The Non-Invasive Brain Biopsy

Implementation and Application of Quantitative Magnetic Resonance Spectroscopy on Healthy and Diseased Human Brain

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DON’T PANIC

– Douglas Adams
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Acknowledgement
Abstract

Introduction: In this thesis, one of the major objectives was to implement a method for (absolute) quantitative magnetic resonance spectroscopy (qMRS) of the human brain, intended for clinical use. The implemented method was based on standard spatially selective MRS sequences. The tissue water was used as an internal reference, which was calibrated using whole brain quantitative magnetic resonance imaging (qMRI). The second objective was to apply the method in clinical neuroimaging investigation, of different disease processes in the human brain.

Materials and Methods: In total, 158 subjects were included and 507 MRS measurements (330 in white matter and 177 in the thalamus) were acquired. In a cross-sectional study of multiple sclerosis (MS), 35 ‘clinically definite MS’ (CDMS) patients were included, of which 15 were atypical CDMS patients with a very low number of white matter lesions (two or fewer), and 20 were typical CDMS patients with white matter lesions (three or more) were included. The metabolite concentrations in normal appearing white matter (NAWM) and the thalamus were assessed using the qMRS method developed in this thesis, and the brain parenchymal fraction (BPF) was calculated from the qMRI data. A cohort of 27 CDMS patients were then treated with Natalizumab and examined both at baseline, and after one year of treatment. Both qMRS and CSF samples for the purpose of assessing intrathecal inflammation were obtained. In addition, the frontal deep white matter (FDWM) and the thalamus were investigated in 20 idiopathic normal pressure hydrocephalus (iNPH) patients using qMRS. Finally, the left thalamus of 14 Kleine-Levin Syndrome (KLS) patients were examined using both qMRS and functional MRI (fMRI) of neurological activation of the left thalamus during a working memory test. Moreover, 63 healthy subjects were included as controls for this work.

Results: A quantitative MRS method based on water referencing was successfully developed, implemented, and evaluated at 1.5 T. Both healthy subjects and MS patients showed a positive correlation between the concentrations of total Creatine (tCr) and myo Inositol (mIns) and age, and also a negative correlation with BPF were observed. Glutamate and Glutamine (Glx) levels were elevated for all MS patient groups compared to healthy controls. In contrast, lower concentrations of total N-acetyl aspartate and N-acetyl aspartate glutamate (tNA) and higher mIns concentrations in NAWM were only observed in MS patients that had developed white matter lesions. Moreover, the change in concentrations of tCr and total Choline (tCho) in MS patients during
Natalizumab-treatment were positively correlated with markers of intrathecal inflammation. The iNPH patients had lower tNA and N-acetyl aspartate (NAA) concentrations in the thalamus compared to the controls. In addition, the NAA concentrations in the left thalamus were inversely correlated to the fMRI activation in the left thalamus during the working memory test in KLS patients.

Discussion: The calculated calibration factors were in good agreement with the results found in the literature, indicating that the calibration factors were accurate.

The observed elevated Glx concentration in MS could be due to increased concentrations of glutamate (Glu), which is neurotoxic at high concentrations, thus the elevated Glx could be linked to the clinically observed neurodegeneration in MS both in patients that have developed lesions and in atypical patients that do not develop any (or extremely few) lesions.

Both tCr and mIns can be used as glia markers, thus the correlations of tCr and mIns concentrations with both age and BPF indicates that the local glia cell density, or tissue fraction, increases with age and atrophy. Moreover, the higher mIns concentrations in the NAWM of MS patients with a substantial white matter lesion load indicate that the glia tissue amount in NAWM is increased in MS patients that develop lesions. NAA is neuronal-specific, thus the lower tNA concentrations indicate that the neurone concentration is lower in the NAWM of MS patients that develop MS lesions. The lack of correlation between tNA with age and BPF in combination with the presence of correlation between tCr and mIns with both age and BPF, might be explained using a model for neurodegeneration. In which, there is a higher neurone loss compared to the glia loss. However, the lost tissue is compensated by compression of the tissue, which keeps the density of neurones more or less constant and the density of glia increased.

The low concentration levels of the neuronal marker NAA in the thalamus of the iNPH patients indicates that the basal ganglia-thalamic-subcortical frontal circuits are damage or at least strongly modulated in the thalamus.

The correlation between strong activation in left thalamus during a working memory test with the neuronal marker NAA indicate that the KLS patients that have low neuronal concentration also needed to utilise the working memory circuitry more heavily in order to perform the task as healthy subjects.

Conclusion: It is possible to use qMRI for accurate and robust determination of qMRS in clinical practice, even at 1.5 T field strength. The tGlx concentration may be an important marker for pathology in the non-lesional white matter of MS-patients. The increased glia and loss of neurones in the NAWM are associated with the formation of white matter lesions.
Sammanfattning

Introduktion: Denna avhandlings huvudmål var att implementera en metod för absolut kvantitativ magnetresonansspektroskopi (MRS) som kan användas på vanliga kliniska MR-system. Metoden baserades enbart på standard-MRS-sekvenser och vattensignalen användes som intern referens, som i sin tur kalibrerades med hjälp av kvantitativ magnetresonanstomografi (MRT).

Ett andra mål med avhandlingen var att använda metoden i kliniska neurobildvetenskapstudier för att undersöka olika sjukdomsprocesser i hjärnan.

Material och Metoder: Totalt inkluderades 158 personer och 507 MRS-mätningar genomfördes (varav 330 i vitsubstans och 177 i thalamus).


Metoden användes dessutom i en studie av sjukdomen idiopatisk Normaltrycks-hydrocefalus (iNPH) där den djupa vitsubstansen i frontalloben och thalamus undersöktes i 20 patienter med iNPH.

Slutligen undersöktes den funktionella aktiviteten i vänster thalamus på 14 Kleine-Levin Syndrom (KLS) patienter när de utförde ett arbetsminnestest. I samma undersökning mättes även metabolitkoncentrationen i vänster thalamus med kvantitativ MRS. Sedan utfördes en korrelationsanalys mellan aktivitet och metabolitkoncentrationer.

Resultat: Det var möjligt att implementera en kvantitativ MRS-metod där vattensignalen användes som en intern referens och kalibrerades med hjälp av kvantitativ MRT.

Både friska kontroller och MS-patienter visade på en positiv korrelation mellan koncentration av total Kreatin (tCr) och myo-Inositol (mIns) med ålder och en negativ korrelation mot BPF. Glutamin och Glutamat (Glx) var högre i vitsubstans
hos samtliga MS-grupper jämfört med friska kontroller. Däremot observerades sänkningen av totalt N-acetylaspartat och N-acetylaspartatglutamat (tNA) i vitsubstans och förhöjningen mIns i vitsubstans jämfört med friska kontroller bara i MS-patienter som hade utvecklat lesioner. Dessutom fanns det en korrelation mellan en ökning av total Kolin och tCr med intratekal inflammation. INPH-patienterna hade lägre tNA och N-acetylaspartat (NAA) i thalamus jämfört med friska kontroller. Slutfilen observerades en korrelation mellan NAA-koncentration i thalamus och aktiveringsgrad under arbetsminnestest i KLS-patienterna.

**Diskussion:** Den beräknade kalibreringsfaktorn var i god relation till resultat baserat på tidigare forskning, vilket indikerar god noggrannhet i metoden.

Den höjda Glx-koncentrationen i MS-patienternas vitsubstans kan bero på förhöjda Glutamat-koncentrationer (Glu) som är neurotoxisk vid höga extracellulära-koncentrationer. Detta kan tyda på att de höjda Glx-koncentrationerna är relaterade till neurodegenerationen i MS. Både i typiska patienter som utvecklar lesioner och atypiska MS-patienter som inte utvecklar (alt. utvecklar väldigt få) lesioner.

Både tCr och mIns är gliacellmarkörer, alltså antyder tCr- och mIns-korrelationerna mot ålder och att PBF gliacelldensiteten ökar med åldrande och atrofi. Dessutom tyder den höjda mIns-koncentrationen på att gliadensiteten är förhöjd i MS-patienter som utvecklar lesioner. NAA är neuronsspecifik, alltså tyder den sänkta tNA-koncentrationen i MS-patienter som har utvecklat lesioner på att neuronndensiteten är lägre i den normalsignalerande vitsubstansen i MS-patienter som utvecklar lesioner. Dock saknades det signifikant korrelation mellan tNA och både ålder och BPF vilket tyder på att neuronndensiteten i vitsubstans är konstant trots åldrande och atrofi. Kombinerat med resultaten att gliadensiteten ökade med ålder och BPF skulle kunna förklaras med att det finns en neuronförlust men att den kompenseras genom att vävnaden komprimeras.

Den sänkta NAA- och tNA-koncentrationen i iNPH-patienter talar för hypotesen att det finns en störning i kretsen basala gangljer-thalamus-subkortikal frontallob.

Den negativa korrelationen mellan NAA och aktivering av thalamus tyder på att de KLS-patienter som har en låg neuronndensitet i thalamus också måste aktivera thalamus starkare än patienter som har högre neuronndensitet.

**Slutsatser:** Det är möjligt att använda kvantitativ MRT för kvantitativ MRS på ett kliniskt MR system. Den förhöjda koncentrationen av Glx i alla undersökta typer av MS skulle kunna vara en viktig markör för MS, även för atypiska MS-patienter. Den förhöjda gliadensiteten i MS-patienters vitsubstans och neuronförlusten var associerad till utvecklingen av vitsubstanslesioner.
List of Papers

This thesis is based on the following five papers, in the text referred to by their roman numerals:

I. **Procedure for Quantitative ¹H MRS and Tissue Characterisation of Human Brain Tissue Based on the Use of Quantitative MRI**
   A Tisell, O Dahlqvist Leinhard, JBM Warntjes, P Lundberg

   My contributions: First author, study design, implementation of MR protocols, MR data analysis, writing/editing/revising manuscript.


II. **Increased Concentrations of Glutamate and Glutamine in Normal Appearing White Matter of Patients with Multiple Sclerosis and Normal MR Imaging Brain Scans**
   A Tisell, O Dahlqvist Leinhard, JBM Warntjes, A Aalto, Ö Smedby, AM Landtblom, P Lundberg

   My contributions: First author, study design, implementation of MR protocols, MR data analysis, writing/editing/revising manuscript.

   Submitted to *PLoS ONE*, 2012, IF5 4.537

III. **MR Spectroscopy in MS: Changes in Non-Lesional White Matter are Correlated with Inflammation**
    J Mellerård*, A Tisell*, O. Dahlqvist Leinhard, I Blystad, AM Landtblom, K Blennow, B Olsson, C Dahle, J Ernerudh, P Lundberg, M Vrethem (*Shared first author)

    My contributions: Shared first author, study design, implementation of MR protocols, MR data analysis, writing/editing/revising manuscript.

    *PLoS ONE*, 2012 September 17;7(9):e44739, IF5 4.537

IV. **Reduced thalamic N-acetylaspartate in Idiopathic Normal Pressure Hydrocephalus: a Controlled ¹H Magnetic Resonance Spectroscopy Study of Frontal Deep White Matter and the Thalamus Using Absolute Quantification**
    F Lundin*, A Tisell*, OD Leinhard, M Tullberg, C Wikkelso, P Lundberg, G Leijon (*Shared first author)

    My contributions: Shared first author, study design, implementation of MR protocols, MR data analysis, writing/editing/revising manuscript.

    *J Neurol Neurosurg Psychiatry*, 2011, January, 82(7) 772-778. IF5 4.953

V. **Low Thalamic NAA-Concentration Corresponds To Strong Neural Activation in Working Memory in Kleine-Levin Syndrome**
    P Vigren*, A Tisell*, M Engström, T Karlsson, O. Dahlqvist Leinhard, P Lundberg, AM Landtblom (*Shared first author)

    My contributions: Shared first author, study design, implementation of MR protocols, MR data analysis, writing/editing/revising manuscript.

    Submitted to *PLoS ONE*, 2012 (minor revision) IF5 4.537
Other related publications not included in the thesis

**Peer reviewed full Papers:**

VI. Preoperative and Postoperative $^1$H-MR Spectroscopy Changes in Frontal Deep White Matter and the Thalamus in Idiopathic Normal Pressure Hydrocephalus


**Peer reviewed conference abstracts:**

i. **Brain Atrophy in MS Patients Correlates with Creatine Concentrations**
   A Tisell, O Dahlqvist Leinhard, JBM Warntjes, A-M Landtblom, P Lundberg

   ISMRM, Melbourne, 2012

ii. **Decreased Cretin in NAWM Suggest a Reduced Gliosis in Natalizumab Treated MS Patients**
    A Tisell, J Mellergård, O Dahlqvist Leinhard, C Dahle, J Ernerudh, M Vrethem, A-M. Landtblom, P Lundberg

   ISMRM, Melbourne, 2012

iii. **Idiopathic Normal Pressure Hydrocephalus Pre -Postoperative 1H -MRS changes in Frontal Deep White Matter and the Thalamus.**
    F Lundin, A Tisell, O Dahlqvist Leinhard, L Davidsson, A Grönkvist, C Wikkelso, P Lundberg, G Leijon

   Hydrocephalus, Copenhagen, 2011

iv. **Multiple Sclerosis Severity Score (MSSS) Correlates With Changes in NAWM Metabolism During Treatment**
    A Tisell, J Mellergård, O Dahlqvist Leinhard, C Dahle, J Ernerudh, M Vrethem, A-M Landtblom, P Lundberg

    ESMRMB, Leipzig, 2011

v. **Increased Glia in Multiple Sclerosis Patients Correlates with Intrathecal Inflammation**
    A Tisell, J Mellergård, O Dahlqvist Leinhard, C Dahle, J Ernerudh, M Vrethem, A-M Landtblom, P Lundberg,

    ESMRMB, Leipzig 2011

vi. **MR Spectroscopy and Quantitative MRI in Multiple Sclerosis Patients Treated with Natalizumab: Changes in Normal Appearing White Matter are Associated to Intrathecal Inflammation and Clinical Variables.**
    J Mellergård, A Tisell, O Dahlqvist Leinhard, C Dahle, A-M Landtblom, J Ernerudh, P Lundberg, M Vrethem

    ECTRIMS, Gothenburg, 2010
vii. Combining fMRI with qMRS for Understanding the Etiology of Periodic Hypersomnia
A Tisell, M Engström, O Dahlqvist Leinhard, T Karlsson, P Vigren, AM Landtblom, P Lundberg
ISMRRM 2009, Hawaii, 2009

viii. Absolute Quantification of \(^1\)H Magnetic Resonance Spectroscopy of Human Brain using qMRI
A Tisell, O Dahlqvist Leinhard, JBM Warntjes, J West, P Lundberg
ISMRRM 2009, Hawaii, 2009

ix. Magnetic Resonance Spectroscopy of INPH-Metabolism in the Frontal Deep White Matter and in Thalamus
F Lundin, A Tisell, O Dahlqvist Leinhard, M Tullberg, C Wikkelso, P Lundberg, G Leijon
Hydrocephalus, Baltimore, 2009

x. Absolute Quantification of LCModel Water Scaled Metabolite Concentration of \(^1\)H Magnetic Resonance Spectroscopy (MRS) Using Quantitative Magnetic Resonance Imaging (qMRI)
A Tisell, O Dahlqvist Leinhard, JBM Warntjes, M Engström, AM Landtblom, P Lundberg.
ESMRMB, Valencia, 2008

