MYELIN WATER MEASUREMENT BY MAGNETIC RESONANCE IMAGING IN THE HEALTHY HUMAN SPINAL CORD: REPRODUCIBILITY AND CHANGES WITH AGE

by

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Abstract

Multi-echo T$_2$ relaxation measurements of the human spinal cord (SC) reveal a short T$_2$ pool of water believed to arise from water trapped between myelin bilayers, where the proportion of this water to the total water signal is called the myelin water fraction (MWF). In the present study, MWF were measured in the healthy human cervical spine at the C4-C6 vertebral levels *in vivo* using a 3D modified 32 echo CPMG sequence to acquire axial slices perpendicular to the cord. Volunteers were recruited in two age ranges, under 30 years old and over 50 years old, and a subset of both groups were scanned twice to test reproducibility. Mean MWF in the dorsal and lateral column WM of the group under 30 years of age was 0.29 (0.01) (mean(SE)), which agrees with previously reported MWF values in the cervical spine. The mean absolute difference between two scans was 0.06 or 26%. A negative correlation between WM MWF and age was hinted at in these findings, however more subjects are required to improve statistical power. This study paves the way for the use of 3D myelin water imaging in the cervical spine at 3.0T for the assessment of SC WM pathology.
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<tbody>
<tr>
<td>3D</td>
<td>Three Dimensional</td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
<td></td>
</tr>
<tr>
<td>AMN</td>
<td>Adrenomyeloneuropathy</td>
<td></td>
</tr>
<tr>
<td>B₀</td>
<td>Static Magnetic Field (along z direction)</td>
<td></td>
</tr>
<tr>
<td>BPP</td>
<td>Bloembergen-Purcell-Pound theory</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
<td></td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
<td></td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
<td></td>
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<tr>
<td>CSM</td>
<td>Cervical Spondylotic Myelopathy</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>Dorsal Column</td>
<td></td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Energy</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
<td></td>
</tr>
<tr>
<td>FID</td>
<td>Free Induction Decay</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Magnetic Field Gradient</td>
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</tr>
<tr>
<td>GM</td>
<td>Grey Matter</td>
<td></td>
</tr>
<tr>
<td>ℎ</td>
<td>Plank's Constant (divided by 2π)</td>
<td></td>
</tr>
<tr>
<td>I/E</td>
<td>Intra- and extra-cellular</td>
<td></td>
</tr>
<tr>
<td>k₈</td>
<td>Boltzmann's Constant</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>Lateral Column</td>
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</tr>
<tr>
<td>LLC</td>
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</tr>
<tr>
<td>M₀</td>
<td>Equilibrium Magnetization</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
<td></td>
</tr>
<tr>
<td>MT</td>
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</tr>
<tr>
<td>MTR</td>
<td>Magnetization Transfer Ratio</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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<td></td>
</tr>
<tr>
<td>MWF</td>
<td>Myelin Water Fraction</td>
<td></td>
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<tr>
<td>$M_{xy}$</td>
<td>Transverse Magnetization</td>
<td></td>
</tr>
<tr>
<td>NAWM</td>
<td>Normal Appearing White Matter</td>
<td></td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
<td></td>
</tr>
<tr>
<td>NNLS</td>
<td>Non-Negative Least Squares</td>
<td></td>
</tr>
<tr>
<td>PNS</td>
<td>Peripheral Nervous System</td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
<td></td>
</tr>
<tr>
<td>RLC</td>
<td>Right Lateral Column</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>Region Of Interest</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>Spinal Cord</td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>Spinal cord injuries</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Tesla, unit of magnetic field strength</td>
<td></td>
</tr>
<tr>
<td>$T_1$</td>
<td>Spin-Lattice Relaxation Time</td>
<td></td>
</tr>
<tr>
<td>$T_{1WI}$</td>
<td>T1 Weighted Imaging</td>
<td></td>
</tr>
<tr>
<td>$T_2$</td>
<td>Spin-Spin Relaxation Time</td>
<td></td>
</tr>
<tr>
<td>$T_{2*}$</td>
<td>Gradient Echo Transverse Magnetization Time</td>
<td></td>
</tr>
<tr>
<td>$T_{2'}$</td>
<td>Transverse Magnetization Relaxation Time Due To Magnetic Susceptibility Differences</td>
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<tr>
<td>$T_{2WI}$</td>
<td>T2 Weighted Imaging</td>
<td></td>
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<tr>
<td>TE</td>
<td>Echo Time</td>
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</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
<td></td>
</tr>
<tr>
<td>VOI</td>
<td>Volume Of Interest</td>
<td></td>
</tr>
<tr>
<td>WM</td>
<td>White Matter</td>
<td></td>
</tr>
<tr>
<td>$x,y,z$</td>
<td>Coordinates in the laboratory frame</td>
<td></td>
</tr>
<tr>
<td>$x',y'$</td>
<td>Coordinates in the frame rotating about the z axis at the Larmor frequency</td>
<td></td>
</tr>
<tr>
<td>$\phi$</td>
<td>Phase</td>
<td></td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Gyromagnetic Ratio</td>
<td></td>
</tr>
<tr>
<td>$\tau_c$</td>
<td>Correlation Time</td>
<td></td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Larmor Frequency</td>
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After the time I have spent working with the UBC MRI Research Centre and the MacKay group, I truly believe I know how it feels to stand on the shoulders of giants.
Dedication

To my family for their love and support, and to Andrew for making my dreams come true.
Co-Authorship Statement


Identification of design and research program: I participated in discussions where the research goals and protocol were decided upon. As well, I wrote the majority of the successful Cervical Spine Research Society 21st Century Development Grant.

Performing the research: I conducted pilot scans to test the implementation of the sequence in the cervical spinal cord, scanned the group of young healthy adults, and was present while older volunteers were scanned by the MRI technologists.

Data analysis: I performed all image registration, placement of regions of interest, data extraction and statistical analyses.

Manuscript preparation: I prepared the manuscript text and figures with constructive comments from all co-authors.
1 Introduction

Magnetic resonance imaging (MRI) is a non-invasive tool with unparalleled capabilities for investigation of the human species’ least understood and most mysterious organ system, the central nervous system. MR images arise from the interaction of water protons with an external magnetic field, and radio-frequency electromagnetic waves, both of which are non-ionizing forms of energy enabling MRI to be used for both diagnostic and research purposes with very little risk to subjects. For investigation of the central nervous system, MRI is unequaled in its ability to image grey and white matter with excellent contrast and good spatial resolution, and to provide quantitative information about tissue structure and chemistry.

1.1 Central Nervous System

The human brain is characterized by some $10^{23}$ synapses. Thus the number of different states of a human brain is 2 raised to this power – i.e., multiplied by itself ten trillion times. This is an unimaginable large number, far greater, for example, than the total number of elementary particles (electrons and protons) in the entire universe, which is much less than 2 raised to the power of $10^3$. In the face of these numbers, the wonder is that there are any regularities at all in human behavior.

