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Author: Ercan, Ayşe Ece

Title: Diffusion-weighted MRS and MRI : methods and neuro applications

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Diffusion-weighted MRS and MRI: Methods and Neuro Applications

Ayşe Ece Ercan

Colophon

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Diffusion-weighted MRS and MRI: Methods and Neuro Applications

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ve canım anneannem Ayşe Nurten Kuleli'ye;
beni her kararımda destekleyen annem Jale Kuleli,
dayım Tanju Kuleli ve kız kardeşim Meriç'e.

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1

Introduction

Preface

SINCE its inception, magnetic resonance imaging (MRI) has become one of the most frequently-used imaging tools in biomedical research and clinical applications. As a neuroimaging tool, MRI is used in numerous ways to probe the human brain non-invasively in vivo. MRI is unique among neuroimaging tools as it offers a wide range of image contrast mechanisms, many of which cannot be obtained by other neuroimaging techniques. Based on these contrast mechanisms, various MRI techniques have been developed to probe structural and functional aspects of the human brain. Among these techniques, diffusion weighted imaging (DWI) and subsequently, diffusion tensor imaging (DTI) allow one to probe brain tissue microstructure in vivo. These techniques exploit the possibility to sensitize the MRI signal with respect to water diffusion within the imaging voxel. Diffusion properties of water in tissue are largely dictated by the restrictions and hindrances posed on molecular motion by cell membranes, organelles, macromolecular structures and other tissue micro-structural components. Voxel-wise quantification of these properties using DWI and DTI allows a unique way to characterize tissue microstructure, and its greatest clinical usefulness is in detecting micro-structural changes in brain tissue caused by disease processes.

A large number of studies have applied DWI in a clinical setting and have shown its ability to detect localized disease-related effects, such as the affected area in ischemic stroke [1–4], lesions in multiple sclerosis and other neurological disorders [5–7], and brain tumors [8, 9]. In combination with functional MRI data, DTI is also used in surgical planning for brain tumors [10]. DTI has shown to be sensitive in detecting more subtle changes in brain tissue caused by psychiatric [11], developmental [12, 13], and autoimmune [14] diseases. Studies on schizophrenia [15] and multiple sclerosis (MS) [16] have shown that DTI can detect group-level diffuse changes related to these diseases in normal-appearing brain.

While DTI is sensitive to changes in tissue microstructure, it lacks specificity in identifying the source of these changes [17]. DTI and DWI probe the diffusion of water, and since water is the major component in tissue, disease-related changes in water diffusion properties reflect changes that may occur in each of the brain tissue compartments: the extracellular and intracellular spaces, as well as the space within the myelin sheath, albeit to a lesser extent because of the shorter T_2 of myelin water, compared to that of water in other tissue compartments. Consequently, disease-related change in DTI metrics, such as mean diffusivity (MD) or fractional anisotropy (FA), cannot be linked to a specific tissue compartment, and thus cannot be linked to a specific underlying disease mechanism that may have caused the observed change. Moreover, the ubiquitous presence of water in all cell-types makes it impossible to distinguish changes in DTI caused by alterations in specific cell types, e.g. neurons or glia. This further limits the explanatory power of DTI in disease-related microstructural alterations.

Magnetic resonance spectroscopy (MRS) can be used to measure concentration of brain metabolites in vivo [18]. Among these metabolites, N-acetylaspartate (NAA) and the excitatory neurotransmitter glutamate (Glu) are neuronal metabolites specifically located in the axons and cell body of neurons; whereas myoinositol and choline compounds (tCho) are predominantly found in glial cells [19]. With MRS, the changes in the metabolite concentrations can be used to follow pathological changes in a specific cell population. For instance, the lower NAA concentration in the brain of MS patients compared to

healthy controls has been shown to relate to neuronal damage in MS [20]. In contrast to diffusion-based methods, MRS does not provide structural information; metabolite concentrations alone do not reflect the effect of pathomechanisms on tissue microstructure in general, or on disease-related cytomorphological changes in particular.

By combining the respective types of information that MRS and diffusion-based MRI provide, it is possible to probe tissue microstructure in a cell-type and compartment-specific manner. For example, by probing the diffusion properties of neuronal metabolites such as NAA, glutamate, and predominantly glial metabolites such as myo-inositol and choline, it is possible to differentiate between disease-related microstructural changes that occur in neurons and axons and those that occur in glia. Diffusion-weighted MR spectroscopy (DW-MRS) allows such compartment-specific assessment of tissue microstructure [21]. The diffusion properties of these metabolites reflect a variety of properties of the intracellular space, including viscosity and molecular crowding of the cytosol, as well as cytomorphological features such as axonal diameter and cell size, and hence potentially provide sensitive markers for compartment-specific microstructural changes caused by disease [21].

Applications of ^1H DW-MRS in animal and human brain have confirmed the ability of the method to obtain compartment-specific information pertinent to disease or to basic neuroanatomy [22–30]. Early studies of the ^1H DW-MRS method in human brain showed the feasibility of applying the method in vivo [23]. DW-MRS was also used for investigating ischemic changes in the rat brain [31, 32]. Further applications of DW-MRS aimed to relate metabolite diffusivity values to brain neuroanatomy [33] [33] and to differentiate metabolite diffusion in gray and white matter [26, 33]. Post-processing and acquisition methods of DW-MRS were further improved for in vivo human brain applications [24, 25, 27]. Applications of DW-MRS to case-control studies, including diseases such as multiple sclerosis [34], brain tumors [35, 36], ischemia [36, 37], schizophrenia [38] and bipolar disorder [39], showed promising results in compartment-specific differentiation of disease-related changes in human brain in vivo.

For DW-MRS to become a widely applied clinical research tool, several challenges need to be addressed. DW-MRS sequences are for the most part not commercially available on clinical MRI scanners, and robust acquisition techniques must be implemented across platforms. Optimized acquisition protocols for DW-MRS, compatible with clinical protocols, are needed to facilitate the integration of DW-MRS into a “clinical” setting. Post-processing and analysis pipelines that combine the cell-type specificity of this technique with the structural sensitivity of DTI to reflect axonal orientation will enhance the utility of DW-MRS in the analysis of neurological disorders. Extending DW-MRS from single volume measurements to robust and reproducible 2D or 3D diffusion-weighted spectroscopic imaging will add the possibility to obtain spatially-resolved information from a large field-of-view, significantly increasing the amount of information obtained from a single scan. Finally, hardware improvements in gradient performance can improve the SNR of DW-MRS measurements, thus adding statistical power to group DW-MRS results.

1.1 Thesis objectives

- (a) Using DW-MRS to investigate the link between the diffusion properties of brain metabolites in white matter and the cytomorphology of their host compartments;
- (b) Developing and evaluating new DW-MRS techniques and acquisition protocols that

enhance the use of DW-MRS in research and clinical settings;

- (c) To use DW-MRS and other MRI microstructural methods, such as DTI and MTI, to characterize the microstructural deficit in a poorly diagnosed disease, neuropsychiatric systemic lupus erythematosus (NPSLE).

1.2 Thesis outline

This thesis is divided into two main parts, preceded by an introductory chapter, and followed by a general discussion of the findings presented here. The first part focuses on the development of DW-MRS methodology, on the assessment of its robustness, and its application to a basic neuroanatomy investigation. The second part consists of the application of MR-based tissue microstructural tools, including DW-MRS, to address clinically relevant questions in one particular disease: neuropsychiatric systemic lupus erythematosus. The introductory chapter (Chapter 1) is a short overview of the technical and scientific concepts used throughout the thesis.

In the first part of the main body of the thesis (Chapters 2-4) three works are presented. In chapter 2, the diffusion properties of tNAA, tCr and tCho are measured in the corpus callosum of healthy human brain in vivo and the relationship of these measures to the structure of their respective host compartments is investigated. In chapter 3, the reproducibility of DW-MRS measurements is assessed in the corpus callosum at two field strengths, 3T and 7T, and an optimized DW-MRS protocol for case-control cross-sectional studies is provided at these two field strengths. In chapter 4, DW-MRS is further extended to include diffusion weighted chemical shift imaging (DW-CSI), which enables obtaining spatially resolved (image) information on diffusion of brain metabolites, and thus can localize the information about metabolite diffusion over a large field-of-view.

The second part of the thesis comprises chapters 5-6. In chapter 5, the microstructural changes found in the brain of SLE and NPSLE patients is investigated using a multimodal approach, in which data from two microstructural MRI tools, DTI and MTI, are acquired and co-analyzed in order to better understand disease-related changes in the brain of NPSLE patients. In chapter 6, DW-MRS and DTI are used to study disease-related compartment- and cell-specific changes in the brain of a cohort of NPSLE and SLE patients. Here the main aim was to quantify intra-axonal and intra-glial changes caused by the disease.

The thesis is concluded with Chapter 7, in which a general discussion is presented. Chapter 8 contains a concise summary of the work presented in this thesis together with the major conclusions drawn from this work.

1.3 MRI and microstructural investigations

MRI is used as a unique non-invasive neuroimaging tool that can provide structural information on the brain. Various MRI techniques can provide details on structures smaller than the nominal spatial resolution of the image voxel [40]. Among these techniques, relaxometry and magnetization transfer can indirectly reflect the microstructure-related properties [41, 42]. With relaxometry, it is possible to differentiate the tissue compartments with different MR relaxation properties [42]. Magnetization transfer can provide information about tissue components with very short transverse relaxation time (T_2) that are in fast exchange with water protons. Protons in these tissue components, such as myelin and cell membranes, cannot be directly detected with other MRI methods, due to

the fast decay of their transverse magnetization [41]. Diffusion-based methods on the other hand can provide quantifiable and direct microstructural measures that can reflect tissue microstructural properties at a mesoscopic scale (0.1 to 100 μm), although the nominal imaging voxel obtained with clinical scanners is usually not higher than ~ 1 mm resolution [43]. This gives a unique way to use MRI to study tissue microstructure in vivo [40].

1.3.1 Diffusion MRI methods

1.3.1.1 Diffusion

Diffusion represents the Brownian motion of molecules in a medium, driven by their thermal energy. In a fluid medium without obstacles, geometric constraints or restrictions, the mean square displacement of the molecules increases linearly with time. The mean square displacement in any one direction is linearly related to D , as stated by Einstein's equation [44]:

$$\langle (r - r_0)^2 \rangle = 2D \cdot t \quad (1.1)$$

where D is the diffusion coefficient, r_0 and r are the initial and final positions of the molecules, respectively, and t is the observation time. In a medium without geometric constraints, the diffusion process is termed Gaussian, and D is referred to as the free diffusion coefficient, which depends on the temperature and viscosity of the solution, as stated by the Stokes-Einstein equation [44]:

$$D = \frac{k_B T}{6\pi\eta R_H} \quad (1.2)$$

where k_B is the Boltzmann constant, T is the temperature of the solution, η is the viscosity of the solution, and R_H is the hydrodynamic radius of the molecule.

In a medium without physical boundaries, the diffusion is isotropic i.e. the molecules diffuse equally in all directions. In more complex media, such as brain tissue, the diffusion is restricted or hindered by geometric constraints, e.g. membranes, proteins, organelles and the cytoskeleton. When the diffusion time t is very short, i.e. $t < a^2/2D$, where a is the typical distance between physical boundaries such as the membranes and hence the resulting molecular displacement, $\sqrt{\langle (r - r_0)^2 \rangle}$, is smaller than a , this effect is negligible and diffusion is still Gaussian [45]. At longer diffusion times ($t \gg a^2/2D$), the molecular displacement is affected by the presence of the geometric restrictions and the diffusion coefficient observed in such situations deviates from the free diffusion coefficient, D . Moreover, because of the presence of anisotropic restrictions - physical constraints in the medium that are organized in a certain direction, the diffusion coefficient can spatially vary and depends on the direction in which it is measured, i.e. it is anisotropic. A new parameter is then defined, the apparent diffusion coefficient (ADC), which results from spatial averaging of the diffusion coefficients measured in several directions. The ADC is thus determined both by the 'intrinsic' diffusion properties of the molecules and by the effect of the restrictions in the medium [46].

1.3.1.2 Diffusion-weighted MRI and Diffusion Tensor Imaging

In MRI, it is possible to measure molecular diffusion using the method introduced by Skejskal and Tanner [47]. This method, which exploits the effect of loss of coherence of transverse magnetization when spins move in an inhomogeneous field [48], is based

on a simple T_2 -weighted Hahn spin-echo sequence, where diffusion-sensitizing gradients are applied in a given direction before and after the 180° pulse. As a consequence of applying these gradients, the signal is attenuated by diffusion-dependent phase dispersion caused by the diffusion of spins along the gradient direction. The attenuation of the signal is determined by the diffusion coefficient of the molecule of interest, as well as by the diffusion-weighting sequence parameters. In the case of Gaussian (free) diffusion, the diffusion weighted signal amplitude is given by:

$$S(b_i) = S(0)e^{-b \cdot D_i} \quad (1.3)$$

where $S(b_i)$ is the measured signal in direction i of the diffusion sensitizing gradient, $S(0)$ is the signal without diffusion-weighting, D_i is the diffusion coefficient for direction i and b is given by [50] [50]:

$$b = \gamma^2 g^2 \sigma^2 \left(\Delta - \frac{\delta}{3} \right) \quad (1.4)$$

where γ is the gyromagnetic ratio of the observed nucleus, g is the gradient amplitude, δ is the gradient duration, and Δ is time between the dephasing and the rephasing gradients. The term in brackets is the effective diffusion time.

DWI combines Skejskal-Tanner's method with echo-planar imaging (EPI) in order to obtain image-based diffusion information. The echo-planar T_2 -weighted spin-echo sequence used in DWI reduces the scan time and subsequent motion artifacts, enabling the measurement of diffusion properties in three orthogonal diffusion directions, and the subsequent calculation of the rotationally invariant ADC [49].

Diffusion tensor imaging (DTI) uses diffusion measurements with at least 6 non-collinear diffusion directions to calculate the diffusion tensor and to obtain information about the directionality of diffusion within each imaging voxel [50]. The diffusion tensor is a 3×3 matrix of the following form:

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \quad (1.5)$$

By diagonalizing the diffusion tensor, one can obtain the three eigenvectors (e_1, e_2, e_3) and corresponding eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) of the matrix. The first eigenvector, e_1 , is the direction that corresponds to the largest eigenvalue λ_1 and represents the direction in which diffusion is dominant. The second eigenvector e_2 corresponds to λ_2 and points along a direction that is orthogonal to the first eigenvector. The third eigenvector e_3 correspond to λ_3 and is orthogonal to both to e_1 and e_2 . If diffusion is isotropic, all eigenvalues are equal, whereas when it is anisotropic, the first eigenvalue is larger than the other two ($\lambda_1 > \lambda_2 > \lambda_3$ or $\lambda_1 > \lambda_2 = \lambda_3$).

Several diffusion metrics can be obtained from the diffusion tensor. Mean diffusivity (MD) is the average of the three eigenvalues and thus represents a rotationally invariant diffusion coefficient. Axial diffusivity (AD) is the first eigenvalue and thus represents the diffusion coefficient along the dominant diffusion direction. Radial diffusivity (RD) is the average of the second and third eigenvalues and thus represents an average of diffusion orthogonal to the dominant diffusion direction. Fractional Anisotropy (FA) [51] is a dimensionless measure of directionality in a voxel, is closely related to the standard

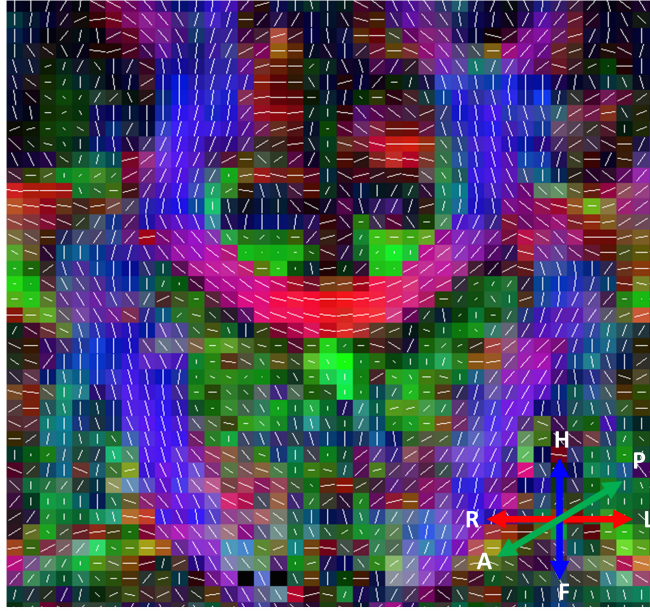


FIGURE 1.1: A coronal slice from a color-coded fractional anisotropy map of a healthy volunteer. The first eigenvector is shown for each voxel with the white lines. The color coding represents the direction of the first eigenvector.

deviation of the eigenvalues and is given by:

$$FA = \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}} \quad (1.6)$$

In the context of the human brain and in particular in white matter, which consists mostly of tightly organized tracts of long-range axons (white matter tracts), AD is assumed to be mainly associated with diffusion along the axonal propagation direction and RD is mainly associated with diffusion perpendicular to the axonal propagation direction, i.e. perpendicular to the axonal walls. The first eigenvector is also assumed to be the direction of the main axonal orientation within the DTI voxel. Figure 1.1 shows an example of a color-coded FA map where the first eigenvector is used to delineate the main fiber orientation per voxel.

DWI and DTI have been applied to many clinical conditions and have been shown to be sensitive both localized and diffuse changes in the brain due to various diseases including stroke, brain tumor, intracranial infections, head trauma, multiple sclerosis, neuropsychiatric systemic lupus erythematosus and many others [49].

The main drawback of DWI and DTI is their lack of compartmental specificity, which stems from the fact that it is a water-based technique. Water is by far the most abundant molecule in the human body, and as such is found in all brain tissue compartments, inside all cells - neurons, all types of glia: astrocytes, microglia and oligodendrocytes, as well as

in the myelin sheet and between cells in the extracellular space. Moreover, water exchange between compartments mixes the compartmental diffusion properties during the diffusion time of the MR measurement. Ultimately, diffusion measures obtained from DTI and DWI represent the summed effect of the diffusion properties of all tissue compartments and cell types, each with its own characteristic structure, tortuosity and viscosity. The lack of compartment- and cell-type specificity of DWI and DTI limits their ability to characterize tissue microstructure and to explain disease-related changes in them, as the results can seldom be unequivocally attributed to specific compartment or cell type.

1.3.1.3 ^1H MR Spectroscopy

Magnetic resonance spectroscopy (MRS) is a spectroscopic technique based on acquisition of nuclear magnetic resonance (NMR) data of a desired nucleus. Nuclei of the same type, e.g. ^1H nuclei, resonate at slightly different frequencies in different chemical environments, due to slight differences in the shielding of the magnetic field exerted by the electron cloud that surrounds them. This gives rise to a frequency spectrum, in which the various resonances can be assigned to known functional groups, and the concentrations quantified. The differences between the resonant frequencies and a reference resonant frequency are expressed by the chemical shift (δ):

$$\delta = \frac{(\omega_s - \omega_{ref})}{\omega_{ref}} \times 10^6 \quad (1.7)$$

where ω_s is the resonant frequency of a substance and ω_{ref} is the reference resonant frequency, which is that of the protons in tetramethylsilane (TMS). The chemical shift δ is a dimensionless quantity which is expressed in parts per million (ppm).

Pulse sequences that are used for MRS mainly aim to localize the MR spectrum within a volume of interest (VOI), where the spectral information can be used to identify different molecules within the VOI. Depending on the echo time, size and location of the VOI, and field strength of the MRI system that is used, the resulting ^1H MRS spectra of the human brain may contain distinct peaks from various brain metabolites including lactate, N-acetylaspartate (NAA), glutamate (Glu), glutamine (Gln), creatine (Cr), choline (Cho) and myo-inositol (myo-Ins) [52]. An example of a spectrum obtained from the human brain with a 7T MR system is shown in Figure 1.2 [53]. The distribution of metabolites across cell types in the brain is not homogenous [54]. Among these brain metabolites NAA and glutamate are mainly found in neurons, and myo-inositol and choline are predominantly found in glial cells [19]. By quantifying the absolute concentrations of the metabolites, it is possible to evaluate the molecular content within different tissue compartments and to follow changes in these concentrations in disease.

One of the earliest clinical in vivo applications of ^1H MRS showed higher choline and lower NAA peaks in brain tumors [55] than in surrounding healthy tissue. Many subsequent MRS studies investigated differences in NAA, Cr, Cho, lipids and lactate in high- and low-grade gliomas [56]. MRS studies of autoimmune diseases such as MS and SLE have shown lower levels of the NAA/Cr ratio in patients compared to healthy controls, providing evidence of neuronal involvement in MS [20, 57]. On the other hand, the higher Cho/Cr ratio found in MS patients compared to healthy controls has been linked to abnormal Cho mobility since this metabolite is generally found in insoluble form in normal tissue [58].

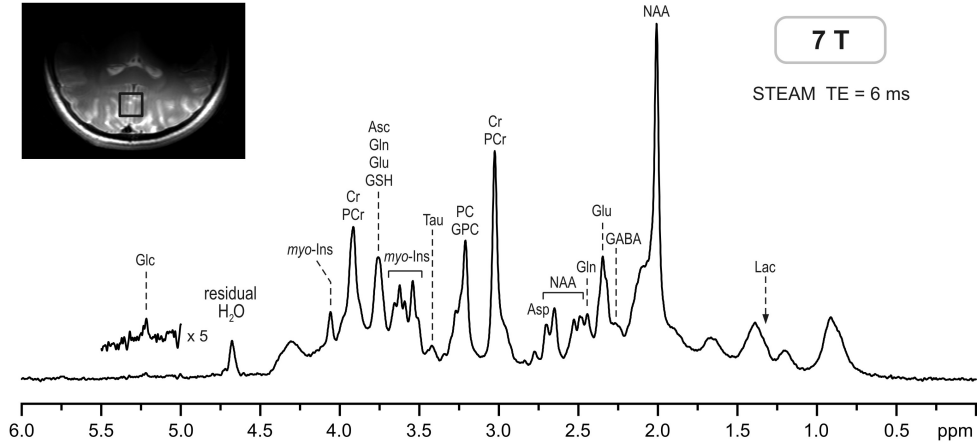


FIGURE 1.2: ^1H NMR spectra acquired with a STEAM sequence from the occipital lobe of human brain at 7T [53].

A limitation of MRS is that it does not provide microstructural information. The spectra obtained only reveal abnormalities in the metabolite concentration and therefore provide compartment-specificity to identify the type of cells that could be affected by a disease mechanism. However, MRS alone cannot reveal what type of disease-related structural alterations occurs in these cells.

1.3.1.4 Diffusion-weighted MR spectroscopy

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) combines the compartmental specificity of MRS with the sensitivity to microstructure of diffusion-based measurements. In this method, standard localized spectroscopy sequences are modified by adding diffusion sensitizing gradients [47]. An example of a DW-MRS sequence based on point resolved spectroscopy (PRESS) is shown in Figure 1.3.

In this sequence, the timing of the diffusion-weighting gradients with respect to the 180° pulses, as well as the choice of echo time, enable the selection of different diffusion times. To reduce the effect of eddy currents, a bipolar gradient scheme is used for all of the DW-MRS studies in this thesis. The implementation of the bipolar diffusion gradients in the PRESS sequence is shown in Figure 1.4.

In the case of bipolar diffusion gradients, the diffusion-weighting depends also on the bipolar gap, τ , and is given by [59]:

$$b = \gamma^2 g^2 \sigma^2 \left(\Delta - \frac{\delta}{3} - \frac{\tau}{2} \right) \quad (1.8)$$

As expected from equation (1.4), metabolite peaks in DW-MRS are attenuated by diffusion-weighting. An example of a set of diffusion-weighted spectra is shown in Figure 1.5, where the spectra acquired within a VOI in the human corpus callosum are shown as a function of b . By acquiring spectra with and without diffusion weighting, or with a set of

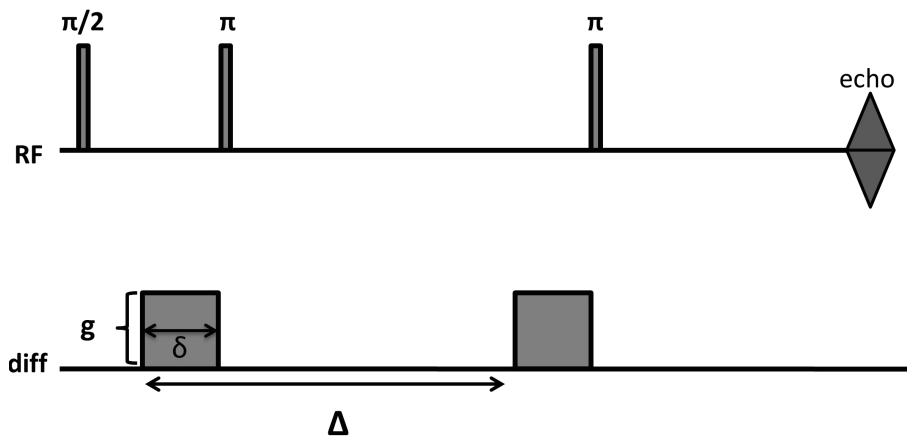


FIGURE 1.3: DW-MRS pulse sequence based on the PRESS method. g is the gradient amplitude, δ is the gradient duration, and Δ is the diffusion time.

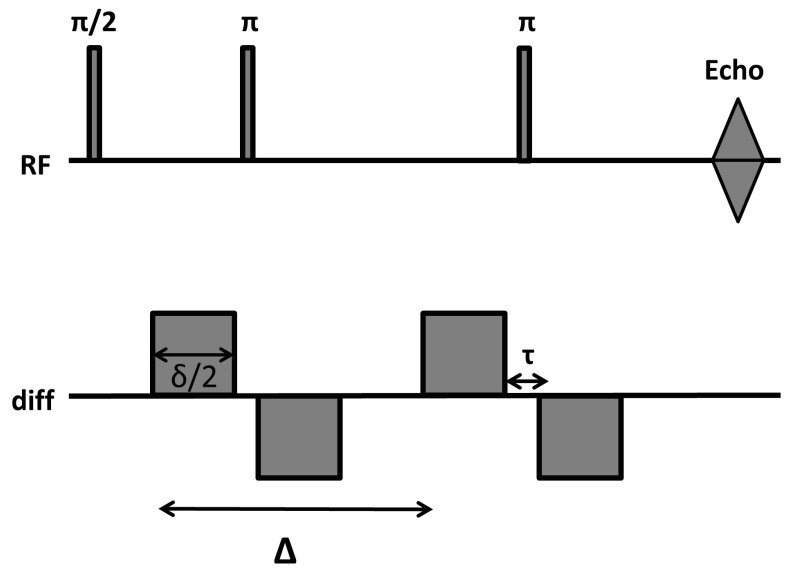


FIGURE 1.4: DW-MRS pulse sequence based on PRESS method with bipolar diffusion-sensitizing gradients.

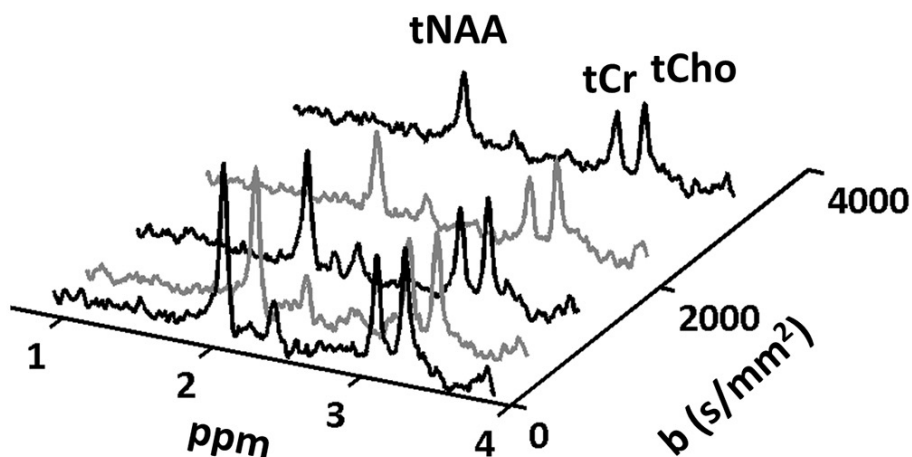


FIGURE 1.5: Typical spectra acquired with DW-MRS as a function of b . Line broadening of 5 Hz was applied to each spectrum for display purposes.

different diffusion weighting values, it is possible to calculate the ADC or other diffusion properties for each metabolite.

Since the first proof-of-concept DW-MRS study in humans [23], a number of studies have applied DW-MRS to study microstructural changes in disease. Two studies of ischemic stroke showed that the ADCs of NAA, Cr and Cho in acute ischemia were lower than those of healthy controls [36, 37]. These data corroborated the results of earlier studies performed on animal models of acute stroke [31, 32] and served as the first evidence of alteration in intracellular space due to stroke. Higher metabolite ADCs found in brain tumors compared to normal-appearing regions suggested changes in the cytosolic viscosity [36]. In MS, DW-MRS in a normal appearing region of the corpus callosum showed that the axial diffusivity of NAA was lower in patients than in healthy controls, suggesting intra-axonal tortuosity changes due to the breakdown of intra-axonal structures such as microtubules and neurofilaments [34]. In schizophrenia, higher ADC values observed for NAA were hypothesized to be associated with axonal abnormalities in white matter tracts associated with the disease [60]. These clinical research studies with DW-MRS thus show the potential of DW-MRS to elucidate disease-related mechanisms reflected in microstructural changes.

While DW-MRS has been shown to reveal compartment-specific disease-related changes in human brain, the number of DW-MRS applications is still relatively small. Significant improvements need to be made in order for the method to become more appealing to the clinical community. The inherently low SNR of DW-MRS, dictated by the lower concentration of brain metabolites compared to that of water [52], causes large variability in the data, especially when relatively small VOIs are used. This may be improved by repeating the same diffusion condition and increasing SNR by averaging at the expense

of longer scan times, which subsequently limit the clinical feasibility of the method. The lower diffusion coefficients of metabolites compared to that of water [21] necessitate the use of higher b -values to obtain a sizeable diffusion-weighting effect on the metabolite signals. These can be achieved by using either high gradient strengths or long gradient durations. Since the maximum gradient strength achievable with most MRI systems is limited, long diffusion gradient durations are required, resulting in long echo times. Macroscopic motion, such as the effect of cardiac pulsation, is another factor that can potentially severely affect the accuracy of DW-MRS measures. Motion causes incoherent phase shifts between successive acquisitions and therefore leads to signal loss when averaging: therefore, special acquisition and post-processing methods have to be devised to correct for these effects. Lack of standardization in the acquisition and post-processing makes it also difficult to evaluate and compare results obtained with DW-MRS. All these issues must be accounted for and resolved for the accurate measurements of metabolite diffusion.

The work in this thesis addresses some of the issues that impede the clinical applications of DW-MRS. Investigations in the following chapters of this thesis, that include both methodological and clinical aspects, are aimed at increasing the future applications of DW-MRS.

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Part I

Method Developments and Neurobiological Applications

2

Axonal and glial microstructural information obtained with diffusion-weighted magnetic resonance spectroscopy at 7T

This chapter was adapted from:

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Abstract

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) offers unique access to compartment-specific microstructural information on tissue, and potentially sensitive detection of compartment-specific changes in disease. The specificity of DW-MRS is, however, offset by its relative low sensitivity, intrinsic to all MRS-based methods, and further exacerbated by the signal loss due to the diffusion weighting and long echo times. In this work we first provide an experimental example for the type of compartment-specific information that can be obtained with DW-MRS from a small volume of interest in brain white matter. We then propose and discuss a strategy for the analysis of DW-MRS data, which includes the use of models of diffusion in compartments with simple geometries. We conclude with a broader discussion of the potential role of DW-MRS in the characterization of tissue microstructure and the complementarity of DW-MRS with less specific but more sensitive microstructural tools such as diffusion tensor imaging.

2.1 Introduction

THE characteristic dimensions of microstructural features of living tissue are several orders of magnitude smaller than the typical spatial resolution obtained by magnetic resonance imaging (MRI). Yet, MRI has become a ubiquitous tool for investigating tissue microstructure *in-vivo*, thanks to the direct relationship between the motional properties of the nuclei that generate the MR signal and the properties of the nuclear magnetic resonance (NMR) signal that ensues. In particular, self-diffusion of spins in a magnetic field gradient generates a signal loss that is controlled by the characteristics of the gradients as well as by geometric factors that hinder or restrict the diffusion process over time. Incorporation of pulsed magnetic field gradients in MR pulse sequences led to the most robust manner of sensitizing the MR signal to self-diffusion [1] and has since been frequently used in the investigation of numerous aspects of tissue microstructure, including characterization of disease processes [2], exploration of white matter connectivity [3, 4] and quantitative evaluation of microstructural features such as axonal diameter and myelination [5–7].

The explanatory power of diffusion weighted (DW) MRI methods is limited, however, by the lack of specificity of the signal source, namely, the hydrogen protons in water molecules. The ubiquity of water in living tissue (~65% in volume) accounts for the high sensitivity of MRI when compared to MR of other tissue constituents, but the lack of compartmental specificity complicates the interpretation of DW-MRI results. For example, competing disease processes that simultaneously act on several tissue compartments may have similar, or opposed effects on diffusion properties of water in those compartments. This, in turn, generates a high degree of ambiguity in the interpretation of the DW-MRI results, or in other instances dilutes the information related to the specific process of interest. Examples include DW-MRI of stroke, where the decrease in apparent diffusion coefficient (ADC) in the ischemic region can be attributed to several competing effects that influence the ADC: increase in restriction in the diffusion of water in the extracellular compartment, influx of water into the restricted intracellular compartment, and the cessation of active transport (cytosolic flux) in the affected cells. In the case of multiple sclerosis and other neurological disorders, damage to myelin may increase water ADC and reduce the fractional anisotropy (FA) of the diffusion tensor, but neuronal/axonal damage may reduce overall ADC. It is thus desirable to devise a MR-based modality with an intrinsic ability to provide compartment specific information about tissue microstructure. Several approaches to achieve this goal have been suggested, including high-level modeling of DW-MRI acquired at a variety of diffusion-weighting values and directions [8, 9]. A more direct way to tackle the issue of compartment specificity is to measure the diffusion properties of endogenous spin species that reside in specific tissue compartments and are concentrated enough to yield a visible MR signal with reasonable signal-to-noise ratio (SNR). In neural tissue, these are intracellular metabolites in the mM concentration range and give rise to distinct spectroscopic patterns in a magnetic resonance spectroscopy (MRS) experiment. Some metabolites are almost entirely cell-specific, e.g. N-acetylaspartate (NAA) and the excitatory neurotransmitter glutamate (Glu), which reside both almost exclusively in neurons/axons, and myo-inositol (mI) and glutamine (Gln), which are thought to primarily reside in astrocytes [10, 11]. Other intracellular metabolites, e.g. creatine and phosphocreatine (Cr and PCr) are less specific and have a well-defined role in cellular metabolism, while others are preferential

to a specific cell type but not exclusively so, e.g. choline-based molecules, which are believed to be more concentrated in glia than in neurons at a ratio of approximately 3:1 [11]. Given the low concentration of these metabolites, the sensitivity of their detection is much lower than that of water, and thus the spatial resolution that can be achieved with diffusion-weighted spectroscopic measurements is similar to that of MRS methods in general, i.e. typical size of a volume of interest (VOI) in human experiments is several milliliters.

DW-MRS studies have been used to probe excised neural tissue [12, 13]; *In-vivo* DW-MRS studies in small animals and primates were performed on muscle [14–16] and brain [17–22]. Robust measurements have been performed in humans, [23–27]. DW-MRS studies at 7T provided tissue-specific information on several metabolites [28] and provided sensitive diffusion tensor MRS (DTS) data on NAA from small portion of the corpus callosum in a group of subjects with multiple sclerosis (MS) [29].

In this work we explore the potential of DW-MRS to provide compartment-specific information by focusing on a set of DW-MRS results from a small portion of the corpus callosum. We show that the DW-MRS data *per-se* dependably reflects microstructural features of the structures populated by the metabolites on which the measurements are performed. We then discuss the potential role that DW-MRS may have in the evaluation of microstructural properties of tissue in conjunction with other methods, and its optimal positioning in the arsenal of MR-based microstructural tools.