A Tisell, M Engström, T Karlsson, P Vigren, O Dahlqvist Leinhard, P Lundberg.
World Molecular Imaging Conference, Nice, 2008

F Lundin, A Tisell, O Dahlqvist Leinhard, M Tullberg, C Wikkelso, P Lundberg, G Leijon
Hydrocephalus, Hannover, 2008
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BPF</td>
<td>Brain Parenchymal Fraction</td>
</tr>
<tr>
<td>( C_{ABS} )</td>
<td>Metabolite absolute concentration</td>
</tr>
<tr>
<td>( C_{AQ} )</td>
<td>Metabolite aqueous fraction concentration</td>
</tr>
<tr>
<td>( C_{met} )</td>
<td>Metabolite concentration</td>
</tr>
<tr>
<td>( \hat{C} )</td>
<td>Calculated concentration</td>
</tr>
<tr>
<td>CDMS</td>
<td>Clinical Definite Multiple Sclerosis</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
<tr>
<td>CSF</td>
<td>CerebroSpinal Fluid</td>
</tr>
<tr>
<td>ERETIC</td>
<td>Electronic REference To access In vivo Concentrations</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Glx</td>
<td>Total Glutamine and Glutamate</td>
</tr>
<tr>
<td>IR</td>
<td>Inversion Recovery</td>
</tr>
<tr>
<td>iNPH</td>
<td>Idiopathic Normal Pressure Hydrocephalus</td>
</tr>
<tr>
<td>KLS</td>
<td>Kleine-Levin Syndrome</td>
</tr>
<tr>
<td>( M_{xy} )</td>
<td>Magnetisation in the transverse plane</td>
</tr>
<tr>
<td>( M_z )</td>
<td>Magnetisation along the main magnetic field</td>
</tr>
<tr>
<td>MEGA-PRESS</td>
<td>J-difference edited PRESS</td>
</tr>
<tr>
<td>( m_{Ins} )</td>
<td>Myo-Inositol</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>NAWM</td>
<td>Normal Appearing White Matter</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PP-MS</td>
<td>Primary Progressive Multiple Sclerosis</td>
</tr>
<tr>
<td>qMRS</td>
<td>Quantitative Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>qMRI</td>
<td>Quantitative Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>QRAPMASTER</td>
<td>Quantification of Relaxation times And Proton density by Multiecho Acquisition of a Saturation-recovery using Turbo spin-Echo Readout</td>
</tr>
<tr>
<td>( R_1 )</td>
<td>Longitudinal relaxation rate</td>
</tr>
<tr>
<td>( R_{1,H2O} )</td>
<td>Water longitudinal relaxation rate</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>----------</td>
<td>--------------------------------------------------------------</td>
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<tr>
<td>( \hat{R}_{1,\text{H}_2\text{O}} )</td>
<td>Estimated water longitudinal relaxation rate</td>
</tr>
<tr>
<td>( \bar{R}_{1,\text{H}_2\text{O}} )</td>
<td>Mean water longitudinal relaxation rate</td>
</tr>
<tr>
<td>( R_2 )</td>
<td>Transverse relaxation rate</td>
</tr>
<tr>
<td>( R_{2,\text{H}_2\text{O}} )</td>
<td>Water transverse relaxation rate</td>
</tr>
<tr>
<td>( \hat{R}_{2,\text{H}_2\text{O}} )</td>
<td>Estimated water transverse relaxation rate</td>
</tr>
<tr>
<td>( \bar{R}_{2,\text{H}_2\text{O}} )</td>
<td>Mean water transverse relaxation rate</td>
</tr>
<tr>
<td>RR-MS</td>
<td>Relapsing Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>semiLASER</td>
<td>Slice Selective Excitation Combined with Localisation by Adiabatic Selective Refocusing</td>
</tr>
<tr>
<td>SP-MS</td>
<td>Secondary Progressive Multiple Sclerosis</td>
</tr>
<tr>
<td>tCho</td>
<td>Total choline</td>
</tr>
<tr>
<td>tCr</td>
<td>Total creatine</td>
</tr>
<tr>
<td>TE varied PRESS</td>
<td>Echo time varied PRESS</td>
</tr>
<tr>
<td>tNA</td>
<td>Total N-acetyl aspartate and N-acetyl aspartate glutamate</td>
</tr>
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</table>
1. Introduction

Nuclear Magnetic Resonance (NMR) was discovered in 1946 by Purcell, Torrey and Pound (Purcell et al. 1946) at MIT and simultaneously by Bloch, Hansen and Packard (Bloch 1946) at Stanford. For the discovery, Purcell and Bloch were awarded the Nobel Prize in physics in 1952. Moreover, in 1950 Proctor, Yu (Proctor et al. 1950) and independently Dickinson (Dickinson 1950) observed that molecular structure affected the resonance frequency which is known as the chemical shift, and it is the chemical shift that makes NMR spectroscopy possible. The first to use NMR relaxation times for a medical application and for characterising different tissues were Odeblad and Lindström who introduced the technique in 1956 (Odeblad et al. 1956).

In 1966, NMR was further developed by Ernst and Andersson (Ernst et al. 1966) who introduced the Fast Fourier transform for interpreting the signal from pulsed NMR experiments. In 1973, Lauterbur (Lauterbur 1973), and Mansfield and Grannell (Mansfield et al. 1973) introduced the gradient magnetic field for image encoding of the Fourier space which enabled NMR imaging in vivo (or Magnetic Resonance Imaging, MRI). For this, Lauterbur and Mansfield shared the Nobel Prize in medicine in 2003. Since then, the technical development has been tremendous and today MRI is an integral part of clinical radiology. One important factor in the success of MRI is its versatility, and although difference in relaxation rates between different tissues is still the foundation of MRI diagnostics, many other properties and functions of tissue can be investigated using MRI, such as; water diffusion, blood flow, blood-oxygen level dependent (BOLD) fMRI, and magnetisation transfer, etc.

NMR spectroscopy or Magnetic Resonance Spectroscopy (MRS) for measuring metabolite concentration was also introduced early (Moon et al. 1973; Hoult et al. 1974). However, the impact of MRS for diagnostics in humans has not yet been as significant as the impact of MRI. One reason is the small magnitude of the metabolite signals, which is typically at least three orders of magnitude smaller that the water signal (0.01 M vs. 55 M). MRI is also lacking quantitative information. However, in a MRI investigation the surrounding tissue can be used as contrast reference when classifying a focal pathology. Unfortunately, in conventional MRS there are no intrinsic reliable references that can be used for accurate chemical shift.
and quantification purposes. Thus to interpret the MRS signal accurate the MRS signal must be quantified using other means, which will be discussed in more detail below.

1.1. Biological Background

Central Nervous System

The central nervous system (CNS) is a complex organ consisting mainly of neurones and glia cells. A neurone consists of a neuronal body and two types of extensions, one type called dendrites which consists of several short branching extensions, and another type called axon which is a single long extension. The dendrites receive input impulses to the neurone and the axon transmits nerve impulses toward another neurone, a muscle fibre, or a gland cell (Tortora 2003). To increase the speed of nerve impulses the axon is warped in electrically insulating sheets of myelin (see Figure 1.1 B). The myelin sheets are produced by a type of glia cells called oligodendrocytes (see Figure 1.1 A). Astrocytes are an other kind of glia cells, the astrocytes contribute to homeostasis by providing the neurones with

![Figure 1.1](image)
energy and substrates for neurotransmission. Moreover, the astrocytes removes excess neurotransmitter molecules from the extracellular space. (Allen et al. 2009).

The myelin give the tissue a whitish colour thus the myelinated part of the brain parenchymal is termed, ‘white matter’, the non myelinated part is termed, ‘grey matter’. The CNS consist also of a third tissue type termed, ‘cerebrospinal fluid’ (CSF). CSF is a clear, colourless liquid that protects the brain against physical and chemical injuries. It also carries oxygen, glucose and other needed chemicals from the blood to neurones and glia cells (Tortora). The CSF is produced by the choroid plexuses in the ventricles and diffuse through the brain and is reabsorbed by the arachnoid villi of dural venous sinuses in to the blood.

Multiple Sclerosis

Multiple Sclerosis (MS) is a multi-focal inflammatory demyelinating disease of the central nervous system (CNS). The cause of MS is not fully understood, but it is thought to be a caused by a combination of certain inherited genes and environmental factors. The geographical distribution of MS is heterogeneous, with high prevalence in northern Europe, North America and Australia and low prevalence at the equator (Compstone et al. 2005). MS affects women 2-3 times more often than men and the peak of onset is typically around the age of 30 (Peterson et al. 2005). The usual clinical presentation, occurring in approximately 85% of patients, is a period of high inflammatory activity in the CNS with neurological deficits that is reversible, termed, ‘relapsing remitting MS’ (RR-MS). The inflammations can be effectively treated using immunomodulators e.g. Natalizumab reduce the cell migration across the blood-brain barrier. However, after around 10-15 years the inflammatory activity in the CNS decline and the

![Figure 1.2](image) (A) Image of axonal transaction in MS leading to Wallerian degeneration. (B) Typical disease course for a MS patient. (Reprinted with permissions (Trapp et al. 1999; Peterson et al. 2005))
disease turns in to stage of continuously progressive neurological deterioration and increased axonal loss, termed, ‘secondary progressive MS’ (SP-MS) (see Figure 1.2 B). The pathogenic mechanisms responsible for the transition from RR-MS to SP-MS is unknown and treatment of SP-MS is unsatisfactory (Trapp et al. 1999). Approximately 15 % of the MS patients have a continuously progressive neurological deterioration from the onset of disease, this type of MS is termed, ‘primarily progressive MS’ (PP-MS).

**Pathology of MS**

Pathological studies of the MS disease are primarily based on autopsies from MS patients after an extended SP-MS phase, but also in some cases after atypical clinical presentations, or in patients that died during a fulminate attack. Thus the main bulk of pathological studies do not represent the typical MS population. Nevertheless, biopsy data can be extrapolated to the general MS population (Filippi et al. 2012). An important process in the ‘normal appearing white matter’ (NAWM) of MS found pathological studies (Bjartmar et al. 2001) is the Wallerian degeneration. Wallerian degeneration occurs if an axon is transacted, then the part of the axon not connected to the neuronal body will disintegrate although myelin will remain in the tissue, and the tissue will therefore visually appear to be normal, it is therefore termed ‘normal appearing white matter’ (see Figure 1.2 A).

**Clinical Assessment of MS**

White matter lesions are often clearly visible on conventional MRI, and active inflammation can be visualised using contrast enhanced MRI. In contrast, cortical lesions and pathologies in NAWM are not visible using conventional MRI, thus the radiological diagnosis of MS is mainly base on characterising white matter lesions (McDonald et al. 2001; Polman et al. 2005; Polman et al. 2011). Clearly the formation of a lesion implies a disease process, but the number and extent of white matter lesions have poor correlation both with long term clinical outcome and the continuous process of neurodegeneration in MS (Trapp et al. 1999; Barkhof 2002).

The level of disability caused by the MS disease is assessed using the ‘expanded disability status scale’ (EDSS) (Kurtzke 1983). The EDSS measures the accumulated disability, however, EDSS do not reflect disease activity and can thus not be used to predict the disease progression. To overcome this problem, the ‘multiple sclerosis severity score’ (MSSS) was introduced (Roxburgh et al. 2005) which is designed to provide a measure of disease severity. The MSSS is calculated
from relating the EDSS score and the disease duration, in such way that, that patients with high EDSS and short disease duration get a high MSSS score while a patient with equally high EDSS but very long disease duration get a low MSSS score. Preliminary reports have indicated that the MSSS may allow the prediction of disease severity over time (Pachner et al. 2009).

**Normal Pressure Hydrocephalus**

Idiopathic normal pressure hydrocephalus (iNPH) is a condition of disturbed CSF dynamics that mainly affect elderly people with the peak of prevalence between 70 and 79 years (Brean et al. 2008). The typical symptoms of gate disturbance, cognitive impairment and urinary incontinence. INPH can be treated with shunt surgery, a drainage is put into the ventricle system and CSF is drained through a valve that opens at a certain pressure.

The pathophysiological mechanisms involved in the development of iNPH is not fully understood. However, it is known that initially there is impaired absorption of CSF through the arachnoid villi in to the blood (Borgesen 1984) resulting in a higher CSF pressure and enlargement of the ventricles. Possibly, the higher CSF pressure leads to increased CSF absorption in the periventricular white matter leading to a new steady state causing the CSF pressure to drop to within normal limits (Deo-Narine et al. 1994). Cerebral blood flow studies in iNHP have showed global and frontal hypoperfusion and have been showed to correlate with CSF pressure in the basal ganglia and the thalamus. This results and the clinical resemblance to Parkinson-like syndromes indicate that subcortical structures, basal ganglia and the thalamus may be involved in the pathogenesis of iNPH (Lundin 2012). There are MRS studies of iNPH have indicated on differences in metabolism in iNPH patines (Lenfeldt et al. 2007; Algin et al. 2010).

**Kleine-Levin Syndrome**

Kleine-Levin Syndrome (KLS) is a rare disorder of periodic hypersomnia associated with other clinical symptoms such as cognitive changes, eating disturbance, hypersexuality, compulsions, and depressed mood. KLS is mainly present adolescence with a median age of onset 15 years and typical duration of eighth years and the mean duration of episodes is typically ten days recurring every 3.5 month (Arnulf et al. 2005). Traditionally, patients have been considered to have
normal functions between hypersomnia periods, regarding sleep patterns as well as cognitive function. However, neuropsychological testing have showed KLS patients have a disturbance in working memory function that is long lasting, perhaps even permanent (Landtblom et al. 2002; Landtblom et al. 2003). Moreover, a functional MRI (fMRI) study has showed that the disturbance in working memory function is associated with a stronger blood oxygen level dependent (BOLD) activation in the left thalamus in KLS patients compared with healthy controls (Engstrom et al. 2009).