~Carl Sagan in The Dragons of Eden (1)

The human nervous system is a “wired” system that relies on the structural arrangement of neurons to receive, process, and transmit information (2). This wired system is conceptually divided into the central nervous system (CNS), comprised of the brain and spinal cord (SC), and the peripheral nervous system (PNS), which are the network of wires that connect the CNS with other parts of the body. Sensory information from the PNS is interpreted and integrated in the CNS, which then sends a response through the PNS to the glands or muscles that carry out the appropriate reaction.

The functional unit of the nervous system, the neuron, is a highly specialized cell that has dendrites for reception of signals from other neurons, a cell body which integrates the incoming signals, and an axon for propagation of the resulting nerve
impulses to other neurons via axon terminals (see Figure 1-1). Impulse transmission is accelerated up to 50 times by the myelin sheath, a lipid bilayer that is wound around segments of the axon by the processes of oligodendrocytes (3). Within the CNS, tissue is visually divided into the aptly named grey and white matters: grey matter (GM) are neuronal cell bodies and white matter (WM) are bundles of axons called “tracts” that connect various grey matter regions. The fatty myelin coating on axons is the source white matter’s milky colour.

**Figure 1-1. The functional unit of the nervous system, the neuron.** The neuron receives input signals through the dendrites, integrates signals in the cell body and propagates signals along the axon to the next neurons through the axon terminals. Oligodendrocytes extend their arms to wrap layers of myelin (inset) around segments of each axon to form the myelin sheath to accelerate nerve impulse conduction.

The SC is the information highway of the nervous system; this 45 cm long cylindrical tissue contains GM centrally located in a butterfly-shaped pattern (Figure 1-2) surrounded by the WM tracts that connect the brain and body. The SC is labelled in the cranial-caudal direction in relation to the vertebral body that it is enclosed within. Most of the information that enters the SC is transmitted to or from the higher processing centres of the brain, however, the GM of the SC is
responsible for the fast-acting reflexes that potentially save us from danger. The WM is responsible for transmitting information as quickly as possible, and so contains tracts of highly myelinated axons. If myelin in the SC is disrupted, transmission can be slowed or lost completely, leading to symptoms of tingling sensations or loss of motor control in the limbs.

Figure 1-2. Motor and sensory WM tracts in the spinal cord. GM is surrounded by WM tracts shown in red and blue. Descending motor pathways are shown in red, including the lateral column (LC), and ascending sensory pathways are shown in blue, including the dorsal column (DC).

1.2 Cervical Spondylotic Myelopathy

As the leading cause of SC dysfunction in North Americans over the age of 55, cervical spondylotic myelopathy (CSM) has an enormous impact on our economy and health care systems. Management of CSM is difficult due to its varied and diverse clinical manifestations and causes. CSM is caused by degenerative changes in the cervical spine that constrict the spinal canal leading to both direct compression damage and indirect compression-induced ischemia of the SC (4).

Clinical and post-mortem studies of CSM suggest that the central GM and medial tracts are the first areas to be affected by gliosis, cystic cavitation, and demyelination (5). Wallerian degeneration occurs above the level of stenosis in the lateral corticospinal tracts, as well as in the dorsal columns of severe cases, while
demyelination is detected below the level of compression. Finally, in cases of anteroposterior compression ratios of less than 20%, local infarction of the GM can occur. The loss of myelin in WM tracts and death of cell bodies in the GM is manifested in symptoms such as altered gait, coordination, and strength, and the feeling of numbness or tingling in the extremities (4).

1.2.1 Conventional Imaging of CSM
The current state of clinical, and the majority of research, imaging of CSM rely on T1 and T2 weighted imaging (T1WI and T2WI, respectively) to identify intramedullary (within the SC tissue) lesions for assessment of damage. However, the prognostic value of intramedullary signal intensity patterns on T1 and T2 WI has been suggested to indicate a “broad spectrum of spinal cord recuperative potentials” (6, 7). A recent study of CSM concluded that T2WI could not predict outcome following surgery, despite the improvements from higher magnetic field, and high resolution T2WI (8). A comparison of intramedullary T2 signal intensity and histopathological findings in autopsy cases reported that the signal intensity changes were based on a variety of pathological processes including edema, inflammation, and demyelination, confounding the use of T2WI as a prognostic tool (6). It is not surprising that in a review article of imaging of CSM the authors concluded without further explanation that the use of “MRI or CT myelography remains the choice of the investigator. We favour MRI” (4).

1.3 Measuring Myelin with MRI
Myelin’s bilayer structure offers a variety of structural and chemical features that distinguish it from the cytoplasm and extracellular spaces of both GM and WM. The high concentration of lipids yields a large pool of mobile lipid protons, and the tight wrapping of the bilayers creates a close association between water molecules and the bilayers. MRI can probe these properties through quantitative techniques including spin-spin (T2) relaxation measures, magnetization transfer (MT) imaging, and diffusion tensor imaging (DTI).

1.3.1 Myelin Water Imaging
An innovative MRI approach, developed at the University of British Columbia, to acquire quantitative information about myelin content in CNS tissues is to perform
a spin-spin ($T_2$) relaxation experiment. Spin-spin relaxation is the loss of magnetization through the process of spin energy dispersion in a pool of protons by energy conserving interactions. The rate at which spin energy is dispersed through spin-spin interactions depends on a variety of factors such as concentration of non-aqueous protons and water diffusion, which are significantly different between cerebrospinal fluid (CSF), intra- and extra-cellular water (I/E water), and water trapped between myelin bilayers (9). Thus, $T_2$ relaxation measurements are able to distinguish myelin-associated water from other water environments. The ratio of myelin water to total water (called the myelin water fraction, MWF) has been shown to correlate strongly with Luxol Fast Blue staining for myelin, suggesting that the MWF is surrogate marker for myelin content (10).

To demonstrate the clinical applications of myelin water imaging (MWI), $T_2$ relaxation measurements have been used to investigate healthy human brain (9), multiple sclerosis (10, 11), schizophrenia (12), attention-deficit hyperactivity disorder (13), and phenylketonuria (14). In healthy human brain, $T_2$ relaxation measurements revealed that: 1) different normal WM structures exhibit different myelin water contents; 2) total water contents for WM and GM structures are in agreement with published values using biochemical techniques; and 3) WM structures demonstrate broader main water peaks than GM structures (9). In healthy adults, MWF in the frontal WM correlated positively with age, suggesting that myelination continues through adulthood (12). A cross-sectional study of the brain from 33 MS patients and 18 controls found that MS lesions exhibited approximately a 50% decrease in MWF and 6% increase in total water content when compared to contralateral normal appearing WM (NAWM) (11). NAWM itself had 16% less myelin water and 2% higher total water content when compared to similar brain regions in controls. When Laule and colleagues interpreted these results using a simple model for water distribution in WM, they concluded that NAWM was globally demyelinated relative to normal WM, highlighting a pattern of increased demyelination from NAWM to MS lesions relative to normal WM.

