reff}}$.

Although a two component exponential fit to our data better than a one component $T_1$ model, which would be a straight line, but at longer $T_{\text{Reff}}$ times (longer than 655ms) the MWFs tend toward unreasonably low values. We know from past experience that MWF is relatively independent of TR for $T_{\text{Reff}}$ greater than about 600ms. Hence, the two component $T_1$ model cannot solely explain our experimental data.

Based on our data one can see two distinct regimes for white matter data: 1) at short $T_{\text{Reff}}$ there is a steep increase in MWF with decreasing $T_{\text{Reff}}$. This behavior might be explained by a slow or intermediate exchange interaction between non-aqueous protons and water. 2) At longer $T_{\text{Reff}}$, MWF does not change significantly with $T_{\text{Reff}}$-elongation and the MWF vs. $T_{\text{Reff}}$ plot simplifies to a straight line which is concordant with the fast exchange regime.
Although the myelin sheath structure and its water compartments in fixed dehydrated tissue has been revealed by electron microscopes, these features are not well known in living myelin. A study by Velumian et al. [37] suggested that living myelin sheath shows two different behaviors. Some parts of the myelin are made of small tunnels known as Schmidt Lanterman clefts SLC. SLCs accelerate the exchange between myelin water and intra/extra water by connecting the outer and inner cytoplasmic layers of the myelin. We call these regions fast exchanging myelin (FEM). Other parts of myelin, in which MW pool and IE water pool are more isolated, are known as slow exchanging myelin (SEM) or compact myelin. This hybrid behavior of myelin can support the results in this study.

2.6. Conclusion

This in vivo study demonstrates that myelin water fraction is a function of TR; MWF decreases as TR\textsubscript{eff} increases. This should be taken into account when devising MWF acquisition schemes which utilize short TR times.

It is not trivial to explain these results. Clearly a simple fast exchange model is not realistic. Furthermore, two T\textsubscript{1} component models using literature T\textsubscript{1} times do not seem appropriate. These results are not capable of unambiguously determining the mechanism for the increase of MWF with decreasing TR. However two plausible models are capable of fitting the data: 1) a hybrid model where myelin exists in two states; one were myelin water exchanges rapidly with intra and extracellular water and another where myelin water exchanges slowly with intra and extracellular water and 2) model where rapid cross-relaxation occurs at short times between non-aqueous protons and myelin water and at longer times myelin water and intracellular water relax with a single T\textsubscript{1}.
Chapter 3

Investigating the dependence of the intra/extra cellular water geometric mean $T_2$ time on the refocusing interval of the CPMG sequence

3.1. Introduction

3.1.1. Longitudinal and transverse relaxation times

In an MRI scanner the powerful magnetic field causes the net magnetization vector of protons in the body to align along the field direction. Radio frequency pulses at the proton resonance frequency can rotate the net magnetization vector away from the field direction. When the radio frequency pulses are turned off, the proton magnetization vector precesses about the field direction and realigns itself with the magnetic field direction. This process is called relaxation. There are two types of relaxation processes: $T_1$ or longitudinal relaxation (describes recovery of magnetization parallel to the magnetic field) and $T_2$ or transverse relaxation (de-phasing of the magnetization perpendicular to the magnetic field). Although relaxation times play a key role in MR image contrast the detailed nature of $T_1$ and $T_2$ relaxation in brain is still not well understood.

For a homogeneous system, the $T_1$ relaxation time is the time required for the longitudinal magnetization to grow and reach 63% of its equilibrium value (assuming that the initial $z$ magnetization was zero). The $T_2$ relaxation time is the time taken for the transverse magnetization to drop to 37% of its initial value.
\( T_1 \) is determined by thermal interactions between spins and the lattice, allowing the energy absorbed by resonating spins to be dispersed to other nuclei in the lattice. In the \( T_2 \) relaxation protons exchange energy with each other.

Unlike \( T_1 \) interactions, \( T_2 \) interactions do not lead to a net loss of spin system energy but only change the phase of individual spins, which leads to a loss of phase coherence. Spin-spin relaxation depends on many variables, including environment, the proximity of non-aqueous molecules and how far the water molecules diffuse during the \( T_2 \) time scale.