1.2. Magnetic Resonance

**A Brief Account of NMR Physics**

The foundation of NMR (Nuclear Magnetic Resonance) is based on the quantum mechanical spin property of atomic nuclei. The ‘N’ in NMR stands for ‘nuclear’; however, the N is often omitted in medical applications and NMR is therefore most often denoted ‘MR’ in medicine. The magnetic nuclear spins have magnetic moments thus they will interact with magnetic fields. In this thesis only hydrogen spins (\(^1\)H), or protons, are used, and the methodology is therefore often denoted ‘proton spectroscopy’, or proton MRS. Quantum theory postulates that a proton can have two different spin “states” either up or down, and when a proton is placed in a magnetic field (\(B_0\)) the spin states therefore split up into two different energy levels with the spin up state (parallel with \(B_0\)) being more energy favourable (higher population) than the down state. Purcell et al. (Purcell et al. 1946) and Bloch et al. (Bloch 1946) showed in 1946 was that if a sample of spins was exposed to electromagnetic radiation with an oscillating magnetic field (\(B_1\)) of a particular angular frequency (\(\omega\)) in the ‘radiofrequency range’ (MHz), and if \(\omega\) exactly matched the energy level difference between the spin up and spin down state (\(\Delta E\)), the spins will interact with \(B_1\). And if the spins are placed in a coil the ensemble of spins will induce a small but detectable current in a coil circuit tuned to the appropriate RF-frequency. The phenomenon that the spins move between energy levels when exposed to radiation of a certain angular frequency is conventionally termed ‘resonance’ and this is the “resonance” in nuclear magnetic resonance. Thus, the so called **angular frequency** at which the spins interacts with \(B_1\) is termed ‘resonance frequency’ or ‘Larmor frequency’ and is denoted \(\omega_0\). For
hydrogen the gyromagnetic ratio ($\gamma$) is $26.75 \times 10^7$ rad/T/s (equivalent to 46 MHz/T) and thus at 1.5 T the precession frequency for hydrogen is 63 MHz ($\omega_0 = -\gamma B_0$).

The signals which were induced in the detection coils in Bloch and Purcell’s experiments, were fundamentally due to the quantum mechanical phenomena of spin precession. The components of the spins that are perpendicular to $B_0$ (i.e. in the transverse $xy$ plane) precess around the $B_0$ field with the Larmor frequency $\omega_0$, (Cohen-Tannoudji 2005). And since each spin has a magnetic moment, the ensemble of spins will lead to an precessing macroscopic magnetic moment (in the transverse plane perpendicular to $B_0$), a process which in turn will induce a current in the detection coil.

It should be pointed out that spins in a magnetic field are always precessing, however, the spin are usually incoherent meaning that the sum of all oscillations average out and no current is therefore induced in the coil. However, what Purcell and Bloch discovered was that a net magnetisation along $B_0$ will arise. And by applying an oscillating magnetic field ($B_1$) (that is oscillating with exactly the angular frequency $\omega_0$) perpendicular to $B_0$ all the net magnetisation will rotate around $B_1$. Since $\omega_0$ is within the radio-frequency band and the application of the oscillating field is applied for a very short time, the exposure is references to as a radio frequency pulses (or ‘RF-pulse’ for short). The pulse is typically described by the angle that they will rotate the magnetisation (e.g. a $90^\circ$ RF pulse will rotate the net magnetisation from a direction along $B_0$ to a direction perpendicular to $B_0$).

The spins will be distributed according to the Boltzmann distribution, and if they are not perturbed by interaction with an $B_1$ field, they will be at thermal equilibrium. For hydrogen at body temperature there are only 0.0005 % more spins in the ‘spin up’ state (than in the state ‘spin down’) resulting yielding an extremely small, but detectable, net-magnetisation.

The rotating XYZ-coordinate system (‘rotating frame of reference’) used for describing MR experiments is conventionally defined with the $B_0$ pointing in the $Z$-direction, thus the spins in equilibrium will be polarised with a net magnetisation in the $Z$-direction. The signal measured in the MR experiment arise as a consequence of the projection of the polarised magnetisation in the transverse plane (perpendicular to $B_0$) which precess with the Larmor frequency $\omega_0$. 

Relaxation
After the application of an excitation RF-pulse, the spins are no longer at equilibrium. However, equilibrium is a dynamic process with a slightly larger preference for the lower energy state, as was originally described by Boltzmann. The process for returning the system to equilibrium is called ‘longitudinal relaxation’ and the equations describing this was introduced by Bloch (Bloch 1946); thus they are known as the ‘Bloch equations’:

\[
\frac{dM_z(t)}{dt} = -R_1(M_0 - M_z(t)) \Rightarrow M_z(t) = M_0 \left(1 - e^{-R_1 t}\right) + M_0(0)e^{-R_1 t},
\]

were \(R_1\) is the longitudinal relaxation rate (often the longitudinal relaxation time \(T_1 = 1/R_1\) is given as in the original description by Bloch (Bloch 1946)).

After excitation using a 90º pulse, a coherent net magnetisation will arise in the XY-plane and this will induce a detectable signal. However, the coherence will degenerated with time in a process called ‘transverse relaxation’, and this transverse process was also described by Bloch as the second part of the Bloch equations:

\[
\frac{dM_{xy}(t)}{dt} = -R_2^* M_{xy}(t) \Rightarrow M_{xy}(t) = M_{xy}(0)e^{-R_2^* t},
\]

where \(R_2^*\) is the effective transverse relaxation rate, which is the sum of the \(R_2'\) and \(R_2\), \(R_2'\) is the relaxation rate due to field inhomogeneity, and \(R_2\) is the intrinsic transverse relaxation rate due to thermodynamic effects (often reported as the transverse relaxation time \(T_2 = 1/R_2\)). For a comprehensive discussion on relaxation see (Levitt 2003; Cowan 2005).

Spin Echo
Since \(R_2'\) is mainly due to \(B_0\) field inhomogeneity (caused both by the sample itself and by imperfections in the magnet), in a sample of spins there will be a distribution of Larmor frequencies thus the spins will eventually lose coherency. However, the effect of the inhomogeneity can be reversed by ‘flipping’, ‘inverting’, or ‘rotating’ the spins 180º using a RF-pulse (this is often called a refocusing pulse), thus the spins will again obtain phase coherence and a so called spin echo will be obtained when all spin are coherent, and then they will lose phase coherence again.
**Chemical Shift**

The chemical structure (or rather the electronic structure defining the chemical bonds) of the molecule will modulate the effect of the local magnetic field around the nuclear spin. Since the electrons are charged particles that surround a specific spin they will produce a magnetic moment that is in the opposite direction of $\mathbf{B}_0$. As a consequence of this process the nuclear spin will be shielded slightly from $\mathbf{B}_0$ by the magnetic moment of the electrons. The shielding effect is important for the appearance of a spectrum and it leads to the ‘chemical shift’ which is conventionally reported in parts per million ppm (which relates it to the Larmor frequency ($\omega_{\text{ref}}$) of a reference solution). A widely used reference compound in MRS of aqueous samples is 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (Provencher 2012), the chemical shift of DSS is assigned to 0.00 ppm (although it is slightly pH dependent). The chemical shift ($\delta$; in ppm units) is defined as:

$$\delta \equiv \frac{\omega - \omega_{\text{ref}}}{\omega_{\text{ref}}} \times 10^6,$$

(1.3)

**Spin Spin coupling (J)**

Besides the shielding effect from the electrons that is responsible for the chemical shift, a resonance signal can also be split into several separate lines (a ‘multiplet’). This process is called ‘spin-spin coupling’, alternatively ‘J-coupling’ or ‘scalar coupling’ (Ramsey et al. 1952). In a typical liquid sample it is the direct spin-spin coupling (dipolar coupling) between different spins that is the main mechanism causing transverse relaxation. The multiplet can be a singlet (no splitting), doublet, triplet, quartet, or a multiplet. Sometimes several multiplets overlap, especially at low $\mathbf{B}_0$, leading to a very complex appearance. The separation constant is called the coupling constant, abbreviated $J$ (in unit of Hz, e.g., $J_{HH} = 7.35$ Hz for lactate).
**MRS Spectra**

The signal measured in a MR scanner is referred to as the ‘Free Induction Decay’ (FID, cf. classical Faraday induction), and it is directly proportional to the magnitude of $M_{xy}$, which according to the Bloch equations can be described as a decaying exponential function (Eq. 1.2). However, the spin-spin coupling effects were not included in the Bloch equations and for finding the true time representation of $M_{xy}$ spin density matrix formalism, or alternatively product operator formalism, must be used. Nevertheless the FID can be described as a sum of complex exponential functions, as in

$$S(t) \propto M_{xy}(t) \Rightarrow S(t) = \sum_{j} A_j \sum_{k} e^{\Omega_{jk} t},$$

(1.4)

where, $S(t)$ is the signal at time $t$, $N_j$ is the number of resonances, $N_{jk}$ is the $k$ number of spectral lines due to spin-spin coupling effects for resonance $j$, $A_j$ is the amplitude of resonance $j$ and $\Omega_{jk}$ is a complex function describing the relaxation and phase of the spectral line $j,k$ and $\text{Real}(\Omega_{jk}) < 0$.

It appears to be a difficult task to separate the different resonances that are mixed together in a FID, and therefore the ‘Fast Fourier Transform’ (FFT) of the FID is therefore often calculated. In Fig. 1.3 the FID and FFT of a singlet resonance of water *in vivo* are presented.

*Figure 1.3 The Free Induction Decay (FID) signal of and the Fast Fourier transform of the FID of a sample of water in vivo.*
**Water suppression**
The concentration of water is by far the grates of all metabolites in CNS with ca. 40 M compared to NAA ca 10 mM (see Figure 1.9). Even if modern AD converters have sufficient bandwidth to correctly digitalis both the water and the metabolite resonance. Mechanic vibration induced side bands in the water resonance will interfere with the metabolite signal (van Der Veen et al. 2000). Therefore, it is standard to suppress the water signal in *in vivo* MRS for quantification of metabolites. Nevertheless, in a standard MRS acquisition a small number of MRS transients are measured without water suppression (non suppressed MRS) prior to the series of water suppressed MRS transients. The non suppressed MRS signal are then used for calibration e.g. eddy current compensations (Klose 1990) and as described later as internal reference.

It should also be noted, there have been successfully implementations of non-water suppressed MRS demonstrated (Dreher et al. 2005).

![Figure 1.4](image)

*Figure 1.4 In panel A a spectrum of the water signal. In panel B the corresponding water suppressed metabolite spectrum. In panel C a magnification of the metabolite spectrum for the “analysing window” from 4 to 0 ppm.*
1.3. Metabolites

In this section the spectral and metabolic properties of the main metabolites that are detected using in vivo MRS of human brain are described briefly.

Creatine

Creatine (Cr) and phosphocreatine (PCr) both have isochronous singlet resonances (at 3.03 ppm and 3.93), thus they are difficult to separate in in vivo MRS. However, the sum tCr can reliably be quantified. Although some controversy remains about the exact role of Cr and PCr, it has been suggested that PCr serves as an energy buffer, retaining constant ATP levels through the creating kinase reaction and as an energy shuttle diffusing from energy producing (mitochondria) to energy utilising sites (nerve terminals) (Graaf 2007).

Both Cr and PCr are present in both neurones and glia cells, however, they are more abundant in glia cells (Brand et al. 1993) thus they could be used as a glia marker.

Myo-Inositol

Myo-Inositol (mIns) is a sugar with six MR detectable protons that give rise to four groups of resonances, a doublet of doublets centred at 3.52 ppm, a triplet centred at 3.61 ppm, another triplet centred at 3.27 ppm and triplet centred at 4.05 ppm (see Figure 1.6). The exact role of mIns in the brain is not known (Graaf 2007).
However, mIns is glia specific (Brand et al. 1993) thus it has often been used as a glia marker.

**Choline**

The singlet resonance at 3.2 ppm is a collective signal representing the co-resonating metabolites free choline (Cho), glycerophosphocholine (GPC) and phosphocholine (PCh), thus the sum tCho is often given.
N-Acetyl Aspartate and N-Acetyl Aspartate Glutamate

The singlet resonance of N-acetyl aspartate (NAA) at 2.008 ppm is the most prominent resonance in a proton MRS of healthy human brain in vivo. However, the resonance is overlapping with singlet resonance at 2.042 ppm from the methyl groups in N-acetyl aspartyl glutamate (NAAG). Thus it is difficult to separate NAA and NAAG, consequently often the sum tNA is quantified and reported. There are also a complex spectral pattern of resonances at 2.677 and 2.486 ppm (See Figure 1.8).

The role of NAA is not known, it has been proposed that it is used for production of myelin, for osmoregulation, and that it is a breakdown product of the neurotransmitter NAAG (Graaf 2007).

NAA is neurone specific (Urenjak et al. 1992; Urenjak et al. 1993; Bjartmar et al. 2002). Thus NAA can be used as a neuronal marker.

Figure 1.8 Molecular structures and high resolution spectra of N-acetyl aspartyl glutamate (NAAG) and N-acetyl aspartate (NAA). As well as corresponding basis spectra obtained at 1.5 T. (11.4 T spectra reprinted with permission (Govindaraju et al. 2000))
Glutamate and Glutamine

Glutamate (Glu) is the major excitatory neurotransmitter in the human brain but it is also a precursor for the major inhibitory neurotransmitter, GABA. The protons in the Glu are strongly coupled, thus Glu has a complex spectral pattern with a doublet of doublets at 3.75 ppm and multiples between 2.04 ppm and 2.35 ppm. These resonances overlap with the resonances of glutamine (Gln), and therefore it is difficult to separate the Glu and Gln. Nevertheless, the sum Glx can be quantified with high accuracy (Graaf 2007). To separate detect Glu from Gln, a MRS sequence called ‘TE averaged PRESS’ can be used (Hurd et al. 2004).

Figure 1.9 Molecular structures and high resolution spectra of Gln and Glu as well as corresponding basis spectra obtained at 1.5 T. (11.4 T spectra reprinted with permission (Govindaraju et al. 2000))
1.4. Magnetic Resonance Spectroscopy

Quantifying the MRS signal

In a bulk tissue sample there are many different metabolites present. Consequently, the MRS signal will be a the sum of all the MRS signals from each metabolite. However, since the chemical shift and spin-spin couplings are dependent on the molecular structure, there will be different signal patterns for different metabolites (see Section 1.3) and it is therefore often possible to separate the signals from different metabolites using specific fitting procedures. In general, there are two main domains in which metabolite quantification can be performed, the time domain or the frequency domain. Moreover, there are some additional hybrid methods, e.g. the Padé transform which is comparable with the Fast Fourier transform, although complex exponential functions are used instead of sine and cosine functions (Belkić et al. 2005). In this thesis linear combinations of model spectra for spectral analysis (LCModel) were used.