In the SC, MWF were measured in vitro from bovine excised tissue and in vivo in humans (15). In the bovine samples, Minty et al. showed that MWF exhibited the characteristic butterfly pattern of low myelin in the GM relative to the surrounding WM. The human in vivo myelin water images were acquired sagittally to
demonstrate the cranial-caudal pattern of myelin content. The first study of SC pathology with MWI reported a significant difference between the MWF in the SC of MS patients relative to healthy age-matched controls between the second and third cervical (C2/C3) vertebral level: (mean(standard error)) 0.257(0.014), as compared 0.198(0.017), respectively (16). However, this study was performed at 1.5T and did not segment the SC into GM and WM regions, raising the question that GM and WM may be affected differently in MS.

1.3.2 Magnetization Transfer Imaging
Magnetization transfer imaging is a technique that probes the ratio of non-aqueous protons (those bonded to macromolecules or membranes) to free water protons. A radiofrequency pulse is applied off resonance from the free water resonance to suppress the magnetization of the non-aqueous protons, and if there is chemical exchange between the non-aqueous and water protons, the water protons will also lose their magnetization. The effect of this saturation pulse is measured by the magnetization transfer ratio (MTR), which compares an image acquired with the saturation pulse to a reference image, and because many of these non-aqueous protons in CNS tissues are bound to myelin phospholipids, the MTR has been suggested to be related to myelin concentration (17).

Until very recently MT imaging was not employed in the investigation of SC disorders due to the size of the cord and motion artifacts. Smith et al. overcame these challenges in 2005 by using the CSF in the MT image as a reference (termed MTCSF), omitting the need for a reference scan (18). This technique successfully identified a difference in the MTCSF pattern along the cord between patients suffering from adrenomyeloneuropathy (AMN, a genetic myelin disorder) and healthy controls, and speculated that this difference was due to non-inflammatory axonopathy followed by demyelination (19). Although MTCSF can identify changes in the SC caused by AMN, the authors admit that MTCSF is not expected to be able to discriminate between demyelination and inflammation effects. To further support this view, an animal study of experimental allergic encephalomyelitis, in which CNS tissues experience inflammation and demyelination, concluded that the MTR was affected by both inflammation and demyelination, while inflammatory activity did not affect the MWF (20).
1.3.3 Diffusion Tensor Imaging

White matter is comprised of the densely packed tubes of myelinated axons, causing water to be much more likely to diffuse along the length of the axon rather than to diffuse through the cell wall and layers of myelin. Diffusion tensor imaging (DTI) is an MRI technique that can measure the fractional anisotropy (FA), the degree to which water is more likely to diffuse in one direction over all others, and the apparent diffusion coefficient (ADC), also called the mean diffusivity which is a measure of the magnitude of water diffusion, in CNS tissues (17). Thus, WM tracts are expected to have a higher FA than GM tissue, which can be influenced by the breakdown of myelin.

In the human SC, Demir et al. compared ADC and T₂WI in relation to clinical symptoms of CSM (21). They found that the ADC maps were more sensitive, but less specific, to myelopathy than T₂WI. Similarly, Mamata et al. noticed that 15 out of 39 cases of cervical spondylosis that exhibited increased ADC combined with decreased FA also presented clinical symptoms of myelopathy (22). However, this study only identified these diffusion abnormalities in 54% of the 72 patients with normal T₂WI. In addition, Mamata et al. investigated the effects of age on DTI measures at the C2/C3 level and found a positive correlation (Spearman r=0.242) between ADC and age, as well as a negative correlation (Spearman r=-0.244) of FA with age. Although DTI does detect changes in the SC due to myelopathy, changes in the diffusion components perpendicular to WM tracts can also be caused by a number of other processes including edema, inflammation, and axonopathy (23).

1.4 Motivation

Conventional T₁WI and T₂WI of CSM fails to predict severity of symptoms, as well as outcome following decompressive surgery. The role demyelination plays in CSM suggests that a measure of myelin could be more specific or sensitive to WM damage caused by stenosis, and perhaps hold a higher diagnostic and prognostic potential. Thus, MWI offers the potential to be a non-invasive technique to measure myelin content for the assessment of cervical spondylotic myelopathy.

To better understand changes in myelination caused by a stenosis, changes in myelination in the normal aging process must first be understood. As mentioned above, previous studies have found a relationship between DTI measures and age.
in the SC, though the correlation was weak, and the subject pool included patients experiencing neurological symptoms. A post-mortem study of age-related changes of the corticospinal tract concluded that small myelinated fibre density decreased significantly in the cervical spine with age (24). It is expected that this drop in fibre density in the cervical WM will be reflected by a decrease in MWF with age.
1.5 References


2 Myelin Water Measurement by Magnetic Resonance Imaging in the Healthy Human Spinal Cord: Reproducibility and Changes with Age

2.1 Introduction

The application of multi-echo T2 relaxation measurements to the investigation of central nervous system (CNS) tissue structures has grown immensely over the last 20 years. The discovery that water trapped between myelin bilayers can be distinguished from intra- and extra-cellular (I/E) water based on their T2 relaxation times gave rise to the myelin water fraction (MWF), the fraction of water with a short T2 component, which has been demonstrated through histopathology to be a robust surrogate marker for myelin content (1). MWF have been successfully applied to investigate myelin contents in the healthy human brain (2) and spinal cord (SC) (3), as well as changes caused by age (4), multiple sclerosis (5), schizophrenia (4), attention deficit hyperactivity disorder (6), and phenylketonurea (7). The advancement of MRI scanners to a higher magnetic field strength, 3.0T, combined with the development of a 3D acquisition sequence, has led to improvements in both spatial resolution and signal to noise ratio. The purpose of the present study was to apply a 3D multi-echo sequence to the lower cervical spine region for the measurement of T2 relaxation, to test reproducibility of the measurement, and to explore whether the resulting MWFs exhibit a trend with age.

2.2 Materials and Methods

2.2.1 Subjects

A total of 31 healthy normal volunteers (12 male, 19 female, mean age 48y, range 21-75), who were screened for a history of neurological and spinal disorders, diseases, and injuries, participated in this study. Approval was obtained from the local institutional review board (see Appendix A), and signed, informed consent was obtained prior to the MRI examination.

1 A version of this chapter will be submitted for publication. MacMillan EL, Mädler B, Curt A, Dvorak MF, Li DKB, MacKay AL. Myelin water measurement in the healthy human spinal cord: Reproducibility and changes with age. Magn Reson Med.
Volunteers were recruited in two groups, an under 30 year old group (5 male, 8 female, mean age 25y, range 21-30) scanned initially, followed by a cohort of subjects over the age of 50 (7 male, 11 female, mean age 59, range 51-75).