Any contribution to spin dephasing will cause the transverse magnetization to decay. Some of these processes are not directly related to the spin-spin interaction, but rather originate with the inhomogeneity of the field (e.g. the inhomogeneity due to imperfections of the magnet, or local inhomogeneities caused by magnetic susceptibility effects, etc.). The time constant of the spin dephasing process caused by field inhomogeneities is called \( T_2' \). To take in to account these inhomogeneities, \( T_2 \) should be replaced by \( T_2^* \) defined as

\[
\frac{1}{T_2^*} = \frac{1}{T_2'} + \frac{1}{T_2}
\]  

Unlike \( T_2 \) relaxation, the \( T_2' \) relaxation process can be reversed in a sequence called the spin echo. The spin echo involves applying a 180 degree pulse, called a refocusing pulse, to invert phase of the spins. As a result, with Larmor precession, the spins are refocused, i.e. come into phase with each other, and generate the so called spin echo signal with the amplitude dependent on the \( T_2 \) only.

\( T_2 \) studies of white matter enable separation of the MR signal into several components; from myelin water (MW, \( T_2 \sim 15\) ms), from the combined intra and extra cellular water signal (IEW, \( T_2 \sim 70\) ms) and from cerebrospinal fluid (\( T_2 \sim 3\) s) [15].
Both in vivo [15] and ex vivo [38] measurements have demonstrated that in brain about 15% of the total white matter tissue water is located in myelin. White matter contains myelinated axons. Myelin is made up of approximately 80% lipid and 20% protein [39, 40, 41]. The myelin sheath is an insulating structure covering and protecting the axons and accelerating action potential propagation (Saltatory conduction). Water trapped between the myelin bilayers is called myelin water.

Grey matter contains mainly unmyelinated axons and hence it has fewer lipids and a higher water content than white matter. Grey matter has about half the amount of white matter’s non-aqueous protons. The average water content in white matter is 10% less than that in grey matter.

In MRI, different contrast in various tissues originates from different transverse relaxation rates in each tissue. Hence, it is important to investigate the factors that influence T2 relaxation.

### 3.1.2. Magnetic susceptibility

Magnetic susceptibility is the capability of a material to be magnetized when placed in a magnetic field.

When a homogeneous magnetic field $B_0$ is applied to a substance the magnetic field inside the material is given by $B$.

$$B_0 = \mu_0 H$$  \hspace{1cm} (3.2)

where $H$ is magnetic field strength and $\mu_0$ is the absolute permeability of free space ($\mu_0 = 4\pi\times10^{-7} \ Tm/A$)

$$B = \mu_0 (H + M)$$  \hspace{1cm} (3.3)

where $M$ is the induced magnetization inside the material which is proportional to the $H$ field.
\[ M = \chi H \]  

(3.4)

\( \chi \) is the magnetic susceptibility constant. Equation (3.3) can be written as

\[ B = \mu_0 (1 + \chi) H \]  

(3.5)

\[ B = \frac{1+\chi}{\chi} \mu_0 M \]  

(3.6)

\[ B_0 = \frac{\mu_0}{\chi} M \]  

(3.7)

Thus, both magnetic fields, inside and outside, depend on induced magnetization. This is important in MRI since the spatial encoding is done by applying a gradient field. When the gradients are in the z magnetic field.

\[ G_x = \frac{dB_x}{dx} \]  

(3.8)

based on the Larmor equation \((\omega = -\gamma B)\)

\[ \omega(x) = -\gamma (B_0 + G_x \cdot x) \]  

(3.9)

In the presence of a magnetic field perturbation along \(\Delta B(x)\),

\[ B(x) = B_0 + \Delta B(x) + xG_x \]  

(3.10)

Using Larmor equation

\[ \omega(x) = -\gamma (B_0 + \Delta B(x) + xG_x) \]  

(3.11)

\[ \omega(x) = -\gamma (B_0 + G_x(x + \frac{\Delta B(x)}{G_x})) \]  

(3.12)
where \( x' = x + \frac{\Delta B(x)}{G_x} \)

Thus, those protons under the influence of the field perturbation \( \Delta B \) are mapped to an incorrect position \( x' \). This field perturbation leads to incorrect localization and causes spins to precess with different frequencies which makes spins become out of phase which effects \( T_2^* \) relaxation resulting in a signal loss.