Linear Combination of Model Spectra (LCModel)

LCModel (Provencher, Canada) is a commercial package for quantification of $^1$H-MRS-spectra (Provencher 1993). The measured spectrum is fitted with a linear combination of model spectra $M_m(\omega)$, and the linear combination can be written as:

$$\hat{Y}(\omega) = \sum_{m=1}^{N} \hat{C}_m M_m(\omega), \tag{1.5}$$

where, $M_m(\omega)$ is a simulated or measured in vitro spectrum of metabolite $m$, $N$ is the number of terms in the linear combination (or number of basis functions in the basis set), $\hat{C}_m$ is the regression coefficient for $M_m(\omega)$ in the linear combination. $M_m(\omega)$ is termed basis function and the set of all $N$ basis function is termed a ‘basis set’. In LCModel the linear combination in (Eq. 1.5) is fitted to the measured spectrum $Y(\omega)$ (see Figure 1.10). The fitting procedure can in principle be described as the minimising problem:

$$\min \left\{ \left( Y(\omega) - \hat{Y}(\omega) \right)^2 \right\}, \tag{1.6}$$
Figure 1.10: LCModels fit of model spectra. The measured MRS signal (black line) is fitted with the measured in vitro model spectra (red line) N-acetyl aspartate (A), N-acetyl aspartate glutamate (B) Glutamate, (C) , Glutamine (D), Creatine (E) Choline (F) myo Inositol (G) and the resulting linear combination of all basis functions.
where, \(Y(\omega)\) is the measured MRS signal divided with the volume of the MRS voxel and \(\hat{Y}(\omega)\) is the model function.

Experimentally measured basis sets are normalised with the concentration of the \textit{in vitro} solution and the volume of the MRS voxel. Thus, if there is no external factors or differences between the measurement of the \textit{in vitro} MRS signal \(Y(\omega)\) and the measurement of the basis sets, the regression coefficients \(\hat{C}_m\) would be equal to the true \textit{in vivo} concentration, thus \(\hat{C}_m\) is used as measured concentration. However, the signal will depend on sequence-parameters such as combinations of echo times (\(t_E\)), repetition times (\(t_R\)), and flip angles (\(\alpha\)). In addition the MRS signal will also be dependent on system-parameters such as receiver gain settings, local coil sensitivity, \textit{etc.} Moreover, subject that is measured will induce inhomogeneities \textit{etc.}, and finally, the relaxation rates of the spin population will effect the signal. All these parameters must be corrected for in order to relate \(\hat{C}_m\) with \(C_m\).

The relation between \(\hat{C}_m\) and \(C_m\) can be describe through separation of the MRS spectrum of metabolite \(m\) into a shape function \(\Gamma_m(\omega)\) with unit and an area function \(\Lambda_m\) (or an amplitude function). The area function \(\Lambda_{m,\text{invivo}}\) of \textit{in vivo} measurement of metabolite \(m\), can then be written as;

\[
A_{m,\text{invivo}} = f_{\text{examination,invivo}} f_{\text{system,invivo}} \Theta_{m,\text{invivo}} C_m, \quad (1.7)
\]

where, \(f_{\text{examination,invivo}}\) is a complicated function that describes the signal modulations that are specific for the specific examination e.g. coil load, temperature, \textit{etc.}, \(f_{\text{system,invivo}}\) is a complicated function describing signal modulations due to systemic factors such as receiver gain settings, local coil sensitivity, \textit{etc.}, \(\Theta_{m,\text{invivo}}\), is a function describing the metabolite relaxation effects, and \(C_m\) is the metabolite concentration. The same separation can be used with the basis function. In addition, the basis function is also normalised with respect to the \textit{in vitro} concentration. The difference between the measurement of the \textit{in vivo} MRS and the basis set will not effect the shape of the spectrum. Consequently, the shape function \(\Gamma_m(\omega)\) will be equal for the \textit{in vitro} and \textit{in vivo} measurement (although it should be noted that there are some differences such as line broadening, and pH dependence, but these effects are included in the fitting procedure). Thus the sum
of squares in Eq. 1.6 will have its minimum if the area function also is equal. Therefore, the solution to the minimisation problem with an experimentally measured basis set will give the estimated concentration:

\[ \hat{C}_m = \frac{f_{\text{examination, in vivo}} f_{\text{system, in vivo}} \Theta_{m, \text{in vivo}}}{f_{\text{examination, in vitro}} f_{\text{system, in vitro}} \Theta_{m, \text{in vitro}}} C_m. \] (1.8)

The examination dependent factors \( f_{\text{examination, in vivo}} \) and \( f_{\text{examination, in vitro}} \) will generally not be equal. The metabolite relaxation rates are generally different \textit{in vivo} and \textit{in vitro} thus \( \Theta_{m, \text{in vivo}} \) and \( \Theta_{m, \text{in vitro}} \) will not be equal. Moreover the repetition time of the \textit{in vitro} experiment is set long (> 10 s) which is not practicable in \textit{in vivo} measurements and it also leads to a difference in \( \Theta_{m, \text{in vivo}} \) and \( \Theta_{m, \text{in vitro}} \). If the \textit{in vivo} and \textit{in vitro} measurements are performed on different systems, or if there have been systemic changes between the \textit{in vitro} and \textit{in vivo} measurement such as, calibration of the system, temporal changes, hardware changes etc., \( f_{\text{system, in vivo}} \) and \( f_{\text{system, in vitro}} \) will not be equal.

**Absolute quantification of MRS**

**External Phantoms**

One way for calibrating of the unknown parameters of (Eq. 1.8) is by measuring an MRS spectrum of a phantom with known concentration of one or more metabolites. By doing this, the difference between the factor \( f_{\text{system, in vivo}} \) and the factors \( f_{\text{examination, in vitro}} f_{\text{system, in vitro}} \) can be calculated. This should be repeated regularly since the performance of the system will vary with time, and it should at least be repeated at each upgrade and service where the system is re-calibrated. Moreover, this method does not calibrate for the examination effects \( f_{\text{examination, in vivo}} \), which needs to be performed as a separate procedure.

To calibrate for \( f_{\text{examination, in vivo}} \) effects, a calibration phantom can be placed in with the subject. This makes it possible to correct for part of \( f_{\text{examination, in vivo}} \). However, due to inhomogeneities in the B1 field this may induce new differences that require additional calibration.

In (Helms 2000) Helms proposed a method for absolute quantification of 1H-MRS using the ‘principle of reciprocity’ (Helms method). An extra calibration scan was added to the MRS examination; the calibration scan consisted of a series of MRS measurement with a range of flip angles. The calibration scan was acquired both on
the MRS ‘volume of interest’ (VOI) and on an external phantom, and each calibration scan took 1.5 min (thus 3 min was added to the examination time plus also 1.5 min for each additional MRS that were measured). Moreover, the implementation presented in (Helms 2000) was specific for GE MR systems. On the Philips MR system an optimisation of transmitter gain is performed for each new MRS VOI, and therefore, the measurements on the external phantom as proposed by Helms, would not work as stated on a Philips system.

**Electronic Reference to Access In Vivo Concentrations**

In *in vitro* NMR it is possible to add a known internal reference to the solution for absolute quantification. Obviously this cannot be done *in vivo*. However it is possible to synthesise a reference signal electronically by the so called ‘Electronic reference to access *in vivo* concentrations’ method (ERETIC). The ERETIC method demands specialised hardware equipment for generating a reference signal, and the method does not add any extra scan time (Barantin et al. 1997; Heinzer-Schweizer et al. 2010).

**Water scaling**

As mentioned above, it is possible to use the unsuppressed water signal as an internal reference. Since the water signal is acquired in the same protocol as the water suppressed MRS signal the coil load, temperature etc. will be equal for the suppressed and unsuppressed MRS signal. In LCModel the water scaling is calculated by scaling the measured signal with a scaling factor $f_{\text{scale}}$. This is calculated as the normalised area of one fitted basis function, divided by the normalised area of the water signal (Provencher 2012). The scaling factor can be written as:

$$f_{\text{scale}} = \frac{A_{\text{Basis}}}{C_{\text{Basis}}} \frac{WCONC \times ATTH2O}{A_{\text{H2O}}} = f_{\text{examination, in vitro}} f_{\text{system, in vitro}} \frac{WCONC \times ATTH2O}{\Theta_{\text{H2O}} C_{\text{H2O}}}, \quad \text{(1.9)}$$

where, $A_{\text{Basis}}$ and $A_{\text{H2O}}$ are the areas of the fitted basis function and the water signal respectively, $C_{\text{Basis}}$ is the *in vitro* metabolite concentration, $C_{\text{H2O}}$ is the water concentration in the tissue, $\Theta_{\text{H2O}}$ is a function describing the relaxation of the water signal, and $WCONC$ and $ATTH2O$ are LCModel parameters. Thus by using the water as an internal reference, both the $f_{\text{examination}}$ and $f_{\text{system}}$ factors cancel out. However, the measured metabolite concentration now depends on the water
concentration \( C_{H2O} \), the water relaxation \( \Theta_{H2O} \), WCONC and ATTH2O. The estimated concentration \( \hat{C}_m \), that can be written as:

\[
\hat{C}_m = C_m \frac{1}{C_{H2O} \Theta_{H2O} \Theta_{m,invivo} WCONC \times ATTH2O},
\]

where, \( \hat{C}_m \) is called the water scaled LCModel concentration.

In this thesis the ‘Point RESolved Spectroscopy’ PRESS sequence was used for volume selective spectroscopy. Assuming monoexponential relaxation the function describing the water relaxation \( \Theta_{H2O} \) can be written as:

\[
\Theta_{H2O} = e^{-R_2,H2Ot_E} \left(1 - e^{-R_1,H2Ot_R}\right),
\]

where, \( R_{1,H2O} \) and \( R_{2,H2O} \) are the water longitudinal and transverse relaxation rates respectively, and \( t_E \) and \( t_R \) are the echo time and repetition time respectively.

WCONC is used for calibration of the water concentrations \( (C_{H2O}) \) (default value 35880 mM) and ATTH2O for calibration of the water relaxation \( (\Theta_{H2O}) \) (default value 0.7). These values were based on healthy white matter (Ernst 1993), thus for accurate absolute quantification both \( C_{H2O} \) and \( \Theta_{H2O} \) should be measured, especially since \( C_{H2O} \) and \( \Theta_{H2O} \) may be altered due to neurological disease (Miller et al. 1989), and regional differences within the brain (Gelman et al. 2001), tissue disintegration or pathology due to ageing or illness, and differences between subject may also occur. Clearly the water signal should be quantified for each individual MRS if it is used as internal reference.

In this thesis it was investigated if it was possible to use quantitative magnetic resonance imaging for measuring \( C_{H2O} \) and \( \Theta_{H2O} \).

**Absolute or Aqueous Fraction Concentrations**

Essentially, all NMR visible metabolites are dissolved in the intracellular fluids. A consequence of this is that the metabolite concentrations can in general be expressed in absolute units of mM (which is the amount in relation to the physical acquisition MRS volume). Alternatively, the metabolite concentrations can be expressed in relation to the volume of the cellular water in the MRS VOI, which in
this thesis is denoted, ‘aqueous fraction concentration’ (mM$_{AQ}$). As a third alternative the amount of metabolites can be expressed in relation to the weight of the solvent termed molality which is measured in the SI unit mol/kg. Quantitative Magnetic Resonance Imaging

**Measuring R1**

Inversion recovery (IR) is the gold standard method for measuring R$_1$. By first applying a 180° inversion of M$_z$ at equilibrium then M$_z$ starts to recover at a rate that is determined by the relaxation rate R$_1$, and after a delay time termed the inversion time (TI), an excitation pulse is applied and the signal is measured. Then M$_z$ should recover to equilibrium before the experiment is repeated using another value for the inversion time. By fitting a model to the measured signal, R$_1$ can then be calculated. An example of an IR experiment is presented in Figure 1.11. To avoid saturation effects, M$_z$ should be allowed to recover to very close to equilibrium between each saturation (five times T1 is required for almost complete

![Inversion Time](image)

Figure 1.11. Inversion recovery experiment, in the top row the MRI series of one slice from an IR experiment. In the bottom panel the IR signal from a white matter region of interest is displayed.
relaxation). Thus, the required repetition time is in practice very long, >10 s for in vivo applications. Hence, an IR experiment takes an exceedingly long time to perform.

**Measuring R2**

It is in principle possible to measure $R_2$ by the use of a series of multiple 180° RF-pulses resulting in multiple spin echoes (Carr-Purcell scheme, or CP). Then, by fitting a model to the measured signal, $R_2$ can be calculated. However, if the excitation pulse or the refocusing pulses are not perfect 90° (or 180°) pulses (which they never are due both to technical and time limitations), there will be a cumulative effect on the signal, typically resulting in an estimated larger $R_2$ than the true $R_2$ (Cowan 2005). To reduce the effect of non-perfect RF-pulses the self correcting Carr-Purcell-Meiboom-Gill (CPMG) sequence can be used instead. The CPMG sequence consists of a series of multiple 180° refocusing pulses that are phased-shifted 90° from the excitation pulse (e.g., along Y). However, this introduces oscillation in the measured signal (see panel C in Figure 1.12) and several echoes are required to calculate an accurate $R_2$ from a CPMG experiment.

![Image](image-url)  

**Figure 1.12.** In panel A a series of MRI acquired using the CPMG sequence are shown. In panel B a plot of the mean signal in a ROI of white matter and a fitted mono exponential decay are shown. In panel C a magnification fit showing the initial oscillations in the CPMG sequence.
Accelerated qMRI

A number of alternative methods have been proposed for accelerated quantification of $R_1$ and $R_2$ (Deoni et al. 2003; Warntjes et al. 2007; Warntjes et al. 2008). In this project the QRAPMASTER sequence (Warntjes et al. 2008) was used (sometimes also referred to as QMAP). QRAPMASTER is a combination of a modified IR experiment with a CPMG experiment. Instead of using a $180^\circ$ inversion pulse, a $120^\circ$ pulse is used for saturation of the signal. Moreover the repetition time is shorter that of a regular IR experiment ca. 3-5 s, and only a limited number of echoes in the CPMG sequence are used. Both $R_1$ and $R_2$ are then calculated from the data by applying a signal model containing $B_1$ inhomogeneity and simulations of the pulse profiles to calibrate for the oscillating first echoes in the CPMG sequence. Furthermore, by the use of internal references in the data, the water concentration ($C_{H2O}$) can be calculated in absolute fraction of 100% water. Hence, by using the QRAPMASTER sequence it is possible to retrieve absolute $R_1$, $R_2$ and $C_{H2O}$ in clinical acceptable scanning times.

Figure 1.13. (A) Raw MRI images acquired using the QRAPMASTER sequence. (B) Mean signal in a region of interest (ROI) in white matter summarised over all echoes. (C) Mean signal in a ROI of white matter summarised over all inversion time points.
2. Aims

The main objective of this project was to implement a quantitative magnetic resonance spectroscopy (qMRS) method suitable for clinical use on the human brain. A standard procedure for “absolute” quantification is to use the tissue water as an internal reference. “Absolute” means obtaining the metabolite concentration in real world physical units, and in as accurate and repeatable a manner as possible. However, the tissue water signal which is used for referencing purposes depends on the water relaxation and concentration, which are generally not known, and also depends on age, brain tissue region and pathology.