2.2.2 Magnetic Resonance Experiment
All MRI scans were performed with a phased array spine coil, using only the first 4 elements, on a Philips 3.0T Achieva system (Philips Medical Systems, Best, The Netherlands). All volunteers were scanned with localizer and sagittal T2WI sequences to orient axial slices perpendicular to the SC as shown in Figure 2-1A; additional axial T2WI were acquired from the over 50 years cohort for improved GM and WM contrast, shown in Figure 2-1B. A 3D 32 echo Carr-Purcell-Meiboom-Gill (CPMG) sequence was acquired from all subjects for T2 measurement of the cervical SC. The 3D volume was positioned so as to span the region of C4-C6, however, in some subjects the stack was raised to avoid phase-wrap artifact from the shoulders. The 3D volume was acquired in one average with a FOV = 180mm × 135 mm × 40mm, and reconstructed into 8 axial slices 5mm thick with an in-plane resolution of 0.7mm×0.7mm. The 32 CPMG echoes were spaced 10ms apart beginning at 10ms, with a TR of 1300ms (effective TR=980ms), and a total scan time of 20min (8). Reproducibility was tested by acquiring the CPMG sequence twice in one session on three subjects from the under 30 years of age group, and twice on different days from eight subjects in the over 50 years age group.

2.2.3 Data Analysis
The 32 echo decay curve was fit on a voxel by voxel basis (9) using a regularized non-negative least squares (NNLS) algorithm (in-house software) using 120 input relaxation times logarithmically-spaced from 15ms to 2s, and no a priori assumptions about the number of exponential components were used in the fit (10). The MWF was defined for each voxel as the area under the T2 distribution between 15 and 35ms relative to the total area, to produce a MWF map, an example of which is shown in Figure 2-1C (2, 11).

For scans from the under 30 years age group, regions of interest (ROI) were placed on the MWF map in the dorsal column (DC), right (RLC) and left lateral column (LLC) WM, as indicated in Figure 2-1C. For scans from the over 50 years age group, these ROIs were placed on the axial T2WI, depicted in Figure 2-1B, which
were mapped by manual 2D rigid registration to the last echo image of the corresponding CPMG sequence for best contrast between the cord and cerebrospinal fluid (CSF). Signal intensities were averaged over both 2 dimensional ROIs, as well as 3 dimensional volumes of interest (VOI) created by combining each region along all slices in each subject. ROIs were not placed on either the first or last slice due to phase wrapping in the through-plane direction.

Reproducibility was measured by calculating the mean absolute difference in MWF for each ROI between scan one and scan two, as well as the mean relative difference:

\[
\text{Mean Relative Difference} = \frac{|\text{mean(scan one)} - \text{mean(scan two)}|}{\text{mean(scan one)}}
\]

All errors are expressed as standard errors. All post-NLNS data analysis was produced using Matlab (The Mathworks, Natick, MA, USA).

**Figure 2-1. Location of 3D CPMG stack and placement of ROIs for data analysis.**

A) Alignment of CPMG stack of slices centered on the C4/C5 intervertebral disc level on a sagittal $T_2$WI.

B) Location of dorsal and lateral column ROIs on axial $T_2$WI.

C) Location of dorsal and lateral column ROIs on a MWF map.
2.3 Results

A representative 32 echo decay curve, NNLS fit, and fit residuals are shown in Figure 2-2A, and the decay shown with a logarithmic time axis in Figure 2-2B. The decay curve is fit very well by the NNLS multi-exponential fit, as indicated by the residuals of around 2%, and the non-linear trend exhibited in logarithmic plot suggests that the data could not be fit accurately by a single exponential component. The corresponding $T_2$ distribution in Figure 2-2C clearly shows the presence of short $T_2$ components that are attributed to myelin water, as well as medium $T_2$ components arising from intra- and extra-cellular water (I/E water). Figure 2-3 below shows an example of a MWF map, where each pixel’s intensity is the fraction of water with a $T_2$ between 15ms and 35ms. The cut-off time of 15ms, which is the first input $T_2$ time, was used because it is the shortest $T_2$ time that can be reliably measured with a first echo at 10ms. The upper boundary for the myelin water $T_2$ window was set to 35ms due to the disappearance of gray matter pixels with zero MWF for cut-offs above this time.
Figure 2-2. Single pixel 32 echo measurement from a healthy subject under 30 years old, and resulting fit.
A) 32 echo decay curve and NNLS fit for a single pixel from deep DC WM with signal amplitude relative to the extracted density (the signal intensity at TE=0).
B) 32 echo decay curve with logarithmic time axis.
C) Corresponding $T_2$ distribution.
2.3.1 Reproducibility

Reproducibility was measured with two different timing schemes. The first method compared two CPMG scans acquired in immediate succession on three healthy subjects under 30 years old, yielding a mean absolute difference was 0.062(0.006), and mean relative difference was 25% (4%). The second method involved scanning eight subjects twice, about a week apart, naturally involving some repositioning. Three subjects’ repeat scans could not be included in the reproducibility analysis, one due to motion artifact, another to poor fit to noise of the NNLS algorithm, and the last due to misalignment to an incorrect vertebral level. For the remaining 5 cases, the mean absolute difference was 0.064(0.005), and the mean relative difference was 26%(2%). The absolute and relative differences did not vary significantly with vertebral level or location of the VOI. Figure 2-4 illustrates the scan-rescan reproducibility and variation in relative difference along vertebral levels for both the under 30 year old subjects and over 50 year old subjects. Figure 2-5 compares the reproducibility between the DC, RLC, and LLC VOI locations.
Figure 2-4. Reproducibility of MWF measurements.
A) Three subjects under 30 years old, scanned twice in one session.  B) Five subjects over 50 years old who were scanned about a week apart. 
Top: Scan one versus scan two MWF scatter plots for all VOIs. 
Bottom: Variation of the relative difference between scan one and scan two with vertebral level.
Figure 2-5. Scan-rescan scatter plot of MWF for DC, RLC, and LLC locations.
Scan one versus scan two MWF scatter plots coloured to highlight variations with VOI location.
Left: Three subjects under 30 years old, scanned twice in one session.
Right: Five subjects over 50 years old who were scanned about a week apart.

2.3.2 MWF variation along vertebral level

MWF were consistent along cervical vertebral levels for both groups, and there was no significant trend with vertebral level as shown in Figure 2-6. Since the position of the stack of slices in the cranial-caudal direction along the spine varied for the volunteers over 50 years old, a greater span of the cervical spine was investigated, however there were only a few subjects whose images contributed to the cervical levels cranial to C4 or caudal to C6.
2.3.3 Effect of Age

There appears to be a negative correlation between MWF and age, however, the differences between the two subject groups are smaller than the lowest estimate of reproducibility. The DC, and LLC, as well as all VOIs combined have Spearman’s rank coefficients of -0.352, -0.416, and -0.352, respectively, which reach significance at the 95% confidence level (Figure 2-7). This trend is reflected in the significant difference between the two age groups for all VOIs combined, as determined by a student’s t test outlined below in Table 2-1.

Table 2-1. Comparison of MWF values between subjects under 30 years old and subjects over 50 years of age.