There is a classification based on materials magnetic properties under the influence of an external magnetic field: Diamagnetism, paramagnetism, and ferromagnetism. The characteristic feature of ferromagnetic substances is that they become permanently magnetized when placed in a magnetic field, i.e. they will be magnetized even after they are removed from the magnetic field. Since ferromagnetic materials have a large positive susceptibility, the induced magnetic field inside the material can be stronger than the external one. Most organic substances are diamagnetic. Diamagnetic materials have a negative susceptibility constant (\( \chi < 0 \)). When a diamagnetic substance is placed in a magnetic field the induced magnetic moments oppose the external field and hence weakens the field. Paramagnetic substances have a small positive susceptibility constant (\( \chi > 0 \)). Applying a magnetic field to a paramagnetic material makes its induced magnetic moments to align with the external magnetic field and strengthen the applied field. The presence of an agent of high magnetic susceptibility shortens the \( T_2^* \) values.

In \( T_2 \)-weighted MRI, contrast between tissues originates from different transverse relaxation rates in each tissue. Hence, it is important to investigate the factors that influence \( T_2 \) relaxation. One of the most common techniques to measure \( T_2 \) times is the Carr-Purcell-Meiboon-Gill sequence. The CPMG sequence consists of a 90 degree excitation pulse followed by a series of 180 degree refocusing pulses. During the interval between 180 degree pulses, water can move between regions with different magnetic susceptibility leading to \( T_2 \) dependence on echo spacing intervals [42]. Many studies have shown that the presence of a high magnetic susceptibility agent may engender \( T_2 \) dependence on the refocusing intervals [43-48].
In the study by Stefanovic et al. [49] which reported on multi spin echo measurements with different inter pulse delays in occipital white and grey matter, $T_2$ was observed to depend on the refocusing interval. Upon increasing the refocusing interval from 8ms to 22ms, $T_2$ times decreased by $3.3 \pm 1.5$ msec in grey matter and by $3.0 \pm 1.5$ msec in white matter. This was accounted for by the presence of partly deoxygenated blood. The existence of paramagnetic deoxyhemoglobin is proposed to give rise to vascular components with different magnetic properties. Water exchange between compartments leads to irreversible Larmor frequency shifts which cause a $T_2$ decrease [49].

The goal of this study was to address two questions: 1) Does intra/extra cellular (IE) water geometric mean $T_2$ (gm$T_2$) of white and grey matter depend upon CPMG refocusing interval? 2) What mechanism is responsible for this dependence: water exchange, iron or the effect of blood oxygenation.

It is important to note that there are some differences between this study and the study by Stefanovic. 1) Stefanovic’s study was done at 1.5T while ours was done at 3T. This is why our $T_2$ times are shorter than Stefanovic’s. 2) Refocusing intervals were shorter in Stefanovic’s study than in ours. They used TE=8, 11 and 22 msec while we used TE=10, 20 and 40 msec 3) To fit the echo decay curves, Stefanovic used mono exponential NNLS while we used a multi exponential NNLS to fit our data. By separating the geometric mean IE signal from the myelin signal at short refocusing times, our analysis may have been better able to better fit the $T_2$ decay curves. 4) Stefanovic’s study focused on occipital grey matter and white matter regions but in this study 11 regions of interest, 5 in white matter and 6 in grey matter were studied.
3.2. Methods

In this study we used a gradient and spin echo GRASE sequence for measuring $T_2$. Five healthy volunteers (2 female, 3 male with mean age: 34 ± 17 years (mean ± SD)) underwent a MR scan on a 3.0T whole body MR scanner (Achieva 3.0T, Philips Medical Systems, Best, The Netherlands) using an eight-element phased-array head coil. All subjects provided signed, informed consent prior to participation and all examinations were performed with approval from the University of British Columbia Clinical Research Ethics Board.

The GRASE sequence was employed with 3 different echo spacings TE=10, 20 and 40 ms. Number of echoes for TE=10, 20 and 40ms was 32, 16 and 8 respectively in order that the all three echo trains ended at 320 ms. This is very important because it maintains the same effective TR. Otherwise, the signals would have different T1 weightings.