2.2 Materials and methods

2.2.1 Human subjects

A total of twelve healthy volunteers (six females, six males, age: 26 ± 4 years) participated in this study. The study adhered to the LUMC Institutional Review Board guidelines and informed consent was obtained from all subjects prior to the study.

2.2.2 MRI scanner/Hardware

All experiments were performed on a 7 Tesla Philips Achieva whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with gradient coils capable of a maximum gradient strength of 40 mT/m and a slew rate of 200 T/m/s. A head coil consisting of a quadrature birdcage transmit and 32-channel phased array receive was used for all measurements (Nova Medical Inc., Wilmington, MA, USA).

2.2.3 MRI/MRS protocols

Anatomical images: A short survey scan and a sensitivity encoding (SENSE) reference scan were followed by a 3D T1-weighted gradient echo acquisition to allow for accurate planning of the experiment. Imaging parameters were: field of view (anterior-posterior (AP), foot-head (FH), right-left (RL)): $246.4 \times 246.4 \times 174.0 \text{ mm}^3$, resolution $1 \times 1 \times 1 \text{ mm}^3$, TR/TE = 4.7/2.1 ms. Total scan time was 3 minutes.

DTI protocol: A single subject (male, 25 years) was scanned. Multi-slice single-shot 2D spin-echo echo planar imaging (EPI) (60 slices of 2 mm thickness, TR/TE = 9100/58 ms), field of view of $224 \times 224 \times 120 \text{ mm}^3$ with an in-plane data matrix of 112×112 , resulting in a data set with resolution of $2 \times 2 \times 2 \text{ mm}^3$, 32 diffusion weighting directions with $b=1000 \text{ s/mm}^2$. Fat suppression was performed using spectral inversion recovery (SPIR). Parallel imaging was performed using SENSE with a reduction factor of 3 along the phase encoding direction (AP). Total scan time was 6 minutes.

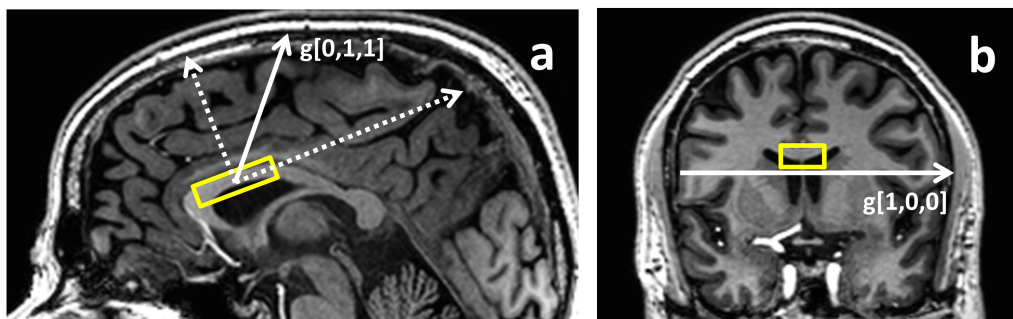


FIGURE 2.1: Typical placement of the DW-MRS VOI (a) on the sagittal plane and (b) on the coronal plane. The gradient directions used in the experiment are $g_{[0,1,1]}$, which is mostly perpendicular to the callosal fibers, and $g_{[1,0,0]}$, which is mostly parallel to them.

DW-MRS: The volume of interest (VOI) for the experiment was the anterior body of the corpus callosum (Witelson segments A3-A4 and part of A2, see Figure 2.1 for a typical VOI positioning). VOI dimensions were 25 mm (AP), 10 mm (RL) and 8 mm (FH) for a total volume of 2 cc. Slight variations in positioning of the VOI across subjects were necessary in order to adjust to slightly different patterns of anterior-posterior curvature. The sequence used for the DW-MRS experiment was a point-resolved spectroscopy (PRESS) sequence (TE = 121 ms) supplemented with a bipolar diffusion weighting scheme [28]. Cardiac synchronization on every third cardiac cycle was achieved via a pulse-oximeter resulting in a TR of about 3 s and was used to avoid strong fluctuations in signal intensity due to cardiac pulsation. Number of time-domain points = 2048, spectral width = 3000 Hz. Shimming up to second order was performed with pencil beam excitation and a typical tNAA line width was about 10 Hz throughout the experiment.

Two directions with respect to the VOI coordinates were chosen for the diffusion weighting: (a) a pure right-left direction, thus mostly parallel to the direction of the callosal fibers); (b) one mostly perpendicular to the callosal fibers, forming a 45° angle between the anterior-posterior axis and the inferior-superior axis of the VOI (see diagram in Figure 2.1). These gradient directions can be denoted in the VOI coordinates as $[1,0,0]$ and $[0,1,1]$, and $g_{[0,1,1]} = \sqrt{2} \cdot g_{[1,0,0]}$ for a specific choice of gradient strength. Diffusion weighting parameters were: gradient pulse duration: 34 ms, diffusion time (Δ) = 60.5 ms, 8 equally spaced gradient amplitude values between 0 and 3.15 gauss/cm resulting in a set of b-values of 0-6594 s/mm² for $g_{[0,1,1]}$ and 0-3297 s/mm² for $g_{[1,0,0]}$. Each of the 15 direction/gradient amplitude conditions (2 directions $\times$ 7 gradient values + one acquisition without diffusion weighting) was acquired 48 times for a total set of 720 individual free induction decays (FIDs) and a total scan time of ~ 35 minutes. “Weak” water suppression was performed in order to preserve enough of the water signal (about 5-10 times the NAA singlet for all conditions) for subsequent phasing and frequency drift correction for each individual acquisition. To help enable this condition for the higher b-values, water suppression was performed in a suboptimal manner by providing only a fraction of the RF power needed for full water suppression. Following the DW-MRS experiment, a short DW-MRS acquisition (~ 3 minutes) with the same diffusion weighting

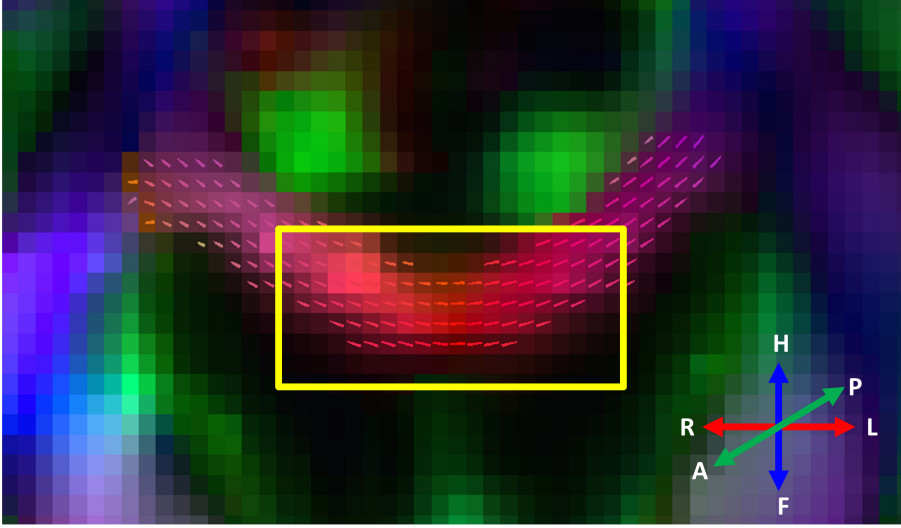


FIGURE 2.2: Color-coded FA map of a coronal slice through the body of the corpus callosum. The DW-MRS VOI dimensions (20 mm RL $\times$ 8 mm FH) in that plane are represented by the yellow rectangle. The ticks in each voxel represent the direction of the main diffusion eigenvector. The resulting directional range is significant, approximately $\pm 20^\circ$.

conditions and 4 acquisitions/condition was performed without water suppression and subsequently used for eddy current correction.

Given the relatively large chemical shift displacement caused by the relatively low bandwidth of the 180° pulses in the PRESS sequence (about 4 mm for the chemical shift difference between the tNAA singlet (2.0 ppm) and the choline singlet (3.2 ppm) in the FH and LR directions), six of the subjects (four females, two males, 25.7 ± 4.8 years) were scanned with the frequency centered around the tNAA peak and the other six (two females, four males, 27.3 ± 3.4 years) centered around the choline peak. For the six subjects scanned with the frequency at 2.0 ppm we report only the tNAA results, and for those scanned with the frequency at 3.2 ppm, results are reported for both tCho and tCr, as the displacement of about 0.5 mm between the choline and the creatine/phosphocreatine singlet was deemed negligible.

2.2.4 Post-processing and Analysis

DTI processing was performed using ExploreDTI [30]. A color coded fractional anisotropy (FA) map and a 2D main eigenvector tick map were generated and displayed in Figure 2.2.

2.2.4.1 DW-MRS data processing

Data processing was performed using a custom written program in Matlab[®] (Mathworks Inc, Natick, USA) as described previously [28]. The main steps in the data processing included: weighted summation and phasing of the individual outputs of the 32 receive coils based on reference water signal, eddy current correction, zero-order phase correction,

frequency drift correction and subsequent averaging. The resulting spectra were analyzed using LCModel [31] with an appropriate simulated set of basis spectra.

2.2.4.2 Modeling of the DW-MRS diffusion data

Analysis of DW-MRS data has been performed in two ways. First, a non-biased analysis was carried out, where general diffusion properties for the three main metabolites were calculated: $D_{||}$, $D^{\perp}$, ADC and pseudo-FA ($D_{||}/D^{\perp}$). These properties were calculated based on the assumption of monoexponential decay with respect to b value:

$$S(b_i) = S(0)e^{-b_i \cdot D_i} \quad (2.1)$$

where $S(b)$ is the measured signal, $S(0)$ is the signal without diffusion weighting, b_i is the b-value in the direction i and D is the calculated diffusion coefficient in that direction.

2.2.4.3 Modeled analysis of the DW-MRS signal

Expressions for signal attenuation of the NMR signal in the presence of magnetic field gradients, when the spin motion is restricted was first formulated by Neuman [32] and applied to the Stejskal-Tanner experiment by Murday and Cotts [33]. The theory was extended to cover a variety of geometries [34, 35] and succinctly formulated in the following expression [36] for planar, cylindrical and spherical pores:

$$\ln(S_{\perp}) = -2\gamma^2 g_{\perp}^2 \sum_{m=1}^{\infty} \frac{1}{\alpha_m^2 (\alpha_m^2 R^2 - n_D)} \times \frac{2\alpha_m^2 D\delta - 2 + 2e^{-\alpha_m^2 D\delta} + 2e^{-\alpha_m^2 D\Delta} - e^{-\alpha_m^2 D(\Delta-\delta)} - e^{-\alpha_m^2 D(\Delta+\delta)}}{(\alpha_m^2 D)^2} \quad (2.2)$$

where $n_D = 2$ for cylinders and 3 for spheres. α_m satisfies the equation $j_1'(\alpha_m R) = 0$, where j_1' is the derivative of the Bessel function (for cylinders) or spherical Bessel function (for spheres) of the first kind and first order, and R is the radius of the cylinder or sphere, respectively.

2.2.4.4 Modeling the tNAA DW-MRS signal

Analysis of the tNAA signal was based on the assumption that in white matter tNAA resides exclusively in axons. An estimate of the typical axonal diameter in the callosal region in which the DW-MRS measurements were performed was obtained from [37]. Accordingly, about 75% of axons in the region of interest possess an inner diameter D_{ax} such that $D_{ax} < 1.5 \mu\text{m}$, and for 24% of these axons $1.5 \mu\text{m} < D_{ax} < 4.5 \mu\text{m}$. These values take into account about 30% post-mortem shrinkage in the reported values, as described in [37]. Axonal geometry inside the VOI is assumed to be a stack of curved cylinders, possessing a macroscopic curvature as well as a microscopic misalignment, or “fanning”. In DTI analysis, macroscopic curvature of axonal tracts can be for the most part neglected, but in the case of DW-MRS the dimensions of the VOI are of the same order of magnitude of the radius of curvature of the tract and thus the curvature cannot be neglected. This can be seen in Figure 2.2, in which a coronal slice of a directionally color-coded FA map is shown, along with a representation of a typical VOI in that plane. The variability of the fiber directionality within the VOI, as represented by the projections of the main diffusion tensor eigenvectors on the plane, is clearly visible. Significant microscopic misalignment has been reported in the corpus callosum of the rat [38] and the macaque monkey [39],

and is reported in humans in this research topic by Budde et al. [40], and thus has to be introduced as well.

We base our formulation of the diffusion of tNAA in the axons on previous works [41, 42]. For a single axon at an angle ψ to the direction of the gradient, the diffusion-weighted signal S is the product of the diffusion perpendicular and parallel to the axonal wall:

$$S(\mathbf{q}_j, \Delta) = S_{\perp}(\mathbf{q}_{j\perp}, \Delta) \cdot S_{\parallel}(\mathbf{q}_{j\parallel}, \Delta) \quad (2.3)$$

where Δ is the diffusion time, $\mathbf{q}_j = \frac{\delta\gamma\mathbf{g}_j}{2\pi}$ (δ is the duration of the pulsed field gradient, γ is the gyromagnetic ratio of protons, $\mathbf{g}_j$ is the diffusion weighting gradient vector in the direction j), $\mathbf{q}_{j\parallel} = \mathbf{q}_j \cdot \cos\psi_{ij}$ and $\mathbf{q}_{j\perp} = \mathbf{q}_j \cdot \sin\psi_{ij}$, and $\pi/2 - \psi_{ij}$ is the angle between $\mathbf{q}_j$ and an axon in direction i . $S_{\perp}$ is given by equation (2.1) expressed for the cylindrical case, and $S_{\parallel}$ is assumed to be governed by non-restricted diffusion, i.e

$$S_{\parallel}(\mathbf{b}_{\parallel}, \Delta) = S_{\parallel}(0) \cdot e^{-\mathbf{b}_{\parallel} \cdot D} \quad (2.4)$$

where $\mathbf{b}_{\parallel} = \gamma^2 \delta^2 \mathbf{g}_{\parallel}^2 (\Delta - \frac{\delta}{3})$, and D is the diffusion coefficient of tNAA in the cytoplasm, from here onward $D(\text{tNAA})$.

To account for both the macroscopic curvature within the VOI and the microscopic axonal misalignment, a distribution of the angle ψ_{ij} is introduced, where $\psi_{ij} = \theta_{ij} + \phi_{ij}$, where θ_{ij} is the macroscopic contribution and ϕ_{ij} accounts for axonal misalignment. It is assumed that the displacement of NAA molecules during the diffusion time Δ is much smaller than the radius of curvature, hence θ_{ij} along this distance remains constant. In this case expression (3) is modified to yield the following form [42]:

$$S(\mathbf{q}_j, \Delta) = \frac{\sum_{i=1}^N p(\psi_{ij}) \cdot \sin(\psi_{ij}) \cdot S_{\perp}(\mathbf{q}_j \cdot \cos(\psi_{ij}), \Delta) \cdot S_{\parallel}(\mathbf{q}_j \cdot \sin(\psi_{ij}), \Delta)}{\sum_{i=1}^N p(\psi_{ij}) \cdot \sin(\psi_{ij})} \quad (2.5)$$

For simplicity, it is assumed that the angles ψ_{ij} belong to a Gaussian distribution with zero mean and standard deviation σ_{ψ} :

$$p(\psi|0, \sigma_{\psi}) = \frac{1}{\sigma_{\psi} \sqrt{2\pi}} \cdot \exp\left(\frac{-\psi^2}{2\sigma_{\psi}^2}\right) \quad (2.6)$$

A more appropriate description of angular distribution for the three-dimensional fanning of axons about a specific direction is the Bingham distribution [43], applied in a number of studies that examined axonal fanning based on diffusion weighted images [39, 44]. Here we chose the Gaussian distribution for simplicity, keeping in mind that the actual angular distribution, and in particular the portion attributed to the macroscopic curvature of the axonal tract, will be best described empirically rather than analytically. As the last step, the diffusion weighted data in both directions was simultaneously fitted to the model in equation (2.5) using two free fitting variables: $D(\text{tNAA})$, the diffusion coefficient of tNAA in the direction parallel to the fibers, and σ_{ψ} , the standard deviation of the total axonal dispersion. A diagram of the analysis procedures is given in Figure 2.3.

2.2.4.5 Modeling the tCr and tCho DW-MRS signal

Analysis of the tCr and tCho signals must assume a significant contribution from glial content of these metabolites. The geometric representation of glia is rather complex,

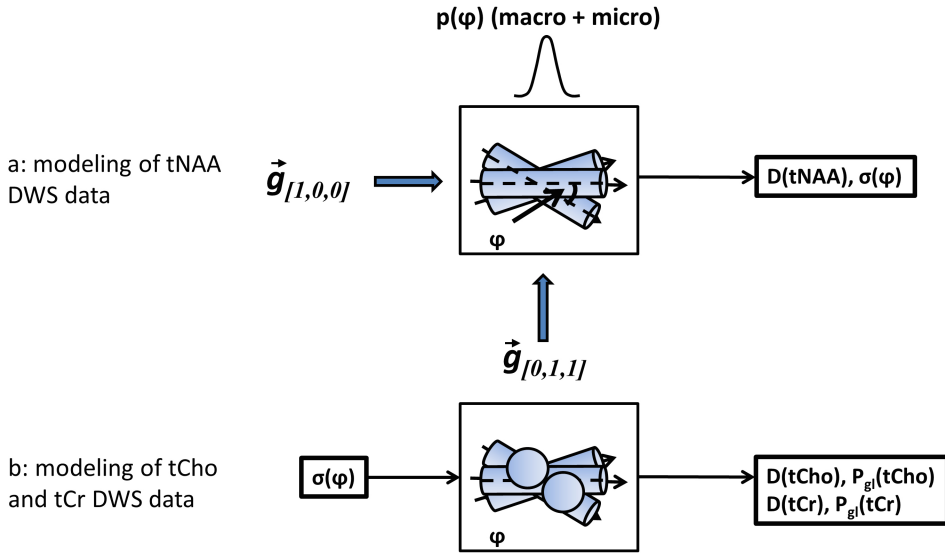


FIGURE 2.3: A schematic representation of the modeling steps. In step (a) the tNAA DW-MRS data in both diffusion-weighting directions is fitted to a model of diffusion in a set of misaligned cylinders with a gaussian angular distribution with standard deviation σ_ψ . The fitting variables are the diffusion coefficient of tNAA and σ_ψ . In step (b), the DW-MRS signal of the two non-axonal specific metabolites, tCho and tCr is fitted to a mixed model of cylinders (axons) and spheres (glia), with σ_ψ obtained earlier as one of the input parameters. The fitting variables for each metabolite data are its diffusion coefficient, $D(\text{tCho})$ or $D(\text{tCr})$, and its glial fraction, $P_{\text{gl}}(\text{tCho})$ or $P_{\text{gl}}(\text{tCr})$, respectively.

as the cells consist of a relatively large, oblong cell body with long, thin projections [45, 46]. One extreme simplification is to assume that most of the cellular volume is represented by the cell body, approximated by a spherical pore. In order to obtain a representative compartment size, it is then possible to use equation (2.2) for the spherical case. Since neither tCho nor tCr are exclusively astroglial, a complete analysis for S_M , the diffusion-weighted signal for the metabolite in question, should include an axonal fraction and a glial fraction, $P_{M,\text{gl}}$ and $P_{M,\text{ax}}$, respectively, weighing the DW-MRS signal contribution from these compartments, $S_{M,\text{gl}}$ and $S_{M,\text{ax}}$:

$$S_M[\delta, \Delta, \mathbf{g}_i] = P_{M,\text{gl}} \cdot S_{M,\text{gl}}(r_{\text{gl}}, D_M, [\delta, \Delta, \mathbf{g}_i]) + P_{M,\text{ax}} \cdot S_{M,\text{ax}}(r_{\text{ax}}, D_M, \sigma_\psi, [\delta, \Delta, \mathbf{g}_i]) \quad (2.7)$$

Here, D_M is the diffusion coefficient of the metabolite, r_{gl} is the typical radius of the glial cell body (assumed to be a sphere), r_{ax} is the typical axonal diameter and σ_ψ is the axonal angular dispersion. If σ_ψ is previously estimated through the DW-MRS of the axonal constituent (tNAA), it can be used in fitting equation (2.7) to the DW-MRS data of the tCr or tCho, for further estimation of $P_{M,\text{gl}}$, $P_{M,\text{ax}}$ and D_m .

2.2.4.6 Statistical analysis of residuals for the modeled data

The quality of the fit of the experimental DW-MRS data to the proposed models was assessed performing a χ^2 goodness-of-fit test for the residuals. For this purpose, we used the Cramér-Rao Lower bounds (CRLB) of the spectroscopic measurements for the value of the variance of the single measurement. Typical CRLB values for the condition $b = 0$ s/mm² (in percent of the estimated peak integral) were 4% for the tNAA, 6% for the tCr and 6% for the tCho. The highest CRLB values were those for the maximum b-value in the [1,0,0] condition and typical values were 18% for the tNAA, 13% for the tCr and 13% for the tCho.

2.3 Results

Typical diffusion weighted spectra are shown in Figure 2.4 for $b = 0$, 6594 and 2422 s/mm². The spectrum in the top panel was acquired without diffusion weighting. The one below was acquired with the largest b value in the direction [0,1,1], which is mostly perpendicular to the callosal fibers. The bottom spectrum was acquired with $b = 2422$ s/mm² in the [1,0,0] direction, which is roughly parallel to the callosal fibers. It is seen that the difference between the tNAA peak heights in the two diffusion weighted spectra is significantly larger than the difference between the peak heights of the tCho and tCr. DW-MRS data for the three metabolites: tNAA, tCho and tCr, averaged over the 6 subjects scanned for tNAA and the 6 subjects scanned for tCho + tCr are shown in Figure 2.5. A qualitative estimate of the diffusion directionality, or anisotropy, for each of the three metabolites is provided by calculating $D_{[1,0,0]}/D_{[0,1,1]}$, the ratio between a single diffusion coefficient calculated for the set of measurements of diffusion parallel, and one calculated for the diffusion perpendicular, to the fibers. Table 2.1 summarizes these data. It can be seen that $D_{[1,0,0]}/D_{[0,1,1]}$ is the highest for tNAA (above 5), roughly twice the value for tCho and tCr. Interestingly, the values for $D_{[0,1,1]}$ are rather similar for all three metabolites, $D_{[0,1,1]}$ for tNAA being slightly lower than the other two, and substantial differences are found between $D_{[1,0,0]}$ of tNAA and those of tCr and tCho.

2.3.1 Modeling of tNAA signal

Figure 2.6 shows the DW-MRS tNAA results from a single subject, to which the model described in the method section was fitted. The model fits the data well, well within the goodness-of-fit acceptance criterion ($\chi^2(14) < 6.3$, $p > 0.9$ for all subjects). The two fitting variables were σ_ψ , the standard deviation of the axonal angular dispersion, and $D(\text{tNAA})$, the diffusion coefficient of tNAA in the cytoplasm. In order to exemplify the effect of σ_ψ , a suboptimal fit is shown in the left panel, in which σ_ψ is about 50% of its optimal value. In that case, the diffusion effect is underestimated for $D_{[0,1,1]}$ (mostly perpendicular to the fibers) and overestimated for $D_{[1,0,0]}$ (mostly parallel to the fibers). The average results for $D(\text{tNAA})$ and σ_ψ for the six subjects are given in Table 2.1.

2.3.2 Modeling of tCho and tCr signal

Choline soluble compounds are found in astrocytes and neuron/axons at a ratio of about 3:1, whereas creatine and phosphocreatine are ubiquitous in neural tissue, regardless of cell type [11]. A comprehensive analysis of the diffusion weighted tCho and tCr signals should thus consider the contribution of both glial and axonal compartments, as is explicitly specified in equation (2.7). In order to fit the data to the model in equation (2.7), the axonal angular dispersion σ_ϕ found for the tNAA was used as an input parameter for

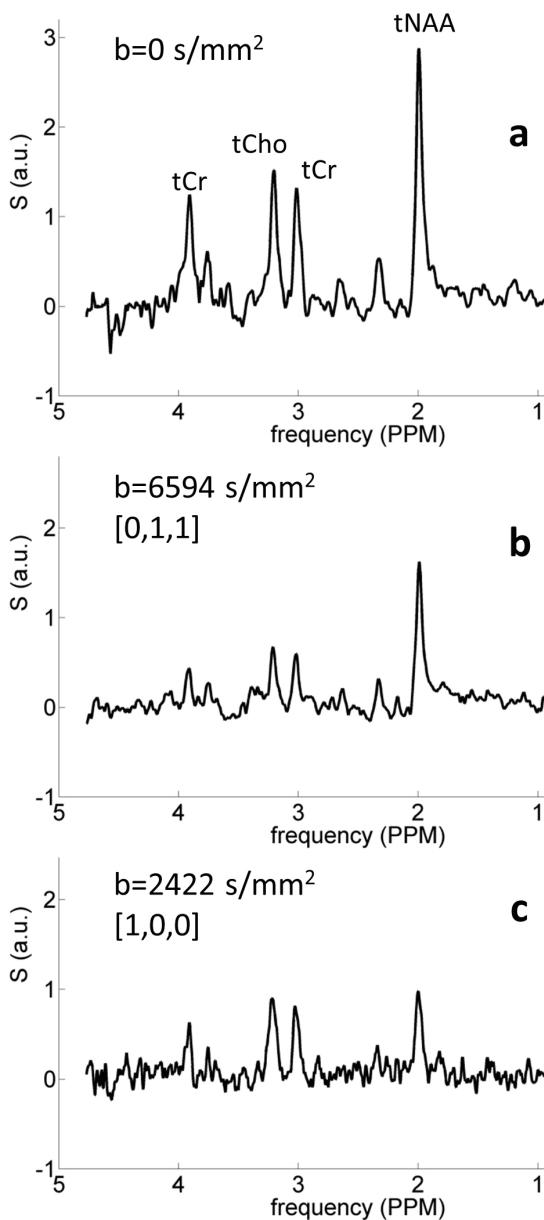


FIGURE 2.4: Typical spectra acquired from one of the subjects. (a) spectrum acquired without diffusion weighting; (b) spectrum acquired in the direction $[0, 1, 1]$ with $b = 6594 \text{ s/mm}^2$, the largest b -value used here; (c) spectrum acquired with $b = 2422 \text{ s/mm}^2$ in the direction $[1, 0, 0]$. The strong diffusional anisotropy of the tNAA signal is clearly seen.

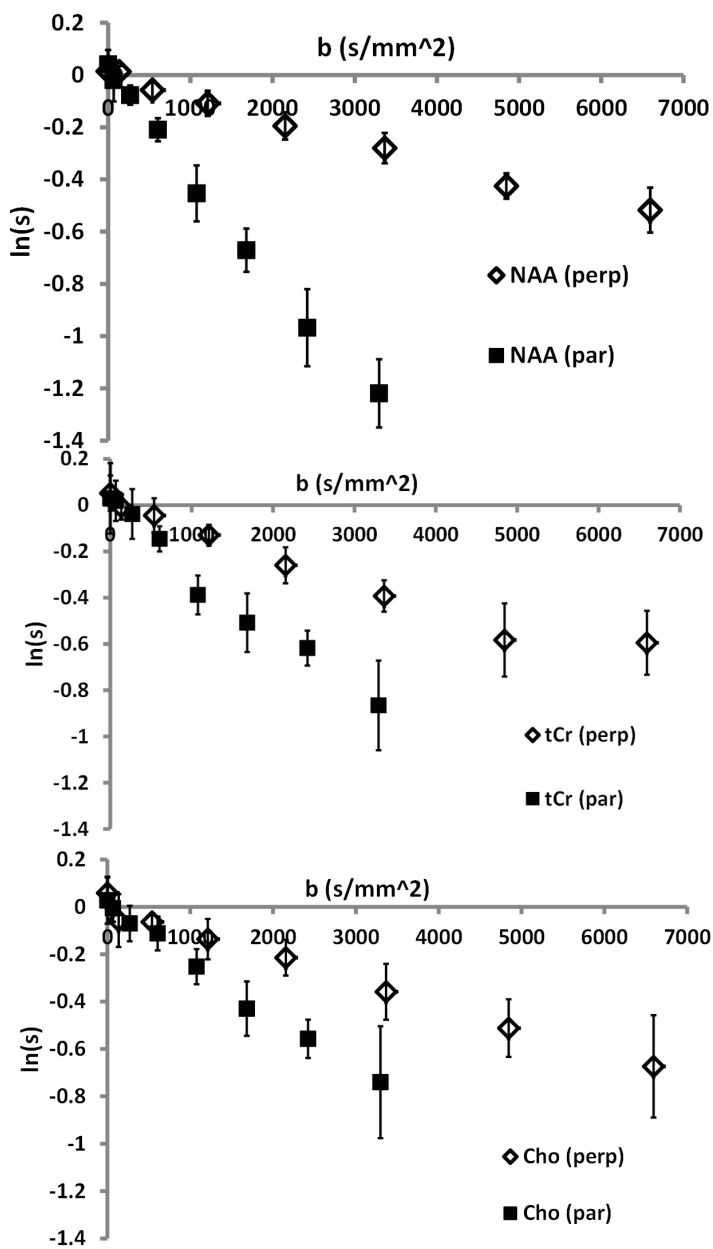


FIGURE 2.5: Group-averaged data for the three main metabolites: top - tNAA, middle - tCr, bottom - tCho.

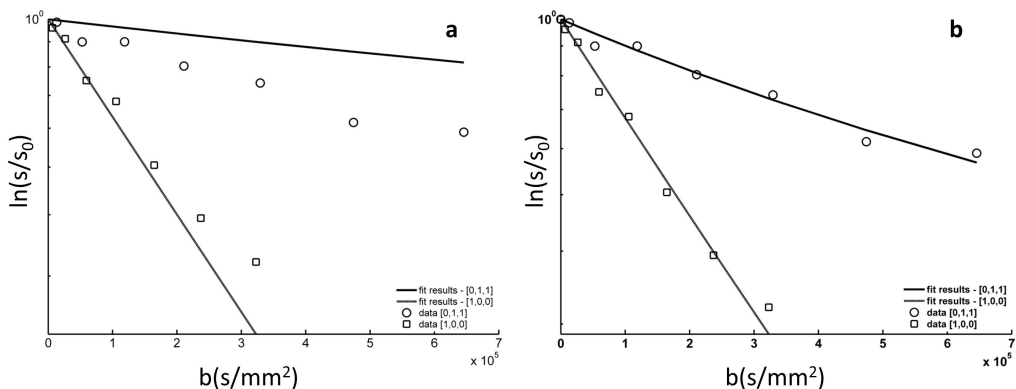


FIGURE 2.6: : tNAA DW-MRS data from a single subject (symbols) fitted to the model described in the methods section and in step (a) in figure 3. (a): the data fitted to the model with one fitting variable, $D(tNAA)$, and $\sigma_\psi = 10.0^\circ$; (b) the data fitted to the model with two fitting variables, $D(tNAA)$, and σ_ψ . In this case $D(tNAA) = 0.43 \times 10^{-3} \text{ mm}^2/\text{s}$ and $\sigma_\psi = 22.3^\circ$.

TABLE 2.1: Group-averaged DW-MRS data ($n = 6$ for tNAA and $n = 6$ for tCr and tCho) for the three metabolites.

	tNAA	tCr	tCho
ADC (mm^2/s)	0.240 ± 0.010	0.180 ± 0.010	0.160 ± 0.020
$D_\perp$ (mm^2/s)	0.079 ± 0.003	0.095 ± 0.004	0.093 ± 0.007
$D_\parallel$ (mm^2/s)	0.410 ± 0.010	0.260 ± 0.020	0.220 ± 0.020
$D_\parallel/D_\perp$	5.200 ± 0.030	2.800 ± 0.040	2.400 ± 0.040
D_{model} (mm^2/s)	0.480 ± 0.050	0.370 ± 0.060	0.420 ± 0.080
$\sigma_\psi (^\circ)$	21.10 ± 2.600	-	-
P_{glia}	-	$0.400 \pm 0.100^*$	$0.500 \pm 0.100^*$

the modeling of the two remaining metabolite data, and the only fitting parameters were the glial fractions for the two metabolites, $P_{\text{gl}}(\text{tCho})$ and $P_{\text{gl}}(\text{tCr})$, and the cytoplasmic diffusion coefficients of tCho and tCr, $D(\text{tCho})$ and $D(\text{tCr})$ respectively. The cell body radius was not set as a free variable, but was gradually incremented until a stable fit with a constant error was obtained. This occurred at $r_{\text{gl}} = 9 \mu\text{m}$, which is well within the range of astrocyte cell body radius [45]. The model fits the data reasonably well, with $\chi^2(14) < 11.7$, $p > 0.5$ for all subjects). The group averages of the diffusion coefficient and the glial fractions for tCho and tCr are given in Table 2.1. The difference between $P_{\text{gl}}(\text{tCr})$ and $P_{\text{gl}}(\text{tCho})$ was statistically significant (p-value = 0.011, paired t-test).

2.4 Discussion

The estimated $D_\parallel$ and $D^\perp$ for the three metabolites measured in this work qualitatively display the most salient difference in compartmentation between tNAA, which is predominantly present in neuronal and axonal cytoplasm, and tCho and tCr, which are not

unique to neurons and are also found in glial cells, mostly in astrocytes. In this work, the DW-MRS data for tCr and tCho were acquired separately from those for tNAA, introducing inter-individual differences as a possible confound, and it is thus desirable to devise a shortened acquisition protocol that allows to acquire these data from the same subject. The good reproducibility of the DW-MRS results for each metabolite, as can be appreciated from the results shown in Figure 2.5, is encouraging, and indicates that inter-subject variability is relatively low. From the structural perspective, it is useful to consider the intra-axonal space as cylinders with an inner diameter that is mostly in the 1 - 5 μm range [37], and astrocytes as represented by slightly oblong oval structures with average diameter of about 10 - 20 μm [45, 47] with thin processes that radiate radially from the soma in a rather isotropic fashion [46]. It has been established that tNAA is almost exclusively intracellular [48], and thus any contribution from “hindered” diffusion in the extracellular matrix can be neglected. The picture may be somewhat more complex for tCr and tCho, which both cross the blood brain barrier and are then cleared from the extracellular space through specialized transporters, although in normal conditions the levels of both extracellular tCr and tCho are low compared to intracellular levels [49–51].

The strong structural anisotropy of axons is reflected by the significantly higher $D_{[1,0,0]}$ value for tNAA than that for tCr and tCho. A strong incentive for an accurate estimation for $D(\text{tNAA})$ is the suggested link between axonal integrity and the diffusion coefficient along the axonal axis, or axial diffusivity as evaluated from the diffusion tensor [52–54]. The axial diffusivity is the first eigenvalue of the diffusion tensor, λ_1 , and represents the diffusion coefficient in the main direction of the axonal tract. In DTI experiments, where the spatial resolution is on the order of a few millimeters, λ_1 is mostly defined by two major factors: the “effective” diffusion coefficient of water molecules in the intra- and extracellular space at the given diffusion time, and by the axonal organization, “fanning”, or microscopic misalignment. The former is tightly related to the tortuosity of both the cytoplasmic as well as the interstitial medium, and it is hypothesized that structural damage to intra-axonal structures such as neurofilaments and microtubules resulting from pathological processes may affect the intra-axonal component of λ_1 . Accurate measurement of $D(\text{tNAA})$ has the potential to provide an additional source of information on intra-axonal structural integrity in white matter tracts, while avoiding the confound of the extra-axonal contribution. The potential sensitivity of $D(\text{tNAA})$ to pathology has been shown in the case of multiple sclerosis, where the axial diffusivity of tNAA in the corpus callosum was significantly lower in a group of MS patients and correlated well with behavioral outcome [29]. The specificity of DW-MRS of tNAA is offset by the low sensitivity of the method, which dictates significantly larger volumes than the typical DTI voxel and longer measurement times. One of the main problems resulting from using a large VOI is the impact of macroscopic curvature of the axonal tracts on the estimation of $D(\text{tNAA})$. In this work we showed that in the relatively simple case of the corpus callosum, it is possible to incorporate in the model a synthetic angular distribution of axonal directions. A simple and robust way to provide a realistic estimate for the macroscopic curvature can be based on an independent DTI data set, where the set of main eigenvectors for the DTI voxels within the DW-MRS volume can be used to generate a realistic distribution of angles between the tract directions and the diffusion weighting gradients. These angular distributions are patently non Gaussian, and are expected to be different for each diffusion weighting direction. We do not present such distributions in this work as we have not acquired DTI data from the subjects on which the reported DW-

MRS measurements were performed, but preliminary estimates based on data collected so far for a current project yield values of $\sigma_{\theta[1,0,0]} \approx 15^\circ$ and $\sigma_{\theta[0,1,1]} \approx 10^\circ$, where θ is the angle between the diffusion weighting direction ($[1,0,0]$ or $[0,1,1]$) and the main eigenvector of a DTI voxel within the DW-MRS VOI. The quantitative incorporation of these angular distributions in the processing pipeline of DW-MRS data of NAA is one of the future directions we propose to investigate. Additionally, the addition of a DTI experiment to the DW-MRS protocol will provide tractographic data for the visualization of white matter tracts of interest that are not as recognizable as the corpus callosum, and help position the DW-MRS VOI on such tracts.