In Paper I, we investigated whether quantitative magnetic resonance imaging (qMRI) could be used for calibration of the internal water signal. Moreover, an estimate of uncertainty in absolute units were compared with the relative Cramér-Rao Lower Bound (CRLB) that has sometimes been used for quality assurance of the data. Additionally, the relation between metabolite concentrations in white matter and the thalamus was correlated with age and qMRI results of white matter and the thalamus respectively.

In Papers II and III, the qMRS method was applied to investigate the disorder Multiple Sclerosis (MS) and to scrutinise the metabolite concentrations of normal appearing white matter (NAWM) in the thalamus of MS patients compared to healthy controls. The method was also used to investigate how the metabolite concentrations were related to age, ‘Multiple Sclerosis Severity Score’ (MSSS), ‘Extended Disability Status Scale’ (EDSS), disease duration, intrathecal inflammation biomolecular factors, brain parenchymal fraction (BPF) and qMRI measurements of the tissue.

Based on the hypothesis that idiopathic normal pressure hydrocephalus (iNPH) patients suffer from disturbed basal ganglia-thalamic-subcortical frontal circuits, our main objective in Paper IV was to investigate to what extent the metabolite concentrations of frontal deep white matter and the thalamus were affected, compared to healthy controls.
In Paper V, our main objective was to investigate how the previously described working memory disturbance, which in turn was associated with higher activation of the thalamus during working memory load, was associated with metabolite concentration levels in the thalamus.
3. Materials and Methods

3.1. Subjects and Data

This thesis includes five different clinical neurological projects (i. NormSprak, ii. qMR in MS, iii. Natalizumab treatment, iv. iNPH, and v. KLS) in which qMRS were used. In total, 507 MRS measurements (330 in the white matter and 177 in the thalamus) were included. Of 158 subjects, 63 were healthy controls, 81 were diagnosed with CDMS, 20 were diagnosed with iNPH, and 14 were diagnosed with KLS. A summary of the projects and the acquisition protocols is presented in Table 1. All measurements were performed on a Philips Achieva 1.5 T MR scanner (Philips Healthcare, Best, The Netherlands). In all projects the MRS signal was acquired using the PRESS sequence with echo time of either (TE) 25 or 30 ms and repetition time (TR) of 3 s. A qMRI volume was acquired either using a standard CPMG sequence, or the QRAPMASTER sequence. For motion detection, T2w coronal image volumes were acquired both prior and after the MRS acquisition on all projects. The different projects are described briefly below.

**NormSprak**

The NormSprak project was performed in combination with an fMRI project to investigate the fMRI activation pattern during a normal language abilities test (van Ettinger-Veenstra et al. 2010; Van Ettinger-Veenstra et al. 2012). Of the 18 subjects included, the qMRI and MRS examinations were performed on 12 of the subjects (age median, min-max, 39, 21-63 years). The examinations were performed using a transmit-receive head coil. One MRS VOI of white matter, and one MRS VOI of the thalamus were acquired with an echo time of 30 ms. The qMRI volume was acquired using QRAPMASTER with four echoes equidistantly spaced, echo times 21-83 ms, repetition time 3.7 s, slice-thickness 3 mm and in-plane resolution 1.1 by 1.3 mm. The data was used as ‘cohort I’ in paper I.
qMRS in Multiple Sclerosis

Thirty-five patients diagnosed with CDMS were included in the project. The patients were divided into two groups: 15 patients (age 57, 32-69 years) with two or fewer T2 hyperintense white matter lesions were included in the atypical non-lesional MS group (MRI_{neg}), 20 patients (age 46, 20-66 years) fulfilling the Barkhof-Tintoré criteria, as described in Table 1 in (McDonald et al. 2001) were included in a typical MS group (MRI_{pos}), and 20 healthy control subjects (age 48, 27-72 years) were included.

The MR examinations were performed using the eight-channel phased array head coil. Two single voxel MRS of NAWM were acquired with an echo time of 30 ms, NSA 128, and one single voxel MRS of the thalamus, NSA 128. The qMRI volume was acquired using QRAPMASTER with five echoes equidistantly spaced, echo times 14-70 ms, repetition time 2.9 s, slice-thickness 4 mm and in-plane resolution 1.1 by 0.9 mm.

The data of the healthy control subjects was used as ‘cohort III’ in Paper I, and when time allowed, the QRAPMASTER sequence and the MRS of white matter were repeated in the control subjects (in six subjects both white matter MRS were repeated and in 14 subjects one white matter MRS was repeated). The clinical assessments EDSS, MSSS, and the disease duration of the patients were retrieved from the National MS register. The qMRS data was analysed in Paper II and the healthy controls was used as cohort III in Paper I and as controls in Paper III.
Figure 3.1 Typical voxel placements and spectra of white matter. In the top panel a control subject (female age 45), in the middle panel MRI_{neg} (female age 57, MSSS 3.54) and in the lower panel MRI_{pos} (female age 45, MSSS 2.95). The spectra are not drawn to the same magnitude scale. Residuals are showed on the of each spectra.
Natalizumab

Twenty-seven CDMS patients (age 40, 22-62 years) with active MS disease and who had been selected for Natalizumab treatment were included. All patients were examined using qMRS before treatment and after one year of treatment, CSF samples were also acquired from 25 patients prior and after one year of treatment (two patients refrained from lumbar puncture). In an initial phase of the project, (the first 20 patients) $R_2$ was measured using a Multi-slice 2D-CPMG sequence with eight echoes equidistantly spaced, echo times 20-160 ms, repetition time 5.3 s, slice thickness 3 mm and in-plane resolution 2 by 2 mm. In the second phase of the project (the last seven patients) $R_2$ was measured using the QRAPMASTER sequence with four echoes equidistantly spaced, echo times 14-66 ms, repetition time 2.7 s, 4 mm slice thickness, and in-plane resolution 1 by 1 mm$^2$. Moreover, in the original protocol, a CSI slice was acquired and a single voxel MRS of white matter. However, during quality control of the data the CSI were found unsuitable for absolute quantification, therefore a second MRS voxel replaced the CSI acquisition. Thus in the 24 first examinations at base line and the nine first one-year follow-ups, one white matter MRS was acquired and in the three last examinations at base line and 18 last one-year follow-ups, two MRS of white matter were

![Figure 3.2 Voxel placement and example spectra showing at baseline and at one year followup, with residual showed on the of each spectra.](image)
acquired. Moreover, sampling of CSF was obtained before start of Natalizumab treatment (mean 1.8 ± 2.5 months) and after one year of treatment. Both levels of cytokine (IL-1β and CXCL8) and chemokine (CXCL10, CXCL11 and CCL22) in the CSF were assessed using a multiplex bead assay (Vignali 2000).

In Paper III, data was pooled from both phases of the Natalizumab project and the healthy controls in the qMR on MS project were used as control subjects.

**Normal Pressure Hydrocephalus**

Twenty iNPH patients (age 77, 49-83 years) and 16 healthy controls (age 74, 62-89 years) were included. The MR-examinations were performed using an eight-channel phased array head coil (SENSE). One single voxel MRS of frontal deep white matter (FDWM) and one single voxel MRS of the thalamus were acquired with an echo time of 30 ms. In a later phase, R₂ was measured using eight echoes equidistantly spaced, echo times 20-160 ms, repetition time 5.3 s, slice-thickness 4 mm and in-plane resolution 2 by 2 mm². Moreover, motor function was assessed by a physiotherapist using a timed up and go test and a 10-metre walk test (w10m). In the TUG test the patient starts by sitting in a chair, then stands up and walks three metres, then turns and walks back and sits down. The performance is measured both in seconds (TUGt) and in number of steps (TUGs). In the w10m test the patient walks 10 metres and the performance is measured both in seconds (w10mt) and in number of steps (w10ms). The project is reported in paper IV.

![Figure 3.3 Voxel placement and example spectrum.](image)
Kleine-Levin Syndrome

Fourteen KLS patients (age 21, 15-37 years) and 15 healthy controls (age 22, 19-40 years) were included. The examination consisted of three parts. First, a working memory test outside the scanner by a neuropsychologist. Second, an fMRI investigation with a paradigm mimicking the working memory task. Third, a qMRS examination. The qMRS examination was performed using the transmit-receive head coil. Two MRS VOIs of white matter and two MRS VOIs of the thalamus were acquired using an echo time 25 ms, NSA 128. The qMRI volume was acquired using QRAPMASTER with echoes equidistantly spaced, echo times 21-83 ms, and repetition time 3.7 s. The data was used in Paper V and the data of the healthy control subjects was used as ‘cohort II’ in paper I.

*Figure 3.4 (A) Location of fMRI activation. (B) Voxel placement, (C) example spectrum.*
Table 1. Overview of included subjects and data.

<table>
<thead>
<tr>
<th>Project</th>
<th>Subjects</th>
<th>Coil</th>
<th>qMRI</th>
<th>qMRS</th>
<th>Included in paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>NormSprak</td>
<td>12 Healthy controls (5/7) (22, 18-40)</td>
<td>Transmit</td>
<td>QRAPMASTER, TR: 3.7, TE: 21-83 ms</td>
<td>2 white matter VOI 2</td>
<td>Paper I</td>
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<tr>
<td></td>
<td></td>
<td>Receive</td>
<td></td>
<td>thalamus VOI, TE 30, TR 3s</td>
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<tr>
<td>qMRS on MS</td>
<td>20 Healthy controls (5/15), (48, 27-72)</td>
<td>Phased Array</td>
<td>QRAPMASTER, TR: 3, TE: 14-56 ms,</td>
<td>2† white matter VOI 1</td>
<td>Paper II, Healthy</td>
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<tr>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td>thalamus VOI, TE 30, TR 3s</td>
<td>controls also in Paper I and III</td>
</tr>
<tr>
<td></td>
<td>15 MRI&lt;sub&gt;loop&lt;/sub&gt; (1/14), (57, 32-69)</td>
<td>Phased Array</td>
<td>CPMG 20 subjects, TR: 5.28 s, TE: 20-160</td>
<td>1-2†† MRS VOI of</td>
<td></td>
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<td></td>
<td></td>
<td>8</td>
<td>ms, QRAPMASTER 7 subjects, TR: 2.7 s, TE:</td>
<td>NAWM examined at base and after on year,</td>
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<td></td>
<td></td>
<td>channels</td>
<td>14-66 ms</td>
<td>TE 30 ms, TR 3s</td>
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<tr>
<td></td>
<td>20 MRI&lt;sub&gt;loop&lt;/sub&gt; (6/14), (46, 20-66)</td>
<td>Phased Array</td>
<td>QRAPMASTER, TR: 3, TE: 21-83 ms</td>
<td>2 white matter VOIS 2</td>
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<td>thalamus VOIs, TE 25, TR 3s</td>
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<td>channels</td>
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<tr>
<td>Natalizumab</td>
<td>27 MS (14/13), (22-62)</td>
<td>Phased Array</td>
<td>CPMG TR: 5.28 s, TE: 20-160 ms</td>
<td>1-2††† NAWM MRS VOIs and 1-2†††</td>
<td>Paper IV</td>
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<tr>
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<td>8</td>
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<td>thalamus MRS VOIs, TE 30 ms, TR 3s</td>
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<td>channels</td>
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<tr>
<td>NPH</td>
<td>16 Healthy controls, (7/9), (74, 62-89)</td>
<td>Phased Array</td>
<td>CPMG TR: 5.28 s, TE: 20-160 ms</td>
<td>1-2††† NAWM MRS VOIs and 1-2†††</td>
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<td></td>
<td>20 NPH (12/8), (74, 49-83)</td>
<td>Phased Array</td>
<td>QRAPMASTER, TR: 3.7, TE: 21-83 ms</td>
<td>2 white matter VOIS 2</td>
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<td>thalamus VOIs, TE 25, TR 3s</td>
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<td>channels</td>
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<tr>
<td>KLS</td>
<td>15 Healthy controls (7/8), (22, 19-40), 14 KLS patients (6/6), (21, 15-37)</td>
<td>Phased Array</td>
<td>QRAPMASTER, TR: 3.7, TE: 21-83 ms</td>
<td>2 white matter VOIS 2</td>
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<td>thalamus VOIs, TE 25, TR 3s</td>
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<td>channels</td>
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</tbody>
</table>

1In the healthy subjects the qMRI volume and one or both MRS of white matter was repeated. †††In the initial phase of the project a single MRS was acquired (the 24 first baseline and the 9 first at one year followup). ††††In the initial phase of the project a single MRS was acquired (the 10 first controls and the 10 first NPH).
3.2. Implementation

**MR Acquisition**

The MRS volume was placed in the intended target area simultaneously using MRI images of three different cardinal projections; sagittal, coronal, and transversal (see panel A Figure 3.5). Whole brain volume qMRI data were also acquired in the same session (using QRAPMASTER (Warntjes et al. 2008)), or a CPMG sequence.

MRS data was acquired using a PRESS sequence with a reference water spectrum and a water suppressed metabolite spectrum acquired in the same acquisition protocol, thus the same coil load and pulse profiles were used for both the water reference and the metabolite spectra.

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**Figure 3.5** Panel A, shows typical MRS VOI placement in white matter using three cardinal planes. Panel B shows qMRI data acquired using the QRAPMASTER sequence. Panel C shows an MRS examination consist of one series of non water-suppressed and one series of water-suppressed spectra.
Patient movement was monitored using two T2w coronal image volumes that were acquired both prior and after the MRS acquisition. Moreover, in examinations in which more than one MRS voxel was measured, the MRS voxel placement was verified using the most recent T2w coronal image volume.

**Post Processing the qMRI Data**

![Figure 3.6. Quantification of QRAPMASTER data using SyMRI Brain studio.](image)

In Papers I, II and V the QRAPMASTER sequence was used and $\hat{R}_1$, $\hat{R}_2$, and $\hat{C}_{H_2O}$ were analysed using SyMRI brain studio (Synthetic MR AB, Linköping, Sweden), both the sequence and software are commercially available and the QRAPMASTER sequence was implemented as a standard imaging protocol on the scanner. In Papers I and II tissue maps of white matter, grey matter and CSF were also calculated.