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>All VOIs (DC+RLC+LLC)</th>
<th>Dorsal Column</th>
<th>Right Lateral Column</th>
<th>Left Lateral Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 30 years</td>
<td>0.290(0.007)</td>
<td>0.30(0.01)</td>
<td>0.28(0.01)</td>
<td>0.28(0.01)</td>
</tr>
<tr>
<td>Over 50 years</td>
<td>0.266(0.006)</td>
<td>0.28(0.01)</td>
<td>0.27(0.01)</td>
<td>0.25(0.01)</td>
</tr>
<tr>
<td>p value</td>
<td>0.008</td>
<td>0.102</td>
<td>0.230</td>
<td>0.088</td>
</tr>
</tbody>
</table>
Figure 2-7. Relationship between MWF and age.
A) MWF for all DC, RLC, and LLC VOIs with age.  B) DC MWF with age.  C) RLC MWF with age.  D) LLC MWF with age.  The Spearman’s rank coefficients for A, B, and D are significant at the 95% confidence level.
2.4 Discussion

2.4.1 Comparison with 1.5T MWF from literature

MWF values reported in this study are in agreement with results previously reported in the literature for the cervical spinal cord, listed below in Table 2-2. Previous MWF measurements have all been collected at 1.5T and calculated with the myelin water $T_2$ window lower boundary at 15ms and an upper boundary at either 40ms (3) or 50ms (12,13). Although Bloembergen-Purcell-Pound (BPP)(14) theory predicts that the $T_2$ of water protons should be increased by changes in magnetic field strength in the range of a few Tesla, multi-echo $T_2$ measurements in the brain demonstrated a significant decrease in mean $T_2$ time of I/E water between 1.5T and 3.0T(15). All brain ROIs exhibited shorter mean I/E water $T_2$ times at 3.0T, as compared to 1.5T. The myelin water $T_2$ upper boundary of 35ms used in the present study arose from the presence of I/E water between 35ms and 40ms, which is consistent with the trend found in the brain (15).

The CPMG sequence employed in the present study differed from those employed at 1.5T in the spine not only in that it was a 3D acquisition, but also by the lack of a CSF-nulling inversion recovery prepulse. At 1.5T the CSF was found to be nulled with an inversion time (TI) of 1.153s (3). At 3.0T we expect the $T_1$ to be longer, requiring a longer inversion time. However, even the inversion time at 1.5T is longer than the effective TR of the 3D sequence, so implementation of an IR prepulse would have required at least doubling the TR time and thus doubling the scan time, which would result in an unpractical scan time for clinical use.

Table 2-2. List of MWF values in the spinal cord and brain, at 1.5 and 3.0T.

<table>
<thead>
<tr>
<th>Field Strength</th>
<th>Cervical Spine MWF Mean (SD)</th>
<th>Overall Brain WM Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.5T</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.27 (0.02) Wu et al.(13)</td>
<td>0.08(0.02) Mädler et al.(8)</td>
</tr>
<tr>
<td></td>
<td>0.26 (0.01) Laule et al.(12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.22 (0.02) Minty et al.(3)</td>
<td></td>
</tr>
<tr>
<td><strong>3.0T</strong></td>
<td>0.29 (0.04) present study</td>
<td>0.10(0.04) Mädler et al.(8)</td>
</tr>
</tbody>
</table>
The cervical spine MWF values reported in this study were obtained from WM regions only, and are closest to those reported by Wu et al (13). In the study by Wu, MWF were calculated voxel by voxel in an axial slice of the cervical SC, and they attempted to select only WM voxels by excluding voxels that did not have any T₂ component between 15ms and 50ms. This means that any pixel with some myelin water was included in the average MWF, including pixels that have both GM and WM. Thus, it is reasonable that this study reports slightly higher MWF as pixels with GM partial voluming were not included. The study by Laule et al. (12) also acquired axial images, however the ROI was drawn around the entire SC, averaging both GM and WM together, yielding a lower MWF. Finally, lower MWF values reported by Minty et al. (3) could also be due to partial voluming of GM and WM, since the images were acquired sagittally.

The trend towards slightly higher MWF at 3.0T reported in this study, as compared to the three studies performed at 1.5T, echoes the trends found in brain WM reported by Mädler et al. (8) and Oh et al. (16). It has been suggested that the iron content in GM may increase local field inhomogeneities, leading to echo train artifacts that could artificially increase the proportion of short T₂ components. As the spinal cord is a very small structure with GM spread throughout the centre, it is possible that the iron in GM can affect T₂ measurements in the WM.

2.4.2 Reproducibility

Estimating reproducibility of MWF measurements is essential for designing studies of MWF in the spine in the future. In the present study, the mean absolute difference in MWF between two scans was 0.06 or 26%. This difference between two scans is probably due to inhomogeneities in the RF field combined with low SNR, as seen in the fit residuals. At this level of reproducibility, future studies would require a sample size of 12 for each population to reach a statistical power of 0.80 in the detection of a difference in mean MWF between 0.29 and 0.22.

The reproducibility in the present study is nearly identical between scans acquired in immediate succession and those acquired a week apart. This suggests that there is very little error in MWF caused by repositioning, which is to be expected because the structure of the SC changes very little in the cranial-caudal direction throughout the cervical levels, and that differences in head tilting can be mitigated by ensuring
axial images are oriented perpendicular to the SC. The exclusion of three repeat
scans from the procedure performed on different days highlights the importance of
minimizing human error while selecting the correct stack vertebral level, and the
inherent risk of motion artifacts due to subject movement or swallowing when
imaging the cervical cord. It is encouraging to note that reproducibility was not
significantly affected by the vertebral level positioning or spinal cord location.

2.4.3 Variation along vertebral levels
The cervical enlargement, which begins at C3 and continues until the upper thoracic
vertebrae, has a consistent structure in the cranial-caudal direction and increased
grey matter relative to lower thoracic regions because of the large number of
innervations connecting the SC to the upper extremities (17). The C4 to C6
vertebral levels were the most common segments covered by the stack of CPMG
slices, which are well within the cervical enlargement. Thus, the lack of a trend of
MWF along the few vertebral levels investigated is concordant with the known
structure of the cervical cord.

2.4.4 Effect of age on MWF
MWF measurements suggest that myelin content decreases with age in both dorsal
and lateral column WM. The Spearman rank correlations of MWF with age reached
significance for all VOIs grouped together, as well as for the DC and LLC
individually. As well, it is interesting to note that MWF below 0.20 were only
measured in volunteers above the age of 50 (see Figure 2-7A). However, the
difference between the age groups only reaches a statistical power of 0.41 for 3
VOIs per subject, or 0.17 for each VOI individually, with the current sample sizes.
For such a small difference between groups (a drop in MWF from 0.29 to 0.27), the
sample size of each age group would need to be 142 to reach a statistical power of
0.80 in each VOI, or sample sizes of 48 with 2 VOIs per subject. Hence, to strongly
support the notion that myelin content decreases with age, more volunteers need
to be recruited.