Imaging parameters were: repetition time TR=1000msec, acquired number of slices = 20, slice thickness = 5 mm, field of view (FOV) = 23 cm, matrix size = 232 x186. For each subject all acquisitions were obtained during a single 40 minute session in the same order.

For each subject, regions of interest (ROI) were drawn on a single slice for 5 white matter structures: splenium, genu, posterior internal capsules, major forceps, and minor forceps and 6 gray matter structures: caudate, putamen, globus pallidus, thalamus, cingulate gyrus, cortical gray matter.

At each ROI the $T_2$ distribution was extracted from the $T_2$ decay curve, using Non Negative least squares, NNLS. The geometric mean $T_2$ (gm$T_2$) of the intra/extra cellular water was calculated for the three refocusing intervals.
3.3. Results

The intra/extra cellular water gmT$_2$ averages of 5 subjects for different white and grey matter regions are listed in table 3.

<table>
<thead>
<tr>
<th>ROI</th>
<th>TE=10ms</th>
<th>TE=20ms</th>
<th>TE=40ms</th>
<th>gmT$_2$ shift from 10-40ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor forceps</td>
<td>65.48 (1.40)</td>
<td>63.64 (1.52)</td>
<td>62.62 (1.71)</td>
<td>2.86</td>
</tr>
<tr>
<td>Genu</td>
<td>63.46 (2.30)</td>
<td>62.47 (1.26)</td>
<td>60.51 (3.42)</td>
<td>2.95</td>
</tr>
<tr>
<td>Internal capsules</td>
<td>66.99 (3.16)</td>
<td>64.53 (2.78)</td>
<td>64.32 (2.78)</td>
<td>2.67</td>
</tr>
<tr>
<td>Splenium</td>
<td>71.87 (2.14)</td>
<td>70.11 (2.94)</td>
<td>69.20 (3.35)</td>
<td>2.67</td>
</tr>
<tr>
<td>Major forceps</td>
<td>75.33 (2.58)</td>
<td>73.73 (2.63)</td>
<td>71.48 (3.73)</td>
<td>3.85</td>
</tr>
<tr>
<td>Caudate</td>
<td>65.74 (2.73)</td>
<td>63.72 (2.46)</td>
<td>61.83 (2.54)</td>
<td>3.91</td>
</tr>
<tr>
<td>Putamen</td>
<td>65.39 (3.77)</td>
<td>63.75 (3.30)</td>
<td>62.04 (4.13)</td>
<td>3.34</td>
</tr>
<tr>
<td>Globus Pallidus</td>
<td>63.20 (4.80)</td>
<td>61.11 (5.21)</td>
<td>59.70 (5.90)</td>
<td>3.50</td>
</tr>
<tr>
<td>Thalamus</td>
<td>65.90 (3.07)</td>
<td>64.70 (1.31)</td>
<td>63.95 (3.31)</td>
<td>1.95</td>
</tr>
<tr>
<td>Cingulate gyrus</td>
<td>77.00 (1.57)</td>
<td>75.20 (1.31)</td>
<td>72.55 (1.85)</td>
<td>4.45</td>
</tr>
<tr>
<td>Cortical GM</td>
<td>72.30 (2.77)</td>
<td>67.75 (2.85)</td>
<td>69.25 (2.48)</td>
<td>3.05</td>
</tr>
</tbody>
</table>

Table 3. Intra/extra cellular water gmT$_2$ averages of 5 subjects for different ROI. The standard error associated with each measurement is shown inside parenthesis.

Averaged gmT$_2$ data for TE=10, 20, 40 msec are shown in Figure 4 and 5 for white matter and grey matter, respectively.
For the 10 to 40 ms refocusing interval, the average Geometric mean $T_2$ drop for white matter and grey matter was $3.0 \pm 0.5$ ms and $3.3 \pm 0.5$ ms respectively. The T-value for
shift in white matter and grey matter from 10-40ms was 0.36 showing that the effect is not different for both tissues.