The similar $D_{[1,0,0]}$ and $D_{[0,1,1]}$ values for tCho and tCr initially suggest that the compartmentation of these two metabolites in white matter is rather similar, and the differences can at least partly be explained by differences in molecular weight and in interactions with the surrounding medium. Upon more detailed analysis of the diffusion data for these two metabolites, a significant difference in their glial fraction is found, namely, the glial fraction of tCho for each subject is significantly higher than the glial fraction of tCr. This finding is supported by the literature [11] and provides an encouraging support for the validity of the suggested model. It should be noted that since the measurements shown here were performed using a single diffusion time, it is impossible to use the data to extract information about compartment size, and in the proposed analysis the compartment size was either provided from histological data, as in the case of the axonal diameter, or given a lower limit in which the data fit well with the model, as in the glial diameter. The estimate of the latter ($r_{gl} = 9 \mu\text{m}$) agrees well with histological data, but there is no doubt that measurements with a set of diffusion times would narrow down the estimates for $D(\text{tCr})$ and $D(\text{tCho})$, as well as those for the glial fraction for those metabolites. Moreover, we assumed here that the prevalent geometry of astrocytes is that of spherical compartments. This is a simplistic assumption that does not take into account astrocytic processes, which comprise a significant fraction of the astrocytic volume [55]. The astrocytic processes are isotropically distributed and thus do not affect the directional anisotropy of the DW-MRS measurements, but since diffusion in fibers significantly differs from diffusion in spheres in its dependence on diffusion time [21] it is expected that introducing astrocytic processes to the model will have an impact on both the estimate of glial size as well as on $D(\text{tCr})$ and $D(\text{tCho})$.

Diffusion properties of intracellular metabolites are not only indicative of cellular microstructure. In a recent study [56] DW-MRS was applied to measure changes in ADC of metabolites in the primary visual cortex upon visual stimulation. In this work, the most salient result was that the ADC of tCr robustly increased with activation, and correlated well with the stimulation paradigm. Although this finding does not exclude the possibility of microscopic neuronal structural changes upon activation such as cell swelling [57], the fact that the ADC of tNAA, a purely neuronal constituent, was significantly less correlated with the stimulus indicate a possible involvement of a metabolic process as responsible to the increase in ADC of tCr, rather than a structural one. The increase in SNR at ultra-high field facilitated the robust separate assessment of ADC of glutamate and glutamine in gray and white matter [28]. It is hoped that measuring the diffusion properties of glutamate and glutamine will provide further useful insights into such processes as glutamate release and the transport of glutamine from astrocytes to neurons upon neuronal activation, and contribute to the understanding of the role of glutamate compartmentation in disorders such as schizophrenia [58, 59].

In this work we provided some insight into the vast potential of obtaining unique compartment-specific information from DW-MRS, and an example of a scaffold for the analysis of such data. There are significant challenges associated with robust acquisition, processing and analysis of diffusion weighted spectroscopy. Additionally, the lower sensitivity of DW-MRS when compared to diffusion-based imaging methods, prevents it from being used for high-resolution mapping of microstructural features of tissue. Conversely, the unique intrinsic compartment-specificity of the metabolites probed with DW-MRS gives DW-MRS a potential role in tissue characterization that water-based diffusion methods cannot fulfill. The challenge ahead is how to combine, in a multi-modal manner, DW-MRS data with more sensitive but less specific high-resolution data from e.g. DTI, to provide stronger explanatory power to both. Early attempts at doing so aimed at better understanding the processes that affect tissue in stroke [17, 60], and recently such attempt was aimed at characterizing tissue damage in normal appearing white matter in multiple sclerosis [29]. DW-MRS will certainly gain from the advent of ultra-high field MRI for humans (7T and higher), as the sensitivity of localized spectroscopy at higher fields is increased, with very few detrimental effects. It is hoped that the increase in availability of ultra-high field MRI scanners will generate an increase in interest in this unique tool, and that DW-MRS will find its place in the growing arsenal of methods used for the non-invasive investigation of tissue microstructure.

2.5 References

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3

Reproducibility and optimization of *in vivo* human diffusion-weighted magnetic resonance spectroscopy of the corpus callosum at 3T and 7T

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Abstract

Diffusion-weighted MR spectroscopy (DW-MRS) of brain metabolites enables the study of cell-specific alterations in tissue microstructure by probing the diffusion of intracellular metabolites. In particular, the diffusion properties of neuronal N-acetylaspartate (NAA), typically co-measured with N-acetylaspartyl glutamate (NAAG) ($\text{NAA} + \text{NAAG} = \text{tNAA}$), have been shown to be sensitive to intraneuronal/axonal damage in pathologies such as stroke and multiple sclerosis. Lacking so far are empirical assessments of the reproducibility of DW-MRS measures across time and subjects, as well as a systematic investigation of optimal acquisition parameters for DW-MRS experiments, both of which are sorely needed for clinical applications of the method.

In this study, we acquired comprehensive single volume DW-MRS data sets of the human corpus callosum at 3T and 7T. We investigated the *inter*- and *intra*-subject variability of empirical and modeled diffusion properties of tNAA ($D_{\text{avg}}(\text{tNAA})$ and $D_{\text{model}}(\text{tNAA})$, respectively). Subsequently, we used a jackknife-like resampling approach to explore the variance of these properties in partial data subsets reflecting different total scan durations. The coefficients of variation (C_V) and the repeatability coefficients (C_R) for $D_{\text{avg}}(\text{tNAA})$ and $D_{\text{model}}(\text{tNAA})$ were calculated for both 3T and 7T, with overall lower variability in the 7T results. Although this work is limited to the estimation of the diffusion properties in the corpus callosum, we show that a careful choice of diffusion-weighting conditions at both field strengths allows accurate measurement of tNAA diffusion properties in clinically relevant experimental time. Based on the resampling results we suggest optimized acquisition schemes of 13 minute duration at 3T and 10 minute duration at 7T while retaining low variability ($C_V \approx 8\%$) for the tNAA diffusion measures. Power calculations for the estimation of $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ based on the suggested schemes show that less than 21 subjects per group are sufficient for detecting a 10% effect between two groups in case-control studies.

3.1 Introduction

DIFFUSION-WEIGHTED MR spectroscopy (DW-MRS) assesses diffusion properties of intracellular metabolites, thus providing specific information about the microstructural properties of the compartments where they reside [1–14]. The compartmental localization of these metabolites, as well as their slow inter-compartmental exchange on the time scale of an MRI-based diffusion experiment, potentially makes them excellent compartment- and cell-specific markers for physiological and microanatomical changes in disease. Among the metabolites accessible to *in-vivo* magnetic resonance spectroscopy (MRS) techniques, N-acetylaspartate (NAA) plays a central role in the evaluation of neuronal and axonal damage resulting from neurological disorders. Approximately 95% of tNAA in the mature CNS is found within neurons [15], and therefore tNAA diffusion properties can provide a unique insight on damage to the intraneuronal/intra-axonal space, separate from confounding contributions such as the extracellular space. Early DW-MRS studies in animal models showed that the amplitude of the diffusion-weighted tNAA peak was modulated by experimentally induced ischemic stroke [16, 17]. More recent studies have demonstrated the importance of compartment-specific diffusion measures derived from DW-MRS in distinguishing disease-related changes in multiple sclerosis [18], acute cerebral ischemia [19, 20], brain tumors [20, 21], and psychiatric disorders [22].

Performing *in-vivo* DW-MRS measurements on a clinical scanner and deriving meaningful information from these experiments remains challenging, and the robustness and reproducibility of the technique still need to be established. The low concentrations of metabolites compared to that of water, coupled with the long echo times (TE) required to accommodate the diffusion-weighting gradients, result in a relatively low signal-to-noise ratio (SNR). This, in turn, necessitates long measurement times and/or large volumes of interest, both detrimental to clinical applications. Other effects, such as strong eddy currents, and inter-shot phase and amplitude fluctuations, may further affect the accuracy and reproducibility of the measurements. Additionally, the diffusion properties of metabolites may require different diffusion-weighting parameters than those used in water diffusion-weighted techniques. Previous work, for example, emphasized the importance of b-value choice for an accurate estimate of fractional anisotropy (FA) of brain metabolites from DW-MRS experiments [23]. In order to assess the viability of DW-MRS as a meaningful diagnostic tool, it is therefore essential to ascertain: (a) the *inter*- and *intra*-subject variability of metabolite diffusion properties across the acquisition parameter space, e.g. the number and range of different b-values used and the number of spectral averages per single diffusion-weighting condition; (b) the optimal parameters for a reliable DW-MRS experiment within an experimental time that is suitable for clinical and clinical research purposes.

We chose to accomplish these goals with a set of DW-MRS measurements performed on the anterior body of the corpus callosum (aCC). The aCC has been thoroughly studied with DW-MRS [11, 18, 24, 25], and the relatively straightforward organization of the cross-hemispheric fibers makes the aCC a suitable site in which to measure diffusion properties roughly parallel and perpendicular to the main fiber direction. These properties are in turn dictated by microstructural variables such as, amongst others, axonal diameter and molecular crowding in the axonal space [4]. Diffusion along the callosal fibers has been linked to axonal degradation in MS [18], and the involvement of the aCC in several

neurological disorders makes it a plausible candidate for clinically relevant measurements [26, 27].

The goal of the work reported here is to help experimenters interested in obtaining robust measures for tNAA diffusion to choose the optimal combination of experimental parameters within the limitations of experimental time available. In the first part, reproducibility of tNAA DW-MRS measurements parallel and perpendicular to the callosal fiber main direction is assessed on two MRI scanners, operating at different magnetic fields of 3T and 7T, using an almost identical protocol. The reproducibility of derived quantities, such as diffusion coefficients of tNAA parallel and perpendicular to the callosal fibers, is estimated across repeated measurements within subjects as well as across subjects. We also test the reproducibility of modeled quantities with potential clinical relevance, such as the cytosolic diffusion coefficient of tNAA within the callosal fibers and the orientation dispersion of axons within the volume of interest (VOI). These quantities are calculated based on a model that accounts for the subject- and position-specific macroscopic curvature of the fibers within the VOI [24]. Once the impact of macroscopic curvature is removed, the cytosolic diffusion coefficient is more directly influenced by hindrances to diffusion within the intracellular space and is thus expected to be more sensitive to alterations in intracellular microstructure caused, for example, by disease processes such as breakdown of microtubules and neurofilaments. We also provide an estimate for the repeatability of the diffusion coefficients of other two metabolites detected within the same VOI selection: total creatine (creatine + phosphocreatine = tCr) and choline compounds (phosphocholine + glycerophosphocholine = tCho). The second part of the study reports the dependence of the statistical properties of the diffusion properties of tNAA obtained from the direct fit and the modeling as a function of scan parameters, such as the number and range of b-values used for each diffusion-weighting direction, as well as the number of spectral averages. Additionally, in order to assess the clinical viability of DW-MRS measurements, power calculations are performed to assess the minimum number of subjects required in a case-control study in order to detect a certain effect due to disease.

3.2 Materials and Methods

3.2.1 Human subjects

Six healthy volunteers (three men, three women, ages: 34 ± 8 years), without known neurological abnormalities, participated in this study. Each subject was scanned in 5 separate sessions. The study adhered to local Institutional Review Board guidelines, and informed consent was obtained from all subjects prior to the study.

3.2.2 MRI Scanner/Hardware

Three of the subjects were scanned on a 3 Tesla Achieva MRI scanner (Philips Medical Systems, Cleveland, OH, USA) at the National Institutes of Health, Bethesda, MD. The scanner was equipped with an 8-channel phased array receiver head coil and gradient coils, which in the selected mode of operation, could deliver a maximum gradient strength of 60 mT/m at a slew rate of 100 T/m/s. The other three subjects were scanned on a 7 Tesla Achieva whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) at the Leiden University Medical Center, equipped with gradient coils capable of a maximum gradient strength of 40 mT/m and a slew rate of 200 T/m/s. A head coil consisting of a

quadrature birdcage transmit and 32-channel phased array receive (Nova Medical Inc., Wilmington, MA, USA) was used for the 7T measurements.

3.2.3 Scan Protocol

3.2.3.1 Anatomical imaging

For each separate scan session, a short survey scan and a sensitivity encoding (SENSE) reference scan were followed by a 3D T_1 -weighted gradient-echo acquisition for positioning the VOI in the DW-MRS experiments and for tissue segmentation in the post-processing stage. Imaging parameters for the T_1 -weighted image acquired at 3T were: field of view (anterior-posterior (AP), foot-head (FH), right-left (RL)): $240 \times 240 \times 180 \text{ mm}^3$, 1 mm isotropic resolution, TR/TE: 7.00 / 3.15 ms, total scan time: 5.30 min. Imaging parameters for the T_1 -weighted image acquired at 7T were: field of view (AP, FH, RL): $246 \times 246 \times 174 \text{ mm}^3$, resolution: $0.85 \times 0.85 \times 1 \text{ mm}^3$, TR/TE: 5.00 / 2.20 ms, total scan time of 1.59 min.

3.2.3.2 Diffusion tensor imaging protocols

A DTI data set was also acquired in each scan session and was used to estimate macroscopic curvature of the axonal tracts in the modeling of the DW-MRS data, as explained in [24]. Single-shot 2D spin-echo echo-planar imaging was performed in both 3T and 7T scan protocols. DTI parameters for 3T acquisitions were: field of view (AP, FH, RL): $224 \times 224 \times 120 \text{ mm}^3$, 2 mm isotropic resolution, TR/TE: 7487 / 85 ms, 32 diffusion weighting directions with $b = 800 \text{ s/mm}^2$, total scan time: 5.50 min. DTI parameters for 7T acquisitions were: field of view (AP, FH, RL): $224 \times 224 \times 120 \text{ mm}^3$, 2 mm isotropic resolution, TR/TE: 7209/67 ms, 15 diffusion weighting directions with $b = 1000 \text{ s/mm}^2$, total scan time: 2.41 min. Parallel imaging was performed for all scans with a reduction factor of 3 along the phase-encoding direction (AP).

3.2.3.3 Diffusion-weighted spectroscopy protocols

The PRESS (Point Resolved Spectroscopy) [28] sequence was chosen as the base spectroscopic sequence for the single volume DW-MRS experiments and was supplemented with a bipolar diffusion-weighting scheme for minimization of eddy currents [29]. The VOI was positioned at the anterior body of the corpus callosum as shown in Figure 3.1. VOI dimensions were $30 \text{ (AP)} \times 15 \text{ (RL)} \times 8 \text{ (FH)} \text{ mm}^3$ for 3T and $25 \text{ (AP)} \times 15 \text{ (RL)} \times 8 \text{ (FH)} \text{ mm}^3$ for 7T experiments. For the diffusion weighting, two directions were chosen for all scans: (1) a pure right-left direction in the VOI frame, which is mostly parallel to the direction of the callosal fibers; (2) a direction perpendicular to the callosal fibers, forming a 45° angle between the anterior-posterior axis and the inferior-superior axis of the VOI. These gradient directions can be denoted in the VOI coordinates as $[1, 0, 0]$ and $[0, -1, 1]$. The position of the gradient directions with respect to the VOI is shown in Figure 3.1 (panels a and d).

In all experiments, the center frequency was set to the tNAA singlet peak at 2.0 ppm. Water suppression was achieved using two frequency-selective excitation pulses centered at the water resonance frequency, followed by dephasing gradients. The water suppression was “de-optimized” for the diffusion-weighting conditions in order to allow sufficient residual water signal for later use in the post-processing stage for zero-order phase correction of individual spectra prior to spectral averaging. A peripheral pulse unit (PPU) was used for cardiac synchronization of the DW-MRS acquisition in order to

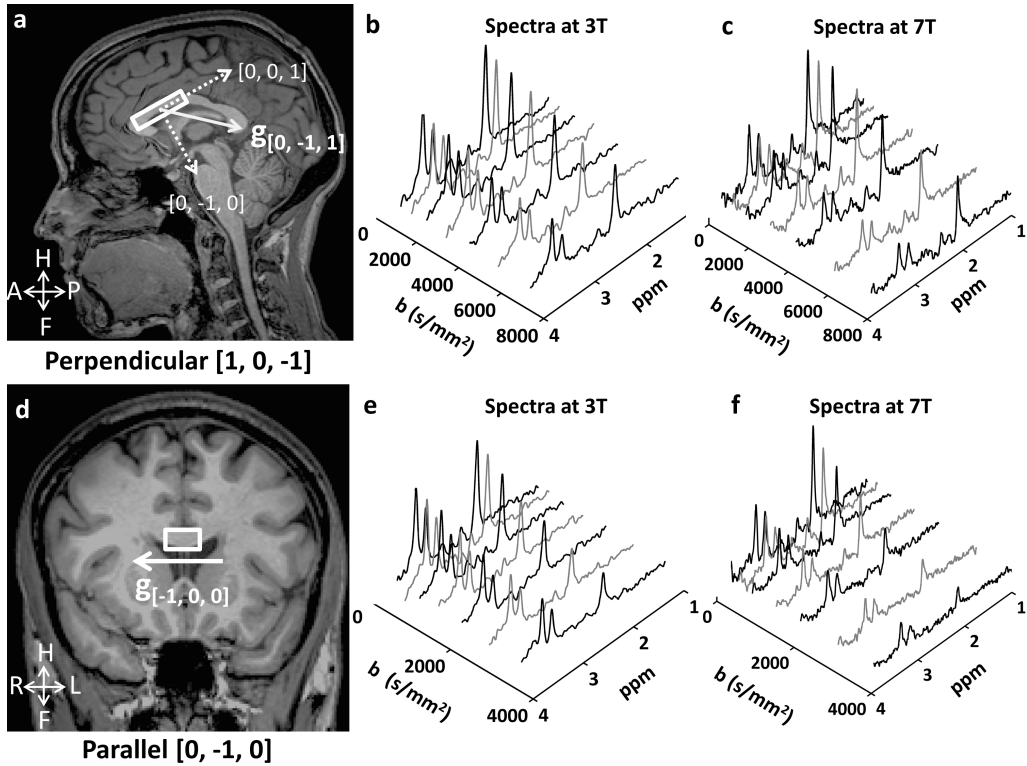


FIGURE 3.1: Planning of the single volume DW-MRS experiment on the anterior body of the corpus callosum as seen on sagittal (a) and coronal (d) T_1 -weighted image slices from a 3T scan. The solid arrows show the diffusion gradient directions perpendicular (a) and parallel (d) to the callosal fibers applied in all scans. Typical spectra acquired at 3T with diffusion-weighting in the $g[0, -1, 1]$ direction and in the $g[-1, 0, 0]$ directions are shown in panels (b) and (e). Spectra acquired at 7T with the same gradient directions are shown in panels (c) and (f). A line broadening of 5 Hz was applied to all of the spectra for display purposes.

minimize signal fluctuations due to cardiac pulsation. Pencil-beam shimming was applied up to second order, resulting in a typical tNAA singlet linewidth of 7 Hz on the 3T scanner and 12 Hz on the 7T scanner, in agreement with 3T and 7T spectra in previous work [30]. Following each scan, a shorter scan with identical VOI position and diffusion conditions was performed with the center frequency set at the water resonance frequency and without water suppression. This scan was subsequently used for eddy-current correction.

The DW-MRS parameters for the 3T acquisitions were: $TE = 110$ ms, $TR = 2$ cardiac cycles (about 2000 ms), trigger delay = 200 ms, number of time-domain points = 1024, spectral width = 1500 Hz, gradient duration (δ) = 22 ms, bipolar gap = 20 ms, diffusion time (Δ) = 55 ms with 7 different gradient amplitudes resulting in b -values of 213, 469, 826, 1285, 1847, 2511, 3277 s/mm^2 in the $[1, 0, 0]$ direction and 410, 917, 1629, 2544, 3665, 4989, 6518 s/mm^2 in the $[0, -1, 1]$ direction. 72 spectra were collected for each diffusion condition (2 directions, 7 b -values). Total DW-MRS scan time ranged from 35-50

minutes, depending upon heart rate of the subject, typically about 60 beats per minute (BPM).

The DW-MRS parameters for the 7T acquisitions were: TE = 121 ms, TR = 3 cardiac cycles (about 3000 ms), trigger delay = 300 ms, number of time-domain points = 1024, spectral width = 3000 Hz, gradient duration (δ) = 37 ms, bipolar gap = 16 ms, diffusion time (Δ) = 60.5 ms with 7 different gradient amplitudes resulting in b-values of 63, 317, 664, 1278, 1912, 2885, 3808 s/mm² in the [1,0,0] direction and 134, 656, 1361, 2602, 3883, 5844, 7700 s/mm² in the [0,-1,1] direction. 40 spectra were collected for each diffusion condition. Total DW-MRS scan time was about 40 minutes.

3.2.4 Image processing

T₁-weighted and DTI volumes were processed in MIPAV (Medical Image Processing, Analysis and Visualization) [31, 32] and JIST (Java Image Science Toolbox) [33]. The T₁-weighted image was rigidly registered to the Montreal Neurological Institute (MNI) brain with the Optimized Automatic Registration (OAR) algorithm [34], intensity inhomogeneity was corrected using N3 [35], and images were skull-stripped with the Simple Paradigm for Extra-Cerebral Tissue Removal (SPECTRE) [36] and segmented into white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF) using the Topology-preserving Anatomy-Driven Segmentation (TOADS) [37, 38]. A 3D mask in the shape of the DW-MRS VOI was applied to the WM segmentation volume to create a mask within the spectroscopic VOI that contains only WM pixels.

The DTI data were processed as follows: the individual diffusion-weighted volumes were rigidly registered to the b = 0 s/mm² image, which was then registered to the T₁-weighted volume in MNI space using affine registration, and the same transformation was then applied to the diffusion-weighted volumes. The diffusion tensor for each voxel was then estimated and diagonalized to yield maps of the primary eigenvector ($\mathbf{E}_1$) and the fractional anisotropy (FA), which are later used in the modeling. The white matter VOI mask obtained from the previous stage was then applied to the diffusion parametric maps to obtain the $\mathbf{E}_1$ and FA values within the VOI used for the DW-MRS experiments. The angles between the main eigenvectors within VOI and the applied diffusion gradients were calculated for each voxel according to the following equation:

$$\theta_{ij} = \cos^{-1} \left(\frac{\mathbf{E}_{1k} \cdot \mathbf{g}_j}{\|\mathbf{E}_{1k}\| \|\mathbf{g}_j\|} \right) \quad (3.1)$$

where $\mathbf{E}_{1k}$ is the principal eigenvector of DTI voxel k , $\mathbf{g}_j$ is the diffusion-weighting gradient in direction j , and $\|\cdot\|$ denotes the norm of a vector. These angles were used later in the modeling to incorporate the macroscopic curvature of the axonal tracks within the VOI.

3.2.5 Spectral processing

All spectral processing was done with custom codes in MATLAB[®] (release R2014b, Mathworks, Natick, MA, USA). DW-MRS data were corrected for eddy currents using the unsuppressed water data. Zero-order phase and frequency drifts were corrected for each shot using the residual water peak, and data for each condition were subsequently averaged. The number of averages was determined separately for inter- and intra-subject analysis, as described below. The residual water peak was removed with Hankel singular value decomposition (HSVD) [39], and a first-order phase-correction was performed

based on the tNAA peak. The spectrum from each condition was then analyzed with LCModel [40] to generate metabolite peak integrals.

3.2.6 Derivation of metabolite diffusion measures

Based on the LCModel data for tNAA, the following empirical quantities were calculated: $D_{\text{par}}(\text{tNAA})$, diffusivity along the $[1, 0, 0]$ direction (roughly parallel to the callosal fibers); $D_{\text{perp}}(\text{tNAA})$, diffusivity along the $[0, -1, 1]$ direction (roughly perpendicular to the callosal fibers); and $D_{\text{avg}}(\text{tNAA})$, the average of the two diffusivities described above (representing the empirical apparent diffusion coefficient of tNAA in the VOI). Diffusivities were calculated assuming monoexponential decay of the signal as a function of b-value in each direction:

$$\ln \left(\frac{S_{b,i}}{S_{b_1,i}} \right) = -b_i \cdot D_i \quad (3.2)$$

where $S_{b,i}$ is the measured signal in direction i , $S_{b_1,i}$ is the signal at the lowest b-value for the same direction, b_i is the b-value in the direction i , and D_i is the calculated diffusion coefficient for direction i .

The LCModel output was also used as an input to a modeling routine that calculates the *intra-axonal*, or *cytosolic*, diffusivity of tNAA. This procedure is assumed to minimize the variability in the DW-MRS measurements introduced by macroscopic factors such as the position of the VOI within the white matter tract, the main direction of the tract with respect to the DW gradients, and the macroscopic curvature of the tract within the VOI. The model, thoroughly described in [24], uses the angles between the main eigenvectors of the DTI data within the DW-MRS VOI and the diffusion-weighting gradient directions. The data are fitted to the model using two fitting variables: $D_{\text{model}}(\text{tNAA})$, the cytosolic diffusion coefficient of tNAA, and σ_ϕ , the standard deviation of the axonal angular dispersion. It is important to note that $D_{\text{model}}(\text{tNAA})$ is independent of the tract geometry within the VOI and thus mostly reflects the effects of the cytosolic medium, e.g. viscosity and molecular crowding, on the diffusion of tNAA inside the axons. The empirical apparent diffusion coefficients of tCr, $D_{\text{avg}}(\text{tCr})$, and tCho, $D_{\text{avg}}(\text{tCho})$, were calculated in a similar way to those of tNAA, based on the LCmodel data for tCr and tCho, respectively.

3.2.7 Inter-subject variability analysis of all diffusion measures

For inter-subject variability analysis, the entire data set from each session was used. All spectra belonging to a single diffusion condition were averaged per session. D_{par} , D_{perp} , D_{avg} for tNAA, tCr and tCho, and $D_{\text{model}}(\text{tNAA})$ were calculated for each session for all subjects.

3.2.8 Intra-subject variability analysis of tNAA diffusion measures

For evaluating the dependence of the variability of the calculated diffusion measures within subject on the number of averages as well as on selected diffusion weighting conditions, a jackknife-like subsampling procedure was performed on the data acquired from all subjects [41, 42]. In this procedure, within-session subsets of these data sets were randomly resampled without replacement prior to averaging. For evaluating the effect of selection of specific sets of diffusion weighting conditions, subsets of n b-values ($3 \leq n \leq 7$) were selected from the full range of 7 b-values. For example, g_{247} corresponds

to a selection of the 2nd, 4th, and 7th b-values for each direction, starting from the lowest b-value. For the 3T case, each subset consisted of 30, 36, 42, 48, 54 and 60 spectra per diffusion-weighting condition (the full number of spectra per condition was 72). For the 7T case, 16, 20, 24, 28 and 32 spectra were used out of the 40 spectra available per condition. For each randomly selected subset, an average spectrum was obtained. These spectra for were then used to calculate D_{par} , D_{perp} , D_{avg} , D_{model} , and σ_{ϕ} . This procedure was then repeated 100 times for each subset size to obtain the jackknife averages and standard deviations of these quantities.

A diagram of an example of the jackknife-like procedure is shown in Figure 3.2, where data from one session is used to generate 100 subsampled data sets, each with 3 b-values (out of the 7 available) and 6 averages (out of the 72 available). The same procedure was applied to all sessions, resulting in 5 sets of diffusion properties and their standard deviations per subject for each jackknife subsampling. These averages and standard deviations are used to generate the *across-session* averages and standard deviations of the *intra-session* averages and standard deviations.

3.2.9 Statistical analyses

All between-group (3T versus 7T) analyses were performed with GraphPad Prism version 6.0b for Mac OS X, GraphPad Software, San Diego, California, USA. Between and within subject analyses were accomplished with STATA release 11, StataCorp, College Station, Texas, USA. A one-way random-effects ANOVA model was used to estimate the between and within subject variance of the DW-MRS measurements. Between-subject variance was used for inter-subject variability analyses whereas within-subjects variance was used for repeatability coefficient and power/sample size calculations. The repeatability coefficient (C_R) within the 95% confidence interval is defined as:

$$C_R = 1.96 \times \sqrt{2} \times \sigma \quad (3.3)$$

Where σ is the within-subject standard deviation [43–46]. Power calculations were done to estimate the sample size required to detect a difference (Δ) in tNAA diffusion measures between two groups based on the variance (σ^2) of our measurements. For this, it was assumed that the means were normally distributed and the variance was the same for both groups ($\sigma_1^2 = \sigma_2^2$). For all calculations, we used a two-sided test with significance level $\alpha = 0.05$, $z_{1-\alpha/2} = 1.96$ and a power of 80% ($1 - \beta = 0.80$, $z_{1-\beta} = 0.84$). The sample size (n) required for each group was estimated as described in [47]. Additionally, the coefficient of variation ($C_V = 100 \times \frac{\sigma}{\mu}$, where μ is the mean and σ is the standard deviation of the resampling results) is reported to allow for comparison between diffusivity measures, which have different mean values.

3.3 Results

3.3.1 Diffusion-weighted spectra and diffusivity calculations

Typical diffusion-weighted spectra from all b-values applied in the two gradient directions are shown in Figure 3.1 for 3T (Panels b and e) and 7T (Panels c and f) scans. Cramér-Rao lower bounds (CRLB) for the tNAA peak from all spectra were between 6% and 20% at 3T and between 3% and 14% at 7T. CRLB for tCr were between 5% and 20% at 3T and 5% and 16% at 7T. CRLB for tCho was between 5% and 17% at 7T. CRLB values for tCho were mostly above 20% at 3T, even at the low b-value conditions and thus precluding an

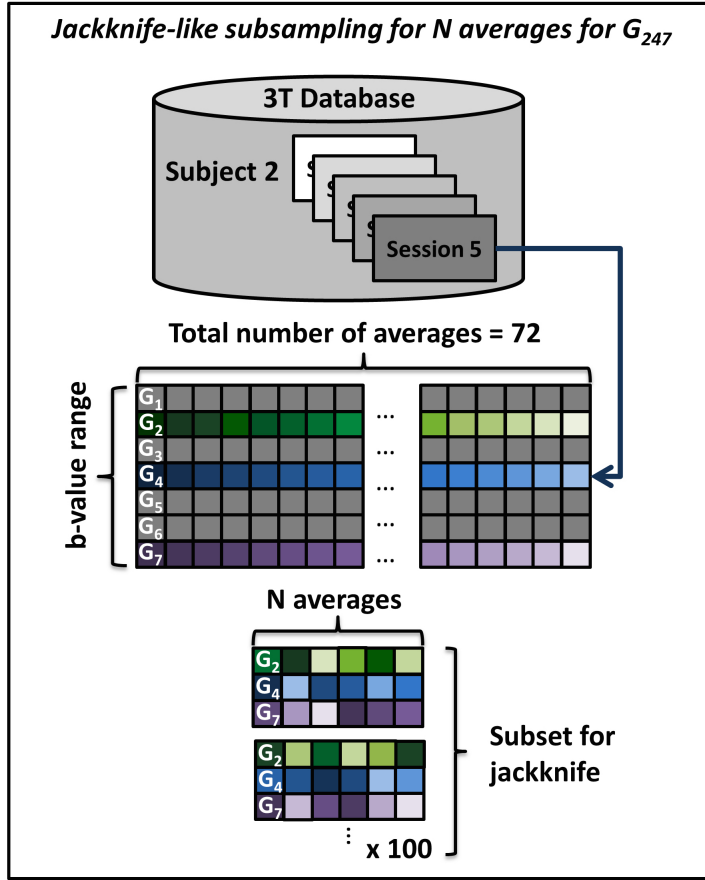


FIGURE 3.2: Schematic description of the method in which data subsets are randomly selected from the full data set of a single session of a specific subject.

accurate calculation of $D_{\text{avg}}(\text{tCho})$ at 3T. SNR and spectral resolution at 7T were higher than those at 3T. The highest CRLB values were typically obtained at the highest b-value in the $[-1, 0, 0]$ diffusion-weighting direction.

In Figure 3.3, the logarithm of the diffusion-weighted tNAA data is plotted as a function of b-value for the two diffusion gradient directions. The data were obtained from a single session of one subject scanned at 3T (panels a and b) and one subject scanned at 7T (panels c and d). In panels (a) and (c), the data acquired with the two gradient directions were separately fitted to two independent monoexponential decay functions (equation 3.2). Panels (b) and (d) show the modeled fit and the resulting parameters D_{model} and σ_{ϕ} for these particular data sets.

3.3.2 Inter-subject variability

Table 3.1 shows the averages, standard deviations, and coefficients of variation of tNAA, tCr and tCho diffusivity measures across subjects and sessions. The mean coefficients of

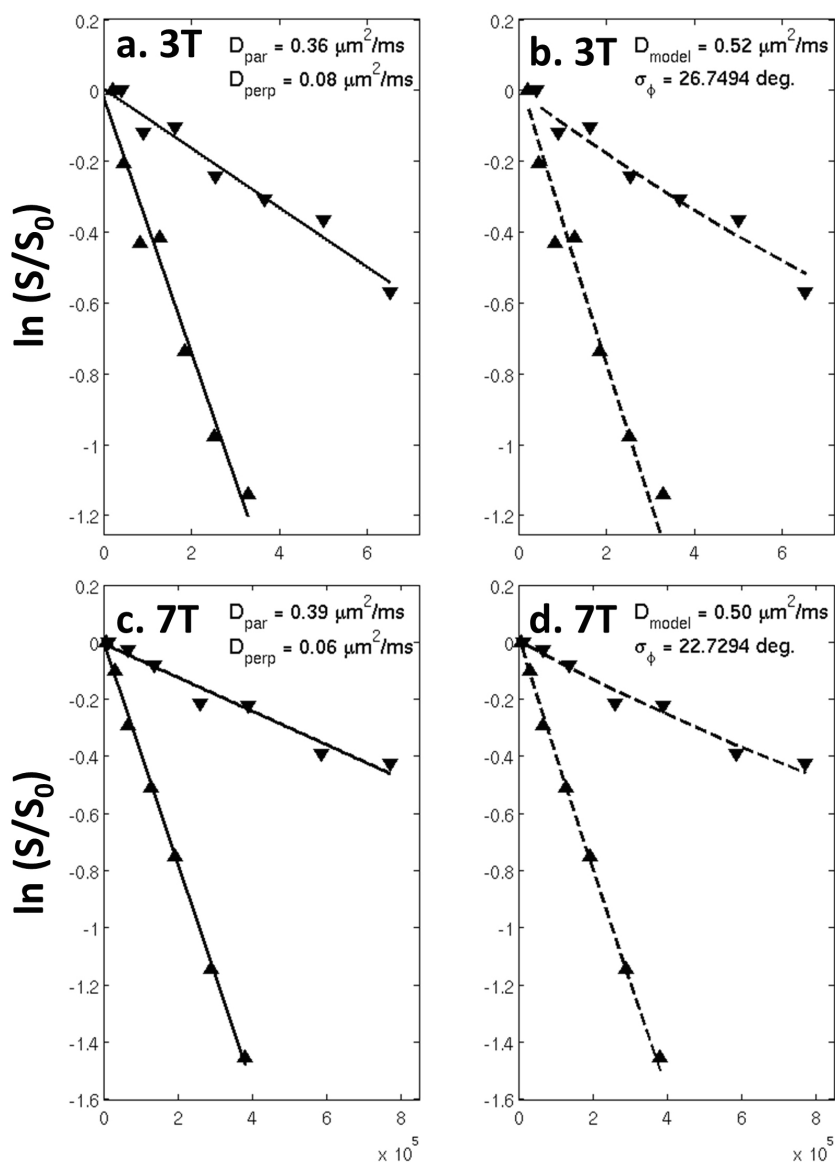


FIGURE 3.3: Logarithm of the diffusion-weighted tNAA signal measured with the diffusion weighting applied along the $[0, -1, 1]$ and $[-1, 0, 0]$ directions as a function of b-value (measured in s/mm^2). Panels a and c show the monoexponential fits used to calculate the parallel ($D_{par}(tNAA)$) and perpendicular ($D_{perp}(tNAA)$) diffusivity values from one data set acquired at 3T (a) and one acquired at 7T (c). Panels b and d show the same data fitted to the model described in the text, which yields the cytosolic diffusivity $D_{model}(tNAA)$ and the standard deviation of the axonal angular dispersion σ_ϕ .