A photomicrograph investigation of the lateral corticospinal tracts (a major tract in
the lateral columns) also found a negative correlation between the density of small
myelinated fibres (those with diameters between 1µm and 7.28µm) at the C6 level
with age (18). The density of large myelinated fibres (those with diameters over
7.28µm) also decreased with age, but the correlation did not reach significance in that report. The effect of age on fibre density may be stronger than its effect on MWF due to the increasing thickness of myelin sheaths with age, as well as the increased formation of myelin balloons (bulging of the myelin sheath)(19), both of which could increase myelin content and the MWF without increasing fibre density. The trend towards lower MWF with age suggests that decreases in myelin content may contribute to the deterioration of motor and sensory function that is often associated with normal aging.

2.5 Conclusions
MWFs were successfully measured in cervical WM isolated from GM for the first time in humans in vivo, and were in agreement with previously reported cervical cord values. The reproducibility of MWF suggests that a larger number of subjects are required to strengthen the statistical power of the negative correlation between age and MWF. The improvements of incorporating 3D axial data acquisition and moving to the higher field strength of 3.0T advance the application of MWF measurements for the in vivo investigation of SC WM diseases and injuries.
2.6 References


3 Conclusion

The findings presented in this study mark the first time that MWF have been measured in SC WM independently from GM in humans in vivo. As expected, the MWF reported in Chapter 3 were slightly higher than previous studies due to the lack of GM partial voluming (1-3), but also possibly due to the move to a higher magnetic field strength MRI scanner (4). This study also helps to usher in the era of 3D multi-echo T2 measurements; this is one of the first studies to employ additional phase encoding along the z-axis, allowing more slices to be acquired with less averages (4). The present 3D technique applied to the cervical spine yielded 6 slices, spanning 3cm of the spinal cord, or roughly 2 vertebral levels. Since the slices were acquired axially, the field of view was kept small to attain a fine resolution for the small cord, but this also limits the use of this protocol to the neck region to avoid phase wrap artifacts from the shoulders. The advantage of acquiring axial slices enables the investigation of WM independent from GM, while the improvement to 3D acquisition means that focal lesions, such as those caused by stenoses, can be investigated throughout the affected vertebral level and possibly above or below the affected levels as well.

Dorsal and lateral column regions of interest in SC WM were investigated for changes with either vertebral level or age. The targeted vertebral levels in this study were within the cervical enlargement, which has a constant structure in the cranial-caudal direction, and did not exhibit any trend in MWF with vertebral level as expected. The MWF measured in this study hint at a negative correlation with age, however, the statistical power with the current sample sizes is low and more volunteers are required to reach an acceptable power level.

It is encouraging that the mean absolute difference in MWF between scans of the same person, acquired either during the same or different sessions, is on the same order as the estimated minimum percent difference in MWF that can be detected with a sample size of 5, as reported in a study of healthy human brain (5). The mean absolute difference of 26% is quite high and suggests that myelin water imaging can detect a MWF decrease to 0.22 in cervical WM with sample sizes of 12. Though this appears to be a large change in myelin content to reliably detect, MWF
below this level were detected in some healthy volunteers, but only those over 50 years of age. Thus, if healthy volunteers present MWF below 0.22, it is possible that symptomatic CSM patients could present with even lower MWF that could reliably be identified as pathologic.

The multi-echo CPMG measurements acquired in this study also offer information about the $T_2$ distribution of I/E water that has not yet been investigated. The geometric mean $T_2$ time of the I/E water may offer another measure to detect changes in SC tissue structure caused by age or pathology. Geometric mean $T_2$ times of normal appearing WM in multiple sclerosis were significantly different than for WM in controls, suggesting diffuse myelin loss (2,6).

The improvements in MWF measures at 3T and in 3D brighten the future of myelin water imaging as a pathology-specific non-invasive technique for the assessment of WM disorders and diseases in the cervical spinal cord. In particular, the role of demyelination in CSM may be related to the deterioration in motor and sensory functions that cause symptoms, and a non-invasive assessment of WM damage would aid in the diagnosis and treatment planning for CSM. In addition, recent research in spinal cord injuries (SCI) suggests that myelin plays an inhibitory role in neurite regrowth following cord transection (7). Future therapies for SCI may involve intentional demyelination of the cord to allow neurite regrowth, where it will be essential to have a non-invasive technique to determine if demyelination does occur, and if remyelination begins following treatment.
3.1 References


Appendices

A. Spin-Spin Relaxation in CNS Tissues

Magnetic resonance imaging theory spans the scale from individuals to individual particles, employing quantum mechanical principles to describe the behaviour of a single proton and classical mechanics to explain changes of ensembles of protons on the order of Avogadro’s number. To understand how MRI measures can distinguish different tissue environments, the behaviour of protons within each environment must first be understood.

A.1. Spin

On the quantum mechanical scale, protons are described as having a quantized spin; they can only occupy either the spin-up or the spin-down state (1). In an external magnetic field, the spin-up state is the lower energy state because it is aligned with the field, while the spin-down state is the higher energy state being aligned opposite to the field. However, the proton’s spin cannot align perfectly with the external field causing it to precess about the external field according to the Larmor equation A.1 and as illustrated in Figure A-1:

\[ \omega_0 = \gamma B_0 \]  \hspace{1cm} (A.1)

where \( \omega_0 \) is the Larmor frequency, \( \gamma \) is the gyromagnetic ratio, and \( B_0 \) is the external magnetic field which is conventionally referred to as being applied along the \( z \)-direction, \( B_z \) and measured in units of Tesla, T (2). The energy difference (\( \Delta E \)) between the spin-up and spin-down states is given by:

\[ \Delta E = \hbar \gamma B_0 \]  \hspace{1cm} (A.2)

with \( \hbar \) representing Plank’s constant divided by \( 2\pi \).
On the scale of tissues, the ratio of the number of spins in the spin-up state \( N^+ \) to the spin-down state \( N^- \) is related to the external magnetic field and temperature \( T \) as described by Boltzmann statistics:

\[
\frac{N^+}{N^-} = e^{\frac{\gamma h B_0}{k_B T}}
\]  

(A.3)

where \( k_B \) represents the Boltzmann constant. At body temperature with a 3T scanner, for every 2 million protons, 21 more occupy the spin-up state than the spin-down state.

The average of all spin orientations within a tissue is the net magnetization, \( M_0 \), which obeys classical mechanics. At equilibrium, protons precess about the external magnetic field with random phases, so that the net magnetization is aligned with the external field, \( M_0 = M_z \propto B_z \) with no transverse magnetization, \( M_{xy} = 0 \). While it is difficult to detect changes in the equilibrium net magnetization, \( M_z \), due to its very small magnitude (~1µT) compared to the external magnetic field (3T), the transverse magnetization can easily be detected by a coil oriented around the x or y axes.

As described by Faraday's law of induction (3), a changing magnetic field induces an electric field in a coil of wire, enabling the detector coil to measure an electric current. To create a non-zero transverse magnetization, a pulse of electromagnetic radiation is applied to the sample with energy \( \Delta E \) according to equation A.2, which, for protons in an external magnetic field on the order of a few Tesla, is in the radiofrequency (RF) range. The RF pulse causes spins to flip from spin-up to spin-down, and is applied for enough time until the population in both states is equal. Such an RF pulse is called a 90° pulse because the net magnetization has been tipped by 90° into the transverse plane; it is also called the excitation pulse because the applied RF field deposits energy into the system resulting in an excited state.