3.4. Discussion

The major finding of this study is IE water gmT$_2$ times of both white matter and grey matter tissue decreased with refocusing interval prolongation. In order to find the reason for this change we discuss four mechanisms:

1. Water exchange between myelin water and intra/extra cellular water
2. Presence of Non-heme iron
3. Deoxyhemoglobin(dHb) in blood
4. Diffusion in local field gradients

3.4.1. Water exchange simulation

To investigate the exchange effect, the GRASE sequence was simulated for three echo times, TE=10, 20 and 40ms.

The GRASE sequence starts with a 90 degree pulse about the x axis. After half of echo time (TE/2) the repetition cycle starts with a 180 degree pulse about y axis, followed by a TE/2 period, signal acquisition, a TE/2 period, -180 degree pulse around y axis, a TE/2 period, signal acquisition and a TE/2 period. This cycle is repeated n times.

Considering the exchange effect on the T$_2$ decay curve, we assumed a two-component exchange model consisting of the two predefined components of myelin water and intra/extracellular water pools. For myelin water pool T$_{2m}$=15ms, MWF=0.15 and for intra/extracellular water pool, T$_{2IE}$=70ms, intra/extracellular water fraction IEWF=0.85.
Both water pools had same and constant T₁ of 1s. Each pool was assumed to be a distinct compartment with uniform magnetization at any time. Whenever spins from different water pools are in close vicinity, cross relaxation can occur between the two spin systems [50].

In the two-component exchange model $\text{IEWF} = (1-\text{MWF})$. Hence, if MWF increases IEWF will decrease. The exchange equilibrium condition is:

$$\text{MWF} \times \frac{1}{t_m} = \text{IEWF} \times \frac{1}{t_{IE}}$$

(3.13)

where $t_m$ is the residence time for myelin water and $t_{IE}$ is the residence time for intra/extracellular water. The residence time is the time that water molecule resides in a water pool before exchange. Simulations were done for nine residence times: infinity (no exchange), 1000ms, 500ms, 300ms, 200ms, 100ms, 50ms, 20ms, 1ms.

The inverse of residence time is called directional exchange rate constant

$$k = \frac{1}{t_m}$$

(3.14)

$$k_{IE \text{ m}} = k_{m \text{ IE}} \times (\frac{\text{MWF}}{\text{IEWF}})$$

(3.15)

The Bloch–McConnell [51] equation describing this two-site exchange system is

$$\frac{dM}{dt} = A \cdot M + C$$

(3.16)

Which can be written in matrix form as [52]
\[
\frac{d}{dt} \begin{bmatrix} M_{xm} \\ M_{xIE} \\ M_{ym} \\ M_{yIE} \\ M_{zm} \\ M_{zIE} \end{bmatrix} = \begin{bmatrix} -\frac{1}{\tau_{2m}} - k_{mIE} & k_{IE} m & \Delta \omega & 0 & 0 & 0 \\ k_{mIE} & -\frac{1}{\tau_{2m}} - k_{IE} m & 0 & 0 & 0 & \Delta \omega \\ -\Delta \omega & 0 & -\frac{1}{\tau_{2m}} - k_{mIE} & k_{IE} m & 0 & 0 \\ 0 & -\Delta \omega & k_{mIE} & -\frac{1}{\tau_{2m}} - k_{IE} m & 0 & 0 \\ 0 & 0 & 0 & -\frac{1}{\tau_{2m}} - k_{mIE} & k_{IE} m & 0 \\ 0 & 0 & 0 & 0 & -\frac{1}{\tau_{2m}} - k_{IE} m & k_{IE} m \end{bmatrix} \begin{bmatrix} M_{xm} \\ M_{xIE} \\ M_{ym} \\ M_{yIE} \\ M_{zm} \\ M_{zIE} \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ M_{WFW} \\ \frac{\tau_{1m}}{\tau_{1E}} \end{bmatrix} \]

(3.17)

where \(\Delta \omega\) is the shift in Larmor frequency. The solution of the Bloch–McConnell equation for the two-component magnetization is

\[
M(t) = e^{At} R M(0) + (e^{At} - I)A^{-1}C
\]

(3.18)

where \(M(0)\) is the equilibrium magnetization, \(I\) is the identity matrix and \(R\) is the rotation matrix.