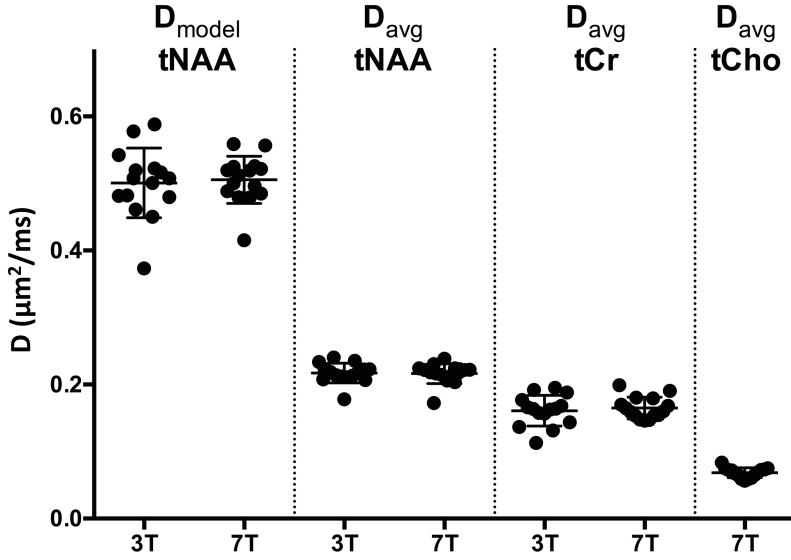


FIGURE 3.4: Diffusivity measures from all subjects and all sessions obtained from the experiments performed at 3T and 7T.

variation of the tNAA diffusivity measures ranged between 2% and 13% for both scanners, with the exception of that of $D_{\text{perp}}(\text{tNAA})$ at 3T, which was 29% across subjects and 22% across sessions. No significant differences in $D_{\text{par}}(\text{tNAA})$, $D_{\text{perp}}(\text{tNAA})$, $D_{\text{avg}}(\text{tNAA})$, $D_{\text{model}}(\text{tNAA})$ were observed between subjects (one-way ANOVA). In Figure 3.4, all measures calculated based on the full data sets from all subjects were grouped. Diffusivity measures obtained from the data acquired at 3T and 7T were not statistically different (Figure 3.4). $D_{\text{model}}(\text{tNAA})$ values had a smaller (not significant) standard deviation (SD) at 7T compared to 3T: mean (SD) values were $D_{\text{model}}(\text{tNAA}) = 0.501$ (0.052) at 3T and $D_{\text{model}}(\text{tNAA}) = 0.506$ (0.035) $\mu\text{m}^2/\text{ms}$ at 7T. For $D_{\text{avg}}(\text{tNAA})$, the mean and SD were the same at 3T and 7T: mean (SD) values were $D_{\text{avg}}(\text{tNAA}) = 0.217$ (0.015) at 3T and $D_{\text{avg}}(\text{tNAA}) = 0.216$ (0.015) $\mu\text{m}^2/\text{ms}$ at 7T. Mean coefficients of variation for the diffusion measures of tCr and tCho ranged between 3% and 22% at 7T. These values were higher for tCr at 3T (13%-27%), and those of tCho at 3T are not reported.

TABLE 3.1: Average, standard deviation and coefficient of variation for D_{model} , D_{par} , D_{avg} , D_{perp} and σ_ϕ values calculated based on complete datasets acquired from 3 subjects at 3T and 3 subjects at 7T

		3T Sub 1	3T Sub 2	3T Sub 3	7T Sub 1	7T Sub 2	7T Sub 3
tNAA D_{model}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.52 (0.05)	0.51 (0.03)	0.48 (0.07)	0.50 (0.06)	0.51 (0.01)	0.50 (0.02)
	C_V (%)	10	5	15	12	2	5
tNAA D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.22 (0.01)	0.22 (0.01)	0.21 (0.02)	0.21 (0.02)	0.22 (0.01)	0.22 (0.01)
	C_V (%)	5	3	11	12	2	3
tNAA D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.36 (0.03)	0.38 (0.02)	0.34 (0.05)	0.35 (0.04)	0.38 (0.01)	0.36 (0.02)
	C_V (%)	7	5	14	13	2	5
tNAA D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.08 (0.02)	0.07 (0.02)	0.08 (0.00)	0.07 (0.00)	0.06 (0.00)	0.07 (0.01)
	C_V (%)	29	22	2	6	7	11
tNAA σ_ϕ	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	26.78 (12.96)	22.57 (4.68)	36.41 (15.37)	23.81 (1.79)	22.02 (1.66)	29.05 (3.59)
	C_V (%)	48	21	42	8	8	12
tCr D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.16 (0.02)	0.16 (0.03)	0.16 (0.02)	0.17 (0.01)	0.16 (0.02)	0.17 (0.02)
	C_V (%)	13	19	14	9	13	10
tCr D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.23 (0.03)	0.23 (0.06)	0.20 (0.04)	0.24 (0.03)	0.24 (0.04)	0.25 (0.03)
	C_V (%)	13	27	18	14	17	11
tCr D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	0.10 (0.02)	0.09 (0.02)	0.12 (0.02)	0.09 (0.01)	0.09 (0.01)	0.09 (0.02)
	C_V (%)	17	22	17	12	13	22
tCho D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	-	-	-	0.10 (0.01)	0.12 (0.01)	0.13 (0.01)
	C_V (%)	-	-	-	9	11	8
tCho D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	-	-	-	0.13 (0.03)	0.15 (0.02)	0.17 (0.00)
	C_V (%)	-	-	-	21	16	3
tCho D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$)	-	-	-	0.07 (0.01)	0.08 (0.01)	0.09 (0.02)
	C_V (%)	-	-	-	17	9	21

3.3.3 Intra-subject variability of tNAA diffusion measures

Figure 3.5 shows the coefficient of variation as a function of number of spectral averages for all of the chosen b-value combinations based on data from all subjects. The relative error is observed to depend much more strongly on the highest b-value used than on the number of b-values. At 3T, the g_{1357} scheme demonstrates low C_V (less than 10%) for both $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ with fewer than 400 total spectra acquired. At 7T, the g_{247} b-value scheme exhibits $C_V = 8\%$ for both $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ using fewer than 200 total spectra.

3.3.4 Reproducibility and sample size analysis

Repeatability coefficients (C_R) are shown in Table 3.2 for $D_{\text{model}}(\text{tNAA})$ and D_{avg} of the three metabolites calculated based on the entire dataset as well as based on the b-value scheme g_{1357} for 3T and g_{247} for 7T. Smaller values of C_R demonstrate greater reproducibility. The measurements from data acquired at 7T using all spectra lead to the lowest C_R values of 21%. Measurements performed 7T using the g_{247} scheme, which utilized fewer than half of the spectra of g_{1-7} , retained low C_R values of 25%. D_{avg} calculated using the entire dataset and D_{avg} calculated based on the b-value scheme g_{1357} yielded similar C_R values of 21% and 24% at 7T and 3T, respectively. D_{model} calculated based on the same schemes at 3T resulted in higher C_R (30% for the entire dataset and 32% for g_{1357} scheme) compared to C_R values of D_{model} at 7T (21% for the entire dataset and 25% for g_{247} scheme). The C_R values for $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ at 7T were similar to those calculated for $D_{\text{avg}}(\text{tNAA})$, whereas at 3T the C_R values for $D_{\text{avg}}(\text{tCr})$ were higher than those for $D_{\text{avg}}(\text{tNAA})$.

Sample size calculations reflect the number of subjects per group required to detect a difference between two groups with power of 80% and significance level of 5%. Sample size (n) values to detect a 10% difference in both D_{model} , D_{avg} , $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ are shown in Table 3.2 while the trends in sample size values for 5, 10, 15 and 20% detectable differences for tNAA are depicted in Figure 3.6.

3.4 Discussion

In this study, we investigated the inter- and intra-subject variability of diffusivity measures of tNAA, both empirical and modeled, derived from DW-MRS experiments performed on the human aCC with 3T and 7T scanners. We also studied the effect of scan parameters, such as number and range of b-values for each diffusion direction and number of spectral averages, in order to suggest optimal scan parameters to perform DW-MRS experiments within a given experimental time limit.

3.4.1 Intra- and inter-subject variability of tNAA DW-MRS measures

The coefficients of variation (C_V) given in Table 3.1 and the reproducibility coefficients (C_R) given in Table 3.2 for D_{avg} and D_{model} indicate acceptable reproducibility ($C_R = 21\%$ for D_{avg} and D_{model} at 7T and $C_R = 32\%$ for D_{model} at 3T) of the DW-MRS measures of tNAA in the corpus callosum. At 7T, all C_V values were less than 13%, and most were in the range of 2 - 8%. The C_V values for the diffusion measures evaluated from the data acquired at 3T were higher than those found at 7T, ranging between 3% and 29%. One should keep in mind that the variability measured in these long sessions (typically about 40 minutes in both scanners) also reflects patient motion and scanner-related instabilities, which are expected to be less pronounced in shorter experiments. Past

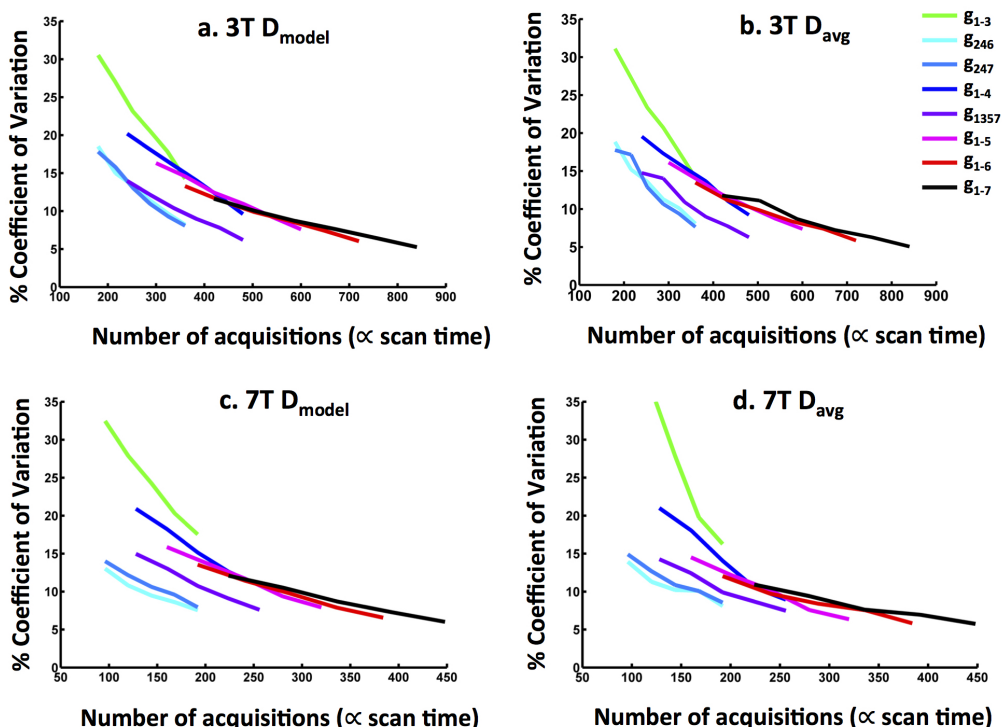


FIGURE 3.5: The coefficients of variation for D_{model} (panels a,c) and D_{avg} (panels b,d) obtained from all data sets acquired at 3T and 7T, respectively, shown as a function of number of acquisitions for different b-value combinations.

DW-MRS studies that explored the effect of disease reported substantial changes in tNAA diffusion measures hypothesized to be related to neuronal/axonal damage. In one study, an increase in $\text{ADC}(\text{tNAA})$ of above 50% was reported in malignant brain tumors, and a decrease in $\text{ADC}(\text{tNAA})$ of about the same magnitude was observed in ischemic stroke [20]. Zheng et al. [19] reported an age-related drop of 27% in $\text{ADC}(\text{tNAA})$ values. In our own study on normal appearing white matter changes in MS at 7T, a decrease of about 20% was observed in the D_{par} of tNAA in the aCC of a small cohort of MS patients compared to age-matched healthy controls [18]. Based on our power calculations, a difference between groups of 10% for D_{model} or D_{avg} can be detected with groups as small as 9-18 subjects each for 3T or 9 subjects each for 7T. This result is particularly reassuring as it suggests the ability of DW-MRS of tNAA to pick up subtle changes in intra-axonal structure in normal appearing white matter. It should be noted that this estimate is achieved using the full acquisition scheme used in our experiments. Such an acquisition would take around 35 minutes at 3T and 30 minutes at 7T, thus reaching the higher range of clinically-feasible scan times.

The average inter-subject variability values in D_{avg} and in D_{model} at 7T were 5.7% and 6.3%, respectively, and thus comparable. At 3T, the variability of D_{avg} was $C_V = 6\%$,

TABLE 3.2: Repeatability and sample size values

Scanner	Measure	σ^{2a} (variance)	n (samples ^b)	C_R^a (% mean)	Scan time ^c
3T g_{1-7}	tNAA D_{model}	2.87×10^{-3}	18	0.15 (30%)	33.6 min
	tNAA D_{avg}	0.22×10^{-3}	9	0.04 (21%)	
	tCr D_{avg}	0.61×10^{-3}	24	0.07 (34%)	
7T g_{1-7}	tNAA D_{model}	1.42×10^{-3}	9	0.10 (21%)	28.0 min
	tNAA D_{avg}	0.22×10^{-3}	9	0.04 (21%)	
	tCr D_{avg}	0.11×10^{-3}	4	0.03 (15%)	
	tCho D_{avg}	0.30×10^{-3}	12	0.05 (24%)	
3T g_{1357}	tNAA D_{model}	3.30×10^{-3}	21	0.16 (32%)	19.2 min
	tNAA D_{avg}	0.29×10^{-3}	11	0.05 (24%)	
	tCr D_{avg}	0.71×10^{-3}	28	0.07 (37%)	
7T g_{247}	tNAA D_{model}	2.01×10^{-3}	13	0.12 (25%)	33.6 min
	tNAA D_{avg}	0.32×10^{-3}	12	0.05 (25%)	
	tCr D_{avg}	0.18×10^{-3}	7	0.04 (18%)	
	tCho D_{avg}	0.27×10^{-3}	11	0.05 (23%)	

C_R , repeatability coefficient; NSA, number of spectral averages; tCho, total choline compounds; tCr, total creatine; tNAA, total N-acetylaspartate.

^aUnit: $\mu\text{m}^4 / \text{ms}^2$.

^bSignificance level (α) = 0.05, power ($1 - \beta$) = 0.80, detectable difference (Δ) = 10%.

^c NSA=72 at 3T and NSA=40 at 7T

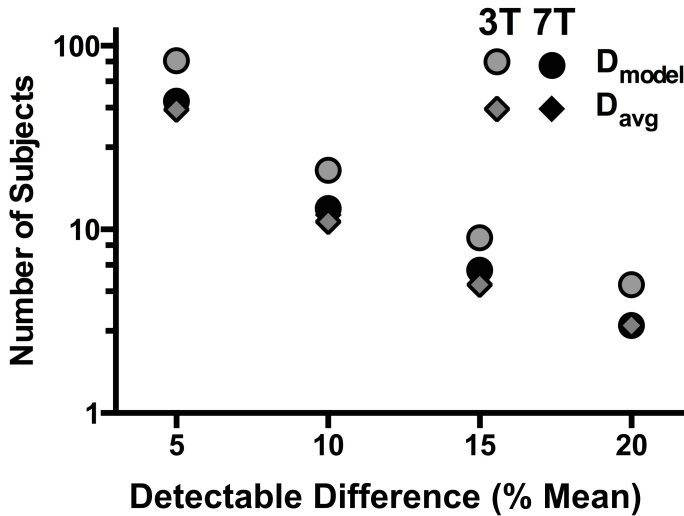


FIGURE 3.6: Number of subjects required to detect difference (in percent of mean) with significance level of $\alpha = 0.05$ and power of $1 - \beta = 0.80$ using the suggested g_{1357} b-value scheme for 3T and the g_{247} scheme for 7T.

whereas the C_V of D_{model} was higher at 10%. D_{avg} is roughly equivalent to the ADC of tNAA within the VOI, i.e. it includes the effect of restrictions on the diffusion of tNAA imposed by axonal membranes. Since the typical DW-MRS measurement is performed on a large volume, these geometric factors introduce a confound that varies across subjects and VOI locations. The model presented in [24] provides a way to remove the impact of macroscopic curvature of white matter tracts within the volume and yields a diffusion measure, D_{model} , which represents the cytosolic diffusion coefficient, and thus includes the impact of tortuous diffusion within the axonal medium. The stability of the resulting D_{model} depends greatly on the SNR of the single DW-MRS measurements that generate the data set needed for the fitting procedure. This hypothesis is also supported by the significantly higher variance at 3T in the second fitting parameter of the model, i.e. the standard deviation of the axonal microscopic misalignment, σ_ϕ (Table 3.1). It can be appreciated that at 7T, where the CRLB values for the individual measurements were relatively low, the variability in D_{model} was comparable to that of D_{avg} . At 3T, where the CRLB were higher, in particular at the high b-value range, D_{model} is found to be less stable.

3.4.2 Effect of b-value scheme on intra-subject variability of tNAA diffusion measures

Figure 3.5 can serve as a guideline for choosing a combination of b-values and number of averages in order to reach a desired variability in both D_{model} and D_{avg} . It is clearly seen that sampling the upper range of b-values is critical for obtaining low variability, e.g. schemes g_{247} , g_{1357} , and g_{1-7} , which all include a balanced sampling of the entire b-value range with 3, 4, and 7 b-values, respectively, quickly converge to low variability values. On the other hand, the scheme g_{123} has almost twice the variability of g_{247} for the same number of acquisitions. There is a substantial difference between 3T and 7T in the number of averages needed to reach the same variability, stemming from the intrinsically higher SNR of spectroscopic measurements at 7T. For example, for the scheme g_{247} about 200 acquisitions are needed to reach 8% variability in both diffusion measures at 7T, whereas a similar number of acquisitions at 3T will result in $C_V = 18\%$. Such an acquisition protocol (3 b-values applied in 2 gradient directions and 32 averages per diffusion weighting condition) will result in an acquisition time of 9.6 minutes at 7T, when a TR of 3 heart beats is used. A similar variability ($\sim 8\%$) can be reached at 3T when the scheme g_{1357} is used with 432 acquisitions (4 b-values applied in 2 gradient directions with 48 averages), with a resulting acquisition time of 14.4 minutes when a TR of 2 heart beats is used. Power calculations based on these schemes suggest that a minimum of 11-13 subjects per group could suffice to detect a subtle difference of 10% in $D_{\text{model}}(\text{tNAA})$ at 7T and $D_{\text{avg}}(\text{tNAA})$ both at 3T and 7T in case-control studies. $D_{\text{model}}(\text{tNAA})$ at 3T will necessitate a minimum of 21 subjects per group in order to detect the same effect.

In Figure 3.5, the differences in the coefficient of variation of $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ are shown to be rather minor, and can only be seen when the variability is high, especially at 7T. Power and repeatability coefficient calculations shown in Table 3.2 imply that $D_{\text{model}}(\text{tNAA})$ is more stable at 7T compared to 3T. $D_{\text{model}}(\text{tNAA})$ successfully accounts for the inter-subject differences in macroscopic and microscopic distributions of axonal directions within the VOI and thus we believe it is a clinically relevant measure for the cytosolic diffusion coefficient of tNAA. However, the non-linear nature of the fitting procedure implies a higher sensitivity to SNR, mainly due to error propagation properties. Thus, at the reduced SNR at 3T, the variability of $D_{\text{model}}(\text{tNAA})$ is markedly higher than

at 7T.

3.4.3 Inter-subject variability of tCr and tCho DW-MRS measures

At 7T, both C_V and C_R for $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ were similar to those obtained for tNAA, while at 3T, the C_R values for $D_{\text{avg}}(\text{tCr})$ were higher than those for $D_{\text{avg}}(\text{tNAA})$, and low SNR for the tCho resonances precluded the reliable calculation of $D_{\text{avg}}(\text{tCho})$. Since the experimental protocol was dictated by the goal of characterizing the diffusion properties of tNAA in a well-defined white matter tract, the small size of the resulting VOI resulted in low SNR for the tCho resonances. This, of course, does not preclude the possibility of robustly measuring the diffusion properties of tCho and other metabolites, as has been shown in several previous studies, but it does emphasize the sensitivity of the calculation of diffusion coefficients to SNR, through propagation of error. Additionally, the low bandwidth of the refocusing pulses used in the PRESS sequence resulted in a large chemical shift displacement for the metabolites other than the tNAA, especially at 7T, causing the effective VOI for tCr and tCho to be significantly shifted from the medial part of the corpus callosum (Figure 3.8 in the supplement). Since our measurement of D_{avg} are based on an assumption of gradient directions that are roughly parallel and perpendicular to the callosal fibers in the medial region of the CC, and not on three mutually orthogonal directions, this shift may have affected the values of $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ reported here.

3.4.4 Limitations of the study

Our study was subject to several challenges and limitations. The five repeated scans per subject allowed examine the long-term repeatability of the metabolite diffusion measures, but the small number of subjects used in our study severely limited the evaluation of across-subject variability. The choice of different subjects for the 3T and 7T measurements, which were performed on different continents, prevents us from performing a more complete comparison of DW-MRS quantities of tNAA across these two field strengths. Moreover, the bigger VOI size at 3T is an additional limiting factor for such a comparison. This choice was dictated by the lower SNR at 3T and the goal of devising protocols with a clinically-relevant scan time. There were several sources of variability that were not properly quantified. Since the experiment was performed using cardiac triggering based on PPU, differences trigger delays, as well as in heart rate and circulation can introduce variability to the TR, and subsequently affecting T_1 saturation. Subject motion across sessions, as well as small differences in the positioning of the VOI in different sessions, can also affect the variability observed in the study, especially in our experimental setup, where the VOI is positioned on the corpus callosum. Our 3T data were acquired with a gradient setting that allowed relatively high gradient amplitude (at the expense of a lower slew rate). More conventional gradient systems may necessitate different choice of diffusion weighting parameters to reach the desired b-values recommended in this work. Finally, we restricted this study to focus on the diffusion properties of tNAA in a specific white matter pathway, the corpus callosum. This choice was guided by the apparent simplicity of the fiber structure in the corpus callosum, its easy identification, and the simplicity of repositioning the DW-MRS VOI in subsequent sessions. The corpus callosum also allows a relatively straightforward implementation of our model for extracting $D_{\text{model}}(\text{tNAA})$ from the two gradient directions. In the future we plan on providing a more general model that will allow an arbitrary choice of DW-MRS VOI with a variety of fiber orientations

within the VOI. Lastly, one should note that the empirical diffusion coefficients in this work were calculated based on fitting the metabolite signal to a single exponential. The diffusion of metabolites in the intracellular space is not expected to be Gaussian, and thus the signal decay is not monoexponential. This directly affects the metabolites' diffusion coefficient, as can be appreciated in Figure 3.7 in the supplementary material. In this figure the $D_{\text{avg}}(\text{tNAA})$ shows a clear dependency on the maximum b-value used. The choice of a monoexponential fit is simple and practical, especially in the b-value range used in this work, but does not reflect the true nature of the diffusion of metabolites in their microenvironments.

3.5 Conclusion

In this study, we evaluated the reproducibility of empirical and modeled diffusion properties of tNAA in the corpus callosum based on DW-MRS data acquired at two MRI scanners operating at 3T and 7T. Statistical assessment of the intra-subject variability shows that DW-MRS experiments can be performed at both field strengths within clinically relevant scan times of about 10-13 minutes while retaining low variance ($\sim 8\%$) for the estimated diffusion properties of tNAA. These measurements provide ample power to detect group mean differences with groups of 13 or fewer subjects.

3.6 References

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3.A Supplementary materials

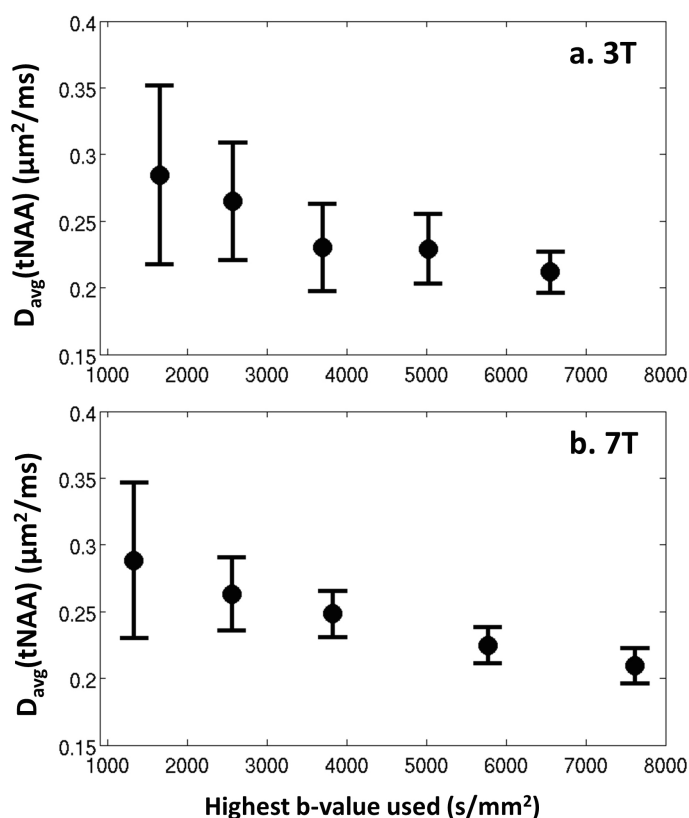


FIGURE 3.7: $D_{avg}(tNAA)$ as a function of the highest b-value used in the experiment. The total number of acquisitions was kept constant for all conditions.

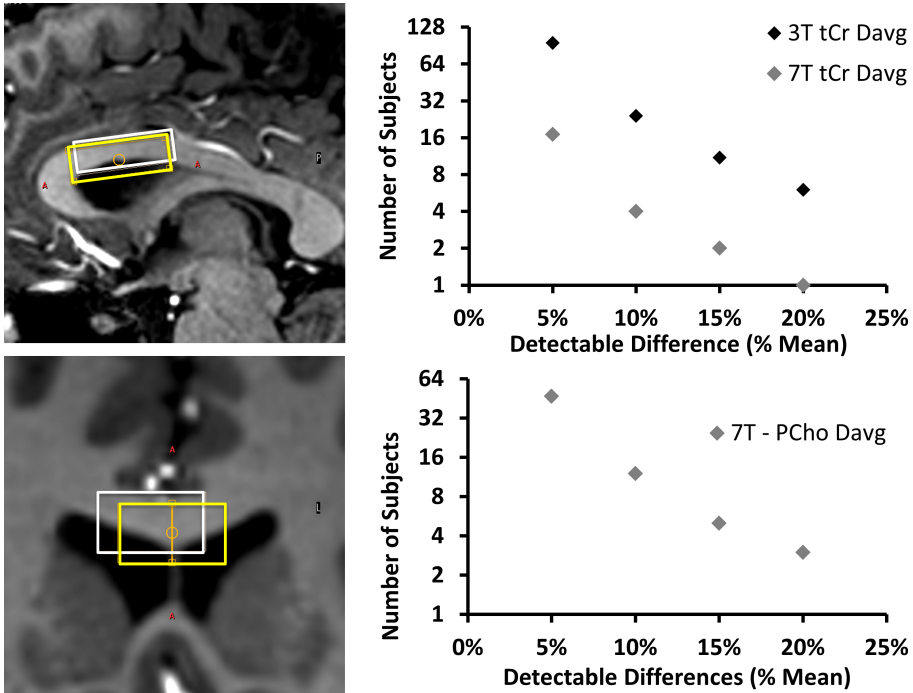


FIGURE 3.8: The effective VOI for the tNAA (yellow) and tCho (white) at 7T. Number of subjects required to detect difference (in percent of mean) with significance level of $\alpha = 0.05$ and power of $1 - \beta = 0.80$ in tCr and tCh using the suggested g_{1357} b-value scheme for 3T and the g_{247} scheme for 7T.

4

Diffusion-Weighted Chemical Shift Imaging of Human Brain Metabolites at 7T

This chapter was adapted from:

A. E. Ercan, A. Techawiboonwong, M.J. Versluis, A.G. Webb and I. Ronen, "Diffusion-weighted chemical shift imaging of human brain metabolites at 7T", *Magnetic Resonance in Medicine*, Volume 73, Issue 6, pages 2053-2061, 2015.

Abstract

Diffusion-weighted chemical shift imaging (DW-CSI) of brain metabolites poses significant challenges associated with the acquisition of spectroscopic data in the presence of strong diffusion weighting gradients. A reproducible DW-CSI acquisition and processing scheme is presented, one that addresses most of the potential sources of instability and which provides reproducible and anatomically-meaningful diffusion-weighted and apparent diffusion coefficient (ADC) metabolite maps. A real-time navigator-based acquisition scheme is used, allowing instantaneous re-acquisition of corrupted k-space data and post-processing correction of gradient-induced phase fluctuations. Eddy current correction based on the residual water resonance is implemented and significantly improves the data quality. Highly reproducible diffusion-weighted metabolite maps of three highest concentration brain metabolites are shown. The navigator-based accept/reject strategy and the post-acquisition corrections significantly improved the stability of the DW-CSI signal and the reproducibility of the resulting DW-CSI maps. Metabolite ADC values obtained in this work could be related to the underlying tissue cellular composition. In conclusion, robust investigation of DW-CSI of brain metabolites is feasible and can potentially provide complementary information to that obtained from more sensitive but less specific methods such as diffusion tensor imaging.

4.1 Introduction

DIFFUSION-WEIGHTED MRI (DWI) and diffusion tensor imaging (DTI) are the most commonly used neuroimaging tools for obtaining information on microscopic structural features of tissue and their modulation by disease processes [1, 2]. The link between diffusion tensor metrics, e.g. fractional anisotropy (FA) and apparent diffusion coefficient (ADC), and tissue microstructural features such as the degree of myelination and cellular/axonal density and size is, however, difficult to establish. DWI lacks compartmental specificity since its signal source, namely water, originates from multiple tissue compartments, and its interpretation is further complicated by rapid exchange of water molecules through cell membranes during the diffusion times used in these experiments [3]. Diffusion-weighted MR spectroscopy (DW-MRS) explores the diffusion properties of intracellular metabolites, such as N-acetylaspartate, creatine and phosphocreatine, and total choline compounds, in the millimolar concentration range. The relatively low concentration of these metabolites dictates a significantly lower sensitivity than that of DWI-based methods, but their cellular and compartmental specificity allows for compartment-specific assessment of tissue microstructure and thus has the potential of providing unique information on cell-specific structure and physiology, and sensitively reporting on specific deficits linked with disease. Single volume (SV) DW-MRS has been shown to yield meaningful and reproducible results in the rodent [4–8], monkey [9, 10], and human brain [11–17], and has been applied to study pathological changes in neural tissue microstructure in various diseases in the human brain, e.g. acute cerebral ischemia [18, 19], brain tumors [19, 20], multiple sclerosis [21], mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome [22] and psychiatric disorders [23].

The single-voxel nature of DW-MRS limits its application to specific white matter tracts or arbitrarily selected regions of interest. An early work showed the possibility of obtaining DW-CSI maps of acetone and water in phantom [24]. Since then, only a few attempts have been made so far at obtaining maps of the diffusion properties of brain metabolites with DW chemical shift imaging (DW-CSI) [25–27]. The long scanning times these experiments require limit their clinical applicability, and so far they have been characterized by a lack of robustness and reproducibility. The instability of DW-CSI can be attributed to several factors including the multi-shot nature of the acquisition, combined with the low signal to noise ratio (SNR) of the metabolite resonances, and the relatively high gradient strength needed for adequate diffusion weighting of the slow diffusing metabolites. These factors result in strong inter-shot phase and amplitude fluctuations as well as strong eddy currents, which degrade the resulting DW spectra.

In this study, we show for the first time a DW-CSI method that yields robust, reproducible and anatomically-meaningful diffusion-weighted maps of the main brain metabolites: N-acetylaspartate + N-acetylaspartylglutamate (NAA + NAAG = tNAA), creatine + phosphocreatine (Cr + PCr = tCr) and total soluble choline compounds (tCho). The method efficiently suppresses the amplitude fluctuations during data acquisition and corrects gradient-induced phase fluctuations as well as the strong eddy currents generated by the diffusion-weighting gradients.

4.2 Materials and methods

4.2.1 Human subjects

A total of five healthy volunteers (two female, three male, age: 34.4 ± 10.1 years) participated in this study. Two of the volunteers were scanned twice for the reproducibility investigation, and one was scanned for demonstration of the navigator-based corrections. The study adhered to the Leiden University Medical Center Institutional Review Board guidelines and informed consent was obtained from all subjects prior to the study.

4.2.2 Data acquisition

4.2.2.1 MRI scanner/Hardware

All experiments were performed on a 7 Tesla Achieva Philips whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with gradient coils capable of a maximum gradient strength of 40 mT/m and a slew rate of 200 T/m/s. A head coil consisting of a quadrature birdcage transmit and 32-channel phased array receive (Nova Medical Inc., Wilmington, MA, USA) was used for all measurements.

4.2.2.2 Anatomical images

A short survey scan and a sensitivity encoding (SENSE) reference scan were followed by a 3D T₁-weighted gradient echo acquisition for accurate planning of the diffusion-weighted experiment and for the assessment of the gray (GM) and white matter (WM) tissue fractions within each CSI voxel via post-processing. Imaging parameters were: field of view (anterior-posterior(AP), foot-head(FH), right-left(RL)): $246 \times 246 \times 174$ mm³, resolution $0.85 \times 0.85 \times 1.00$ mm³, TR/TE=4.94/2.17 ms. Total scan time was 3 minutes.

4.2.2.3 CSI Pulse Sequence

The two-dimensional diffusion weighted chemical shift imaging (DW-CSI) pulse sequence is shown in Figure 4.1. The PRESS (Point Resolved Spectroscopy, [28]) sequence was chosen as the base spectroscopic sequence. Diffusion weighting used bipolar gradients for minimization of eddy currents [29]. Based on our experience in developing the single volume DW-MRS sequences, the implementation of bipolar gradients has several beneficial aspects in addition to the minimization of eddy currents; the use of available time for gradients is maximized, so higher b-values can be reached for the same gradient strength and TE, and the cross-terms with background gradients is efficiently compensated. The phase-encoding gradient, originally located immediately after the excitation pulse in the sequence supplied by the vendor, was repositioned after the trailing edge of the second lobe of the refocusing diffusion gradient, allowing 25-30 data points to be acquired immediately following the second lobe of the refocusing diffusion weighting gradient and before the echo maximum. These data points were used as a phase and amplitude navigator, described later. A supra-callosal axial slice was selected for the DW-CSI experiment (see Figure 4.2 for the planning of the CSI experiment). Field of view: 96×96 mm², matrix size: 12×12 with standard elliptical k-space filtering, zero-filled to a 16×16 matrix. The PRESS sequence was applied in volume-selective mode, where the dimensions of the volume of interest (VOI) were $48 \times 42 \times 8$ mm³ (AP, RL, FH). This resulted in a voxel size of $6 \times 6 \times 8$ mm³ in the reconstructed CSI data set, and a matrix of $8 \times 7 = 54$ encoded voxels within the selected VOI.