The spins that have been flipped land in their new state in phase creating a coherent transverse magnetization, as shown in Figure A-2A. The transverse magnetization vector then precesses about \( B_z \) at the Larmor frequency, and from
this point forward it is convenient to describe the magnetization in the frame of reference rotating at the Larmor frequency (called the x’ and y’ axes) about the z axis.

Figure A-2. Evolution of a spin echo in the rotating frame at the Larmor frequency.
A) A 90° RF pulse applied around the x’ axis tips the net magnetization $M_0$ into the transverse plane with coherent phases. B) Immediately after the 90° pulse spins dephase naturally. C) A 180° RF pulse is applied to rotate the spins about the y’ axis. D) Spins continue to dephase in the same direction. E) Spins have converged together, generating an echo. F) After the echo, the spins continue to dephase. Steps C through F are repeated during a multi-echo train such as the sequence depicted in Figure A-3.

A.2. Spin Echoes

Once the net magnetization is in the transverse plane, spins begin to precess at different Larmor frequencies due to slightly different local magnetic fields, causing dephasing of $M_{xy}$ as in Figure A-2B. Next, a 180° pulse is applied to rotate spins about the y’ axis, which flips their phase angles without changing their precession
frequencies (Figure A-2C). This phase reversal allows the lower frequency spins to catch up to the higher frequency spins (Figure A-2D) causing a return of phase coherence (Figure A-2E), however, this coherence is short lived as the phases continue to accumulate and $M_{xy}$ dephases once again (Figure A-2F).

The dephasing of spins in the transverse plane after the excitation pulse is detected in the receiver coil as an oscillating decay of voltage called a free induction decay (FID) depicted in Figure A-3. The 180° pulse is applied after a time $\frac{TE}{2}$ (the time between the excitation pulse and the first echo is the echo time (TE)), which causes a signal maximum at time TE in the receiver coil, this voltage pattern is called an “echo”. A train of 180° RF pulses spaced time TE apart would result in an “echo train” detected by the coil, an example of which is the Carr-Purcell-Meiboom-Gill (CPMG) sequence. In practice, the excitation pulse and train of refocusing 180° RF pulses would be measured for each pixel in an image, and repeated after a time TR (repeat time) for the next pixel.

The rate at which signal in the receiver coil decays due to the dephasing of each echo is influenced by relaxation of the transverse magnetization (also called spin-spin or $T_2$ relaxation), water diffusion, and differences in tissue magnetic susceptibility. The rate constant of this exponential decay is termed $T_2^*$. However, the decay in the maximum amplitude of the echoes depends only on diffusion and spin-spin relaxation, since the effects of susceptibility differences are cancelled out during the phase reversal of each echo (4).
A 90° RF pulse (in blue) is applied about the x’ axis to tip the net magnetization into the transverse plane. Immediately following the 90° pulse, the receiver coil measures a free-induction decay (FID, in black) which decays exponentially due to $T_1$ relaxation. After a time TE/2, a 180° pulse is applied about the y’ axis to refocus the dephasing spins. At time TE, the spins have completely returned to coherence and immediately after TE begin to dephase again, resulting in an echo detected by the receiver coil. Subsequent 180°y’ pulses at time $\frac{TE}{2} + n \times TE$ yield more echoes, with exponentially decaying peak heights caused by spin-spin ($T_2$) relaxation.

**A.3. Spin-Spin Relaxation**

After the 90° excitation pulse, a system of spins will be precessing about $B_z$ at the Larmor frequency with the same phase in the rotating frame. Following the excitation pulse, water protons will dephase relative to each other via two different mechanisms: diffusion through a magnetic susceptibility gradient, and proton exchange with large molecules. As a water molecule diffuses through tissue it may diffuse through a change in magnetic susceptibility, often caused by changes in iron concentration in the tissue. Thus, the proton experiences a small magnetic field gradient and accrues phase according to equation A.4:
where x represents the distance travelled, G is the magnetic field gradient, and t is the length of time (2). Protons can also dephase if they are exchanged from a molecule that experiences fast $T_2$ relaxation due to the dipole-dipole interaction, such as large molecules or structures.

Dipole-dipole interactions occur when two protons come close enough together that their magnetic dipole moments interact, and each proton experiences a slightly higher or lower local magnetic field. Each proton’s precession frequency will change slightly to match the new local field, resulting in a dephasing away from the Larmor frequency. As the two protons leave each other’s local field, they return to precessing at the Larmor frequency, however the phase gained through their interaction is not reversible. The time interval between these collisions is called the correlation time, $\tau_c$, which is very long for protons on solid and semi-solid structures because they are motionally restricted. Conversely, protons on water molecules are nearly free and have shorter correlation times. The inverse of the correlation time is the tumbling frequency ($\omega$), and the number of protons at each tumbling frequency yields a spectral density function as illustrated in Figure A-4.

**Figure A-4.** Spectral density functions of three proton pools with different correlation times.

Molecules that tumble slowly (small $\omega$) have a longer correlation time, meaning more time between collisions with other molecules. Molecules that tumble quickly (large $\omega$) spend less time between collisions, and thus dephase faster. The Larmor frequency ($\omega_0$) of water protons at clinical MRI field strengths is in the middle of this frequency range.
Protons with a short correlation time, (i.e. free water protons that tumble rapidly) experience the sea of dipoles from other water protons fluctuating very rapidly compared to the measurement time, and effectively average together to appear as a homogeneous magnetic field. This effective homogeneous field causes little dephasing and thus slow spin-spin relaxation. Conversely, protons bound to semi-solid structures or large molecules will tumble more slowly, with more time to experience the dipole magnetic fields of only their close neighbours, causing more dephasing. Thus, the more restricted the proton’s motion, the more it dephases and the faster its spin-spin relaxation (2). The motion of protons is a random process, leading to an exponential decay in transverse magnetization over time, which is called the spin-spin relaxation described by the decay constant $T_2$ (2):

$$M_{xy}(t) = M_0 e^{-\frac{t}{T_2}} \quad (A.5)$$

Protons do not stay bonded to a particular molecule for as long as it takes to dephase, and are free to exchange between chemicals causing the short $T_2$ structures to share their short $T_2$ time with their neighbouring water molecules. Hence, the greater the proportion of water molecules in a hydration layer around macromolecules or large structures as compared to the proportion of bulk water, the shorter the water proton’s average $T_2$ time.

Spin-spin relaxation is often called the “true $T_2$” because although it leads to the loss of transverse magnetization, it does not lead to a net loss of spin energy. The $T_2^*$ dephasing includes the effects of spin-spin relaxation, as well as differences in magnetic susceptibility that are reversed by the $180^\circ$ pulses. These decay constants are related by (4):

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \quad (A.6)$$

where $T_2'$ describes the relaxation due to tissue susceptibility differences.