The detected signal will be

\[
S = \sqrt{[(M_x)_m + (M_x)_{IE}]^2 + [(M_y)_m + (M_y)_{IE}]^2}
\]

(3.19)

The detected signal is the amplitude of the signal at the readout point. In this case we have 32 echoes for TE 10, 16 echoes for TE 20 and 8 echoes for TE 40. Using NNLS, \(T_2\) distribution can be extracted from \(T_2\) decay curve. Using the gmT2 equation (1.25) the geometric mean T2 for intra/extra-cellular water was calculated for three echo intervals and nine \(\tau_m\) s (Table 5).
Table 4. Calculated IE water $g\text{mT}_2$ (all in msec) from the simulation of different TE with different exchange times

<table>
<thead>
<tr>
<th></th>
<th>TE=10ms</th>
<th>TE=20ms</th>
<th>TE=40ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exchange</td>
<td>0.0698</td>
<td>0.0698</td>
<td>0.0697</td>
</tr>
<tr>
<td>$\text{tauM}=1000\text{ms}$</td>
<td>0.0690</td>
<td>0.0690</td>
<td>0.0689</td>
</tr>
<tr>
<td>$\text{tauM}=500\text{ms}$</td>
<td>0.0684</td>
<td>0.0683</td>
<td>0.0682</td>
</tr>
<tr>
<td>$\text{tauM}=300\text{ms}$</td>
<td>0.0675</td>
<td>0.0674</td>
<td>0.0674</td>
</tr>
<tr>
<td>$\text{tauM}=200\text{ms}$</td>
<td>0.0663</td>
<td>0.0662</td>
<td>0.0660</td>
</tr>
<tr>
<td>$\text{tauM}=100\text{ms}$</td>
<td>0.0634</td>
<td>0.0633</td>
<td>0.0631</td>
</tr>
<tr>
<td>$\text{tauM}=50\text{ms}$</td>
<td>0.0598</td>
<td>0.0596</td>
<td>0.0595</td>
</tr>
<tr>
<td>$\text{tauM}=20\text{ms}$</td>
<td>0.0538</td>
<td>0.0536</td>
<td>0.0536</td>
</tr>
<tr>
<td>$\text{tauM}=1\text{ms}$</td>
<td>0.0457</td>
<td>0.0457</td>
<td>0.0457</td>
</tr>
</tbody>
</table>

As expected, the calculated IE water $g\text{mT}_2$ from the simulation of different refocusing intervals TE with exchange effects shortened as exchange became more rapid; however the extent of change was less than 1ms and negligible compared to the experimentally measured changes.

3.4.2. Non-heme Iron

There are two forms of iron in the brain: heme iron (hemoglobin) and non-heme iron (ferritin, ionic iron) [53]. Ferritin is a spherical shell protein that stores excess iron in body and releases it as needed. Since ferritin has paramagnetic characteristics, it creates signal changes which are related to the amount of stored iron inside ferritin. Iron is essential for oxidative metabolism, synthesis of myelin and neurotransmitters; yet accumulation of iron in the human brain can cause oxidative damage and neurodegenerative diseases such as Hallervorden-Spatz Syndrome (HSS), [54,55] Alzheimer’s disease (AD) [56-57], Parkinson’s disease(PD) [58-62] and other demyelinating disorders. Previous studies [53-65] reported that there is a correlation...
between age and iron accumulation in human brain. In the first two decades of life there is a rapid increase in the iron level following a slower increase rate for the rest of the life. Iron concentration in brain is different from region to region. Iron concentration in brain is highest in the globus pallidus 21mg/100 g fresh weight and lowest in meninges 1mg/100 g fresh weight [66].

The table below shows the iron concentration from Hallgren and Sourander [66] and the measured gmT₂ of thalamus, caudate, putamen and globus pallidus for different echo times.