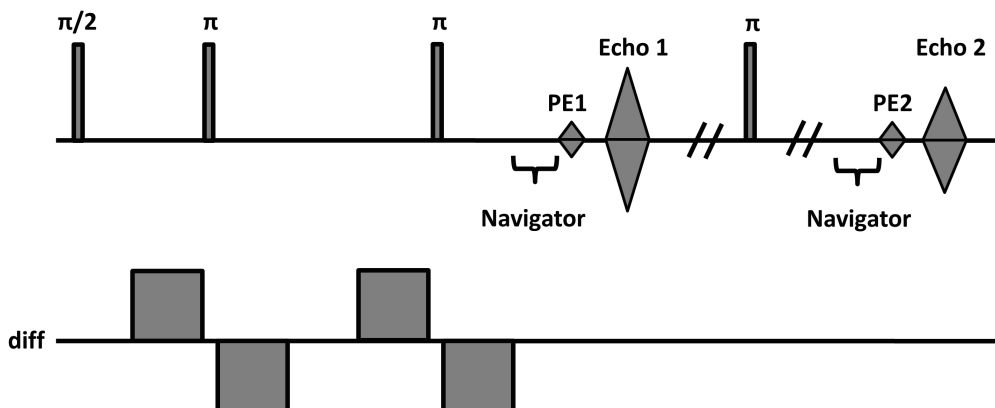


FIGURE 4.1: Schematic representation of the PRESS-based 2D DW-CSI pulse sequence, where bipolar gradients are used for diffusion weighting. The phase encoding (PE) gradient is placed after the second lobe of the refocusing diffusion gradient and before the echo maximum such that 25-30 navigator data points are acquired prior to the phase-encoding step.

Data were collected with cardiac gating using a peripheral pulse unit (PPU). The delay between the trigger and the excitation pulse at the beginning of the sequence was set to 230 ms in order to minimize fluctuations induced by cardiac pulsation. The resulting duration of the water suppression scheme was 223 ms. TR was set to three cardiac cycles (about 3 seconds), and the center RF frequency was set to that of tNAA (2.0 ppm). A turbo spectroscopic imaging (TSI) mode with two echo acquisitions ($TE = 100/205$ ms) was used, and 256 sample points were acquired for each of the two echoes with a spectral bandwidth of 5000 Hz. Water suppression with variable pulse power and optimized relaxation delays (VAPOR) [30] was used. The efficacy of the water suppression was de-optimized during the diffusion-weighted acquisitions by increasing the delay between the last pulse of the VAPOR block and the excitation pulse to 250 ms. This was done so that sufficient residual water signal was present for the navigator and for eddy current correction. For each DW-CSI scan, 5 consecutive CSI data sets were acquired: one without water suppression with $b = 0$ s/mm², to allow for accurate local frequency corrections; one data set with water suppression and no diffusion weighting ($b = 0$ s/mm²) and three diffusion-weighted data sets where the diffusion weighting was applied in three orthogonal directions aimed to maximize available gradient strength: $[-0.5, 1, 1]$, $[1, -0.5, 1]$ and $[1, 1, -0.5]$ [31]. The gradient duration was set to 20 ms, the time between the two bipolar gradients was 14 ms and the diffusion time (time between the first lobe of the dephasing group and the second lobe of the rephasing group) was 50 ms. The gradient amplitude was 35 mT/m, resulting in a b-value of 2870 s/mm². The total scan time for each subject was about 15-20 minutes.

One phantom experiment was performed at room temperature on the GE-MRS "Braino" phantom, which contained the following metabolites: NAA (12.5 mM); creatine (10 mM); choline (3 mM); Ins (7.5 mM); Glu (12.5 mM) and lactate (5 mM). One diffusion weighting direction was used, TR was set to 2 seconds, and the b-value was lowered to

936 s/mm². An additional SV-DWS experiment was performed, with the same conditions and experimental parameters and the VOI for this experiment was 2×2×2 cm³.

Navigator-based data reacquisition scheme: For each diffusion-weighted condition, 5 consecutive measurements with no phase encoding were made prior to acquisition of the phase-encoded data. The sum of the moduli of 6 navigator data points (points 5 - 10) was calculated for each of these measurements, and the highest sum was selected as the *amplitude reference*. An empirically-determined threshold of 85% of the amplitude reference was defined as the amplitude threshold for the following acquisitions. This threshold was then used in real-time as an accept/reject criterion for subsequent acquisitions for that diffusion condition such that for each k-space location, data that fell below the threshold were re-acquired. An upper limit for the number of reacquisitions was set to 100 for each diffusion condition.

4.2.3 Post-Processing and Data Analysis

4.2.3.1 CSI data post-processing

All data analyses were performed off-line. Raw data (k-space/time domain data sets from each individual receive coil) were exported from the scanner and an in-house MATLAB[®] (Mathworks, Natick, MA, USA) routine was used to perform the following operations: (a) individually phase and combine the data from each of the 32 receiver coils, weighted according to the SNR of each channel; (b) use the phase information from the navigator to correct for phase fluctuations introduced by the diffusion gradients (see later for details); (c) perform Hanning filtering on the k-space data, spatial and temporal Fourier transforms, voxel-wise frequency shift corrections using the non-water suppressed data; (d) perform voxel-wise eddy current correction using the residual water peak present in the free induction decay (FID), isolated by performing a linear prediction singular value decomposition (LPSVD) procedure on the FID and using the spectral components in a 1ppm region around the water resonance [32]; (e) remove the residual water resonance using the water spectral information obtained with the LPSVD routine in the eddy current correction stage; (f) export the 5 data matrices (data without water suppression, water-suppressed data with $b = 0$ s/mm² and the 3 diffusion-weighted data sets) in Philips format (SDAT/SPAR) for further spectral analysis.

4.2.3.2 Removal of diffusion-induced phase fluctuations using the phase navigator

The navigator time-domain data were zero-filled to 128 points and line-broadened. Following a Fourier transformation, a coarse spectrum of the navigator was obtained. The phase for each acquisition was then estimated from the residual water, and diffusion induced phase fluctuations were removed using the formula:

$$S_{ij,m}(corr) = S_{ij,m} \cdot \frac{\exp(i\theta_{nav}(ij, b=0))}{\exp(i\theta_{nav}(ij, m))} \quad (4.1)$$

where $S_{ij,m}$ is the FID obtained at the k-space location ij and diffusion condition m , and $\theta_{nav}(ij, m)$ is the navigator phase at this condition. It should be noted that at the condition $b = 0$ the phase includes the effect of the shift of the CSI matrix in image space.

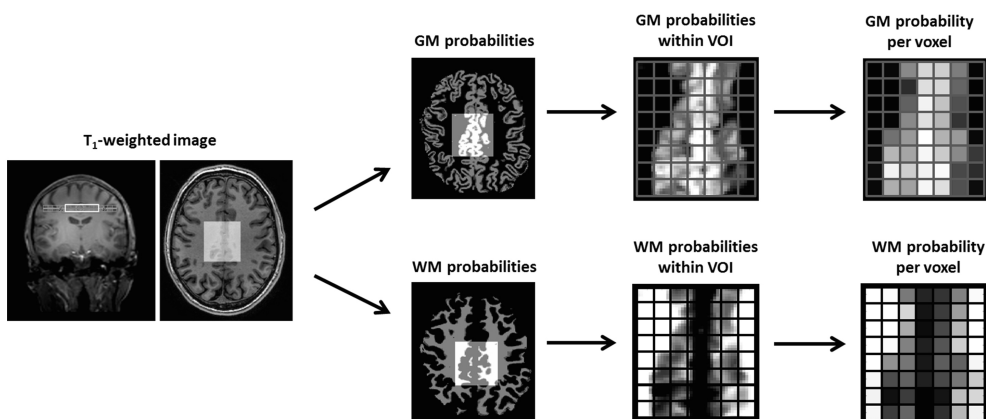


FIGURE 4.2: The procedure of voxel-wise estimation of gray and white matter probabilities within the VOI. The gray and white matter tissue probabilistic maps are obtained following the brain extraction from the T_1 -weighted image. The DW-CSI VOI is then overlaid on the corresponding location in the tissue maps such that the gray and white matter tissue probabilities within the VOI can be assessed. The gray and white matter tissue probabilities were then down-sampled to the resolution of the voxels of the DW-CSI within the VOI.

4.2.3.3 Tissue segmentation and estimation of gray and white matter fractions in CSI voxels

Voxel-wise estimation of white matter, grey matter and cerebrospinal fluid (CSF) fractions was performed with in-house MATLAB[®] routine, using tissue probability maps generated from the high resolution T_1 -weighted image. Brain-confined tissue probability maps were generated by first applying the FSL tools Brain Extraction Tool (BET) [33] and FMRIB's Automated Segmentation Tool (FAST) [34] to the T_1 -weighted volume. The MATLAB routine then matched the voxels in the tissue probability maps with the corresponding CSI voxels, and the final step was to average the tissue probabilities within each CSI voxel to yield an average voxel-wise gray matter and white matter fraction. Tissue fractions were defined according to: $f_{GM} = \frac{p_{GM}}{p_{GM} + p_{WM}}$ and $f_{WM} = \frac{p_{WM}}{p_{GM} + p_{WM}}$, where f_{GM} and f_{WM} are the GM and WM fractions, p_{GM} and p_{WM} are the gray matter and white matter probabilities. p_{CSF} , the CSF probability, was excluded since the concentration of metabolites in the CSF is negligible. The process is illustrated in Figure 4.2.

4.2.3.4 Spectral quantification and ADC calculation

Quantification of the CSI spectra for each condition was done using LCModel [35]. The LCModel output table for each condition, containing the peak estimation and Cramér-Rao lower bounds (CRLB) for each metabolite for each voxel location, was read into MATLAB for calculation of the ADC for each metabolite of interest according to:

$$ADC_{i,j} = -\frac{1}{3b} \sum_{n=1}^3 \ln \left(\frac{S_{i,j,n}(b)}{S_{i,j}(0)} \right) \quad (4.2)$$

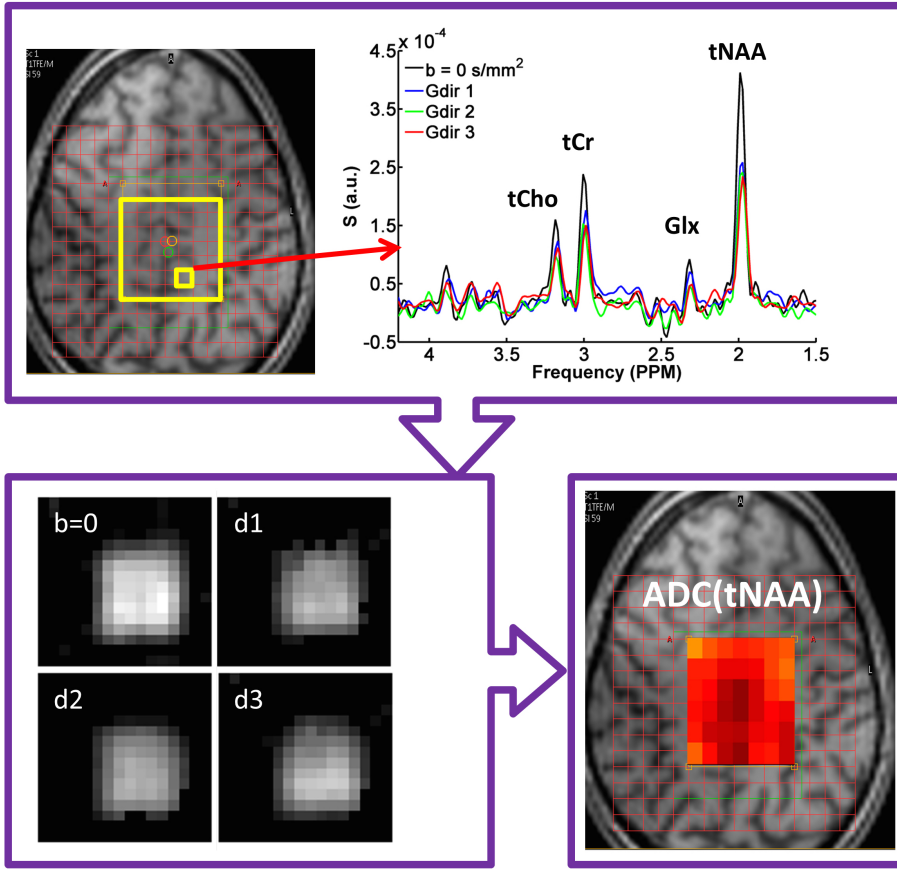


FIGURE 4.3: A schematic description of the acquisition and processing pipeline for DW-CSI.

where $S_{i,j,n}(b)$ is the signal intensity at the CSI location (i, j) obtained with the diffusion-weighting gradient in the direction n , $S(0)$ is the signal intensity without diffusion-weighting, and b is the b-value used in the experiment. Voxels with CRLB values higher than 10% for any of the four diffusion-weighting conditions were discarded.

Figure 4.3 describes the overall process from planning and acquisition to the generation of metabolite ADC maps, in this case for tNAA.

4.3 Results

4.3.1 Artifact removal from DW-CSI data using navigator information

The effects of the navigator-based artifact correction schemes used in our experiments on the DW-CSI data are shown in Figure 4.4, panels a-d, where representative tNAA DW-CSI maps for $b = 0 \text{ s/mm}^2$ and one diffusion weighted condition acquired with $b = 2870 \text{ s/mm}^2$ are shown. Panel (a) shows the CSI map of tNAA acquired at $b = 0 \text{ s/mm}^2$. It is seen that the tNAA signal is well localized within the selected VOI represented by a

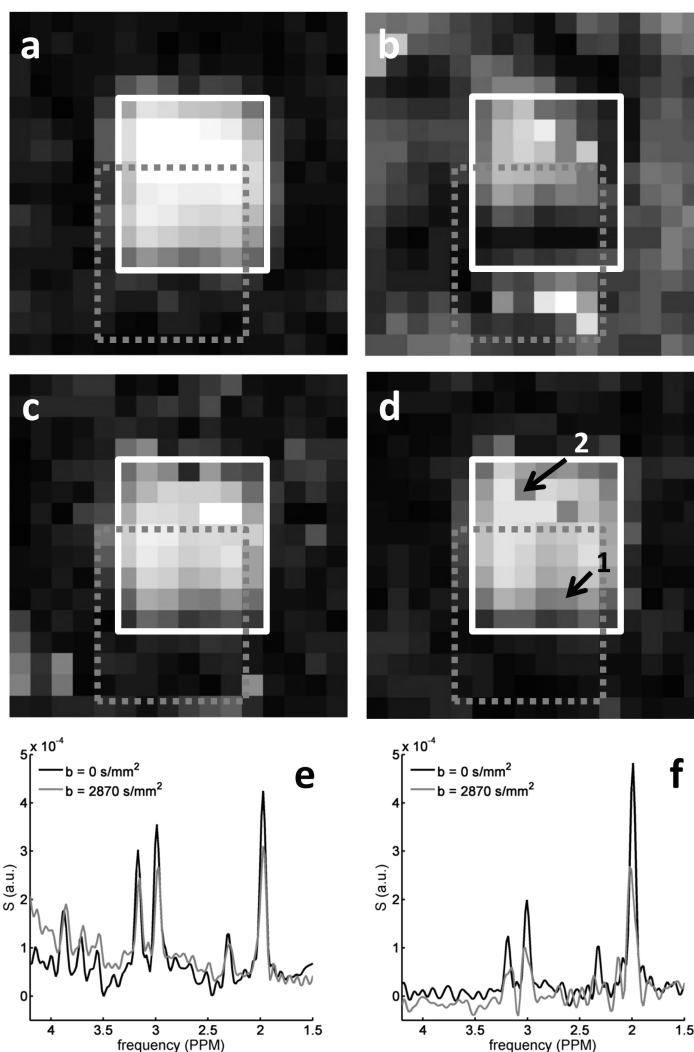


FIGURE 4.4: (a-d) DW-CSI maps of tNAA obtained at $b = 0 \text{ s/mm}^2$ (a) and $b = 2870 \text{ s/mm}^2$ from one diffusion-weighting condition processed in three different ways: (b) no navigator-based re-acquisitions and no removal of phase fluctuations are performed (c) removal of phase fluctuations is performed, without using the navigator-based re-acquired data (d) navigator-based reacquisitions are used, and removal of phase fluctuations is performed. Solid white rectangle: selected VOI at the tNAA singlet resonance frequency (2.0 ppm). Dashed gray rectangle: shifted VOI at the water resonance frequency (4.65 ppm). (e-f) The spectra acquired at $b = 0 \text{ s/mm}^2$ (black line) and with diffusion weighting at $b = 2870 \text{ s/mm}^2$ applied in one direction (gray line). Voxel 1 lies within the intersection of metabolite and water VOIs where sufficient water signal is obtained, thus an efficient eddy correction could be performed. Voxel 2 is located outside the water VOI, and the water-based eddy current correction was not effective. Line-broadening of 5 Hz and zero-filling to 1024 points were applied to all spectra.

solid rectangle. The dashed rectangle represents the shifted VOI that comprises the water signal. Panels b-d show DW-CSI tNAA maps obtained for the first diffusion weighting direction, $[1, 1, -0.5]$, at $b = 2870 \text{ s/mm}^2$ and processed in three different ways: Panel (b) shows the map obtained without using navigator-based re-acquisitions and without removal of the phase fluctuations induced by the diffusion weighting gradients. It can be seen that the localization of the signal to the preselected VOI is severely compromised and significant portion of the signal within the VOI is lost as well. In panel (c) removal of the phase fluctuations was performed, but the navigator based re-acquired data were not used. Localization of the tNAA signal within the selected VOI is improved, but there are still visible artifactual signal contributions from outside the selected VOI. Panel (d) shows the tNAA DW-CSI map obtained following both phase fluctuation removal and use of reacquired data. The number of reacquired excitations was 15 and the total number of k-space encoded excitations was 56. The tNAA data is now firmly localized within the preselected VOI. It should be noted that this data set was acquired without cardiac triggering so the degrading effects of both phase and amplitude fluctuations could be made clearly visible. In our standard protocol, the addition of cardiac triggering further reduces the typical number of reacquired excitations, and helps improve the overall quality of the DW-CSI data.

The effect of the eddy current correction based on the water signal is shown in Figure 4.4, panels (e-f). Panel (e) shows the spectra obtained from voxel 1 in Figure 4.4 panel (d), which lies within the intersection region between the VOI selected at the tNAA frequency (2.0 ppm) and the one selected at the water frequency (4.65 ppm). Spectra are shown for data acquired at $b = 0 \text{ s/mm}^2$ (black) and $b = 2870 \text{ s/mm}^2$ (gray). There is no discernible difference in the metabolite line shape and the quality of the spectrum is sufficiently restored by the eddy current correction procedure. The presence of the residual water peak shoulder can be seen in both spectra. Panel (f) shows spectra obtained from voxel 2 in Figure 4.4 panel (d), which lies inside the tNAA VOI, outside the water VOI and on the margin of the tCr/tCho VOI. The diffusion weighted spectrum is severely distorted by eddy currents, and as expected, the tCr, tCho peaks and other peaks at lower resonance frequencies are attenuated. For further analyses, voxels were chosen from the region where the water-based eddy current correction was effective.

4.3.2 DW-CSI Spectra and Metabolite ADC maps

Figure 4.5 shows typical spectra obtained from a CSI voxel within the preselected VOI where sufficient water signal was obtained. The four spectra shown in each panel were obtained with $b = 0 \text{ s/mm}^2$ and $b = 2870 \text{ s/mm}^2$ applied in the three diffusion gradient directions specified in the methods section. Typical CRLB values for tNAA, tCr and tCho were 3%, 4% and 5% at $b = 0 \text{ s/mm}^2$ and 5%, 6% and 7% at $b = 2870 \text{ s/mm}^2$, respectively. Typical ADC maps for tNAA, tCr and tCho are shown in Figure 4.6. The maps show the voxels within the preselected VOI for which the CRLB values for all four conditions were lower than 10%. Overlaid in solid line is the shifted VOI for the water peak. The intersection of the two regions contains the voxels in which the eddy current correction based on the water peak was reliable, and the ADC values from these voxels are used in the reproducibility and the tissue-based ADC analyses shown later. ADC values for the three metabolites calculated from the single volume and CSI phantom experiments ensured the accuracy of the CSI results; For the SV DW-MRS experiments the ADC values were $0.55 \times 10^{-3} \text{ mm}^2/\text{s}$, $0.76 \times 10^{-3} \text{ mm}^2/\text{s}$ and $0.84 \times 10^{-3} \text{ mm}^2/\text{s}$ for tNAA, tCr and tCho,

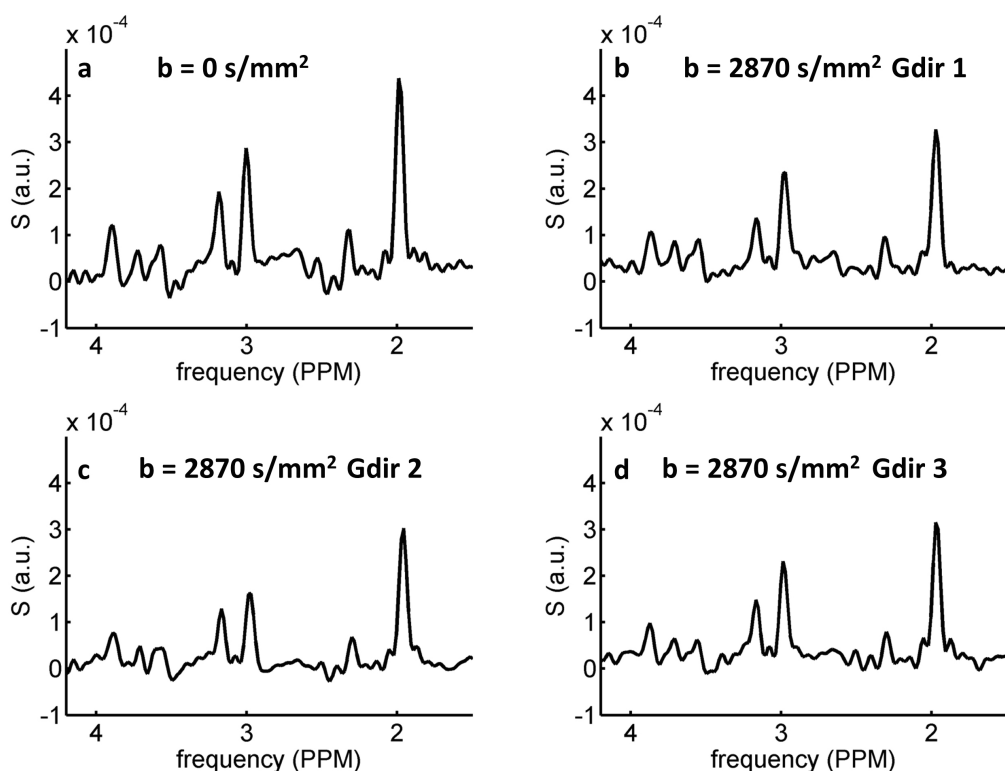


FIGURE 4.5: Typical spectra obtained from a single CSI voxel within the VOI at $b = 0 \text{ s/mm}^2$ (a) and $b = 2870 \text{ s/mm}^2$ applied in 3 diffusion gradient directions (b-d). Line-broadening of 5 Hz and zero-filling to 1024 points were applied to all spectra.

respectively, and for the CSI experiments the ADC values over the preselected VOI were $0.57 \times 10^{-3} \pm 0.02 \text{ mm}^2/\text{s}$, $0.73 \times 10^{-3} \pm 0.03 \text{ mm}^2/\text{s}$ and $0.87 \times 10^{-3} \pm 0.03 \text{ mm}^2/\text{s}$ for tNAA, tCr and tCho, respectively.

4.3.3 Reproducibility of DW-CSI data in repeated acquisitions

Pearson's linear coefficient (r) was calculated as a measure of correlation between the tNAA values obtained from the two scans from one subject, and the resulting values were $r = 0.99, 0.96, 0.97$ and 0.95 for $b = 0 \text{ s/mm}^2$ and the three diffusion-weighted acquisitions ($b = 2870 \text{ s/mm}^2$), respectively for this subject. The correlation between the ADC values calculated from these two data sets resulted in $r = 0.76$. For the second subject who had the two consecutive DW-CSI acquisitions, r values corresponding to the correlation between the tNAA values obtained from the two scans were $r = 0.96, 0.97, 0.94$ and 0.97 for $b = 0 \text{ s/mm}^2$ and the three diffusion-weighted acquisitions, respectively. The correlation between the ADC values calculated from these two data sets resulted in $r = 0.68$.

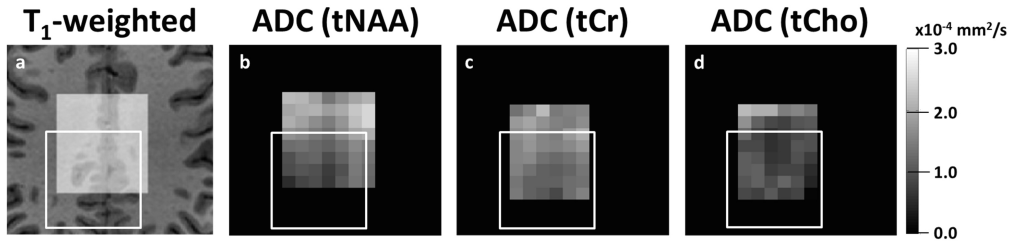


FIGURE 4.6: Metabolite ADC maps for tNAA (b), tCr (c) and tCho (d) and the corresponding T₁-weighted image (a) from one of the subjects. The shifted VOI at the water frequency is shown in white. Only voxels from the regions intersecting with the water VOI were selected for further analyses.

4.3.4 Tissue-based analysis of metabolite ADC

Metabolite ADC values from the DW-CSI data sets were co-analyzed with information on gray/white matter tissue fractions from the corresponding voxels to yield tissue specific information on metabolite ADC values. In Figure 4.7, ADC values for tNAA, tCr and tCho are shown as a function of the GM fraction in the corresponding voxels for one of the subjects. For this data set, Pearson's r values for the correlation between ADC(tNAA), ADC(tCr) and ADC(tCho) and white matter fraction were 0.69, 0.60 and 0.04, respectively.

Extrapolation of the linear fit, such as the one shown in Figure 4.7, to gray matter fractions of 0 and 1 allowed the estimation of metabolite ADC in white matter and gray matter, respectively. Table 4.1 shows metabolite ADC values for GM and WM estimated for each subject and for the entire cohort, as well as the correlation coefficients between the metabolite ADC and the GM fraction. ADC(tNAA) values were significantly negatively correlated with gray matter fraction, and less so were the ADC(tCr) values. The ADC(tCho) showed no significant correlation with gray matter fraction.

The group-averaged metabolite ADC values for GM and WM are shown in Figure 4.8. ADC(tNAA) was significantly higher in white matter than in gray matter (p -value < 0.001, paired 2-tail t -test). ADC(tCr) was also significantly higher in white matter than in gray matter (p -value < 0.005). No significant difference was found between ADC(tCho) in GM and WM.

4.4 Discussion

In this study we have introduced a robust and reproducible DW-CSI acquisition and processing scheme that yields spatially resolved diffusion weighted maps of tNAA, tCr and tCho in the human brain. The results are in agreement with previous tissue-based explorations of metabolite ADC using SV DW-MRS, and are anatomically meaningful, as will be later discussed. To our knowledge, this is the first study to fully demonstrate the feasibility of DW-CSI in humans in which instabilities in signal acquisition associated with the multi-shot nature of DW-CSI acquisition have been successfully resolved. Additionally, the combination with tissue probability maps obtained from high resolution T₁-weighted images allowed estimation of metabolite ADC values in gray and white matter.

Both phase and amplitude signal fluctuations during acquisition required the introduc-

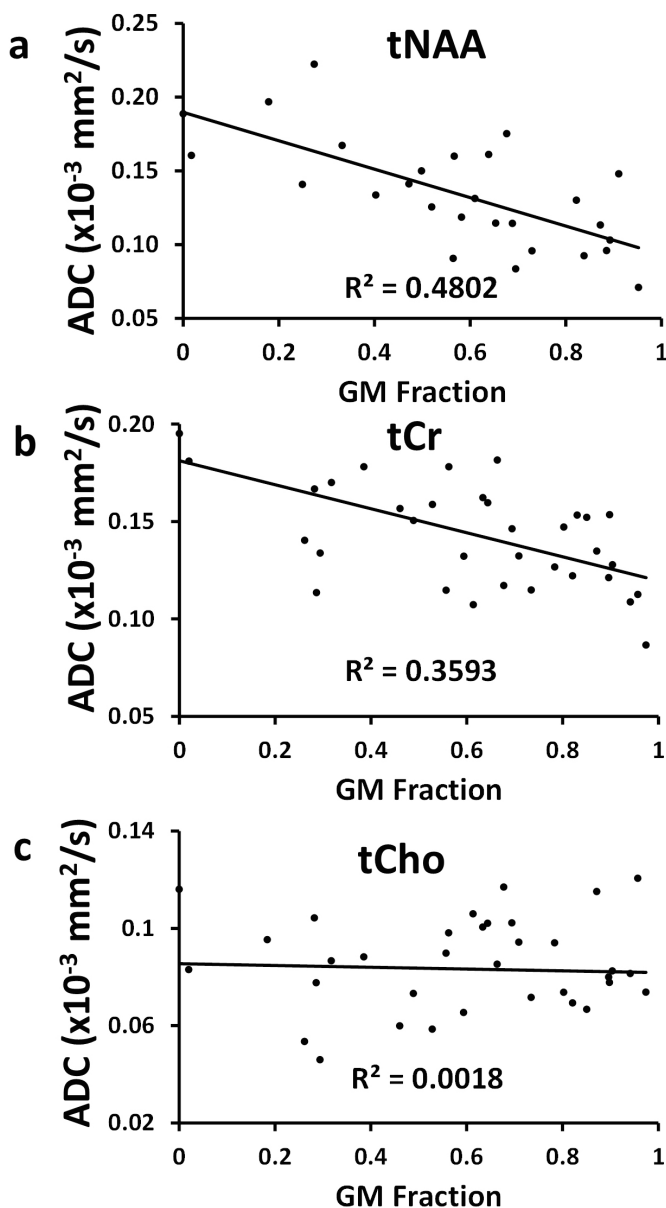


FIGURE 4.7: Correlation between voxel-wise gray matter tissue fraction with the corresponding tNAA (a), tCr (b) and tCho (c) ADC values, taken from one subject. The linear fit (black line) and resulting R^2 are displayed for each metabolite.

TABLE 4.1: Metabolite ADC values estimated for pure gray matter and pure white matter tissues for each subject and for the cohort. Pearson's r value represents the linear correlation of the ADC values as a function of gray matter fraction in the DW-CSI voxels and is presented with the corresponding p-value. NS, not significant.

ADC ($\times 10^{-3}$ mm ² /s)			
tNAA			
Subject	GM	WM	$r(P)$
S1 # 1	0.10	0.14	-0.47 (0.004)
S1 # 2	0.13	0.16	-0.66 (<0.001)
S2	0.11	0.16	-0.48 (0.004)
S3	0.11	0.17	-0.56 (<0.001)
S4 # 1	0.12	0.17	-0.32 (<0.001)
S4 # 2	0.09	0.19	-0.48 (<0.001)
Group	0.11 ± 0.01	0.17 ± 0.02	-0.57 ± 0.10
tCr			
Subject	GM	WM	$r(P)$
S1 # 1	0.10	0.14	-0.54 (<0.001)
S1 # 2	0.14	0.19	-0.59 (<0.001)
S2	0.13	0.14	-0.10 (NS)
S3	0.17	0.18	-0.14 (NS)
S4 # 1	0.15	0.17	-0.24 (NS)
S4 # 2	0.12	0.18	-0.60 (<0.001)
Group	0.13 ± 0.02	0.17 ± 0.02	-0.37 ± 0.23
tCho			
Subject	GM	WM	$r(P)$
S1 # 1	0.08	0.10	-0.22 (NS)
S1 # 2	0.12	0.14	-0.30 (NS)
S2	0.12	0.13	-0.01 (NS)
S3	0.11	0.12	-0.11 (NS)
S4 # 1	0.10	0.18	-0.43 (0.02)
S4 # 2	0.08	0.09	-0.04 (NS)
Group	0.10 ± 0.02	0.13 ± 0.03	-0.19 ± 0.15

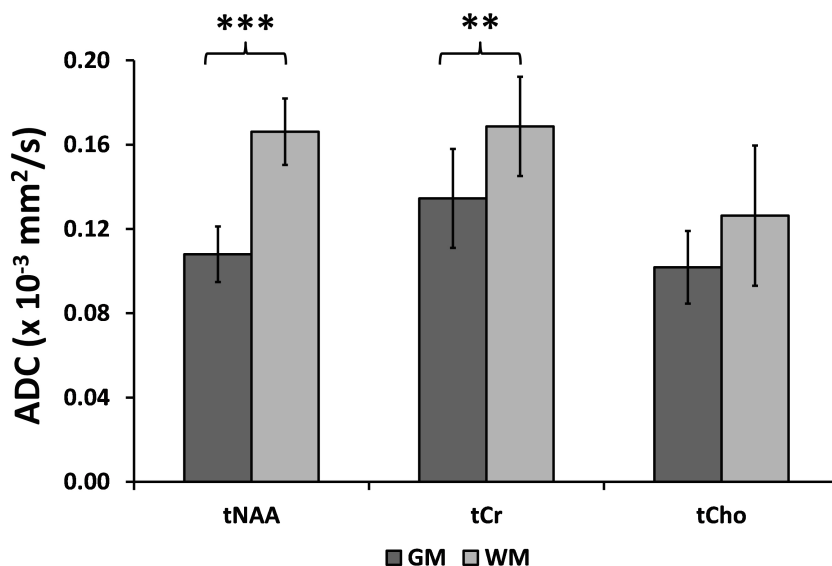


FIGURE 4.8: Group-averaged ($n=6$) metabolite ADC values in mm^2/s based on the extrapolation of the linear fit to unity tissue fractions for gray matter (dark gray) and white matter (light gray). Significant differences were found between gray and white matter ADC(tNAA) values at $p\text{-value} < 0.001$ (***) and ADC(tCr) at $p\text{-value} < 0.01$ (**). No significant difference was found between gray and white matter ADC(tCho). The error bars indicate the standard deviation.

tion of a navigator, positioned immediately after the diffusion weighting gradients and before the phase encoding step. The need to correct the phase of individual acquisitions in DW-MRS to avoid over-estimation of metabolite ADC has been shown in the case of SV DW-MRS [11, 13, 14]. In SV DW-MRS, phase correction is typically performed on the residual water peak. Applying this approach in DW-CSI required shifting the phase encoding gradient from its original position in the vendor-supplied sequence (immediately following the excitation pulse) to a position immediately before the echo creation, such that there are sufficient data points for which the signal phase and amplitude are dominated by the gradient-induced fluctuations. The dramatic effect of the phase fluctuations induced by the diffusion-weighting gradients is seen in Figure 4.4 panel (b), where the phase correction is not performed.

Our approach for minimizing the amplitude fluctuations in the DW-CSI signal was to introduce a user-controlled real-time reacquisition scheme for individual acquisitions, combined with cardiac triggering. Tight control over the amplitude fluctuations was crucial for the robustness and reproducibility of the resulting data. This is especially true for the acquisition of data near the central k -space region, where most of the signal energy is located and thus where signal drops will have the highest impact on the resulting CSI data quality. The effect of amplitude fluctuations on the data is shown in Figure 4.4 panel (c).

Much of the variation in the diffusion-weighted signal amplitude is caused by cardiac

pulsation [36–38], and in our experience, adding cardiac triggering to the acquisition scheme significantly reduced the number of navigator-based reacquisitions. It is possible to rely solely on the reacquisition scheme for filtering out strong signal fluctuations, but in that case acquisition of the complete data set would become significantly longer. Choosing a higher signal threshold for the re-acquisitions will result in an overall improvement of the resulting DW-CSI data, at the cost of a higher number of re-acquisitions and therefore an increase in scan time. In our work we set the threshold so that signal level below 85% of the reference triggered a re-acquisition. This resulted in reacquisition of about 10% of the total data. The threshold was empirically determined based on a compromise between the increase in total experimental time caused by increased number of re-acquisitions when the threshold was set too high, and the extent of signal fluctuations. The extent of the fluctuations varied across subjects as well as across diffusion weighting directions. This suggests that the orientation of major blood vessels within the VOI as well as the properties of CSF flow may have a strong impact on these fluctuations.