In Papers III, and IV a CPMG sequence was used for measuring $\hat{R}_{2, H_2O}$ which was calculated using a least square fitting algorithm implemented in Matlab (MathWorks Inc., Natick, MA, USA). The CPMG data was fitted using the function:

$$Y(t_E) = \hat{A}_{H_2O} \exp(-\hat{R}_{2, H_2O} t_E),$$

where, $Y(t_E)$ is the measured signal at echo time $t_E$ and $\hat{A}_{H_2O}$ is proportional to the water concentration however, $\hat{A}_{H_2O}$ is also $R_1$, and coil load dependent.
MRS VOI Registration in the qMRI Volume

The geometric information of the MRS VOI and the qMRI volume was given in the MRS and MRI file headers respectively. The centres of the MRS VOI and the centre of the qMRI volume were given in a coordinate system defined fixed in the scanner with origin in the scanner isocentre. The angulation of the MRS VOI and the qMRI volume were given as three Euler angles. Using the distance to the isocentre and the Euler angles, the two coordinate transformation matrixes $T_S$ and $T_I$ were calculated. The transformation matrixes $T_S$ and $T_I$ described the coordinate transformations from the scanner coordinate system to the MRS and the MRI coordinate system respectively. The coordinate of a voxel is given as a vector $X_I$ where the index $I$ denotes that the coordinate is given in the MRI coordinate system. The coordinate $X_I$ was calculated using the FOV and resolution of the images, then the coordinate of the qMRI voxels were transformed to the MRS coordinate system by the coordinate transformation: $X_S = T_ST_I^{-1}X_I$. Now all qMRI voxels that were inside the MRS VOI will have coordinates smaller than the size of the MRS VOI. Thus all qMRI values with coordinates smaller than the size of the MRS VOI were stored and subsequently used for calculation of scaling factors.

Figure 3.7. In panel A: qMRI maps of $\hat{R}_{1,\text{H}_2\text{O}}$, $\hat{R}_{2,\text{H}_2\text{O}}$, $\hat{C}_{\text{H}_2\text{O}}$, WM, GM, and CSF. In panel B: histogram for the registered MRS VOI of $\hat{R}_{1,\text{H}_2\text{O}}$, $\hat{R}_{2,\text{H}_2\text{O}}$, $\hat{C}_{\text{H}_2\text{O}}$, WM, GM, and CSF.
Calculating Scaling Factors

In Paper I and Paper II two scaling factors were calculated; $F_{\text{ABS}}$ for calculation of absolute concentrations in mM and $F_{\text{AQ}}$ for calculations of metabolite concentrations in aqueous fraction concentrations.

$F_{\text{AQ}}$ was calculated as:

$$F_{\text{AQ}} = \left( \sum_{j}^{N_{\text{VOI}}} \left( \hat{C}_{\text{H2O},j} \left( 1 - e^{-t_{R} \hat{R}_{1,\text{H2O},j}} \right) e^{-t_{E} \hat{R}_{2,\text{H2O},j}} \right) \right) / \left( \sum_{j}^{N_{\text{VOI}}} \hat{C}_{\text{H2O},j} \right),$$

(3.1)

where $\sum_{j}^{N_{\text{VOI}}}$ was the sum over all the $N_{\text{VOI}}$ qMRI voxels within a separate MRS VOI, $t_{R}$ was the MRS repetition time, and $t_{E}$ the MRS echo time, $\hat{C}_{\text{H2O},j}$ was the measured fraction of water, $\hat{R}_{1,\text{H2O},j}$ was the measured longitudinal water relaxation, and $\hat{R}_{2,\text{H2O},j}$ was the measured transverse water relaxation rate of the qMRI voxel $j$ (where $j$ is the qMRI voxel index number within the MRS VOI).

Similarly in Paper I and II, $F_{\text{ABS}}$ was calculated as:

$$F_{\text{ABS}} = \frac{1}{N_{\text{VOI}}} \sum_{j}^{N_{\text{VOI}}} \left( \hat{C}_{\text{H2O},j} \left( 1 - e^{-t_{R} \hat{R}_{1,\text{H2O},j}} \right) e^{-t_{E} \hat{R}_{2,\text{H2O},j}} \right)$$

(3.2)

where $\sum_{j}^{N_{\text{VOI}}}$ was the sum over all the $N_{\text{VOI}}$ qMRI voxels within a separate MRS VOI, $t_{R}$ was the MRS repetition time, and $t_{E}$ the MRS echo time, $\hat{C}_{\text{H2O},j}$ was the measured fraction of water, $\hat{R}_{1,\text{H2O},j}$ was the measured longitudinal water relaxation, and $\hat{R}_{2,\text{H2O},j}$ was the measured transverse water relaxation rate of the qMRI voxel $j$ (where $j$ is the qMRI voxel index number within the MRS VOI).

In Paper III and IV the scaling factor $F_{\text{AQ,CPMG}}$ was calculated using $\hat{A}_{\text{H2O}}$ and $\hat{R}_{2}$ maps estimated from the CPMG data.

$$F_{\text{AQ,CPMG}} = \left( \sum_{j}^{N_{\text{VOI}}} \left[ \hat{A}_{\text{H2O},j} e^{-t_{E} \hat{R}_{2,\text{H2O},j}} \right] \right) / \left( \sum_{j}^{N_{\text{VOI}}} \hat{A}_{\text{H2O},j} \right),$$

(3.3)

where $\hat{A}_{\text{H2O},j}$ and $\hat{R}_{2,\text{H2O},j}$ was the measured transverse water relaxation rate of the qMRI voxel $j$. 

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Correcting for Tissue Volume

Since essentially all MRS visible metabolites are dissolved in the intracellular fluid, contamination of CSF in the MRS VOI will therefore lead to an underestimation of the metabolite concentration. To compensate for this effect Ernst et al. (Ernst 1993) proposed to scale the measured contamination with a scaling factor $P_{TW}$. In Paper II the metabolite concentrations were scaled using this $P_{TW}$. $P_{TW}$ was calculated from the qMRI data as:

$$P_{TW} = \frac{1}{N_{VOI}} \sum_{j=1}^{N_{VOI}} \frac{1}{1 - \hat{p}_{j,CSF}},$$  \hspace{1cm} (3.4)

where $\hat{p}_{j,CSF}$ was the estimated CSF fraction in voxel $j$ calculated using the SyMRI brain studio. In Paper IV $P_{TW} = 1/(1 - P_{CSF})$ where $P_{CSF}$ was calculated as fraction of $R_2$ values within the MRS VOI that were < 2.5 s$^{-1}$.

Calculating Absolute Concentrations

In Paper I and Paper II the LCModel parameter for 100% WCONC was set to 55047 mM for ‘physiological water’ (in saline, at 310 K). In Paper III-V WCONC was set to 55555 mM to represent 100% pure water as given in the LCModel manual (Provencher 2012).

Calculating Uncertainty Estimates in Absolute Units

The uncertainty of the estimated metabolite concentration was calculated in the LCModel software using the Cramér-Rao-Lower-Bound (CRLB) (Provencher 2012). The CRLB is given in percentage of the estimated concentration, thus the CRLB will both depend on the noise in the signal and on the metabolite concentrations. In Paper I three types of uncertainty estimates were analysed. (1) Absolute standard deviation $SD_{ABS}$, (2) aqueous fraction standard deviation $SD_{AQ}$ and (3) Relative standard deviation $SD_{\%}$ (as the CRLB reported by LCModel). $SD_{ABS}$ and $SD_{AQ}$ were calculated by multiplication of Absolute and Aqueous fraction concentration with the CRLB respectively.
3.3. Statistical methods

Mixed Linear Models

In paper I, II, IV and V JMP 8.0 (SAS Institute Inc., Cary NC., USA) was used for analysing the data using mixed linear model (MLM) analysis. MLMs are regression models with both fixed effects and random effects. The fixed effects work as ordinary independent regression variables, and can be nominal, ordinal or continuous. In this thesis variables such as group, tissue, coil design, echo time have been treated as nominal fixed effects, and variables like age, MSSS, EDSS, disease duration and motor score have been treated as continuous fixed effects. The random effects are assumed to represent random samples from a population with independent normal distributed variables. In this thesis the subjects were treated as random effects.

Orthogonal Regression

In Paper V, orthogonal regression analysis was used to assess the relation between fMRI activation and NAA concentration, in addition, to the MLMs that were used to assess group difference. The orthogonal regression was calculated with PRISM 5.0 (GraphPad Software, San Diego Ca. USA). In orthogonal regression the regression line is calculated by minimising the sum of square of the orthogonal distance between the fitted line and the data points. In contrast in ordinary linear regression the regression line is calculated by minimising the sum of square of the vertical difference between the fitted line and the dependent variable.

Non Parametric Correlation

We assumed that the relation between change in metabolite concentration in the NAWM could have a highly non linear-relation to the levels of inflammation markers in the CSF. Consequently, non-parametric Spearman correlations were used to assess the relation between change in metabolite concentration and CSF inflammatory markers. The correlations were calculated with SPSS 20.0 (SPSS inc., Chicago, IL., USA).
4. Results

4.1. Validation

The 95% confidence intervals (CI) of the mean $F_{ABS}$ and $F_{AQ}$ in white matter were 0.456-0.465 and 0.690-0.701 respectively, and the 95% CI of mean $F_{ABS}$ and $F_{AQ}$ in the thalamus were 0.483-0.495 and 0.677-0.690 respectively. The default LCModel values were $F_{ABS} = 0.452$ and $F_{AQ} = 0.7$ (Provencher 2012) corresponding to white matter based on the work by Ernst et al. (Ernst 1993). The result showed that mean $F_{ABS}$ and $F_{AQ}$ were significantly different between white matter and the thalamus in healthy controls. Moreover, $F_{ABS}$ in white matter was significantly positively correlated with age.

Coil and Echo Time

$F_{ABS}$ and $F_{AQ}$ were significantly lower for an echo time of 30 ms compared with an echo time of 25 ms. Moreover, $F_{ABS}$ and $F_{AQ}$ were significantly lower for the phased array coil compared with the transmit-receive coil for the thalamus. In contrast, no significant difference in scaling factors was observed between the coils in white matter.

In the supplementary Table 2 in Paper I the differences in mean metabolite concentration calculated using different echo times and coils are presented. The main results were that significantly higher Glx$_{ABS}$ and a trend for higher Glx$_{AQ}$ in white matter were observed for an echo time of 30 ms compared to an echo time of 25 ms. In contrast, trends of lower tNA$_{ABS}$ and tNA$_{AQ}$ in white matter were observed for an echo time of 30 ms compared to an echo time of 25 ms. Moreover, higher tNA$_{ABS}$ and tNA$_{AQ}$ were observed using the phased array coil compared to the transmit receive coil. In contrast, lower tGlx$_{ABS}$ and tGlx$_{AQ}$ were observed using the phased array coil compared to the transmit receive coil.
Repeated measurements

In Table 2 mean standard deviation (SD) from the repeated measurements and the mean of SD_{ABS} are presented. The SD was calculated as the square root of the squared difference between repeated measurement divided by 2. The calculated SD were approximately equal or lower to the mean SD_{ABS}.

<table>
<thead>
<tr>
<th>White Matter</th>
<th>Repeated SD</th>
<th>LCM SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=24^2)</td>
<td>(n=48)</td>
</tr>
<tr>
<td>tCr_{ABS}</td>
<td>0.27</td>
<td>0.30</td>
</tr>
<tr>
<td>mIns_{ABS}</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>tCho_{ABS}</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>tNA_{ABS}</td>
<td>0.33</td>
<td>0.42</td>
</tr>
<tr>
<td>Glx_{ABS}</td>
<td>0.90</td>
<td>0.95</td>
</tr>
</tbody>
</table>

SD in mM
4.2. Uncertainty Estimations in Absolute Units

The results of the linear regression models for absolute uncertainty estimations are presented in Table 3. $r^2_{\text{Adjusted}}$ are presented for both model 1 and model 2. For model 2 the linear regression coefficient for $C_{\text{met,ABS}}$ ($\beta$) is also presented. The main results were that the SD% were negatively correlated with $C_{\text{met,ABS}}$. In contrast, $SD_{\text{ABS}}$ and $SD_{\text{AQ}}$ were positively correlated with $C_{\text{met}}$. Moreover, $r^2_{\text{Adjusted}}$ were higher for SD% compared to $SD_{\text{ABS}}$ and $SD_{\text{AQ}}$.

The estimated SD for different echo times and coils are presented in the supplementary Table 3 in Paper I. All SD estimates were significant higher or indicated a trend for higher SD for an echo time of 30 ms compared to an echo time of 25 ms. Moreover, all SD estimates were significantly lower (or indicated a trend for lower) SD for the phased array coil compared to the transmit receive coil.

### Table 3. Linear models for uncertainty estimates for concentrations in white matter. SD$_{\text{ABS}}$, SD$_{\text{AQ}}$ and SD% as dependent factors. $r^2$ adjusted are presented for both the reduced model (Model 1) and the extended model with ‘Metabolite concentration’ as independent factor (Model 2). SD$_{\text{ABS}}$ are in [mM], SD$_{\text{AQ}}$ are in [mM]$_{\text{AQ}}$ and SD% are in percentage of estimate concentrations. Significant effects are emphasized in **bold**.

<table>
<thead>
<tr>
<th>White matter</th>
<th>$r^2_{\text{Adjusted}}$ Model 1</th>
<th>$r^2_{\text{Adjusted}}$ Model 2</th>
<th>$\beta$ (SE)</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD$_{\text{ABS,tCr}}$</td>
<td>0.52</td>
<td>0.61</td>
<td>0.035 (0.007) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{AQ,tCr}}$</td>
<td>0.54</td>
<td>0.61</td>
<td>0.046 (0.011) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD%$_{\text{tCr}}$</td>
<td>0.52</td>
<td>0.65</td>
<td>-1.116 (0.175) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{ABS,mIns}}$</td>
<td>0.46</td>
<td>0.45</td>
<td>0.004 (0.009) &gt;0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{AQ,mIns}}$</td>
<td>0.49</td>
<td>0.49</td>
<td>0.005 (0.012) &gt;0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD%$_{\text{mIns}}$</td>
<td>0.13</td>
<td>0.71</td>
<td>-3.516 (0.248) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{ABS,tCho}}$</td>
<td>0.65</td>
<td>0.66</td>
<td>0.009 (0.005) 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{AQ,tCho}}$</td>
<td>0.67</td>
<td>0.68</td>
<td>0.015 (0.008) 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD%$_{\text{tCho}}$</td>
<td>0.32</td>
<td>0.71</td>
<td>-3.965 (0.335) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{ABS,InA}}$</td>
<td>0.18</td>
<td>0.32</td>
<td>0.032 (0.007) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{AQ,InA}}$</td>
<td>0.20</td>
<td>0.31</td>
<td>0.041 (0.010) ***</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>SD%$_{\text{InA}}$</td>
<td>0.48</td>
<td>0.55</td>
<td>-0.365 (0.091) ***</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{ABS,Glx}}$</td>
<td>0.29</td>
<td>0.29</td>
<td>0.009 (0.009) &gt;0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD$_{\text{AQ,Glx}}$</td>
<td>0.30</td>
<td>0.31</td>
<td>0.015 (0.013) &gt;0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD%$_{\text{Glx}}$</td>
<td>0.09</td>
<td>0.62</td>
<td>-2.096 (0.176) ***</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significance level of F test of independent factors.
4.3. Application of qMRS on Human Brain

Normal White Matter and Thalamus Metabolite Concentrations

Calculated white matter and thalamus metabolite concentrations, and the correlation between metabolite concentration and age are presented in Paper I. Both tCr and mIns in white matter and mIns in the thalamus were positively correlated with age. In Fig. 4.1, the calculated white matter concentrations of healthy controls are presented in comparison to results found in the literature (Brex et al. 1999; Leary et al. 1999; Sarchielli et al. 1999; Helms 2000; Kapeller et al. 2001; Fernando et al. 2004; Gustafsson et al. 2007; Brief et al. 2009).