**A.4. Measuring Spin-Spin Relaxation in CNS Tissues**

As mentioned above, while the signal decay of the FID and each individual echo depends on $T_2^*$ processes, the decay in peak amplitude across all echoes is
dependent only on the spin-spin relaxation and diffusion. For a 32 echo CPMG pulse sequence, the peak height can be measured 32 times along the $T_2$ decay curve. In pure water, the signal decay curve ($S(TE)$) is a single exponential according to equation 1.7 and as illustrated in Figure A-5 below.

$$S(TE) = S(0)e^{\frac{TE}{T_2}}$$  \hspace{1cm} (A.7)

In complex biological systems such as wood, collagenous tissues and CNS tissues, varying proportions of hydration layer water to bulk water can be found, yielding a distribution of $T_2$ times. A 32 echo measurement from the WM in the SC is shown in Figure A-5 as an example of a multi-exponential $T_2$ decay curve, and fit by the single exponential model from equation A.7. The single exponential model yields a systematic trend in the residuals and a maximum residual of around 10%.
Figure A-5. Multi-echo data from pure water and CNS tissue.
Left: A 32 echo decay curve from a region of interest containing pure water, analyzed with a monoexponential fit according to equation A.7.
Middle: A 32 echo decay curve from deep WM in the human spinal cord in vivo, analyzed with a single exponential fit according to equation A.7.
Right: A 32 echo decay curve from an ROI deep within human spinal cord WM in vivo, analyzed with a multiexponential NNLS fit.
The varying amounts of motionally restricted water that exists in association with large molecules and semi-solid structures leads to a continuum of spin-spin relaxation rates, which for the purpose of computer modeling, are expressed as a sum of discrete $T_2$ decay components (5):

$$S(t) = \sum A(T_2) e^{-\frac{t}{T_2}}$$  \hspace{1cm} (A.8)

where the MR signal as a function of time, $S(t)$, is the sum over all components with amplitude $A(T_2)$. Each component describes a pool of protons with a given $T_2$ decay constant. This model is applied to fit 32 echo decay measurements from CNS tissues by using a regularized non-negative least squares (NNLS) algorithm with 120 input relaxation times spaced logarithmically between 15ms and 2s, and no a priori assumptions about the number of components (5). An example of this fit is shown in Figure A-5, which accurately fits the multiexponential decay with a maximum residual around 1% and without any systematic error. The resulting distribution of exponentials that contributed to the NNLS fit in Figure A-5 is shown in Figure A-6. From this $T_2$ distribution it is clear that there are at least two distinct pools of water protons, each pool with a characteristic $T_2$ decay constant (6). The fraction of the $T_2$ distribution between 15 and 35ms is believed to originate from water trapped between myelin bilayers, and this fraction relative to the total area under the distribution curve is called the myelin water fraction (MWF). Most of the water trapped between myelin bilayers is part of a hydration layer associated with the head groups of the phospholipid bilayer, and thus most protons in the myelin water have been exchanged with short $T_2$ protons on the phospholipids. This proton exchange leads to a much shorter $T_2$ of myelin water than the $T_2$ time of the intra/extra-cellular water (I/E water).
Figure A-6. Distribution of $T_2$ decay constants contributing to the multiexponential fit in Figure A-5.
The area under the distribution curve between 15 and 35ms relative to the total area under the distribution curve is called the myelin water fraction (MWF).

To be able to measure the multiexponential behaviour of $T_2$ relaxation in a particular proton pool, such as the myelin water pool, the water molecules must be present in that pool long enough compared to their $T_2$ relaxation rate (7). Otherwise, if water molecules are diffusing through more than one pool as fast as their MR signal is decaying, the decay rate measured will be an average of the decay rates from each pool. This effect is noticeable in spin-lattice ($T_1$) relaxation measurements, where water diffusion is fast enough that protons have sampled both the myelin water and I/E water pools well before the regrowth of the longitudinal magnetization. However, it is fortunate that in CNS tissues, the water diffusion is sufficiently slow in comparison to the spin-spin relaxation rate, allowing $T_2$ measurements to detect distinct water pools.

### A.5. Myelin Water Imaging

Spin-spin relaxation measurements of CNS tissue in vitro demonstrated that all of the water in GM and WM had long enough $T_2$ decay times to be visible with MRI methods; i.e. the $T_2$ was longer than the shortest TE time of 10ms (8). Thus, the 32 echo measurement is sensitive to the short $T_2$ myelin water pool (9). Nuclear
magnetic resonance (NMR) experiments on excised garfish nerves demonstrated that the short T₂ component was detected only in myelinated nerves, while a medium-relaxing component, attributed to water in the axoplasm or extracellular space, was present in both myelinated and unmyelinated nerves (10). The short T₂ component was first detected in vivo in the human brain using MRI at 1.5T in 1994 (6), and the MWI technique has since been successfully applied to the study of multiple sclerosis (11, 12), schizophrenia (13), attention-deficit hyperactivity disorder (14), phenylketonuria (15) and cervical spondylotic myelopathy (16).
A.6. References


B. Clinical Research Ethics Board Certificates of Approval

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue,
Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD APPROVAL

PRINCIPAL INVESTIGATOR: Armin Curt
INSTITUTION / DEPARTMENT: UBC/Graduate Studies

UBC CREB NUMBER: H06-00282

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

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CO-INVESTIGATOR(S):
Marcel F. S. Dvorak
Alexander L. MacKay

SPONSORING AGENCIES:
Cervical Spine Research Society

PROJECT TITLE:
New imaging and diagnostic modalities: a new quantitative magnetic resonance imaging technique for the assessment of cervical spondylotic myelopathy.

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: October 24, 2007

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

REB FULL BOARD MEETING REVIEW DATE:
October 24, 2006

DOCUMENTS INCLUDED IN THIS APPROVAL:

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DATE DOCUMENTS APPROVED:
January 24, 2007
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**CERTIFICATION:**

**In respect of clinical trials:**

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

**Approval of the Clinical Research Ethics Board by:**
ETHICS CERTIFICATE OF EXPEDITED APPROVAL:
RENEWAL WITH AMENDMENTS TO THE STUDY

PRINCIPAL INVESTIGATOR: Marcel F.S. Dvorak
DEPARTMENT: UBC/College for Interdisciplinary Studies
UBC CREB NUMBER: H06-00282

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Other locations where the research will be conducted: N/A

CO-INVESTIGATORS:
Armin Curt
Alexander L. MacKay

SPONSORING AGENCIES:
Cervical Spine Research Society
Michael Smith Foundation for Health Research - "Correlation Between Results from a New Magnetic Resonance Imaging Technique to Measure Myelin in the Spinal Cord and Somatosensory Evoked Potentials"

PROJECT TITLE:
New imaging and diagnostic modalities: a new quantitative magnetic resonance imaging technique for the assessment of cervical spondylotic myelopathy.

The current UBC CREB approval for this study expires: October 24, 2008

AMENDMENTS:

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**CERTIFICATION**

**In respect of clinical trials:**

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

**Approval of the Clinical Research Ethics Board by:**

[Signature]

[Position]

[Date] October 18, 2007