Eddy current correction is crucial in DW-MRS, even when a bipolar diffusion weighting scheme is used. This is shown in Figure 4.4 where voxels with and without efficient eddy current correction are shown. We based our eddy current correction scheme on the residual water signal in each voxel instead of acquiring a full DW-CSI dataset without water suppression and carrier frequency set to the water frequency due to time limitations. To ensure the availability of a sufficiently large water signal in the spectra across the selected VOI, we weakened the water suppression in the diffusion-weighted condition by increasing the delay between the VAPOR scheme and the excitation pulse. This approach is much less affected by inhomogeneity of the B_1 than alternative approaches such as modulating the RF power for the water suppression pulses [16], and thus more suitable for a large preselected VOI. A limitation of the PRESS sequence used in our study is the relatively low bandwidth of the two refocusing pulses, which in turn results in a large chemical shift displacement between the water signal and the other metabolites, shown e.g. in Figure 4.4. Efficient eddy current correction for each metabolite is thus possible in the intersection between the metabolite and the water VOIs, but voxels outside this volume are not properly corrected. Replacing the PRESS sequence with a STEAM partially addresses this issue, since the bandwidth of selective 90° pulses is somewhat higher, albeit with the well-known property of only detecting 50% of the available magnetization. A proper solution will be to incorporate a diffusion weighting scheme with a fully or partially adiabatic spectroscopic sequence, e.g. LASER or semi-LASER [39, 40] where the large bandwidth of the adiabatic pulses will result in a much smaller chemical shift displacement for the water resonance. Another solution is to acquire a full DW-CSI data set without water suppression, with the carrier frequency set to the water frequency. This water data set can be acquired with a shorter TR as well as with a lower spatial resolution, and thus may be incorporated in the protocol if scanning time allows.

The reacquisition and correction strategy implemented here resulted in good reproducibility of the DW-CSI results, as shown in the comparison of two subsequent DW-CSI acquisitions from the same subject. High voxel-by-voxel correlations are reported between acquisitions with the same diffusion weighting conditions, as well as high correlation between the two calculated ADC set of values. Several factors affect the reproducibility of the data, among which are the error in the estimation of the metabolite signal in each voxel (expressed e.g. by the CRLB of the peak estimation), the b-value used for the diffusion weighting, the threshold set on the reacquisition scheme, and patient motion

between the two consecutive scans. The error in signal estimation propagates to the error in estimating the metabolite ADC values (see [2], p.269). The correlation between the two consecutive sets of ADC values was somewhat lower than the correlation between the actual DW-CSI sets possibly due to error propagation properties, as well as due to the smaller relative range of ADC values with respect to their mean, $\Delta ADC / \overline{ADC}$, compared to $\Delta S(CSI) / \overline{S(CSI)}$, the relative range of the DW-CSI signal.

A strong negative correlation of ADC(tNAA) with GM fraction (or alternatively-stated as a strong positive correlation with WM fraction) was found. ADC(tCr) values were negatively correlated with GM fraction as well, albeit to a lesser extent. No correlation was observed between ADC(tCho) and GM fraction. Conversely, the ADC for the three metabolites in white matter, obtained through extrapolation of the ADC data to $f_{GM} = 0$, were higher than their ADC in gray matter, although in tCho this difference did not reach statistical significance. We have reported similar trends in a previous work performed on large single volumes positioned on regions with different content of gray and white matter [16]. A plausible explanation for these findings is the different cellular localization of these metabolites. In white matter, tNAA is almost exclusively axonal, tCr is present in similar concentrations in axons and astrocytes, whereas tCho is predominantly astrocytic [41]. The open-ended axonal geometry and the more “restricted”, close-ended astrocytic geometry could thus provide an explanation for the higher ADC(tNAA) and the lower ADC(tCho) in white matter. In gray matter the prevalence of neuronal cell bodies over long range fibers may serve to explain the lower ADC of all three metabolites, and in particular that of tNAA, in gray matter compared to their corresponding ADC values in white matter.

The metabolite ADC values in this study were obtained from a set of relatively small voxels (0.3 cc) and combined with estimated tissue probabilities that provided a wide range of voxel-wise tissue fractions. This allowed us to more accurately estimate tissue-specific metabolite ADC values and in particular metabolite ADC in gray matter, where previous studies, including our own, reported values obtained from large VOIs where partial volume of white matter is unavoidable. In our study, GM metabolite ADC values of all three metabolites were lower than values previously reported [16, 17, 22]. Values previously reported for metabolite ADC in white matter, e.g. for tNAA, are comparable to those reported here [11, 16, 17, 21]. Due to time limitations, the k-space encoding matrix was relatively small (12×12). Even though the matrix was significantly larger than the selected VOI and Hanning filtering was used to reduce sinc-like intervoxel bleeding, this choice has significant implications on the point spread function of the voxel-wise spectroscopic data, which then becomes contaminated by signal from adjacent voxels [42]. For simplicity, the average tissue probabilities within a CSI voxel were calculated assuming the voxels to be perfect rectangular cuboids with the same size as the reconstructed voxel size of $6 \times 6 \times 8 \text{ mm}^3$. This limits the tissue specificity of the ADC values calculated here, and emphasizes the need for a more efficient k-space sampling strategy.

The significant increase in sensitivity associated with data acquisition at 7T is offset by several challenges. One is the relative increase in chemical shift displacement of the selected VOI, resulting in inefficient eddy current correction in certain regions. Significant inhomogeneity in the transmit B_1 field at 7T result in large spatial differences in the resulting signal generated by spectroscopic sequences such as PRESS, due to the large flip angles used. This limits the effective field of view to a relatively small region, and for this reason the preselected VOI was set to a relatively small section of the encoded area. Some

of these detrimental effects can be alleviated by using B_1 -insensitive adiabatic pulses [39], multiple transmit techniques and padding the head with dielectric material [43]. A bigger VOI selection could also be possible at lower fields such as 3T. One limitation in the way we implemented our DW-CSI acquisition is that CSI data sets under different diffusion weighting conditions are acquired consecutively, or in other words, the inner loop is the k-space position and the outer loop is the diffusion-weighting condition. It is possible to reduce the sensitivity to long-term motional drifts by switching the loop order, and we intend to test this approach in the future. Although the re-acquisition scheme significantly reduced the variance caused by the various instabilities described above, some inconsistencies in our limited data sets, e.g. those in the ADC(tCr) in the repeat scans of subject #4 suggest that there are some sources of variability that our method currently does not account for. For example, we observed that the chosen threshold was not optimal for all three gradient directions in all subjects, and the amplitude fluctuations in a single gradient direction could significantly increase the variability in the resulting metabolite ADC (data not shown). Further investigations are needed to optimize the trigger delay and the corresponding amplitude thresholds, for further improving the robustness of the method. Another limitation of the current implementation is that at this point we have not made use of the parallel imaging capability provided by the multiple receive coil array and the availability of a sensitivity encoding (SENSE) reference scan, resulting in long acquisition times that could limit clinical investigations. Given the relatively high SNR obtained in this study, the acquisition could be shortened by combining the acquisition with SENSE at a reasonable reduction factor [44]. This will also allow the acquisition of full diffusion tensor CSI data sets in a reasonable time, thus opening the way for a methodical comparison between metabolite and water tensor data.

4.5 Conclusions

In summary, we have implemented and showed the feasibility of DW-CSI acquisition and processing scheme in the human brain. The navigator-based corrections implemented in this scheme significantly improve the stability of the DW-CSI signal and the overall quality, reproducibility and robustness of the resulting DW-CSI maps. The method has been shown to work with a two-echo CSI acquisition, allowing for significant shortening of the experimental time. Metabolite ADC values obtained in this work related meaningfully to the underlying tissue cellular composition. Based on previous successful applications of SV DW-MRS to the investigation of neurological disorders, DW-CSI can play a role in increasing the specificity of MR to compartment-specific tissue alterations in disease and lead to identification of new biomarkers for intracellular damage.

4.6 References

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Part II

Clinical Applications

5

A multimodal MRI approach to identify and characterize microstructural brain changes in Neuropsychiatric Systemic Lupus Erythematosus

This chapter was adapted from:

E. Ercan, C. Ingo, O. Tritanon, C. Magro-Checa, A. Smith, S. Smith, T. Huizinga, M.A. van Buchem and I. Ronen, "A multimodal MRI approach to identify and characterize microstructural brain changes in neuropsychiatric systemic lupus erythematosus", *NeuroImage: Clinical*, 8: 337-344, 2015.

Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease with multi-organ involvement and results in neurological and psychiatric (NP) symptoms in up to 40% of the patients. To date, the diagnosis of neuropsychiatric systemic lupus erythematosus (NPSLE) poses a challenge due to the lack of neuroradiological gold standards. In this study, we aimed to better localize and characterize normal appearing white matter (NAWM) changes in NPSLE by combining data from two quantitative MRI techniques, diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI). 9 active NPSLE patients (37 ± 13 years, all females), 9 SLE patients without NP symptoms (44 ± 11 years, all females), and 14 healthy controls (HC) (40 ± 9 years, all females) were included in the study. MTI, DTI and fluid attenuated inversion recovery (FLAIR) images were collected from all subjects on a 3T MRI scanner. Magnetization transfer ratio (MTR), mean diffusivity (MD), fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) maps and white matter lesion maps based on the FLAIR images were created for each subject. MTR and DTI data were then co-analysed using tract-based spatial statistics and a cumulative lesion map to exclude lesions. Significantly lower MTR and FA and significantly higher AD, RD and MD were found in NPSLE compared to HC in NAWM regions. The differences in DTI measures and in MTR, however, were only moderately co-localized. Additionally, significant differences in DTI measures, but not in MTR, were found between NPSLE and SLE patients, suggesting that the underlying microstructural changes detected by MD are linked to the onset of NPSLE. The co-analysis of the anatomical distribution of MTI and DTI measures can potentially improve the diagnosis of NPSLE and contribute to the understanding of the underlying microstructural damage.

5.1 Introduction

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is a relapsing-remitting female-predominant autoimmune disease affecting multiple organs [1–4]. Neuropsychiatric (NP) events are common in SLE but only in 40% of these cases the symptoms can be directly attributed to SLE. [2, 5, 6]. SLE-associated NP syndromes are very heterogeneous, ranging from mild symptoms, such as headaches and mood disorders, to severe NP syndromes, such as seizures, and psychosis [1, 2, 6, 7]. The presentation of NP complaints in a patient with SLE continues to pose a diagnostic challenge to practicing physicians, mainly due to the absence of adequate diagnostic tests for NPSLE, resulting in an inconclusive diagnosis in a significant number of cases [6, 8, 9].

Magnetic Resonance Imaging (MRI) has emerged as a promising technique to study NPSLE, as T₂-weighted and fluid-attenuated inversion recovery (FLAIR) scans have revealed focal hyperintense lesions, which are present in deep, periventricular and subcortical white matter (WM) in up to 70% of NPSLE patients [2, 5, 8, 10, 11]. Although they are common among NPSLE patients, these white matter hyperintensities (WMH) are non-specific and can be found in a variety of groups of SLE patients, including those with active NPSLE (SLE patients with active NP symptoms), SLE patients with NP episodes in their past (inactive NPSLE), and SLE patients with no NP symptoms as well as in many other conditions unrelated to SLE, such as aging, multiple sclerosis, cerebral small vessel disease [10]. Recent studies also showed that 42-47% of the patients with NPSLE do not have any visible MRI abnormalities, suggesting that the link between FLAIR abnormalities and the etiology of NPSLE is tenuous [10, 12].

Quantitative MRI techniques such as diffusion weighted imaging (DWI), diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI) have been shown to be sensitive to *diffuse* alterations in the brain of NPSLE patients, and can be evaluated by quantitative maps of e.g. mean diffusivity (MD) and fractional anisotropy (FA) of DTI, or other methods, such as histograms of these quantities and of magnetization transfer ratio (MTR) [9, 13–20]. Previous MTI studies of NPSLE patients showed lower peak heights in both whole brain and tissue specific MTR histograms compared to healthy controls (HC), suggesting either demyelination or an increase in free water resulting from cytotoxic or vasogenic edema [18, 19]. These alterations were also shown to correlate with reversible changes in outcome of patients with active symptoms [18]. In a separate DTI study that used tract based spatial statistics (TBSS), higher MD and lower FA in WM were reported for NPSLE patients compared to HC, also suggesting a diffuse microstructural basis for the NP symptoms [16].

Although MRI is sensitive to changes associated with NPSLE, no single technique is shown to be specific enough to serve as a diagnostic tool for both the diffuse and focal changes in the brain. A few studies have utilized multimodal analysis of several MRI techniques, including FLAIR, T₁-weighted and T₂-weighted images, MTI and DTI, to understand the etiology of NPSLE [21–24]. These studies, however promising, were limited to showing only global changes in the whole brain or in total normal appearing brain tissue. No study to date employed a voxel-based combination and comparison of different quantitative MRI maps to study NPSLE to investigate the anatomical distribution of tissue damage in NPSLE as reflected by DTI and MTI, as well as the relationship between microstructural damage in normal appearing white matter (NAWM) and WMH.

In this study we simultaneously assessed and combined quantitative information from

MTI, DTI and FLAIR images to better localize and characterize WM changes in SLE and in NPSLE. In this way we aim to obtain more information on the underlying pathological processes driving NPSLE, and compare the diagnostic potential of FLAIR, DTI and MTI whether separately applied or combined.

5.2 Materials and methods

5.2.1 Patients and healthy controls

Nine NPSLE patients (37 ± 13 years), nine SLE patients without NP symptoms (44 ± 11 years) and fourteen age-matched HC without known neurological abnormalities or autoimmune diseases (40 ± 9 years) were included in the study. All subjects were female. The study was in compliance with Helsinki Declaration and approved by Institutional Review Board of Leiden University Medical Center (LUMC). All subjects provided a written informed consent. All SLE and NPSLE patients were recruited from the Department of Rheumatology at our institution and had been diagnosed with SLE according to the 1982 revised American College of Rheumatology (ACR) criteria [25, 26]. NPSLE diagnosis was made after exclusion of other causes by multidisciplinary consensus. NP diagnoses were classified according to the 1999 ACR case definitions for NPSLE syndromes and each NPSLE patient was categorized according to the presence of inflammatory and/or ischemic syndromes [27, 28]. The NPSLE subjects had at least one of the following central nervous system NP syndromes: psychosis ($n=1$), headache ($n=5$), movement disorder ($n=1$), transverse myelitis ($n=1$), seizure ($n=1$), anxiety ($n=1$), cognitive disorder ($n=4$) and mood disorder ($n=3$). Demyelinating syndrome, aseptic meningitis and acute confusional state were not present in our group of NPSLE patients. Table 5.1 shows the clinical characteristics, the autoantibody profile and the medication of the study subjects at the time of the MRI.

5.2.2 Data acquisition

MRI scans were performed on a 3 Tesla Achieva Philips MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with an 8-channel receive head coil. MRI scan protocol consisted of (a) Axial 3D T_1 -weighted images (FOV: $224 \times 177 \times 144$ mm³, resolution: $0.88 \times 0.88 \times 1.20$ mm³, TR/TE = $9.75/4.59$ ms); (b) Sagittal FLAIR images (FOV: $220 \times 173 \times 220$ mm³, resolution: $0.98 \times 0.98 \times 1.12$ mm³, TR/TE/TI = $4800/274/1650$ ms); (c) Axial Diffusion Tensor Images (DTI) (FOV: $224 \times 224 \times 120$ mm³, resolution: $2 \times 2 \times 2$ mm³, TR/TE = $8500/95$ ms, one $b=0$ s/mm² volume and 15 diffusion-weighted volumes with a b -value of 1000 s/mm²); (d) Axial MTI (FOV: $230 \times 230 \times 132$ mm³, resolution: $1.44 \times 1.44 \times 1.5$ mm³, TR/TE = $57.07/10.00$ ms, flip angle: 15° , two sets of images acquired with and without a sinc-gauss radio frequency saturation pulse of 25 ms duration, peak B_1 amplitude of 5.283 μ T and offset frequency of 2000 Hz).

5.2.3 Image Processing and analysis

5.2.3.1 FLAIR Lesion Segmentation

WM hyperintensities were manually segmented from the FLAIR images by an expert reader (O.T.) and binary lesion masks were created for each subject using MIPAV (Medical Image Processing, Analysis and Visualization) software [29, 30]. All FLAIR images were affine registered to MNI152 standard space using FSL FLIRT [31, 32]. The same transformation matrix was applied to align individual lesion masks to the standard space.

TABLE 5.1: Demographics of the study subjects and patient characteristics from SLE and NPSLE patients.

		NPSLE patients (n=9 females)	SLE patients (n=9 females)	Healthy controls (n = 14 females)
Patient characteristics	Age (years)	37 $\pm$ 13	44 $\pm$ 11	40 $\pm$ 9
	SLE disease duration (years)	10 $\pm$ 9	11 $\pm$ 8	-
	NPSLE disease duration (years)	1 $\pm$ 2	-	-
	SLEDAI	4 $\pm$ 5	2 $\pm$ 2	-
	Antiphospholipid syndrome	3 (33%)	1 (11%)	
	Active NPSLE patients	7 (78%)	-	
	Inflammatory phenotype	6 (67%)	-	
	Ischemic phenotype	6 (67%)	-	
Auto-antibodies	Antinuclear antibody	9 (100%)	9 (100%)	
	Anti-DNA	2 (22%)	7 (78%)	
	Anti-RNP	2 (22%)	1 (11%)	
	Anti-SSA	3 (33%)	4 (44%)	
	Anti-SSB	1 (11%)	1 (11%)	
	Anti-Smith	1 (11%)	0	
	Anticardiolipin autoantibodies	1 (11%)	2 (22%)	
	Lupus anticoagulant	3 (33%)	2 (22%)	
ACR criteria	Anti-B2 glycoprotein	2 (22%)	0	
	Malar rash	3 (33%)	2 (22%)	
	Discoid lupus	3 (33%)	3 (33%)	
	Photosensitivity	4 (44%)	5 (56%)	
	Aphthosis	5 (56%)	3 (33%)	
	Arthritis	8 (89%)	7 (78%)	
	Serositis	1 (11%)	3 (33%)	
	Lupus nephritis	1 (11%)	3 (33%)	
	NPSLE	9 (100%)	0	
	Hematologic disorder	6 (67%)	7 (78%)	
	Immunologic disorder	6 (67%)	9 (100%)	
	Antinuclear antibodies	9 (100%)	9 (100%)	
Medication	Prednisone	7 (78%)	3 (33%)	
	Azathioprine	1 (11%)	4 (44%)	
	Methotrexate	0	1 (11%)	
	Hydroxychloroquine	5 (56%)	8 (89%)	
	Rituximab	1 (11%)	0	

Cumulative lesion masks were created for NPSLE patients, SLE patients and HC by adding all individual lesion masks from each group in standard space.

5.2.3.2 DTI post-processing

The diffusion-weighted images were brain-extracted with the BET tool in FSL¹. Using an estimate of the noise variance in CSF signal intensity of the right ventricle, the data was denoised using an algorithm described in [33] and then Rician noise corrected using the algorithm in [34]. The data were corrected for motion and eddy currents by co-registering diffusion weighted images to the image acquired with $b = 0 \text{ s/mm}^2$ using the SPM8 toolbox². The same transformation matrix was applied to the diffusion gradient directions to rotate them according to the registration algorithm. DTI metrics were estimated on a voxel-wise basis using Diffusion Toolbox (FDT) [35]. In order to have an initial alignment between all subjects, each subject's FA map was first registered to the FA map of one representative HC through a rigid registration with 6 degrees of freedom. FA maps were then used as an input to the TBSS process [36] where all FA maps were registered to the FSL FA template in MNI standard space through non-linear registration, and a mean FA image was created from the average of all individual FA images. The mean FA image was then skeletonized, and a threshold of 0.25 was applied to the skeleton to exclude non-WM voxels. All individual FA maps were then projected onto this common skeleton by using the maximum FA value perpendicular to the skeleton voxels. The same transformations and projections were applied to the other DTI maps, such as MD, radial diffusivity (RD) and axial diffusivity (AD).

5.2.3.3 MTI post-processing

An in-house FSL script was used for the processing of the MTI data. The image without saturation (M_0) was brain-extracted using BET and the binary brain mask obtained from BET was applied to the image with saturation (M_s). M_s was then aligned to M_0 with normalized mutual information algorithm using 7 degrees of freedom in FSL FLIRT. The magnetization transfer ratio (MTR) was calculated as a percentage on a voxel-wise basis according to the following equation:

$$MTR = \frac{M_0 - M_s}{M_0} \times 100 \quad (5.1)$$

where M_s is the voxel intensity with saturation and M_0 is the voxel intensity without saturation. In order to align the MTR images with the FA images, the unsaturated image was co-registered to the image at $b = 0 \text{ s/mm}^2$ through affine registration using normalized mutual information, following the procedure described in [37]. The same transformation matrix was then used to align the MTR images with the FA images. Finally, the same MNI transformations and projections used in the DTI post-processing were applied to MTR.

5.2.3.4 Statistical Analysis

Permutation tests were applied to 4D skeletonized FA, MD, AD, RD and MTR images in order to assess voxel-wise statistics between NPSLE patients, SLE patients, and HC. 5000 permutations were used and corrected for multiple comparison based on TFCE

¹<http://www.fmrib.ox.ac.uk/fsl>

²<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>

(Threshold-Free Cluster Enhancement) [38]. Age related changes between groups were corrected by using demeaned age of each individual as a covariate in the TBSS process. In order to assess the statistical differences between NPSLE, SLE, and HC on WM voxels, ANOVA tests were performed with a threshold of p-value < 0.05 on FA, MD, AD, RD and MTR maps. Mann-Whitney U tests was applied to the statistical comparisons of the number and normalized volume of WMH between NPSLE, SLE and HC groups in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp. Released 2011, Armonk, NY, US).

5.2.3.5 Co-analysis of DTI and MTR data

An in-house developed MATLAB® (Mathworks, Natick, MA, USA) code was used to assess the overlap of significant differences between AD, FA, MD, RD and MTR in the different subject cohorts. FSL volumes containing the voxel-wise statistics corrected for age and multiple comparisons were used as an input for the co-analysis. Voxels with a p-value < 0.05 that overlapped in any two of the above metrics were counted and divided by the total number of voxels in the skeletonized WM in order to compute the ratio of overlap between significantly different regions in these two metrics. The mismatch between MTR and each DTI metric was also similarly assessed in order to find the regions with only MTR or DTI differences between groups.

5.2.3.6 Histogram analysis

In order to relate the current data to the histogram findings in previous works, we generated histograms based on the MD and MTR voxel-wise values in the skeletonized WM of the three subject groups. The following steps were followed, using an in-house developed MATLAB script: in order to generate histograms based only on NAWM, MTR, and MD, skeletonized images from all subjects were individually masked in MNI space with the lesion masks. Subsequently, MTR and MD histograms were created for each subject. All histograms were normalized by the total number of NAWM voxels in the skeleton for each individual subject. Peak height and locations were then calculated for each normalized histogram. Average histograms of MD and MTR in NAWM were then created for SLE, NPSLE and HC groups separately. The statistical comparisons of the histogram peak height and locations between NPSLE, SLE and HC groups were performed with Mann-Whitney U tests in SPSS software.

5.3 Results

5.3.1 Co-analysis of MTR and DTI data

Figure 5.1 shows the comparison of FA, MD, AD, RD, and MTR between NPSLE patients and HC (top row), and between SLE and NPSLE patients (bottom row) on one axial slice in MNI152 space. NPSLE patients showed significantly higher AD, RD and MD, and lower FA and MTR than both HC and SLE patients. Cumulative WM lesion maps computed for NPSLE patients and SLE patients are the top and bottom rightmost panels in Figure 5.1 respectively.

Table 5.2 shows the fractions of the total number of voxels in the skeletonized WM with significant differences in the various DTI measures and in MTR between NPSLE and HC (top rows) and between NPSLE and SLE (bottom rows). The diagonal elements in the Table represent the volume fraction of significant voxels of any single modality, and the off-diagonal elements represent the volume fractions of overlap between two modalities. For example, voxels with significant differences in MD between NPSLE and HC comprised

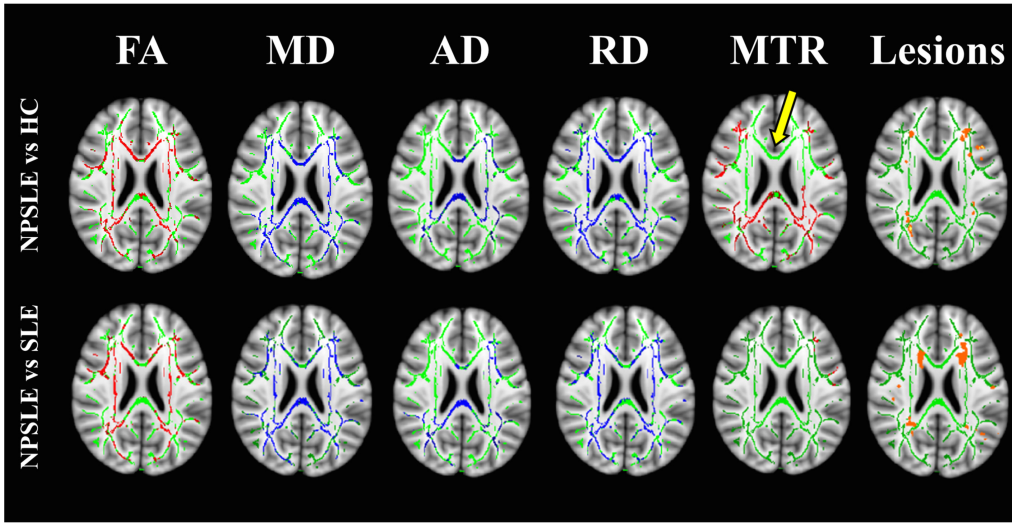


FIGURE 5.1: Comparison of statistically significant differences ($p\text{-value} < 0.05$) in FA, MD, AD, RD, and MTR between NPSLE patients and healthy controls (top row), and between SLE and NPSLE patients (bottom row) on one axial slice in MNI152 space. The mean FA skeleton is shown in green, regions with higher values in the NPSLE patients compared to SLE or HC are shown in blue and regions with lower values in NPSLE patients compared to SLE or HC are shown in red. Yellow arrow shows one of the regions (i.e. genu of the corpus callosum) where extensive differences were observed in RD, MD and FA but not in MTR. Cumulative WM lesion maps from NPSLE patients (top rightmost image) and SLE patients (bottom rightmost image) are shown in orange.

TABLE 5.2: Ratio of significantly different voxels in between NPSLE vs HC and NPSLE vs SLE in AD, FA, MD, RD and MTR to the total number of voxels in the skeleton (highlighted in gray) and the ratio of significantly different voxels overlapping in the significant regions in two of these metrics to the total number of voxels in the skeleton. Overlaps between DTI metrics and MTR are shown as a ratio of significantly different voxels in MTR. Overlaps between all metrics with MD are shown as a ratio of significantly different voxels in MD.

		AD	FA	MD	RD	MTR	Overlap MTR	Overlap MD
NPSLE vs HC	AD	0.18	0.07	0.17	0.14	0.05	0.19	0.40
	FA		0.39	0.24	0.34	0.15	0.52	0.57
	MD			0.42	0.37	0.14	0.51	1.00
	RD				0.49	0.17	0.61	0.87
	MTR					0.28	1.00	0.34
NPSLE vs SLE	AD	0.29	0.08	0.24	0.20	0.00	0.10	0.54
	FA		0.34	0.22	0.29	0.01	0.47	0.48
	MD			0.46	0.39	0.01	0.38	1.00
	RD				0.49	0.02	0.56	0.86
	MTR					0.03	1.00	0.83

42% of the skeletonized WM, whereas voxels with significant differences between NPSLE and HC in *both MD and MTR* comprised 14% of the skeletonized WM.

5.3.1.1 MTR results

Widespread differences in MTR values ($MTR(NPSLE) < MTR(HC)$ in 28% of the WM skeleton) were observed between NPSLE and HC. Strikingly, as opposed to the extensive differences in DTI metrics between NPSLE and SLE subjects, few differences in MTR were found between these two groups. There were no statistically significant differences in MTR between the SLE and the HC groups (results not shown).

5.3.1.2 DTI results

The most significant differences in DTI measures between NPSLE and HC were in RD (49% of WM skeleton), MD (42%) and FA (39%). The AD differences were significantly less extensive (18%). The differences in DTI measures between the NPSLE and SLE groups were as extensive as those found between the NPSLE and HC. This is in stark contrast to the relatively minor MTR differences detected between these groups. There were no significant differences in any of the DTI measures between SLE and HC (results not shown).

5.3.1.3 MTR/DTI overlap

MTR differences between NPSLE and HC, although extensive *per se*, had only a moderate overlap with the widespread DTI differences between the two groups (rightmost column of Table 5.2). This can be also qualitatively appreciated on Figure 5.1. For example, extensive differences in RD, MD and FA were observed in the genu of the corpus callosum (yellow arrow), but were not accompanied by any MTR differences. Overlap between regions with significant MTR and DTI differences between NPSLE and SLE was negligible, as the regions with significant MTR differences between these populations were in themselves very small (3% of skeletonized WM).

5.3.2 Lesion analysis results

Figure 5.2 shows the median, 25% and 75% quartile range for WM lesion volumes and lesion counts in NPSLE patients, SLE patients and HC. Both WM lesion volume and number of lesions were significantly different between NPSLE patients and HC (p -value < 0.01). No significant difference was found between SLE and NPSLE patients or SLE patients and HC.

5.3.3 Histogram analysis results

Figure 5.3 shows the average MD (panel a) and MTR (panel b) histograms for NPSLE, SLE and HC. Table 5.3 shows the average and standard deviation of peak heights and peak locations as well as the results of the non-parametric statistical tests on the normalized histograms from all three groups. MTR histogram peak heights of NAWM were significantly lower in NPSLE compared to HC and SLE, whereas MD histogram peak heights were significantly higher. MTR histogram peak heights of NPSLE patients were also significantly lower than both those of SLE and HC. MTR peak location was significantly lower in NPSLE compared to HC and not significantly different from that of SLE. No significant differences were observed in MD and MTR peak heights and locations of SLE histograms compared to those of HC.

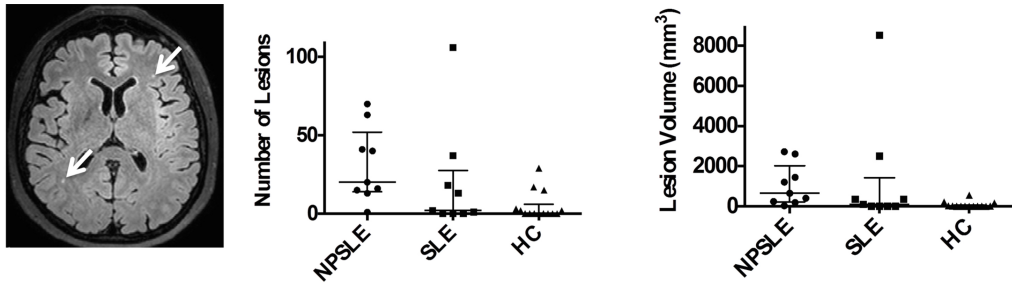


FIGURE 5.2: White matter lesions shown with white arrows on one axial slice of the FLAIR image from an NPSLE patient (left panel). Scatter plots of WM lesion counts (middle panel) and volumes (right panel) in NPSLE patients, SLE patients and healthy controls shown with the median, 25% and 75% quartile range.

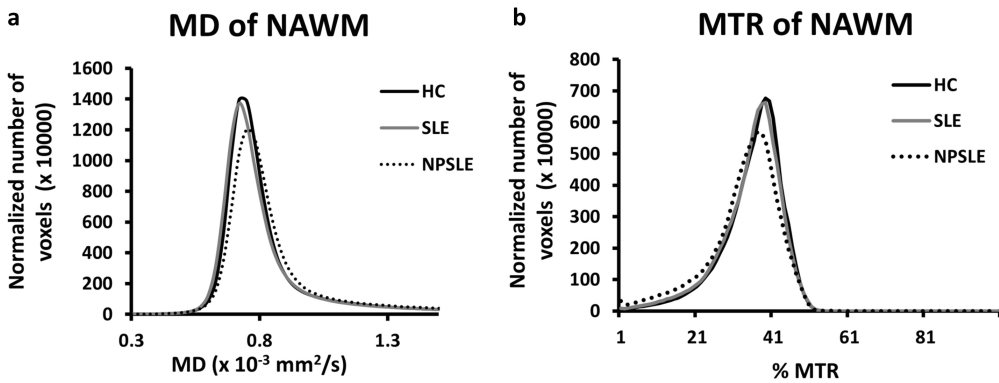


FIGURE 5.3: Average MD (a) and MTR (b) histograms from normal appearing white matter in the skeleton.

TABLE 5.3: MTR and MD histogram peak heights in NPSLE patients, SLE patients and healthy controls.

	NPSLE patients	SLE patients	Healty controls
MTR peak height in NAWM	$63.30 \pm 8.70^{**(*)}$	77.80 ± 8.30	80.00 ± 12.4
MTR peak location in NAWM	$37.11 \pm 2.26^{**}$	38.78 ± 2.22	39.64 ± 2.41
MD peak height in NAWM	$126.7 \pm 21.7^*$	146.7 ± 20.6	147.9 ± 17.2
MD peak location in NAWM ($\times 10^{-3} \text{ mm}^2/\text{s}$)	$0.760 \pm 0.03^{(*)}$	0.730 ± 0.04	0.740 ± 0.02

* p-value < 0.05 versus healthy controls.

** p-value < 0.01 versus healthy controls.

(*) p-value < 0.05 versus SLE

5.4 Discussion

In this study we showed for the first time that two different quantitative MRI tools sensitive to microstructural properties of brain WM reflect in a significantly different manner the underlying tissue damage in NPSLE. This is also the first study to investigate localized MTR changes in NPSLE patients compared to SLE patients with no NP symptoms and HC, and to co-analyze these changes with DTI results from the same patients. Our results strongly suggest that DTI and MTI reflect pathogenic processes in NPSLE that are only partially overlapping, hinting that more than a single process is involved in the etiology of NPSLE. The systematic exclusion of hyperintense lesions from the analysis confines the results of this study to NAWM and emphasizes the relationship between the “invisible” tissue damage revealed by quantitative MRI methods and the disease, in addition to the “visible” damage seen in the lesions.

One of the most striking findings here is that while in the DTI measures, especially in MD and RD, there were significant and spatially extensive differences between NPSLE and SLE, and between NPSLE and HC, differences in MTR, albeit also spatially extensive, were found only between NPSLE and HC, while almost no MTR differences were detected between NPSLE and SLE (Figure 5.1). This, taken with the fact that no differences, either in DTI or MTR, were found between HC and SLE, suggests that MTR is sensitive to a microstructural pathomechanism that already acts in WM in SLE patients but is not distinctive to NPSLE patients. Conversely, it is possible that DTI is more specifically sensitive to a microstructural feature that correlates only with NPSLE and not with SLE. Moreover, the regions in which DTI differences between the groups were observed only partially overlapped with the regions indicating MTR differences, even though both methods showed roughly the same sensitivity to the disease, expressed e.g. by the fraction of voxels in both methods exhibiting significant differences between NPSLE and HC.

MTR reflects the amount of exchange processes between “free” water protons (or molecules) and water protons (or molecules) bound to macromolecular structures in tissue, e.g. myelin, cytoskeleton, cell membranes, proteins and the extracellular matrix [39]. In NPSLE, previous studies that reported MTR findings assigned the drop in MTR in NPSLE patients to several potential mechanisms: axonal injury, demyelination, microvascular ischemia, parenchymal edema and gliosis [19, 22, 23]. Emmer et al. [19] suggested that edema was a more likely cause for MTR changes in NPSLE patients since the reversible changes in the MTR of the NPSLE patients corresponded to the improved clinical status of the patients, and edema is more easily reversible with treatment than e.g. axonal damage, demyelination and gliosis. Histological evidence for many of the mechanisms mentioned above was reported in a previous study that correlated pre-mortem conventional MRI observations with post-mortem histopathology. In this study, the most frequently observed microstructural damages were global ischemic changes and parenchymal edema [40]. This also supports the hypothesis that parenchymal edema, either cellular or interstitial, potentially contributes to the changes in MTR observed in this study. It is also possible that inflammation-driven microglial activation is linked to MTR changes in NAWM as has been hypothesized with regards to M_s [41].