<table>
<thead>
<tr>
<th>Iron concentration (mg iron/100g fresh weight)</th>
<th>Structure</th>
<th>TE=10ms</th>
<th>TE=20ms</th>
<th>TE=40ms</th>
<th>TE= 10 - 40ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.76</td>
<td>Thalamus</td>
<td>66.32</td>
<td>64.59</td>
<td>63.91</td>
<td>2.41</td>
</tr>
<tr>
<td>9.28</td>
<td>Caudate</td>
<td>65.74</td>
<td>63.72</td>
<td>61.83</td>
<td>3.91</td>
</tr>
<tr>
<td>13.32</td>
<td>Putamen</td>
<td>65.39</td>
<td>63.75</td>
<td>62.046</td>
<td>3.344</td>
</tr>
<tr>
<td>21.3</td>
<td>Globus pallidus</td>
<td>63.2</td>
<td>61.11</td>
<td>59.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 5. Iron concentration of four grey matter regions from ref [65] and our measured gmT₂ times

The Thalamus has less iron and also had longer gmT₂ values whereas the globus pallidus, which has the highest iron concentration, had the shortest gmT₂ values. This T₂ shortening is due to the antiferromagnetic character of iron which induces local magnetic field perturbations [67].

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Figure 8. gmT$_2$ values vs. iron concentration [66] for 4 grey matter tissues for 3 different echo times

As expected figure 8 shows that at all TE times T$_2$ values decreased with increasing iron concentration in the grey matter regions (p>0.01)

Figure 9. $\Delta T_2$ for TE=10ms to TE=40ms vs. iron concentration of four grey matter tissues
On the other hand, Figure 9 shows that from TE=10ms to TE=40ms the shift in gmT₂ values were largely independent on tissue iron content ($R^2 = 0.25228$, $P=0.08504$).

Despite large differences in non-heme iron content between white matter and grey matter regions [66] the gmT₂ drop with TE increase was almost equal for both tissues, which indicates that non-heme iron is not the source for this effect.

### 3.4.3. Deoxy-hemoglobin

Red blood cells contain a protein known as hemoglobin which carries oxygen to tissues. Hemoglobin gives red blood cells their characteristic red color. The chemical combination of hemoglobin and oxygen is called oxy-hemoglobin. When oxyhemoglobin releases its oxygen to the tissues it changes to deoxy-hemoglobin. Oxy-hemoglobin and deoxy-hemoglobin are diamagnetic and paramagnetic respectively. Variable amounts of paramagnetic deoxy-hemoglobin in blood vessels create field gradients which de-phase water protons and change the signal [48,68]. This is the basis of contrast in BOLD fMRI measurements [69].

Deoxy-hemoglobin in blood engenders two vascular components: the intra-erythrocytic and the plasmatic. There is a water exchange between these two magnetically different environments which causes a Larmor frequency shift.

$$\Delta \omega = \eta \left(1 - \frac{Y}{100}\right) \omega_0 \quad (3.20)$$

where $\omega_0=128$ MHz is the Larmor frequency at 3 T, $\eta=0.7$ ppm is a constant which depends on the shape, spacing and the susceptibility of dHb, and $Y$ is the blood percent oxygenation and it is about 60% for venous blood. [70]
According to Luz and Meiboom’s model of chemical exchange between multiple sites at different frequencies [71], a Larmor frequency shift results in enhancement of the blood $R_{2b}$ ($R_2 = \frac{1}{T_2}$)

$$R_{2b} = R_{2o} + Hct (1 - Hct) (\Delta \omega)^2 \tau_{ex} (1 - \frac{2 \tau_{ex}}{\tau_{180}} \tanh \frac{\tau_{ex}}{2 \tau_{180}})$$  \hspace{1cm} (3.21)

where $R_{2o}$ is the intrinsic $R_2$ of blood, Hct= 40% is the volume percentage of red blood cells in blood, $\tau_{ex}$ is the average exchange time which is about 6 to 8 ms [70], and $\tau_{180}$ is echo spacing times. The $R_2$ of venous blood (having oxygen saturation of ~60% [70]) decreases by 36% between the 10 and 40 ms refocusing interval acquisitions. $R_2$ is directly related to deoxy-hemoglobin [72].

The normalized signal from a voxel with a slow exchange across the capillary wall, is:

$$\frac{S}{S_0} = x_t \exp\left(-\frac{R_{2t}}{T_E}\right) + x_b \exp\left(-\frac{R_{2b}}{T_E}\right)$$  \hspace{1cm} (3.22)

where $x_t$ and $x_b$ are tissue and blood water volume fractions, respectively.

The volume of blood vessels is larger in grey matter than in white matter [73]. From our calculations the $T_2$ estimate decreases by about 2ms in both GM and WM between the 10 and 40 ms refocusing interval acquisitions.