Correlation between qMRS and qMRI

In Paper I the qMRI values of healthy controls were compared with the qMRS results of the same tissue. The main results were the following: tCr<sub>ABS</sub>, tNA<sub>ABS</sub> and tNA<sub>AQ</sub> in white matter were inversely correlated with $R_1$. Moreover, tNA<sub>ABS</sub> in white matter was also inversely correlated with $R_2$. In contrast, tCr<sub>ABS</sub>, mIns<sub>ABS</sub>, tNA<sub>ABS</sub> and tNA<sub>AQ</sub> in white matter were positively correlated with $C_{H_2O}$. Furthermore, mIns<sub>ABS</sub>, mIns<sub>AQ</sub>, tCho<sub>ABS</sub>, and tCho<sub>AQ</sub> in thalamus were inversely correlated to $R_2$. In contrast, tNA<sub>ABS</sub> in thalamus was positively correlated with $C_{H_2O}$.
Figure 4.1 Comparison of estimated white matter metabolite concentration (of healthy controls) found in the literature: Fernando (F) (Fernando et al. 2004); Paper I, (bars highlighted in grey); Kapeller (K) (Kapeller et al. 2001), Brex (Be) (Brex et al. 1999), Leary (L) (Leary et al. 1999), Helms (H) (Helms 2000), Brief (Bi) (Brief et al. 2009), Pouwels (P) (Pouwels et al. 1998), Gustafsson (G) (Gustafsson et al. 2007), Sarchielli (S) (Sarchielli et al. 1999).
4.4. Application of qMRS on Multiple Sclerosis

Metabolite Group Differences

In Paper II, the group differences in metabolite concentration in the white matter and the thalamus between the control, MRI_{neg} and MRI_{pos} groups are presented. In Paper III, the group differences in metabolite concentration in white matter are presented. The results are summarised in Table 4. The main results were that the Glx in white matter was elevated in all MS groups compared to healthy controls. Moreover, tNA in white matter was lower and mIns in white matter was higher in the MRI_{pos} group and the Natalizumab-treated MS patients compared to the controls, and tCho in white matter was higher in the Natalizumab-treated MS patients compared to the controls.

Table 4. Comparison of group difference in white matter metabolite concentration for the patients in paper II and paper III compared to the control group in the qMRS on MS project. Age corrected 95% confidence intervals of mean difference are presented between each group within paper II and paper III respectively. Absolute concentrations [mM] are presented above the aqueous fraction concentrations [mM]AQ. Significant effects are emphasized in bold.

<table>
<thead>
<tr>
<th>White matter</th>
<th>MRI_{neg} - Controls</th>
<th>MRI_{pos} - Controls</th>
<th>MRI_{neg} - MRI_{pos}</th>
<th>MRI_{nat} - Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td>MRI_{neg}</td>
<td>(N_{neg}=15, N_{ctrl}=20)</td>
<td>(N_{pos}=20, N_{ctrl}=20)</td>
<td>(N_{neg}=15, N_{ctrl}=20)</td>
<td>(N_{neg}=27, N_{ctrl}=20)</td>
</tr>
<tr>
<td>Glx_{ABS}</td>
<td>[ 0.66 , 2.07 ] ***</td>
<td>[ 0.57 , 1.85 ] ***</td>
<td>[ -0.55 , 0.86 ]</td>
<td>[ 0.13 , 3.08 ] *</td>
</tr>
<tr>
<td>Glx_{AQ}</td>
<td>[ 1.07 , 2.95 ] ***</td>
<td>[ 0.65 , 2.35 ] ***</td>
<td>[ -0.43 , 1.46 ]</td>
<td></td>
</tr>
<tr>
<td>tNA_{ABS}</td>
<td>[ -0.41 , 0.63 ]</td>
<td>[ -0.96 , -0.02 ] *</td>
<td>[ 0.08 , 1.13 ] *</td>
<td></td>
</tr>
<tr>
<td>tNA_{AQ}</td>
<td>[ -0.58 , 0.83 ]</td>
<td>[ -1.61 , -0.33 ] ***</td>
<td>[ 0.38 , 1.80 ] **</td>
<td></td>
</tr>
<tr>
<td>tCr_{ABS}</td>
<td>[ -0.26 , 0.30 ]</td>
<td>[ -0.09 , 0.41 ]</td>
<td>[ -0.42 , 0.14 ]</td>
<td></td>
</tr>
<tr>
<td>tCr_{AQ}</td>
<td>[ -0.34 , 0.36 ]</td>
<td>[ -0.22 , 0.41 ]</td>
<td>[ -0.43 , 0.27 ]</td>
<td></td>
</tr>
<tr>
<td>mIns_{ABS}</td>
<td>[ -0.27 , 0.47 ]</td>
<td>[ 0.35 , 1.02 ] ***</td>
<td>[ -0.96 , -0.22 ] **</td>
<td></td>
</tr>
<tr>
<td>mIns_{AQ}</td>
<td>[ -0.42 , 0.70 ]</td>
<td>[ 0.40 , 1.42 ] ***</td>
<td>[ -1.34 , -0.21 ] **</td>
<td></td>
</tr>
<tr>
<td>tCho_{ABS}</td>
<td>[ -0.19 , 0.13 ]</td>
<td>[ -0.04 , 0.25 ]</td>
<td>[ -0.30 , 0.02 ]</td>
<td></td>
</tr>
<tr>
<td>tCho_{AQ}</td>
<td>[ -0.31 , 0.19 ]</td>
<td>[ -0.12 , 0.33 ]</td>
<td>[ -0.42 , 0.09 ]</td>
<td></td>
</tr>
</tbody>
</table>

* P > 0.05, ** P < 0.01, *** P < 0.001. N_{ctrl} (Number of Controls), N_{neg} (Number of Non-lesional MS patients), N_{pos} (Number of lesional MS patients), MRI_{nat} (Number of Natalizumab treated patients)
Scaling Factors Group Differences

The mean $F_{\text{ABS}}$, $F_{\text{AQ}}$ and qMRI results of white matter and the thalamus of controls and MS patients in Paper II are presented in Table 5. The MRI$_{\text{neg}}$ MS group did not show any significant differences compared to the controls. In contrast, the MRI$_{\text{pos}}$ MS group showed significantly lower $R_1$ and $R_2$, and higher $C_{\text{H}_2\text{O}}$ in white matter, which also led to significantly higher $F_{\text{ABS}}$ and a trend for higher $F_{\text{AQ}}$ in white matter compared to the controls. Moreover, lower $R_2$ and higher $F_{\text{AQ}}$ in the thalamus of MRI$_{\text{pos}}$ compared to the controls was observed.

Table 5. Scaling factors and qMRI result of white matter and thalamus of healthy controls, MRI$_{\text{neg}}$ MS patients and MRI$_{\text{pos}}$ MS patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean (SE)</th>
<th>MRI$_{\text{neg}}$ Mean (SE)</th>
<th>p</th>
<th>MRI$_{\text{pos}}$ Mean (SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White matter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_1$</td>
<td>1.59 (0.016)</td>
<td>1.58 (0.02)</td>
<td>&gt;0.2</td>
<td>1.53 (0.02)</td>
<td>0.017</td>
</tr>
<tr>
<td>$R_2$</td>
<td>12.27 (0.089)</td>
<td>12.22 (0.10)</td>
<td>&gt;0.2</td>
<td>11.99 (0.09)</td>
<td>0.035</td>
</tr>
<tr>
<td>$C_{\text{H}_2\text{O}}$</td>
<td>0.666 (0.004)</td>
<td>0.667 (0.004)</td>
<td>&gt;0.2</td>
<td>0.679 (0.004)</td>
<td>0.013</td>
</tr>
<tr>
<td>$F_{\text{ABS}}$</td>
<td>0.457 (0.003)</td>
<td>0.458 (0.004)</td>
<td>&gt;0.2</td>
<td>0.469 (0.003)</td>
<td>0.014</td>
</tr>
<tr>
<td>$F_{\text{AQ}}$</td>
<td>0.686 (0.002)</td>
<td>0.686 (0.002)</td>
<td>&gt;0.2</td>
<td>0.690 (0.002)</td>
<td>0.072</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_1$</td>
<td>1.44 (0.01)</td>
<td>1.41 (0.02)</td>
<td>&gt;0.2</td>
<td>1.42 (0.02)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>$R_2$</td>
<td>12.97 (0.09)</td>
<td>12.80 (0.10)</td>
<td>&gt;0.2</td>
<td>12.64 (0.09)</td>
<td>0.011</td>
</tr>
<tr>
<td>$C_{\text{H}_2\text{O}}$</td>
<td>0.710 (0.004)</td>
<td>0.715 (0.004)</td>
<td>&gt;0.2</td>
<td>0.711 (0.004)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>$F_{\text{ABS}}$</td>
<td>0.474 (0.003)</td>
<td>0.479 (0.004)</td>
<td>&gt;0.2</td>
<td>0.479 (0.003)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>$F_{\text{AQ}}$</td>
<td>0.667 (0.002)</td>
<td>0.670 (0.002)</td>
<td>&gt;0.2</td>
<td>0.674 (0.002)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Significants level of Student’s t test for difference in mean compared to the control group, $R_1$ and $R_2$ in $1/s$, $C_{\text{H}_2\text{O}}$ in fraction of water $F_{\text{ABS}}$ and $F_{\text{AQ}}$ dimensionless scaling factors.
Mixed linear models for EDSS, MSSS, and disease duration are presented with estimate and standard error (SE) of for the factors. Absolute concentrations [mM] are presented above the aqueous fraction concentrations [mM]AQ. Significant effects are emphasized in **bold**.

Table 6. Mixed linear models for EDSS, MSSS, and disease duration are presented with estimate and standard error (SE) of for the factors. Absolute concentrations [mM] are presented above the aqueous fraction concentrations [mM]AQ. Significant effects are emphasized in **bold**.

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{met}} = \beta \cdot \text{Age} + m_0$</th>
<th>$C_{\text{met}} = \beta \cdot \text{MSSS} + m_0$</th>
<th>$C_{\text{met}} = \beta \cdot \text{EDSS}$</th>
<th>$C_{\text{met}} = \beta \cdot \text{Duration}$</th>
<th>$C_{\text{met}} = \beta \cdot \text{BPF}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White matter</strong></td>
<td>((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19))</td>
<td>((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19))</td>
<td>((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19))</td>
<td>((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19))</td>
<td>((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19))</td>
</tr>
<tr>
<td>GlxABS</td>
<td>-0.8E-2 (\pm 1.2E-2)</td>
<td>0.158 (\pm 0.064)</td>
<td>0.177 (\pm 0.083)</td>
<td>-0.003 (\pm 0.016)</td>
<td>1.263 (\pm 4.009)</td>
</tr>
<tr>
<td>GlxAQ</td>
<td>-2.4E-2 (\pm 1.6E-2)</td>
<td>0.183 (\pm 0.082)</td>
<td>0.201 (\pm 0.107)</td>
<td>-0.012 (\pm 0.020)</td>
<td>5.448 (\pm 5.537)</td>
</tr>
<tr>
<td>tNAABS</td>
<td>-5.7E-3 (\pm 9.0E-3)</td>
<td>0.081 (\pm 0.056)</td>
<td>0.120 (\pm 0.074)</td>
<td>-0.005 (\pm 0.013)</td>
<td>-1.924 (\pm 3.050)</td>
</tr>
<tr>
<td>tNAO</td>
<td>-2.0E-2 (\pm 1.2E-2)</td>
<td>0.081 (\pm 0.078)</td>
<td>0.122 (\pm 0.106)</td>
<td>-0.016 (\pm 0.018)</td>
<td>0.358 (\pm 4.269)</td>
</tr>
<tr>
<td>tCrABS</td>
<td>2.0E-2 (\pm 0.5E-2) **</td>
<td>0.063 (\pm 0.029)</td>
<td>0.078 (\pm 0.046)</td>
<td>0.018 (\pm 0.008)</td>
<td>-6.219 (\pm 1.634) **</td>
</tr>
<tr>
<td>tCrAO</td>
<td>2.5E-2 (\pm 0.6E-2) **</td>
<td>0.067 (\pm 0.038)</td>
<td>0.079 (\pm 0.058)</td>
<td>0.022 (\pm 0.010) **</td>
<td>-7.286 (\pm 2.133) **</td>
</tr>
<tr>
<td>mIlnsABS</td>
<td>3.1E-2 (\pm 0.6E-2) **</td>
<td>0.024 (\pm 0.033)</td>
<td>0.028 (\pm 0.059)</td>
<td>0.026 (\pm 0.009) **</td>
<td>-7.708 (\pm 2.435) **</td>
</tr>
<tr>
<td>mIlnsAQ</td>
<td>4.1E-2 (\pm 1.0E-2) **</td>
<td>0.014 (\pm 0.051)</td>
<td>0.010 (\pm 0.086)</td>
<td>0.034 (\pm 0.013)</td>
<td>-9.621 (\pm 3.603) **</td>
</tr>
<tr>
<td>tChoABS</td>
<td>6.7E-3 (\pm 2.8E-3) *</td>
<td>-0.030 (\pm 0.017)</td>
<td>-0.033 (\pm 0.023)</td>
<td>0.001 (\pm 0.004)</td>
<td>-0.536 (\pm 0.987)</td>
</tr>
<tr>
<td>tChoAQ</td>
<td>7.9E-3 (\pm 4.4E-3) *</td>
<td>-0.054 (\pm 0.027)</td>
<td>-0.063 (\pm 0.035)</td>
<td>0.001 (\pm 0.006)</td>
<td>-0.056 (\pm 1.526)</td>
</tr>
</tbody>
</table>