Diffusivity measures obtained with DTI, on the other hand, reflect the displacement of water molecules on a microscopic scale, and changes in these measures reflect changes in tissue microstructure as well as in properties of the diffusing medium, e.g. viscosity and molecular crowding of the cytosol or the extracellular fluid [42]. Higher MD, RD and

AD, as well as lower FA in NPSLE patients compared to HC and SLE patients found in this study and in previous ones [14, 16, 17, 20, 21] point to various underlying mechanisms, including axonal loss, demyelination, edema, as well as microinfarcts caused by the effect of corticosteroids or antirheumatic drugs and acute inflammation in small vessels [14, 16, 17, 20]. However, a salient finding in our study is that the differences in RD between NPSLE and SLE, and between NPSLE and HC, were much more extensive than the differences in AD between groups. It should be noted that MD is linearly dependent on both RD and AD: $MD = \frac{2RD+AD}{3}$, and thus most of the extensive inter-group differences observed in MD in our study can be attributed to the differences in RD. It has been argued that changes in RD mostly reflect the creation of more space for water to diffuse perpendicularly to the axon propagation direction in the extracellular space in WM, caused e.g. by demyelination [43]. Although the hypothesis of an exclusive link between radial diffusivity and demyelination has been challenged in conjunction with multiple sclerosis [44], there is still a significant body of evidence to support the notion that an increase in RD reflects mostly changes in the interstitial space. Thus, with demyelination only as a secondary mechanism for WM microstructural damage in NPSLE [40], it is possible that the DTI changes we observed in our study stem mostly from, e.g. edema. Differences in AD between groups observed in this study were smaller than those observed in RD but are still highly significant. Changes in AD can be related to the interstitial space, or represent intra-axonal changes that interfere with diffusion along the axonal propagation direction caused by, e.g. axonal degeneration and axonal loss, but these will most likely cause a decrease in AD rather than an increase [45, 46]. A recent investigation of the DTI correlates of vasogenic edema using both Monte-Carlo simulations and tissue phantom data showed that increased extent of vasogenic edema could result in decreased FA, increased RD and less significantly increased AD, similarly to the results in our study [47].

From the diagnostic standpoint, the NPSLE subject group in our study consisted exclusively of patients with active complaints at the time of the MRI examination. This may explain the more extensive changes observed in our study, compared to previous DTI-TBSS studies on NPSLE [14, 16, 20]. Several regions which showed significant decreased FA were also reported in previous studies, such as the inferior fronto-occipital fasciculus, the fornix, the posterior limb of the internal capsule (corticospinal tract), the anterior limb of the internal capsule, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, cingulum, corpus callosum, superior longitudinal fasciculus, and the anterior corona radiata [14, 16, 24], while some were observed here for the first time: the external capsule, cerebral peduncle, and posterior thalamic radiation. It is thus plausible that the microstructural tissue damage revealed by the DTI measures is non-specific and based on our results, has a sharp onset correlated with the NPSLE symptoms.

histograms of MTR and DTI measures in the skeletonized WM offer a non-spatially specific assessment of the differences among the various groups. histograms of MTR and DTI measures have been used to evaluate changes in several previous studies [13, 18, 18, 19, 22]. Peak heights in normalized histograms are typically inversely related to the variance (peak width) of the measure represented in the histogram, while the peak location, or the most frequently observed value, is typically correlated (but not identical) to the mean value of the measure in the histogram, and thus is more directly related to the values observed in a spatially-resolved manner in the difference maps in Figure 5.1. Here we show histogram results both for comparison purposes to previous studies, as well as to present more accurate global changes in DTI and MTR than previously shown,

in that they are specific to the core of WM tracts, i.e. clean from partial volume with gray matter and CSF, as well as to NAWM, thus avoiding the confound of WM lesions, which were included in histograms shown in most previous studies, except for [19]. The significant difference between the peak location of the MD histogram of NPSLE and that of both SLE and HC corroborates the spatially-resolved results shown earlier in our TBSS analysis. Similarly, the MTR histogram peak location in NPSLE was significantly different from that of HC but not from that of SLE, although this can be attributed to the variance in the MTR measures. Differences observed in histogram peak heights in both MTR and DTI were in line with previous studies [13, 18, 18, 19].

Although this study mostly focused on microstructural changes in NAWM in NPSLE and SLE, we performed a detailed focal lesion analysis, which confirms previous findings of significant differences in NPSLE patients compared to SLE and HC [2, 11]. It is impossible to exclude the effect that WM lesions have on the disease outcome of NPSLE, although direct relationship is difficult to establish [10]. In the context of our study, individual mapping of WMH and their systematic exclusion from the skeletonized WM analyses helped demonstrate the significant role that alterations in NAWM have in NPSLE.

This study was subject to many limitations and challenges. Besides the small cohort size of this study, which limits its statistical power, one of the most severe limitations of this study is the lack of diagnostic differentiation between ischemic and inflammatory NPSLE patient groups. It has been hypothesized that these phenotypes may result in a different set of disease effects on the brain, and by combining these groups, we measured an averaged overall effect of SLE and NPSLE on WM microstructure, without having the specificity to assign the resulting outcomes to inflammation or ischemia. We intend to separately investigate the effect of inflammatory and ischemic NPSLE in a longitudinal study in order to provide a multimodal MRI characterization of the damage caused by inflammation or ischemia. Another limitation of this study was the heterogeneity of NPSLE syndromes observed in our patients, which subsequently may lead to a large variability in the location and the severity of the tissue microstructural correlates. In the future, we plan to continue our multimodal investigation of SLE and NPSLE with more homogenous subgroups of patients, in the hope that this could lead to a better characterization of the link between NPSLE, and tissue damage in specific areas of the brain resulting in specific symptoms.

Lastly, only 6 NPSLE patients out of the total 9 were scanned within a year of the first onset of NP symptoms, while the other 3 patients were diagnosed as NPSLE 3 to 6 years prior to the MRI scans. Longer disease durations may have probably added confounding factors such as accumulated tissue damage caused by previous NPSLE episodes as well as effects caused by longer treatment. This study could have indeed benefitted from a tighter range of disease durations, however it should be mentioned that given the relative low incidence of NPSLE in the general population, we focused on scanning NPSLE patients with active symptoms, rather than limiting it further to patients with first occurrence of NP symptoms.

5.5 Conclusions

In conclusion, this study supports the notion that microstructural tissue changes in NAWM in NPSLE are heterogeneous, and that these changes give rise to a complex MRI picture as seen by two distinct quantitative MRI tools. We showed the potential usefulness of spatially-resolved co-analyses of MTR and DTI data, which we hope that in the future,

may help detect local effects of the different pathological mechanisms in NPSLE and their link to specific neuropsychiatric symptoms, and can thus be useful for designing better diagnostic procedures for NPSLE.

5.6 References

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6

Glial and axonal changes in systemic lupus erythematosus measured with diffusion of intracellular metabolites

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Abstract

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease with multi-organ involvement. Central nervous system involvement in SLE is common and results in several neurological and psychiatric (NP) symptoms that are poorly linked to standard MRI outcome. MRI methods sensitive to tissue microstructural changes, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), show some correlation with NPSLE symptoms. Histological examination of NPSLE brains reveals presence of cerebral edema, loss of neurons and myelinated axons, microglial proliferation and reactive astrocytosis, microinfarcts and diffuse ischemic changes, all of which can affect both DTI and MTI in a non-specific manner. Here we investigated the underlying cell-type specific microstructural alterations in the brain of SLE patients with and without a history of CNS involvement. We did so combining DTI with diffusion-weighted magnetic resonance spectroscopy (DW-MRS), a powerful tool capable of characterizing cell-specific cytomorphological changes based on diffusion of intracellular metabolites. We used a 7 tesla MRI scanner to acquire T_1 -weighted images, diffusion tensor imaging (DTI) datasets, and single volume DW-MRS data from the anterior body of the corpus callosum of 13 SLE patients with past neuropsychiatric SLE (NPSLE), 16 SLE patients without past NPSLE, and 19 healthy controls. Group comparisons were made between SLE patients with/without past NPSLE and healthy controls on DTI metrics and on diffusion coefficients of three brain metabolites: the exclusively neuronal/axonal N-Acetylaspartate (NAA), and the predominantly glial creatine + phosphocreatine (tCr) and choline compounds (tCho). In SLE patients with past NPSLE, significantly higher DTI mean and radial diffusivities were accompanied by a significantly higher intracellular diffusion of tCr ($0.20 \pm 0.03 \mu\text{m}^2/\text{s}$, $p\text{-value} < 0.01$) and tCho ($0.14 \pm 0.03 \mu\text{m}^2/\text{s}$, $p\text{-value} < 0.05$) compared to healthy controls ($0.17 \pm 0.02 \mu\text{m}^2/\text{s}$, $0.12 \pm 0.02 \mu\text{m}^2/\text{s}$, respectively). tNAA, tCr and tCho diffusion values from all SLE patients correlated positively with SLE disease activity index score ($p\text{-value} < 0.05$, $p\text{-value} = 0.02$, $p\text{-value} < 0.05$, respectively). Our results indicate that intracellular alterations, and in particular changes in glia, as evidenced by increase in the average diffusivities of tCho and tCr, significantly correlate with SLE activity. The higher diffusivity of tCr and tCho in NPSLE patients, as well as the positive correlation of these diffusivities with the SLE disease activity index are in line with cytomorphological changes in reactive glia, suggesting that the diffusivities of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation.

6.1 Introduction

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is a female predominant autoimmune disease that affects multiple organs [1]. Central nervous system involvement in SLE is common and results in several neurological and psychiatric symptoms. These symptoms are poorly characterized by standard magnetic resonance imaging (MRI), which appears normal in about 50% of neuropsychiatric systemic lupus erythematosus (NPSLE) patients. Focal lesions and vascular infarcts, visible on MRI of NPSLE patients, are non-specific and often do not correlate with clinical outcome and with symptom severity [2].

MRI methods sensitive to tissue microstructural changes, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), show diffuse white matter changes that correlate with the clinical status of NPSLE patients [3–7]. Histological examination of NPSLE brains has revealed the presence of cerebral edema, loss of neurons and myelinated axons, microglial proliferation and reactive astrogliosis, microinfarcts and diffuse ischemic changes, all of which can affect the image contrast in DTI, and MTI [8]. Therefore, although clinically informative, due to their lack of specificity, these imaging modalities provide limited insight into the microstructural deficit in NPSLE.

Magnetic resonance spectroscopy (MRS) reports on concentrations of cell-specific metabolites, and MRS studies have shown differences in the concentrations (relative to total creatine) of several brain metabolites, including significantly lower N-Acetylaspartate (NAA) and significantly higher choline and myo-inositol levels in SLE and NPSLE patients compared to healthy controls (HC) [9, 10]. In addition, one study reported significantly lower NAA in SLE patients with high disease activity compared to those with low disease activity [11]. Although MRS provides cell-type specific information, it does not provide any structural information.

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) combines the cell-type specificity of MRS with the microstructural sensitivity of diffusion-weighted imaging (DWI), and allows studying cell- and compartment-specific properties of tissue microstructure by probing the diffusion of intracellular brain metabolites [12, 12, 13]. Of these metabolites, N-acetylaspartate typically co-measured with N-acetylaspartylglutamate (NAAG) ($\text{NAA} + \text{NAAG} = \text{tNAA}$) resides almost exclusively in neurons/axons; creatine and phosphocreatine ($\text{Cr} + \text{PCr} = \text{tCr}$), pivotal in aerobic cell energetics, are found in all neural cells, but their astrocytic concentration is twice their neuronal one, and soluble choline-containing compounds (tCho) are predominantly glial, with a glial/neuronal concentration ratio of 3:1 [14, 15]. The diffusion properties of these metabolites are strongly dictated by the structural and physiological features of their respective intracellular space, and thus provide a unique in-vivo probe for pathology affecting intracellular structures, such as ischemia [16, 16], tumors [17, 18], and axonopathy in multiple sclerosis [19], as well as making accurate vivo cell-specific characterization of tissue microstructure possible [13, 20].

In this study we utilize for the first time the sensitivity of DW-MRS to selectively report on axonal and glial microstructure (a) to investigate the underlying microstructural alterations in a normal appearing portion of the corpus callosum in the brain of SLE patients with and without history of NPSLE and (b) to assess the relationship between DW-MRS indices and SLE activity in the patient population in this study. These studies were performed at ultrahigh field (7 Tesla) in order to obtain the sensitivity required for

robust DW-MRS measurements.

6.2 Materials and Methods

6.2.1 Human subjects

A total of twenty-nine SLE patients (one male, twenty-eight females, age: 43 ± 10 years) and nineteen age and sex matched healthy volunteers (one male, eighteen females, age: 41 ± 11 years) were included in the study. The study adhered to the Helsinki Declaration and was approved by the institutional review board of our institution. Written informed consent was obtained from all subjects prior to the study. Of the twenty-nine SLE patients, thirteen had a history of NPSLE and sixteen had no history. For convenience, patients with past NPSLE incidence are referred to as “NPSLE patients” throughout the text. All SLE patients were diagnosed according to the 1982 revised American College of Rheumatology criteria [21, 22]. All NPSLE patients were diagnosed at the Leiden NPSLE-clinic after a standardized multidisciplinary medical examination [23]. NP diagnoses were classified according to the 1999 American College of Rheumatology case definitions for NPSLE syndromes [24]. The NP syndromes in our NPSLE cohort included cerebrovascular disease (5 patients), seizures (3 patients), cognitive disorder (3 patients), movement disorder (1 patient), headache (2 patients), acute confusional state (3 patients), psychosis (1 patient), transverse myelitis (1 patient), polyneuropathy (1 patient), anxiety (1 patient), and radiculopathy (1 patient). In order to categorize the patients according to SLE disease activity, we calculated the systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K) for each patient [25]. Permanent and irreversible damage due to SLE was assessed with the systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology damage index (SDI) [25, 26]. SLE patients with a SLEDAI-2K ≥ 8 were considered to have high SLE activity and were categorized as SLE-active [11], while the remaining SLE patients were categorized as SLE-inactive. The demographics of the study and the clinical characteristics of the SLE patients are shown in Table 6.1.

6.2.2 Data Acquisition

All subjects were scanned on a 7 Tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel receive head coil (Nova Medical Inc., Wilmington, MA, USA). The scan protocol consisted of a short survey scan and a sensitivity encoding reference scan followed by (a) sagittal 3D T_1 -weighted images (FOV: $246 \times 246 \times 174$ mm³, resolution: $0.85 \times 0.85 \times 1$ mm³, TR/TE: 4.00 / 1.84 ms, total scan time: 1.59 min); (b) axial multislice diffusion tensor images (FOV: $224 \times 224 \times 150$ mm³, resolution: $1.75 \times 1.75 \times 2.20$ mm³, TR/TE: 10000/65 ms, fifteen diffusion weighting directions with $b = 1000$ s/mm², total scan time: 3 min) and (c) single-volume diffusion-weighted spectroscopy scans (detailed protocol below).

6.2.2.1 DW-MRS Protocol

The DW-MRS sequence was based on the PRESS (Point Resolved Spectroscopy) sequence with bipolar diffusion-weighting gradients added on both sides of the 180° pulses. A 3 cc volume of interest (VOI) (25 (AP) $\times$ 15 (RL) $\times$ 8 (FH) mm³) was positioned on the anterior body of the corpus callosum as shown in Figure 6.1. The diffusion-weighting gradients were applied in two directions: a right-left direction in the VOI frame, mostly parallel to the direction of the callosal fibers (direction [1, 0, 0]), and a direction mostly

TABLE 6.1: Patient characteristics.

		NPSLE patients (n=13)	SLE patients (n=16)
Patient characteristics	Age (years)	43 ± 8	42 ± 11
	SLE disease duration (years)	12 ± 9	8 ± 5
	SLEDAI - 2K	7 ± 6	3 ± 2
	SDI	2 ± 2	1 ± 1
	Antiphospholipid syndrome	3 (23%)	1 (6%)
Auto-antibodies	Incidence of past NPSLE	13 (100%)	0
	Antinuclear antibody	12 (92%)	14 (88%)
	Anti-ENA	9 (70%)	7 (44%)
	Anti-DNA	1 (8%)	6 (38%)
	Anti-RNP	3 (23%)	3 (19%)
	Anti-SSA	4 (31%)	7 (44%)
	Anti-SSB	1 (8%)	2 (13%)
	Anti-Smith	2 (15%)	2 (13%)
	Anticardiolipin autoantibodies	3 (23%)	1 (16%)
	Lupus anticoagulant	6 (46%)	2 (13%)
ACR criteria	Anti-B2 glycoprotein	2 (15%)	0
	Malar rash	5 (38%)	8 (50%)
	Discoid lupus	2 (15%)	0
	Photosensitivity	5 (38%)	7 (44%)
	Ulcers	7 (54%)	7 (44%)
	Arthritis	12 (92%)	9 (56%)
	Serositis	4 (31%)	6 (38%)
	Lupus nephritis	4 (31%)	5 (31%)
	Neurological disorder	4 (31%)	0
	Hematologic disorder	6 (46%)	8 (50%)
Medication	Immunologic disorder	9 (70%)	13 (81%)
	Antinuclear antibodies	13 (100%)	16 (100%)
	Prednisone	9 (70%)	9 (56%)
	Azathioprine	3 (23%)	6 (38%)
	Methotrexate	1 (8%)	1 (16%)
	Hydroxychloroquine	11 (85%)	12 (75%)
	Mycophenolate mofetil	3 (23%)	2 (13%)

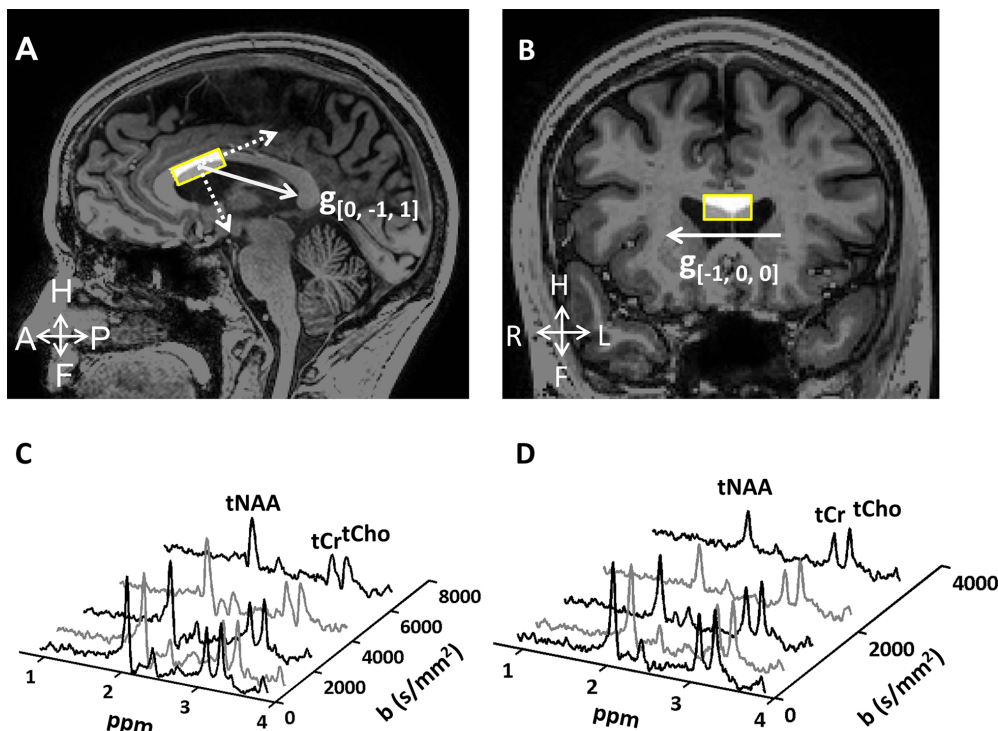


FIGURE 6.1: The position of the volume of interest in sagittal (a) and coronal (b) views. Gradients applied in directions approximately perpendicular (a) and parallel (b) to the callosal fibers are shown in solid lines. Typical spectra acquired with diffusion weighting in the $[0, -1, 1]$ and the $[-1, 0, 0]$ directions are shown as a function of b -value in panels (c) and (d), respectively. Line broadening of 5 Hz was applied for display purposes.

perpendicular to the callosal fibers (direction $[0, -1, 1]$), as shown in panels a and b of Figure 6.1.

The center frequency was set to the tNAA singlet peak at 2.0 ppm. Water suppression was performed using two frequency-selective excitation pulses, each followed by a dephasing gradient before metabolite excitation. Pencil beam second-order shimming was performed, resulting in a typical tNAA line width of 10 Hz. A peripheral pulse unit was used in order to gate data acquisition to the cardiac cycle, thereby minimizing signal fluctuations due to cardiac pulsation. The parameters for DW-MRS acquisitions were: TE = 121 ms, TR = 3 cardiac cycles (about 3000 ms), cardiac trigger delay = 300 ms, number of time-domain points = 1024, spectral width = 3000 Hz, gradient duration (δ) = 37 ms, bipolar gap = 16 ms, diffusion time (Δ) = 60.5 ms with 5 different gradient amplitudes resulting in b -values of 212, 651, 1335, 2262, and 3462 s/mm² in the $[-1, 0, 0]$ direction and 440, 1336, 2718, 4586, and 6945 s/mm² in the $[0, -1, 1]$ direction. The total number of spectra per diffusion condition was 32, resulting in a total scan time of 10~15 minutes. Following this scan, a shorter scan (fewer signal averages) with identical VOI

position and diffusion conditions was performed without water suppression and with the center frequency set at the water resonance frequency. These spectra were used for eddy-current correction in the post-processing stage.

6.2.3 DW-MRS Processing

All spectral pre-processing was performed with custom codes in MATLAB® release R2014b (Mathworks, Natick, MA, USA). Spectral pre-processing consisted of correcting DW-MRS data for eddy currents, zero-order phasing, correction of frequency drift for individual acquisitions, and removal of the residual water peak: averaged spectra were generated for each condition [27]. The resulting spectra were quantified with LCModel [28]. Cramér-Rao lower bound (CRLB) values were used to evaluate the quality of the spectra for each diffusion condition, and the acceptance threshold for DW-MRS data inclusion was set at $CRLB < 20\%$. Based on this, data sets from one NPSLE subject and one SLE subject were excluded from the tNAA analysis.

The LCModel spectral estimates were used to calculate the diffusivity (D_{par}) along the $[1, 0, 0]$ direction (roughly parallel to the callosal fibers) and diffusivity (D_{perp}) along the $[0, -1, 1]$ direction (roughly perpendicular to the callosal fibers) for tNAA, tCr and tCho. These were calculated by performing a linear fit of the natural logarithm of the DW-MRS signal amplitudes as a function of the diffusion weighting value b , assuming a monoexponential decay of the signal as a function of b in each direction:

$$\ln\left(\frac{S_{b,i}}{S_{b_0,i}}\right) = -b_i \cdot D_i \quad (6.1)$$

where $S_{b,i}$ is the measured signal in direction i , $S_{b_0,i}$ is the signal without diffusion weighting, b_i is the value of b in the direction i , and D_i is the calculated diffusion coefficient for direction i . Even though it is possible that the metabolite diffusion-weighted signal decay displays non-monoexponential behavior at very high values of b , our previous work has shown that in the range of b values used in this study the assumption of monoexponentiality is valid and diffusivity values are reproducible [12, 27]. An average of D_{par} and D_{perp} was calculated to assess the average diffusivity (D_{avg}) for tNAA, tCr and tCho. The quality of the linear fittings was evaluated via calculation of the coefficient of determination and an acceptance threshold was set at 75%, leading to exclusion of D_{avg} (tCr) values obtained from two NPSLE patients and one SLE patient.

6.2.4 Image Processing

DTI volumes were motion-corrected with ExploreDTI [29] and further processed with the DTI toolbox [30] of the FMRIB Software Library¹ to obtain the following DTI measures for each subject: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). These DTI metrics were further analyzed with tract based spatial statistics (TBSS) [31]. Statistical differences between NPSLE patients, SLE patients and HC were assessed in FA-MNI152 standard space using 5000 permutations and were corrected for multiple comparison based on threshold-free cluster enhancement [32]. One SLE patient data set was excluded due to poor registration to FA-MNI152.

T_1 -weighted images were used for tissue segmentation within the VOI [27]. FA maps were registered to the T_1 -weighted image of the same subject first by affine transformation

¹FSL release 5.0, <http://www.fmrib.ox.ac.uk/fsl/>

using FSL FLIRT [33, 34] and subsequently by non-rigid transformation using FNIRT [35]. The inverse transformation matrices generated were used to register the DW-MRS VOI to the DTI space. Subsequently, the registration procedure in TBSS was applied to transform each VOI to MNI152 space.

T_1 -weighted volumes were further processed in FreeSurfer² and the intracranial volume, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were calculated for each subject. To evaluate whole brain and callosal atrophy due to SLE and NPSLE, total brain volume, center corpus callosum volume and mid-anterior corpus callosum volume were normalized according to the intracranial volume of the same subject.

6.2.5 Statistical analyses

All statistical analyses were performed with GraphPad Prism 5 for windows (version 5.01, GraphPad Software, USA). Unpaired t-tests were used to assess differences between two groups: a p-value < 0.05 was considered to represent statistically significant differences. Correlation between the metabolite D_{avg} measurements and clinical measures such as SLEDAI-2K score, SDI score and SLE duration were assessed with Pearson's correlation test.

6.3 Results

6.3.1 DW-MRS results

Figure 6.1 shows typical sets of diffusion-weighted spectra obtained with diffusion-weighting in the $[1,0,0]$ direction (c) and the $[0,-1,1]$ direction (d) from a healthy control subject. It can be seen that the attenuation of the tNAA peak is significantly more pronounced in the direction approximately parallel to the axonal fibers, along which diffusion of intra-axonal molecules is much less restricted than in the direction perpendicular to the fibers. Figure 6.2 shows the logarithm of the tNAA, tCho and tCr peak estimates from a DW-MRS data set of a healthy control subject as a function of b , and the linear fit to these data based on the assumption of monoexponential decay in both diffusion weighting directions. The strong anisotropy of the tNAA diffusion is reflected by the sharp difference between the decay rates in the two diffusion-weighting directions, in sharp contrast to both tCho, and tCr, for which the difference between the signal decay in the two directions is significantly smaller.

Figure 6.3 shows D_{avg} data of all three metabolites for HC, SLE and NPSLE patients. In NPSLE patients, $D_{avg}(tCho)$ and $D_{avg}(tCr)$ were significantly higher than in HC (p-value < 0.05 and p-value = 0.006, respectively). The difference in $D_{avg}(tNAA)$ values between NPSLE and HC was slightly above the threshold for statistical significance (p-value = 0.052). Average metabolite D_{avg} values for NPSLE, SLE and HC groups are shown in Table 6.2. No significant differences were observed in any metabolite D_{avg} between NPSLE and SLE or between SLE and HC. When all SLE patients, with and without past CNS involvement, were grouped together and compared to HC, $D_{avg}(tCr)$ remained significantly higher in SLE than in HC ($0.19 \pm 0.03 \mu m^2/s$ in SLE, $0.17 \pm 0.02 \mu m^2/s$ in HC, p-value < 0.05).

Average diffusion coefficients of each of the metabolites showed a link to disease activity in both the SLE and NPSLE patients. When all SLE patients were pooled together,

²<http://surfer.nmr.mgh.harvard.edu/>

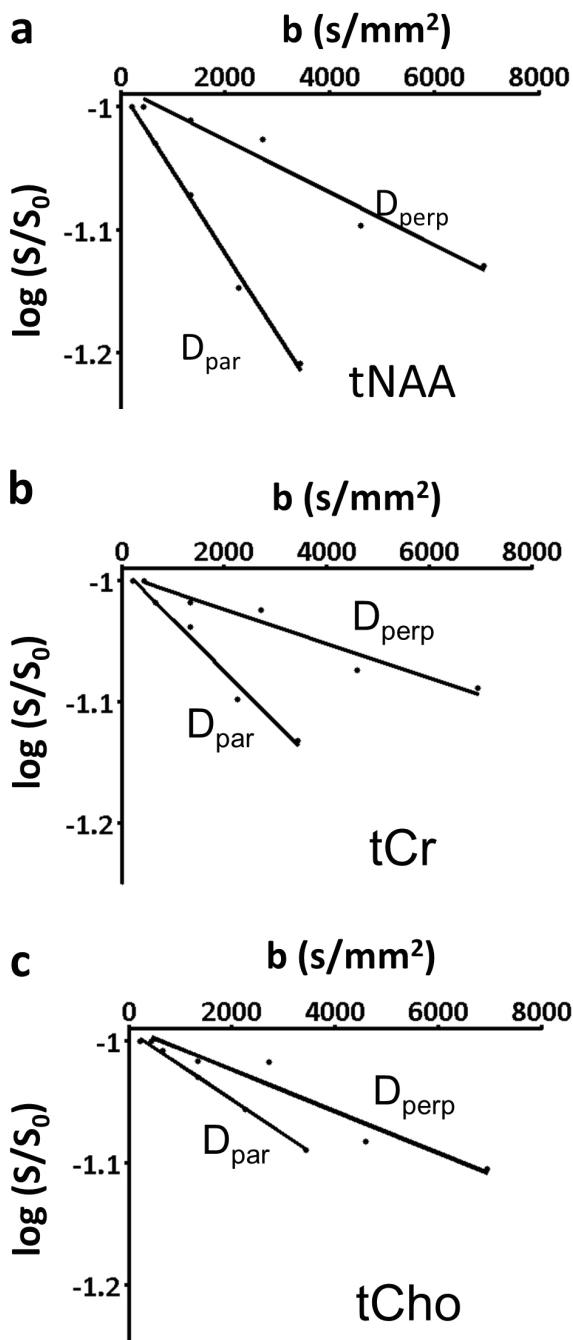


FIGURE 6.2: Logarithm of (a) tNAA, (b) tCr, and (c) tCho values from a single subject as a function of b-value. Linear fits used to extract parallel (D_{par}) and perpendicular (D_{perp}) metabolite diffusivities are shown by the solid lines.

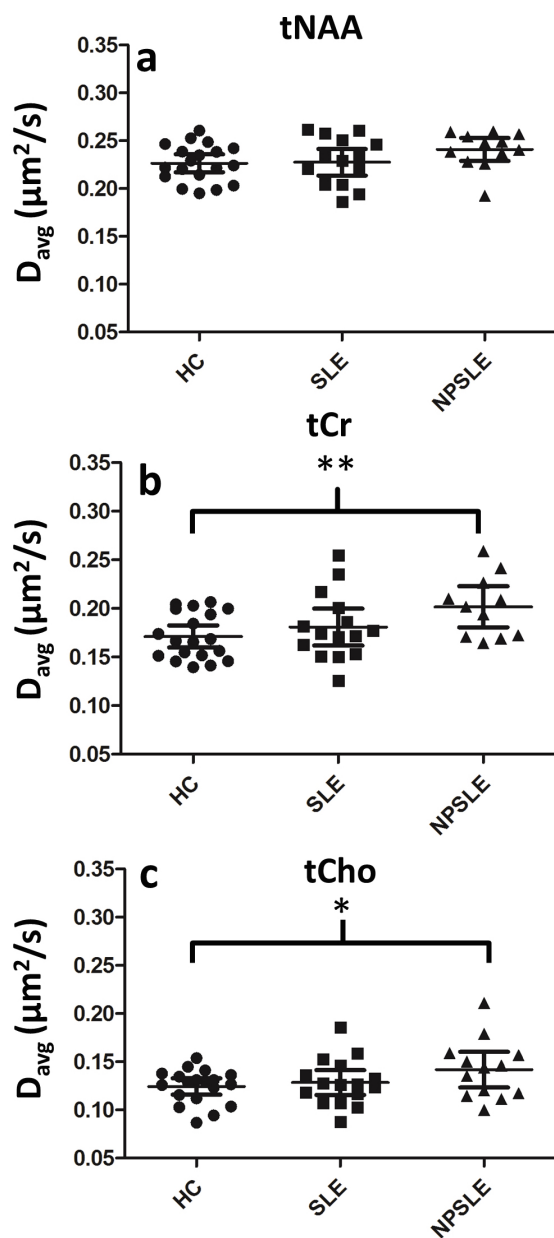


FIGURE 6.3: Metabolite D_{avg} values for healthy controls (HC), SLE patients and NPSLE patients. $D_{avg}(tNAA)$, $D_{avg}(tCr)$ and $D_{avg}(tCho)$ data are shown in panels (a), (b), and (c), respectively. Statistically significant differences with p-value < 0.05 are indicated by *, and by ** for p-value < 0.01. No significant differences were found between SLE and HC, or between NPSLE and SLE in any of the metabolite D_{avg} values.

TABLE 6.2: Metabolite D_{avg} values for NPSLE patients, SLE patients and healthy controls.

	NPSLE patients (n=13)	SLE patients (n=16)	Healthy controls (n=19)	% increase in NPSLE vs HC
D_{avg} (tNAA) $\mu\text{m}^2/\text{s}$	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	7%
D_{avg} (tCr) $\mu\text{m}^2/\text{s}$	$0.20 \pm 0.03^{**}$	0.18 ± 0.03	0.17 ± 0.02	18%
D_{avg} (tCho) $\mu\text{m}^2/\text{s}$	$0.14 \pm 0.03^*$	0.13 ± 0.02	0.12 ± 0.02	14%

regardless of their NP status, SLEDAI-2K scores positively correlated with D_{avg} (tNAA) (p-value = 0.045, $r = 0.39$), D_{avg} (tCr) (p-value = 0.021, $r = 0.45$) and D_{avg} (tCho) (p-value = 0.042, $r = 0.38$). Scatter plots of the SLEDAI-2K scores of all patients as a function of metabolite D_{avg} values are given in Figure 6.4.

When SLE and NPSLE patients categorized as SLE-active (SLEDAI-2K ≥ 8) were compared to HC, the differences in D_{avg} (tCr) and D_{avg} (tCho) were significant (D_{avg} (tCr) = $0.22 \pm 0.03 \mu\text{m}^2/\text{s}$, D_{avg} (tCho) = $0.15 \pm 0.03 \mu\text{m}^2/\text{s}$, p-value = 0.003 and p-value = 0.014, respectively), while the difference in D_{avg} (tNAA) between the two groups was only close to statistical significance (D_{avg} (tNAA) = $0.25 \pm 0.01 \mu\text{m}^2/\text{s}$, p-value = 0.051). No correlation was found between metabolite D_{avg} values and SLE duration or SDI scores.

6.3.2 DTI and volumetric results

Significantly lower FA, higher MD and higher RD in the NPSLE patient group compared to the HC group were found throughout white matter, including the DW-MRS VOI. Similar differences in FA, MD and RD were found between NPSLE and SLE patients. Figure 6.5 shows maps of statistically significant differences in DTI measures overlaid on the MNI152 T₁-weighted image, and the cumulative DW-MRS VOI (i.e. the sum of all the VOIs which are positioned slightly differently for each patient). No significant differences were found in any DTI measure between SLE patients (with and without past CNS involvement) and HC. No significant differences were found in corpus callosum volumes or total brain volumes between SLE or NPSLE patients and HC.

6.4 Discussion

This is the first study to address cell-specific microstructural alterations in the brain of SLE patients with DW-MRS at ultrahigh field. This study focused on measuring the diffusion properties of two predominantly glial metabolites, tCr and tCho, and one exclusively axonal/neuronal metabolite, tNAA. The most salient finding in this study is the strong and consistent link between both D_{avg} (tCr) and D_{avg} (tCho) and disease state, with respect to disease activity and to past CNS involvement, suggesting glial involvement in the brain of these patients. Two potential pathological mechanisms that can explain the significantly higher tCr and tCho diffusivities found in NPSLE patients are inflammation-mediated morphological changes in microglia and astrocytes, and intracellular edema, which would affect both glia and neurons/axons.