A greater decrease of $T_2$ with refocusing interval increase is expected in grey matter than white matter. Our results showed similar changes in gm$T_2$ for white matter and grey matter between 10 to 40 ms refocusing intervals. Hence, $T_2$ dependence on the refocusing interval cannot be fully explained by the effect of deoxy-hemoglobin in blood.

### 3.4.4. Diffusion in local field gradients

Diffusion in inherent magnetic field gradients is a potential explanation for the change in $T_2$ with refocusing interval which would be independent of field strength.
\[ S = S_0 \exp(-\gamma^2 G^2 \tau^3 D) \] (3.23)

where \( \gamma = 42.57 \text{ kHz/Tesla} \), \( \tau = 30 \text{ ms} \) and \( D = 3.0 \exp(-9) \text{ m}^2/\text{s} \) for free water or 1/10 of that for tissue water [74].

This simple calculation indicates that a gradient of about 30mT/m is required to change the measured \( T_2 \) time by about 3ms over a 30 ms refocusing interval; i.e. the difference between a 10 and 40 ms refocusing interval. Unfortunately, we expect internal field gradients of about 0.1 ppm which about 100 times too small to produce the observed effect. Therefore, local field gradients can be eliminated as a mechanism for the observed loss of signal with increased refocusing interval.

### 3.5. Conclusion

The major finding of this chapter is that IE water gm\( T_2 \) values of both white matter and grey matter tissue decrease with refocusing interval prolongation. Four probable mechanisms were studied: water exchange between myelin water and intra/extra cellular water, presence of non-heme iron, deoxy-hemoglobin (dHb) in blood and diffusion in local field gradients. None of these could fully explain the dependence of IE water gm\( T_2 \) on echo spacing.

Although we used different number of echoes for NNLS analysis for different echo times having 8 versus 32 echoes should not make much difference to the extracted \( T_2 \) but it might have introduced some errors.
Chapter 4

Conclusion

The work presented in chapter two promised to distinguish the dependence of MWF on repetition time of scanning since this has significant consequences for the design of pulse sequences for measuring MWF. In order to achieve this goal, MWF was measured from five white matter and three gray matter of 4 healthy subjects at various effective TR times. Measured myelin water fraction increased substantially as TR\textsubscript{eff} was shortened and the rate of this increase was larger at shorter TR\textsubscript{eff}. We tried to fit our data to both fast and slow exchange regime but surprisingly none of them could fully explain the relaxation in white matter. We left with a potential model for myelin in white matter in which myelin has a hybrid character whereby part of the myelin is in a fast exchange regime and the rest of the myelin is in a slow exchange regime. Since we had a limited range of TR times and a small group of subjects more work will be required and more sophisticated models (e.g. four pool model [75]) may be needed to justify myelin behavior.

In chapter 3 our results were almost identical to those found by Stefanovic et. al [49] but there are some differences between these two studies; in particular the Stefanovic data was collected at 1.5T. The values obtained by the Stevanovic and Pike study for T2 changes with echo spacing changes were similar to the values we obtained, suggesting that there was little field dependence in this tissue temperature range.

The proposed magnetization exchange between MW and IEW is unlikely because if MW/IEW exchange was the primary mechanism for changes in IEW gmT\textsubscript{2} with refocusing interval, then the T\textsubscript{2} changes would be larger in white matter than grey matter.
since there is more myelin water in white matter. Furthermore, theoretical predictions of
the impact of exchange suggest that this may be a minor effect.
The observed gmT\textsubscript{2} drop with TE increase was present for both white matter and grey
matter, despite large differences in their iron content, which indicates that iron can not be
the only source of this drop.
Alternatively, if the changes are primary due to dephasing by deoxyhemoglobin, the T\textsubscript{2}
changes should be larger in grey matter.
This study demonstrated that the effect was largely field independent and also hence not
likely to be caused by susceptibility changes.
Finally diffusion in local field gradients also cannot account for the results.

In summary, T\textsubscript{2} values are dependent on the echo time intervals but the origin of the
change in measured T\textsubscript{2} with echo spacing is not clear and further studies are needed.


References


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