Astrocytic and microglial reactivity in response to inflammation and/or ischemia are both highly consistent with an increase in intracellular diffusivity in glia. Reactivity-related cellular hypertrophy and thickening of the processes near the soma (especially in astrocytes) [36] would result in an increase of the intracellular space, and a decrease

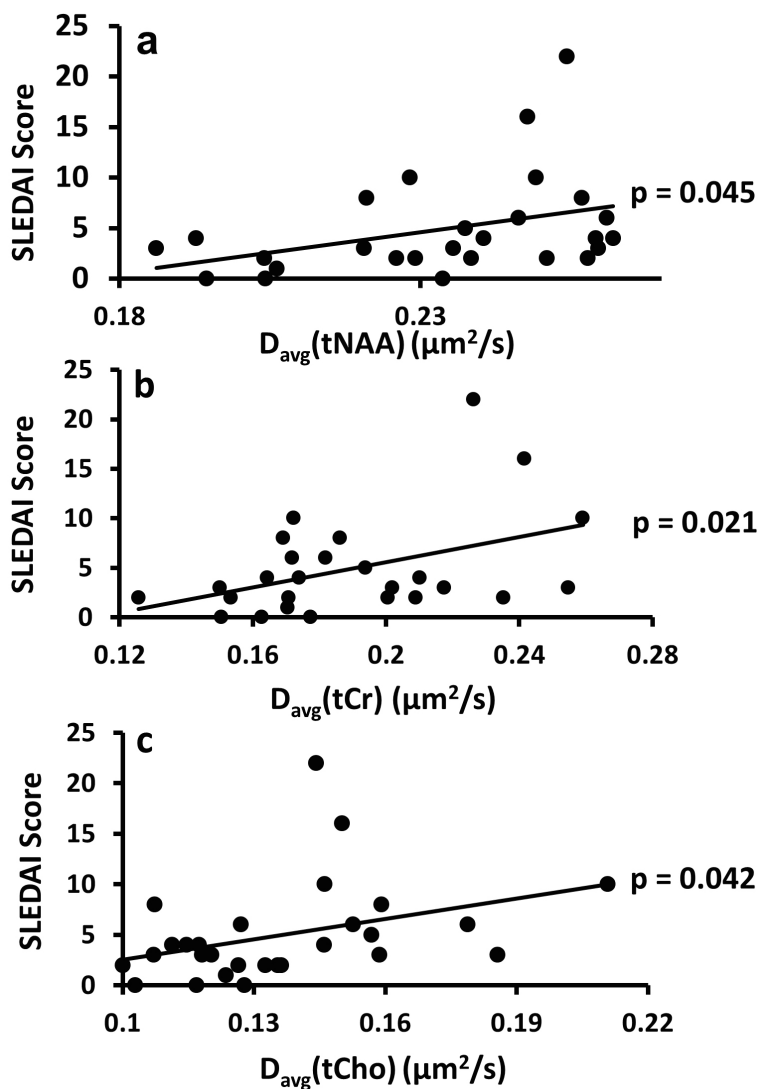


FIGURE 6.4: Correlation of metabolite D_{avg} values with patient SLE disease activity index 2000 (SLEDAI-2K) scores. The resulting linear regression line and significance of the correlations are shown for $D_{avg}(tNAA)$ (a), $D_{avg}(tCr)$ (b) and $D_{avg}(tCho)$ (c).

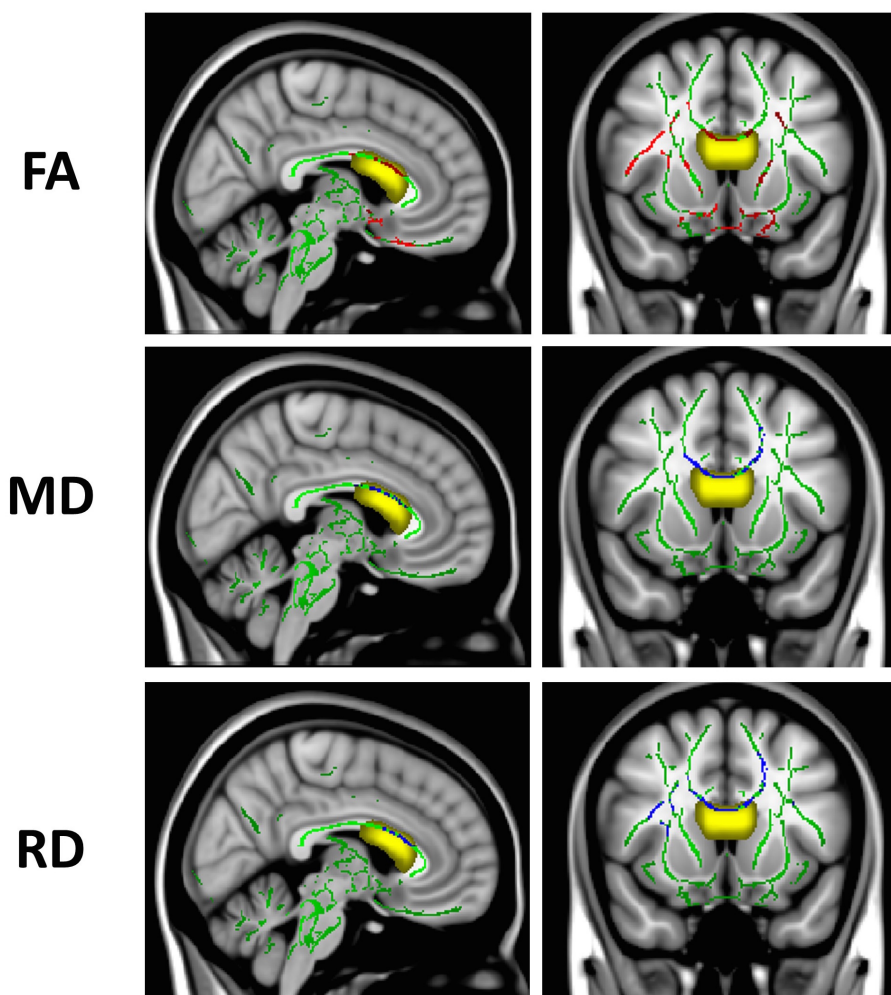


FIGURE 6.5: TBSS results showing regions with statistically significant differences in DTI measures in the white matter skeleton of NPSLE patients and healthy controls (p -value < 0.05). Maps are shown for one sagittal (left column) and one coronal (right column) slice in MNI152 space. The mean FA skeleton is shown in green, regions with higher values in the NPSLE patients compared to HC are shown in blue and regions with lower values in NPSLE patients compared to HC are shown in red. Cumulative VOIs chosen for DW-MRS of NPSLE patients and healthy controls are shown in yellow.

in molecular crowding and intracellular tortuosity, leading to increased diffusivity in the cytosol. The pathogenesis of NPSLE is thought to involve various immune and inflammatory processes that can lead to neuronal injury and vasculopathy [37]. The inflammatory response to injury results likely in glial reactivity and cellular hypertrophy, especially in microglia and astrocytes [38, 39]. Histopathological investigations of brains of NPSLE patients, confirm the widespread presence of reactive microglia and astrocytes, as well as of lipid-laden macrophages among the heterogeneous pathological phenomena [40]. Furthermore, the correlation of *in vivo* MRS results with histological results from the same patients [40] suggest: (a) an association between an increase in tCho concentrations and gliosis, vasculopathy and edema; (b) possible association of tCr with gliosis and reduced neuronal/axonal density; and (c) an association between lower tNAA concentrations and a decrease in neuronal/axonal density [40].

The higher $D_{\text{avg}}(\text{tNAA})$ values found in NPSLE patients compared to HC may be attributed to changes in cytosolic viscosity in axons due either to neuronal/axonal damage or to cytotoxic edema, both of which are seen in the histopathology of brains of NPSLE patients [8]. Higher $D_{\text{avg}}(\text{tNAA})$ has also been observed in a study of patients with schizophrenia where it was hypothesized that inflammatory processes may play a role [41]. On the other hand, *lower* tNAA parallel diffusivity values were found in multiple sclerosis (MS) patients compared to HC in a study focused on myelin and axonal changes in the corpus callosum [19]. It is likely that the different behaviors in tNAA diffusivity seen in MS and in NPSLE reflect different intra-axonal pathological mechanisms associated with these two diseases. Central to MS are demyelination and axonopathy [42, 43]. Since demyelination has no direct effect on diffusion in the intra-axonal space, it has been hypothesized that in MS the decrease in tNAA axosolic diffusivity stemmed from axonal damage that included unusual patterns of neurofilament phosphorylation and packing compared to normal tissue, and a less organized axoskeleton and/or problems with axonal transport [44]. In contrast to findings in MS, histology of NPSLE patients have shown that cerebral edema occurs much more frequently than axonal/neuronal loss [8] and is thus more compatible with the increase in axosolic diffusivity, as evidenced by the increase in $D_{\text{avg}}(\text{tNAA})$ observed in our study.

The high correlation in all SLE/NPSLE patients between SLE disease activity, as quantified by the SLEDAI-2K score, and $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ suggests that the SLE-related peripheral inflammation and autoimmune response may have effect on the brain, independent of overt clinical CNS involvement in SLE. Additionally, significant correlation of $D_{\text{avg}}(\text{tNAA})$ values with SLEDAI-2K scores suggests a permanent or continuous damage to axons correlated with high SLE activity. This is further corroborated by the finding that patients with higher disease activity (those we defined as SLE-active) have higher metabolite diffusivity levels and higher statistical significance in the difference in metabolite diffusivity compared to HC. A previous MRS study in NPSLE/SLE has shown a significantly lower tNAA/tCr level in SLE patients with a high SLEDAI-2K score, and that the level of tNAA/tCr was renormalized in follow-up for patients who were no longer SLE-active, regardless of their NP status [11]. This finding suggests a pathological mechanism, attributed by the authors to neuronal dysfunction, that affects both neurons and axons, the degree of which depends on SLE disease activity, but which is essentially reversible in nature. In our view, and based on our corroborative findings, we attribute this finding to intracellular/intraaxonal edema. It has been suggested that inflammation outside the brain can prime microglia and result in microglial activation for several weeks

[45]. Our findings, as well as those described by Appenzeller et al. [46] support the view that systemic inflammation affects the brain in NPSLE, and the underlying mechanism by which this occurs, e.g. potential disruption of the blood brain barrier in SLE [47], should be further investigated.

Metabolite ADC values found in HC in this study are similar to those reported in previous DW-MRS studies performed on a similar region of the corpus callosum at 7 Tesla and 3 Tesla [13, 27]. A recently published robustness and reproducibility study of DW-MRS in the anterior body of the corpus callosum, aimed to provide guidelines for DW-MRS acquisition for clinical studies such as the one presented here. Using power calculations based on actual data it was estimated that in order to observe a 10% difference in D_{avg} values for tNAA, tCr and tCho in a case-control study, nine, four and twelve subjects, respectively, are sufficient [27]. In this current study we observed a 7% increase in D_{avg} (tNAA), 19% increase in D_{avg} (tCr) and 14% in D_{avg} (tCho) in NPSLE patients compared to HC, with thirteen and nineteen subjects per group respectively. This suggests that it is feasible to observe reliably the disease effect on metabolite D_{avg} reported here. Moreover, based on our power estimation, the number of subjects required to observe reliably a difference in D_{avg} (tCho) is higher than that required for D_{avg} (tNAA). This further supports the notion that our findings indicate a larger effect size for glial than for axonal involvement in SLE/NPSLE. The DTI results are also consistent with previous studies on SLE and NPSLE [5, 6, 48]. Atrophy measurements did not show any significant corpus callosum or total atrophy in the patient population, which could be a type II error due to the small size of the cohort.

There were several challenges and limitations to the study. The main (unmet) challenge of this study was to scan patients with active NP symptoms at the time of the scan, as well as to include more patients with high SLE disease activity. Low incidence of active NPSLE was a factor, as well as significant potential discomfort to patients with clinically overt NP symptoms, preventing these patients from being involved in a research study that is not part of the routine clinical procedure. In our patient cohort, the relative low number of patients with antiphospholipid syndrome made it difficult to evaluate the effect of ischemic/vascular changes on metabolite diffusion. Future studies will focus on separate evaluation of the effects of NP activity, ischemic and inflammatory effects. Missing in this study is an evaluation of the concentrations of the different metabolites, and the study would have benefitted from a separate MRS acquisition at short TE, for an accurate evaluation of the metabolite concentrations in the same VOI, together with additional cell-specific metabolites such as glutamate, glutamine and myo-inositol.

6.5 Conclusions

The results presented in this paper show for the first time that intracellular metabolite diffusion reflective of glial and neuronal/axonal involvement can be measured in a complex autoimmune inflammatory disorder such as SLE/NPSLE, and can thus help unravel pathogenic mechanisms in this complex disease. To our knowledge, DW-MRS of brain metabolites is the only clinical neuroimaging method capable of non-invasive cell-specific detection of cellular morphological changes in disease. We believe that the DW-MRS technique has great potential for the study of the etiology of disease-related changes in tissue microstructure, and that its importance in clinical applications will continue to grow if incorporated in a comprehensive diagnostic scanning protocol together with existing microstructural MRI tools, such as DTI, MTI and susceptibility weighted

imaging.

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7

**General Discussion: DW-MRS current state,
major challenges and future directions**

7.1 Introduction

WITHIN the realm of MRI microstructural tools DW-MRS has a unique role, due to its compartment specificity. In this thesis, metabolite diffusion values in *in vivo* human brain white matter were linked to tissue neuroanatomical properties, new methods were proposed for the acquisition and analysis of DW-MRS data, and DW-MRS was evaluated and optimized for basic neuroscience and clinical research studies. In contrast to the proliferation of applications of DWI and DTI, DW-MRS has so far found only few applications in human brain [1–19]. In this thesis, some of the challenges preventing the DW-MRS method from being more frequently applied were addressed. The results presented in this thesis point towards the use of the DW-MRS method for both basic neuroanatomical research and for research in clinical settings. Some challenges related to DW-MRS still remain unmet. This chapter focuses on the current state of DW-MRS and DW-CSI, discusses some of most salient challenges, and indicates potential future directions.

7.2 Variability of metabolite diffusivity measures across sites: implications on diagnostic and research DW-MRS

The DW-MRS literature shows a high variability of metabolite diffusivity measures across studies and sites. For instance, ADC(tNAA) values obtained from brain white matter of healthy control subjects varied between 0.14 mm²/s and 0.26 mm²/s across studies [1–3, 6, 7, 10, 13, 18, 19]. The main reason for this variability is the differences in acquisition parameters such as echo time, diffusion time, number of diffusion directions, size and the location of VOI, and maximum b-value used in different studies. All these parameters influence the resulting ADC value in different ways. Echo time has been shown to correlate with the ADC(tNAA) values measured in human brain white matter [12]. ADC(tNAA) values decrease with increasing diffusion time [20]. Calculating ADC with one diffusion direction results in rotationally variant ADC(tNAA) values which can be influenced by the axonal orientation within the VOI. This causes variability in the results since ADC(tNAA) can change depending on the choice of gradient direction orientation. The size of the VOI can impact the SNR and consequently deteriorate the accuracy of the obtained ADC values. The inclusion of gray matter tissue in a predominantly white matter VOI volume can result in decreased ADC(tNAA) depending on the extent of the partial volume effect from the gray matter since gray matter ADC(tNAA) is significantly lower than white matter ADC(tNAA) values [6]. A large effect on ADC is also observed based on the choice of b-value since relatively low b-values ($b \ll 1/\text{ADC}$) leads to error propagation [4], and non-monoexponentiality of the diffusion-weighted signal decay needs to be taken account when higher b-values are used [20].

Where clinical studies are concerned, it is important to choose adequate DW-MRS acquisition parameters to prevent unnecessary source of errors (resulting from insufficient number of diffusion directions or low b-values) which can impact the reliability of the DW-MRS results. In the DW-MRS reproducibility study presented in chapter 3 of this thesis, an optimized acquisition scheme is proposed to obtain robust diffusivity values. In this work, a high degree of reproducibility was achieved in a DW-MRS protocol applied at two different sites and two different field strengths. Such an optimized protocol can help resolving inconsistencies in acquisition and post-processing of the DW-MRS across sites. For future clinical studies, it is recommended to establish a baseline clinical DW-MRS

protocol which can enable obtaining reliable results and comparable metabolite diffusivity values across different clinical sites. Moreover, since many diseases also affect the gray matter, exploration of suitable protocols and evaluation of metabolite diffusion properties in gray matter tissue is also needed for future clinical applications of DW-MRS. While conducting such investigations, one should be careful about taking partial volume effect from white matter into account due to the difficulty of planning a VOI that consists of only gray matter tissue, since the cortical volume is relatively small compared to VOI sizes used in DW-MRS.

7.3 Diffusion-weighted CSI: promises and challenges

A reproducible and robust implementation of 2D DW-CSI has been shown in this thesis, within a supra-callosal axial slice. 2D DW-CSI provides spatially-resolved information from a large field-of-view and increases the amount of information obtained from a single scan. This allows simultaneous investigation of multiple brain regions and can subsequently lead to direct comparison of regions with visible disease-effects, such as brain tumors, to regions with normal appearing brain tissue from the same subject. This is advantageous for clinical research since it can help understand intracellular changes leading to visible lesions in various diseases including brain tumors, MS, ischemia and many others. Moreover, since these visible effects are often heterogeneous, investigation of metabolite diffusivity values from different voxels within these visible lesions could help surgery or treatment planning.

Another potential advantage of DW-CSI is that it can be co-analyzed with imaging methods such as FLAIR, DTI and MTI, which provide complementary information to that of DW-MRS. Co-analysis of DW-CSI with T_2 -weighted and/or FLAIR images can help to explore cell-specific microstructural differences between normal appearing white matter, lesions and non-affected tissue, as mentioned above. Co-analysis of DW-CSI with DTI can provide a more comprehensive microstructural exploration of white matter tracts. By applying DW-CSI with at least six non-collinear diffusion directions, it is possible to calculate diffusion tensor for different metabolites. Combining the information from water and tNAA diffusion tensors can give more complete information about both intraneuronal and extracellular space. This, in turn, can provide better insight about disease-related changes in the brain. For instance, correlating water and metabolite diffusivities along white matter tracts can help differentiating axonal damage from extracellular edema and demyelination which occur in disease that specifically affect these tracts. This can have an impact on the treatment strategies as well as improve the understanding of the relation these disease processes have with specific white matter tracts.

A major challenge of DW-CSI is the long acquisition time. This is detrimental for clinical applications, where longer scan times cause discomfort to vulnerable clinical populations. Moreover longer scan times can also lead to subject motion and subsequently result in increase in the variance of data across subjects. Longer acquisition times limit the possibility of including more diffusion directions, or a separate acquisition with water as the planned metabolite, which can subsequently be used for a more efficient eddy-current correction than the one shown in this thesis. Several strategies for accelerating the acquisition of DW-CSI data can be explored. Past studies show that the acquisition time of MR spectroscopic imaging can be reduced by an acceleration factor of 7 by employing information from different coil elements [21, 22]. By integrating parallel imaging in DW-CSI, it is possible to accelerate future DW-CSI applications and reduce the scan time of a

regular DW-CSI scan (with 4 conditions: one without and three with diffusion-weighting) down to about 3 minutes from the current scan time of about 20 minutes. It should be noted that this reduction in time is accompanied by a loss in SNR, and so a suitable compromise must be found. Compressed sensing is another recent technique used to reduce the acquisition time [23]. A recent study showed the possibility to preserve spectral resolution with a six-fold reduction of the acquisition time by implementing compressed sensing in MR spectroscopic imaging. Implementing this technique can therefore further accelerate the scan time of DW-CSI. With such reductions in the acquisition time, clinical applications of DW-CSI method can be facilitated.

7.4 DW-MRS at ultrahigh field: advantages, challenges and proposed future directions

The advent of ultrahigh field (7 Tesla and beyond) for human MRI scanners provides higher SNR and increased spectral resolution in *in vivo* MRS, both of which positively affect spectral quality in DW-MRS. A particularly relevant example is shown in the reproducibility study presented in chapter 3 of this thesis, where ADC values obtained at 7 Tesla had significantly smaller variability compared to those obtained at 3 Tesla.

Besides the advantages of ultrahigh field, there are also some significant drawbacks and challenges. A major drawback of ultrahigh field for brain research is the B_1 transmit field inhomogeneity across the brain. This is mainly caused by the shortened electromagnetic wavelength of the radio frequency (RF) signal at the higher field strengths, where the wavelength is roughly the size of the imaged object. The B_1 inhomogeneity at 7 Tesla is shown to be about 42% across the brain, causing variations in the tip angle at the lateral regions of the brain compared to the medial parts [24]. B_1 inhomogeneity can cause severe reduction of the acquired signal intensity [24], especially in spectroscopic sequences, where at least 3 pulses are consecutively applied with large tip angles (of 90° - 180° - 180° for PRESS sequence and 90° - 90° - 90° for STEAM sequence). In most of the DW-MRS studies presented in this thesis, the corpus callosum was chosen as the region of interest. This choice avoided most of the B_1 -related issues since the central location of this structure does not suffer significant B_1 inhomogeneity. Single volume DW-MRS applications in other brain regions can significantly suffer from degradation of B_1 in that region. For DW-CSI acquisitions, non-uniformity of the B_1 field is more detrimental since the aim is to cover as large an area as possible. B_1 inhomogeneity across a large field of view was the reason for the preselection of a relatively small VOI for the DW-CSI study presented in chapter 4 of this thesis. Various approaches exist to resolve the B_1 inhomogeneity. These approaches include using pads with dielectric material that can increase the B_1 homogeneity [24], implementation of adiabatic RF pulses that are intrinsically less sensitive to the strength of the B_1 transmit field [25, 26] and optimizing the phase and magnitude of individual elements of multi-transmit RF coils to obtain more homogeneous B_1 [27].

The PRESS sequence used for DW-MRS and DW-CSI measurements suffers from large chemical shift displacement at 7T. This is caused by the small bandwidth of the refocusing 180° pulses used in the sequence for slab selection in two directions. In the typical diffusion-weighted PRESS sequence that we used for 7T experiments, a 54% overlap had been observed between tNAA and water resonances due to chemical shift displacement. For single volume DW-MRS this effect can result, for example, in different gray/white matter fractions in the VOI, and add a confound to the ADC values of different metabolites.

In the DW-CSI implementation shown in chapter 4, chemical shift displacement leads to a more challenging situation, since in addition to the shift among metabolites, the water peak is used for eddy current correction. In the regions where the VOI of water did not overlap with the metabolite VOI eddy current correction could not be applied, the metabolite peaks could not be estimated properly and thus metabolite ADC values could not be calculated. This results in unnecessary loss of information from a significant portion of the field of view. Implementing a DW-CSI with adiabatic pulses can resolve some of these problems in the future applications of the method [26], but may result in an increase in SAR and thus require longer TR values, resulting in a longer scan time.

7.5 Dependence of the performance of DW-MRS on gradient coil hardware specifications

The maximum gradient strength of the system is an intrinsic limiting factor for DW-MRS experiments. This, in turn, imposes a limit on the minimum TE value for a given b value in a DW-MRS experiment. For example, the gradients of the 7 Tesla system used in this thesis are limited to a maximum gradient strength of 40 mT/m. In order to obtain high b-values (on the order of $b = 8000 \text{ s/mm}^2$), it was therefore necessary to use long gradient durations ($\sim 37 \text{ ms}$). This in turn resulted in long echo times ($\sim 121 \text{ ms}$) and subsequently resulted in lower SNR at such b-values, limiting the quantification of metabolites such as myo-inositol, glutamate and other unique intracellular markers. By using stronger gradient systems, such as those used for the human Connectome project [28] (with a maximum gradient strength of 300 mT/m), one can reach similar b values with much shorter TE values of about 60 ms. Assuming a typical T_2 for brain metabolites at 7T to be of the order of 150 ms in human brain [29, 30], the expected gain in signal in this case is given by,

$$\frac{e^{-\frac{0.060}{0.150}}}{e^{-\frac{0.121}{0.150}}}$$

and is equivalent to an increase in 50% in SNR.

7.6 References

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8

Summary and Conclusions

8.1 Summary

The main purposes of this thesis were (i) to investigate neuroanatomy *in vivo* with DW-MRS, (ii) to develop methodology to enable future clinical applications of the technique in human brain *in vivo*, and (iii) to characterize the microstructural deficit in neuropsychiatric systemic lupus erythematosus (NPSLE) with DW-MRS and other microstructural tools such as DTI and MTI. The first part of the thesis, consisting of chapters 2 to 4, related to the first purpose, in which: (a) the link between diffusion properties of brain metabolites and the cellular morphology of the compartments that they are found in was investigated, (b) acquisition and post-processing protocols were evaluated and optimized, and (c) a new method was introduced to provide spatially resolved metabolite diffusion properties. In the second part of the thesis, two clinical research applications conducted on NPSLE were described (chapters 5 and 6), where disease-related microstructural changes were assessed by applying DTI together with two separate methods: MTI and DW-MRS.

In **Chapter 2**, the neuroanatomical correlate of metabolite diffusion properties was investigated within the context of their host compartment. DW-MRS was applied in a small VOI in the anterior body of the corpus callosum using a 7 Tesla MRI system. A new strategy was proposed for analyzing the DW-MRS data. The results of the study showed that metabolite diffusion calculated for tNAA, tCr and tCho reflects neuroanatomical features of the anterior body of the corpus callosum, highlighting the potential of DW-MRS to obtain compartment-specific information from human brain *in vivo*.

Chapter 3 presented an investigation of the reproducibility of DW-MRS and optimization of the acquisition scheme for *in vivo* human brain applications. For this purpose, DW-MRS and DTI data were obtained through five repeated scan sessions from six healthy volunteers. Three of these volunteers were scanned on a 3 Tesla MRI system and the remainder on a 7 Tesla MRI system in order to assess and compare the reproducibility and the reliability of metabolite diffusion values obtained at different magnetic field strengths. The inter-subject analysis showed that robust diffusivity results can be obtained at 3T and 7T with a lower variability for 7T scans. An investigation of optimal b-value scheme and number of averages showed that with an optimum acquisition scheme, it is possible to reduce the scan time for DWS to 10 minutes for 7T and 13 minutes for 3T with a variability of 8%. Power calculations showed that DW-MRS could provide reliable information to detect a 10% effect in case-control studies with 11-13 subjects per group (to obtain p-value < 0.05 and a power of 80%).

In **Chapter 4**, a new method, diffusion-weighted chemical shift imaging (DW-CSI) is introduced to obtain spatially-resolved metabolite diffusion properties. The feasibility of DW-CSI acquisition and processing schemes to measure metabolite diffusion from human brain was shown for the first time. Methodological developments that were implemented in this study enabled robust and reproducible results that are in line with the results from single volume DW-MRS experiments in the literature. Metabolite diffusion values calculated with DW-CSI were shown to relate to the underlying tissue gray and white matter decomposition.

Part two of the thesis, consisting of Chapters 5 and 6 related to clinical applications on NPSLE. In **Chapter 5**, DTI and MTI data were co-analyzed to investigate disease-related changes in the brain of the NPSLE patients. MTI changes observed in white matter of NPSLE patients were localized for the first time and were combined with the localized changes observed in DTI. Significantly lower MTR and FA and significantly higher AD,

RD and MD values were found in NPSLE patients compared to healthy controls. Most of these changes were observed in normal-appearing white matter of the brain. The changes observed with MTI and DTI metrics were only moderately co-localized suggesting that the underlying changes in DTI metrics are different than those observed by MTR. DTI metrics were found to be more sensitive to changes in the brains of active NPSLE patients compared to SLE patients and healthy controls, whereas MTI was sensitive to SLE-related changes, regardless of the presence of NP symptoms. Although informative, the combination of DTI and MTI still lacked the specificity needed to explain the source of these changes.

In **Chapter 6**, microstructural changes in the brains of SLE patients with a history of NPSLE were investigated by applying DW-MRS and DTI. The results indicated that intracellular alterations, and in particular changes in glia, significantly correlated with SLE activity. This is the first time in which such intracellular changes in the brain have been observed *in vivo* in relation to an autoimmune inflammatory disease. These findings suggested that diffusion properties of choline compounds and of total creatine are potentially unique markers for glial reactivity in response to inflammation.

8.2 Conclusions

DW-MRS can play a key role in understanding neurobiological mechanisms of diseases that affect the human brain. The specific changes that occur within neurons can be reflected as changes in the diffusivity of tNAA, whereas the changes in glial cells can cause pronounced changes in the diffusivities of tCr and tCho. This information combined with that obtained from DTI and other MRI tools can help elucidate various disease processes in the future. The studies presented in this thesis show the robustness and clinical relevance of microstructural information obtained via DW-MRS. The contributions of this thesis such as the optimized acquisition protocols for single volume (SV) DW-MRS, the robust DW-CSI and DW-MRS post-processing pipelines that comprise information from DTI, will all facilitate the applications of DW-MRS both for basic neuroscience research and clinical research studies and well as to increase the number of applications of DW-MRS in human brain *in vivo*.

Nederlandse samenvatting

De hoofddoelen van dit proefschrift waren: (i) het *in vivo* onderzoeken van de neuroanatomie met behulp van diffusie gewogen magnetische resonantie spectroscopie (DW-MRS), (ii) het ontwikkelen van methodologie die toekomstige klinische applicaties van deze techniek mogelijk maakt, en (iii) het karakteriseren van microstructurele afwijkingen in de hersenen van patiënten met neuropsychiatrische systemische lupus erythematosus (NPSLE) met behulp van DW-MRS en andere microstructurele modaliteiten zoals diffusie tensor MRI (DTI) en magnetisatie-transfer MRI (MTI). Het eerste deel van dit proefschrift, bestaande uit de hoofdstukken 2 tot en met 4, beschrijft studies gerelateerd aan het eerste hoofddoel: hierin (a) werd de link onderzocht tussen diffusie-eigenschappen van cerebrale metabolieten en de cellulaire morfologie van de compartimenten waarin zij zich bevinden, (b) werden de acquisitie en post-processing protocollen van DW-MRS geëvalueerd en geoptimaliseerd, en (c) werd een nieuwe methode geïntroduceerd om de diffusie eigenschappen van de metabolieten in meerdere voxels tegelijkertijd te meten. In het tweede deel van dit proefschrift worden twee klinische studies in patiënten met NPSLE beschreven (hoofdstuk 5 en 6), waarbij aan de ziekte gerelateerde microstructurele veranderingen bestudeerd werden met behulp van DTI in combinatie met respectievelijk MTI en DW-MRS.

Hoofdstuk 2 onderzoekt welke neuroanatomische oorsprong ten grondslag liggen aan de diffusie-eigenschappen van metabolieten waarbij rekening gehouden wordt met het compartiment waarin zij zich bevinden. Op een 7 Tesla MRI scanner werd DW-MRS toegepast op een klein volume van het voorste gedeelte van het corpus callosum en voor het analyseren van de DW-MRS data wordt een nieuwe strategie geïntroduceerd. De resultaten van de studie laten zien dat de diffusie van tNAA, tCr en tCho een afspiegeling zijn van de neuroanatomische kenmerken van het voorste gedeelte van het corpus callosum. Deze bevinding bevestigt de mogelijkheid van DW-MRS om *in vivo* compartiment specifieke informatie van de menselijk hersenen te verkrijgen.

In **Hoofdstuk 3** wordt een onderzoek naar de reproduceerbaarheid en betrouwbaarheid van DW-MRS gepresenteerd alsmede de optimalisatie van het acquisitie schema voor *in vivo* toepassingen in humane hersenen. Voor dit doel werden DW-MRS en DTI data gemeten in zes gezonde vrijwilligers gedurende vijf, herhaalde scansessies. Drie van deze vrijwilligers werden gescand op een 3 Tesla MRI scanner en de overige twee vrijwilligers werden op een 7 Tesla MRI gescand om tevens de invloed van de magneetveldsterkte op de reproduceerbaarheid en betrouwbaarheid van DW-MRS te onderzoeken.

De analyse van de resultaten tussen de deelnemers laat zien dat robuuste diffusie-resultaten gemeten kunnen worden op zowel 3 als 7 Tesla, waarbij 7 Tesla scans een lagere variabiliteit geven. Uit een onderzoek naar het optimale b-waarde schema in combinatie met de hoeveelheid middelingen kan geconcludeerd worden dat het met een optimaal meetschema mogelijk is om de scantijd van DWS te reduceren tot 10 minuten op 7 Tesla en 13 minuten op een 3 Tesla scanner als een variabiliteit van 8% getolereerd kan worden.

Power berekeningen laten zien dat DW-MRS betrouwbare informatie oplevert zodat een 10% effect gedetecteerd zal worden in een case-control studie met 11-13 deelnemers per groep (voor het verkrijgen van een p-waarde < 0.05 en een power van 80%).

Hoofdstuk 4 introduceert een nieuwe methode om de diffusie eigenschappen van de metabolieten in meerdere voxels tegelijkertijd te meten: diffusie-gewogen chemische verschuiving afbeelding (DW-CSI). De haalbaarheid van acquisitie van DW-CSI en de bijbehorende uitwerkingsmethodes werd voor de eerste keer in deze studie aangetoond. De methodologische ontwikkelingen die in dit onderzoek werden geïmplementeerd zorgden voor robuuste en reproduceerbare resultaten die overeenkomen met resultaten uit de literatuur zoals gemeten in een enkel volume. Metaboliet diffusie-waarden die berekend werden met DW-CSI konden direkt gerelateerd worden aan de onderliggende weefselsamenstelling, dat is de hoeveelheid grijze en witte stof.

Deel twee van dit proefschrift, bestaande uit hoofdstukken 5 en 6, demonstreert de toepassing van nieuwe MRI-technologie in patiënten met NPSLE. In **Hoofdstuk 5** worden DTI en MTI data gezamenlijk geanalyseerd om de aan de ziekte gerelateerde veranderingen in de hersenen van NPSLE patiënten in kaart te brengen. Voor de eerste keer werd aangetoond waar in de witte stof MTI-veranderingen optreden en deze werden vergeleken met de gelokaliseerde veranderingen zoals gevonden met DTI. Significant lagere magnetische transfer ratio (MTR) en fractionele anisotropie (FA) en significant hogere diffusiewaarden (zowel AD, RD als MD) werden gevonden in NPSLE patiënten in vergelijking met gezonde controles. De meeste van deze veranderingen werden vastgesteld in de normaal uitzijnde witte stof in de hersenen. De veranderingen zoals gezien met MTI en DTI lieten slechts matige co-lokalisatie zien wat suggereert dat de onderliggende processen leidend tot veranderingen in DTI-waardes anders zijn dan de processen die invloed hebben op de MTR. De DTI-metingen lieten grotere veranderingen in de hersenen van actieve NPSLE patiënten zien in vergelijking tot SLE patiënten en gezonde controles, terwijl de veranderingen in MTI-parameters gerelateerd waren aan SLE, ongeacht of NP-symptomen aanwezig waren. Hoewel deze studie nieuwe informatie opleverde, heeft de combinatie van DTI en MTI nog steeds te weinig specificiteit om precies de onderliggende oorzaak van de waargenomen veranderingen te identificeren.

Hoofdstuk 6 beschrijft de toepassing van DW-MRS en DTI om de microstructurele veranderingen in de hersenen van SLE patiënten met een geschiedenis van NPSLE te onderzoeken. De resultaten laten zien dat intracellulaire veranderingen, en dan met name veranderingen in de glia, significant correleerden met SLE-activiteit. Dit is de eerste keer dat zulke intracellulaire veranderingen in de hersenen *in vivo* geobserveerd werden in relatie tot een auto-immuunziekte. Deze resultaten suggereren dat de diffusie eigenschappen van choline-bevattende complexen en totale creatine mogelijk unieke bio-markers kunnen zijn voor gliale reactiviteit als reactie op een ontsteking.

Conclusie

DW-MRS kan een sleutelrol spelen in het begrijpen van neurobiologische mechanismes van ziekten die de menselijke hersenen beïnvloeden. Specifieke processen die invloed hebben op neuronen kunnen zich uiten als wijzigingen in de diffusiteit van tNAA, terwijl veranderingen in gliale cellen gereflecteerd worden in de diffusiteit van tCr en tCho. Deze informatie gecombineerd met die verkregen met behulp van DTI en andere MRI-methodes kan helpen om verschillende ziekteprocessen in de toekomst beter te begrijpen. De onderzoeken gepresenteerd in dit proefschrift laten de robuustheid van DW-MRS

zien alsmede de klinische relevantie van de microstructurele informatie die hiermee verkregen wordt. De nieuwe technieken die in dit proefschrift zijn beschreven, zoals de geoptimaliseerde acquisitie protocollen voor volume (SV) DW-MRS, de robuuste implementatie van DW-CSI en de uitwerkprotocollen voor DW-MRS die informatie vanuit de DTI-opname incorporeren, zullen de toepassing van DW-MRS faciliteren voor zowel basaal neurowetenschappelijk en klinische onderzoek alsmede het aantal toepassingen vergroten van DW-MRS in de humane hersenen.

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Curriculum Vitae

Ayşe Ece Ercan was born on 31st of July 1983 in Istanbul, Turkey. In 2004, she graduated from Robert College of Istanbul. In 2008, she completed her BSc. degree in Physics at Boğaziçi University with honors and started the Biomedical Engineering Master Program at Delft University of Technology. She conducted her master thesis research at the division of image processing (LKEB), Department of Radiology, Leiden University Medical Center. In 2010, she received her MSc. degree in Biomedical Engineering from Delft University of Technology. In 2011, she started her PhD at C.J. Gorter Center for high field MRI, Department of Radiology, Leiden University Medical Center. Upon the completion of her PhD, she will start conducting post-doctoral research at the Radiology Department of University of Texas Southwestern Medical Center.

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