**Thalamus** \((N_{\text{ctrl}}=10, N_{\text{neg}}=15, N_{\text{pos}}=19)\)

| GlxABS            | 2.7E-2 \(\pm 2.3E-2\)                        | 0.050 \(\pm 0.127\)                          | 0.077 \(\pm 0.164\)                          | 0.009 \(\pm 0.027\)                         | -6.695 \(\pm 7.800\)                         |
| GlxAO             | 3.0E-2 \(\pm 3.1E-2\)                        | 0.073 \(\pm 0.164\)                          | 0.113 \(\pm 0.213\)                          | 0.013 \(\pm 0.035\)                         | -7.542 \(\pm 10.469\)                        |
| tNAABS            | 1.7E-3 \(\pm 6.7E-3\)                        | -0.026 \(\pm 0.038\)                         | -0.033 \(\pm 0.050\)                         | -0.001 \(\pm 0.009\)                        | 0.729 \(\pm 2.294\)                         |
| tNAO              | -6.1E-3 \(\pm 8.9E-3\)                       | -0.037 \(\pm 0.052\)                         | -0.051 \(\pm 0.068\)                         | -0.002 \(\pm 0.011\)                        | 3.272 \(\pm 3.133\)                         |
| tCrABS            | 7.3E-3 \(\pm 6.4E-3\)                        | -0.024 \(\pm 0.037\)                         | -0.075 \(\pm 0.045\)                         | -0.016 \(\pm 0.008\)                        | 1.453 \(\pm 2.101\)                         |
| tCrAO             | 5.5E-3 \(\pm 8.5E-3\)                        | -0.034 \(\pm 0.048\)                         | -0.107 \(\pm 0.059\)                         | -0.027 \(\pm 0.011\)                        | 3.265 \(\pm 2.791\)                         |
| mIlnsABS          | 4.0E-2 \(\pm 7.4E-3\) **                      | 0.014 \(\pm 0.040\)                          | -0.028 \(\pm 0.079\)                         | 0.022 \(\pm 0.013\)                         | -4.034 \(\pm 3.327\)                        |
| mIlnsAQ           | 5.2E-2 \(\pm 1.0E-2\) **                      | 0.020 \(\pm 0.056\)                          | -0.036 \(\pm 0.107\)                         | 0.031 \(\pm 0.017\)                         | -4.643 \(\pm 4.603\)                        |
| tChoABS           | 6.9E-3 \(\pm 2.9E-3\) *                      | -0.028 \(\pm 0.017\)                         | -0.023 \(\pm 0.024\)                         | 0.004 \(\pm 0.004\)                         | -1.527 \(\pm 1.020\)                        |
| tChoAQ            | 8.2E-3 \(\pm 4.0E-3\) *                      | -0.038 \(\pm 0.023\)                         | -0.031 \(\pm 0.032\)                         | 0.006 \(\pm 0.005\)                         | -1.752 \(\pm 1.393\)                        |

* P < 0.05, ** P < 0.01, *** P < 0.001
Metabolite Concentration in Relation to Age, Clinical Status and Brain Parenchyma Fraction

The Mixed Linear Models (MLMs) for age, MSSS, EDSS, disease duration and BPF are presented in Table 6. Glx was positively correlated with MSSS and Glx\textsubscript{ABS} was significantly correlated with EDSS. Moreover, the interaction term Group*BPF was significant for Glx in white matter. Glx in white matter was significantly inversely correlated to BPF for the MRI\textsubscript{pos} group.

Both mIns and tCr in white matter were positive correlated with age and with disease duration. In contrast, mIns and tCr in white matter were inversely correlated with BPF. Moreover, tCr\textsubscript{ABS} in white matter was positively correlated with MSSS, and tCho\textsubscript{ABS} in white matter was positively correlated with age. Furthermore, tCr in the thalamus was negatively correlated with disease duration, and mIns and tCho in the thalamus were positively correlated with age.

Longitudinal Changes in Natalizumab Treated MS (Paper II)

The Natalizumab-treated MS patients showed a significant decrease in inflammation markers Il-1β, CXCL8, CXCL10, CXCL11 and CCL22 in CSF after one year. However, no significant changes in metabolite concentrations between baseline and one year of treatment were observed on a group level. On the other hand there was a large variation between different patients in metabolite concentration between baseline and one year of treatment. To follow up this result we performed a correlation analysis between the change in metabolite concentration and inflammation markers. The results showed a significant positive correlation between change in tCr\textsubscript{AQ} (ΔtCr\textsubscript{AQ}) with CXCL8 and Il-1β levels both at baseline and after one year of treatment, and a significant positive correlation between change in tCho\textsubscript{AQ} (ΔtCho\textsubscript{AQ}) and Il-1β at both baseline and after one year of treatment. Moreover, tCho\textsubscript{AQ} (ΔtCho\textsubscript{AQ}) was significantly correlated with CXCL8 after one year of treatment and showed a trend of correlation with CXCL8 levels at baseline.
Correlation qMRS and qMRI

In Table 7 the results of the linear regression models with metabolite concentration $\hat{C}_{\text{met}}$ dependent variables and $\bar{R}_1$, $\bar{R}_2$ and $\bar{C}_{\text{H}_2\text{O}}$ as independent variables are presented. In the white matter $\text{tNA}$, $\text{tCr}$ and $\text{mlns}$ showed significant or trends of inverse correlations to $\bar{R}_1$ and $\bar{R}_2$ and positive correlation to $\bar{C}_{\text{H}_2\text{O}}$. In contrast, in thalamus $\text{Glx}_{\text{ABS}}$ and $\text{tCho}_{\text{ABS}}$ in thalamus were inversely correlated with $\bar{R}_1$ and $\bar{R}_2$ and positive correlated with $\bar{C}_{\text{H}_2\text{O}}$.

**Table 7.** Linear regression model for qMRS as dependent variable and mean qMRI results as the independent variable. The regression coefficient $\beta$ and standard error (SE) for the absolute and aqueous fraction concentrations models: $[\text{met}]\text{ABS}(R_1)$, $[\text{met}]\text{ABS}(R_2)$, $[\text{met}]\text{ABS}(\text{C}_\text{H}_2\text{O})$ and $[\text{met}]\text{Aq}(\text{C}_\text{H}_2\text{O})$. Significant effects are emphasized in **bold**.

<table>
<thead>
<tr>
<th></th>
<th>$\hat{C}_{\text{met}} = \beta \cdot \bar{R}_1 + m_0$</th>
<th>$\hat{C}_{\text{met}} = \beta \cdot \bar{R}_2 + m_0$</th>
<th>$\hat{C}<em>{\text{met}} = \beta \cdot \bar{C}</em>{\text{H}_2\text{O}} + m_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White matter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Glx}_{\text{ABS}}$</td>
<td>$-1.31 \ (1.73)$</td>
<td>$-0.35 \ (0.32)$</td>
<td>$5.33 \ (7.94)$</td>
</tr>
<tr>
<td>$\text{Glx}_{\text{Aq}}$</td>
<td>$1.79 \ (2.56)$</td>
<td>$-0.02 \ (0.48)$</td>
<td>$-9.39 \ (11.83)$</td>
</tr>
<tr>
<td>$\text{tNA}_{\text{ABS}}$</td>
<td>$-5.49 \ (1.04)$ ***</td>
<td>$-0.83 \ (0.20)$ ***</td>
<td>$22.04 \ (5.03)$ ***</td>
</tr>
<tr>
<td>$\text{tNA}_{\text{Aq}}$</td>
<td>$-4.33 \ (1.59)$ **</td>
<td>$-0.72 \ (0.29)$ *</td>
<td>$15.02 \ (7.51)$ *</td>
</tr>
<tr>
<td>$\text{tCr}_{\text{ABS}}$</td>
<td>$-3.36 \ (0.62)$ ***</td>
<td>$-0.38 \ (0.13)$ **</td>
<td>$16.34 \ (2.79)$ ***</td>
</tr>
<tr>
<td>$\text{tCr}_{\text{Aq}}$</td>
<td>$-2.80 \ (0.90)$ **</td>
<td>$-0.26 \ (0.17)$</td>
<td>$14.18 \ (4.14)$ ***</td>
</tr>
<tr>
<td>$\text{mlns}_{\text{ABS}}$</td>
<td>$-3.14 \ (0.97)$ **</td>
<td>$-0.37 \ (0.18)$ *</td>
<td>$16.83 \ (4.44)$ ***</td>
</tr>
<tr>
<td>$\text{mlns}_{\text{Aq}}$</td>
<td>$-2.69 \ (1.43)$ **</td>
<td>$-0.28 \ (0.26)$</td>
<td>$15.94 \ (6.63)$ *</td>
</tr>
<tr>
<td>$\text{tCho}_{\text{ABS}}$</td>
<td>$0.16 \ (0.35)$</td>
<td>$0.03 \ (0.06)$</td>
<td>$-0.21 \ (1.65)$</td>
</tr>
<tr>
<td>$\text{tCho}_{\text{Aq}}$</td>
<td>$1.09 \ (0.52)$ *</td>
<td>$0.17 \ (0.10)$</td>
<td>$-4.24 \ (2.46)$</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Glx}_{\text{ABS}}$</td>
<td>$-10.43 \ (3.74)$ ***</td>
<td>$-1.57 \ (0.73)$ *</td>
<td>$38.43 \ (14.22)$ **</td>
</tr>
<tr>
<td>$\text{Glx}_{\text{Aq}}$</td>
<td>$-9.75 \ (5.24)$</td>
<td>$-1.75 \ (0.99)$</td>
<td>$34.10 \ (19.94)$</td>
</tr>
<tr>
<td>$\text{tNA}_{\text{ABS}}$</td>
<td>$-1.01 \ (1.18)$</td>
<td>$0.17 \ (0.22)$</td>
<td>$7.45 \ (4.38)$</td>
</tr>
<tr>
<td>$\text{tNA}_{\text{Aq}}$</td>
<td>$2.87 \ (1.58)$</td>
<td>$0.67 \ (0.28)$ *</td>
<td>$-6.43 \ (6.13)$</td>
</tr>
<tr>
<td>$\text{tCr}_{\text{ABS}}$</td>
<td>$-1.94 \ (1.08)$</td>
<td>$-0.16 \ (0.21)$</td>
<td>$9.57 \ (3.90)$ *</td>
</tr>
<tr>
<td>$\text{tCr}_{\text{Aq}}$</td>
<td>$-0.17 \ (1.49)$</td>
<td>$0.02 \ (0.28)$</td>
<td>$3.38 \ (5.53)$</td>
</tr>
<tr>
<td>$\text{mlns}_{\text{ABS}}$</td>
<td>$-2.07 \ (1.72)$</td>
<td>$-0.62 \ (0.31)$</td>
<td>$6.92 \ (6.51)$</td>
</tr>
<tr>
<td>$\text{mlns}_{\text{Aq}}$</td>
<td>$-0.77 \ (2.40)$</td>
<td>$-0.65 \ (0.44)$</td>
<td>$1.23 \ (9.07)$</td>
</tr>
<tr>
<td>$\text{tCho}_{\text{ABS}}$</td>
<td>$-1.26 \ (0.51)$ *</td>
<td>$-0.23 \ (0.10)$ *</td>
<td>$4.62 \ (1.93)$ *</td>
</tr>
<tr>
<td>$\text{tCho}_{\text{Aq}}$</td>
<td>$-0.98 \ (0.71)$</td>
<td>$-0.24 \ (0.13)$</td>
<td>$3.32 \ (2.71)$</td>
</tr>
</tbody>
</table>

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance level of F test of independent factor. $[\text{met}]\text{ABS}(R_1)$ and $[\text{met}]\text{ABS}(R_2)$ are in mM/$s^{-1}$, $[\text{met}]\text{ABS}(\text{C}_\text{H}_2\text{O})$ are in mM / fraction of water, $[\text{met}]\text{Aq}(R_1)$ and $[\text{met}]\text{Aq}(R_2)$ are in mM $aq./s^{-1}$, $[\text{met}]\text{Aq}(\text{C}_\text{H}_2\text{O})$ are in mM $aq. / fraction of water.
4.5. Application of qMRS on Normal Pressure Hydrocephalus

In Paper IV, the mean metabolite concentration and $R_2^*$ of FDWM and the thalamus for iNPH patients and controls are presented. The main findings were reduced NAA$_{AQ}$ and tNA$_{AQ}$ in the thalamus (Figure 4.2). In a follow-up study after shunt surgery, the non-normalisation of NAA$_{AQ}$ or tNA$_{AQ}$ was observed (Lundin et al. 2012). Moreover, no difference in $R_2^*$ between iNPH patients and controls was observed. In addition, the correlation between motor score and metabolite concentrations was investigated. The main finding was that tCr$_{AQ}$ in the frontal deep white matter was significantly positively correlated with all motor scores (Table 8). Moreover, NAA were not correlated with these. However, the metabolite ratio NAA$_{AQ}$/tCr$_{AQ}$ was negatively correlated with the motor scores.

Table 8. Correlation coefficient r for the linear regression model for gait parameter as dependent variable time to walk 10 m in second (w10mt), number of steps for 10 m walk (w10ms), Timed up and go in seconds (TUGt) and Timed up and go number of steps (TUGs). Significant effects are emphasized in bold.

<table>
<thead>
<tr>
<th></th>
<th>tCr</th>
<th>NAA</th>
<th>NAA/tCr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w10mt</td>
<td>0.73</td>
<td>0.001</td>
<td>0.18</td>
</tr>
<tr>
<td>w10ms</td>
<td>0.54</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>TUGt</td>
<td>0.56</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>TUGs</td>
<td>0.53</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>TUG s</td>
<td>-0.10</td>
<td>0.73</td>
<td>-0.61</td>
</tr>
</tbody>
</table>

TUG s

Figure 4.2 95% confidence intervals of mean difference between idiopathic Normal pressure hydrocephalus and healthy controls.
4.6. Application of qMRS on Kleine-Levin Syndrome

No significant differences in mean metabolite concentrations were observed between the KLS group and the control group in the thalamus or in white matter (Paper V). Moreover, the fMRI whole brain level analysis confirmed earlier published results of a stronger BOLD response in the left thalamus of the KLS patients compared to the controls during the working memory task (Engstrom et al. 2009).

An orthogonal linear regression analysis showed an inverse correlation between fMRI activation in the thalamus and NAA concentration ($r = -0.61$, $\beta = -0.30 \pm 0.11$, $p = 0.02$) Figure 4.3.

Figure 4.3 Orthogonal regression between NAA concentration and fMRI activation in left thalamus. Patient with and active disease were denoted with $\times$, KLS patients that were in remission $\Delta$. 
5. Discussion

5.1. Implementation

The implemented qMRS methods presented in this thesis were based on the combination of conventional MRS and qMRI, which are both implemented as standard sequences on our 1.5 T MR system. This design was based on our ambition to introduce (absolute) quantitative MRS in clinical practice. All quantification steps were user-independent and could be implemented as a fully automated process. In addition, significant advantages were obtained from using a qMRI method with whole brain coverage, i.e., any number and angulation of MRS VOIs could be placed within the same single qMRI volume, and thus multi-voxel MRS techniques could be calibrated in the same manner. Furthermore, the qMRI data could be used for characterising the tissue within the MRS VOI.

Accuracy and Precision

No reference method was used for determining the accuracy of the implemented qMRS method. The main reason was that to our knowledge no practicable method for absolute quantification had successfully been implemented for our MR system. However, the calculated $F_{ABS}$ and $F_{AQ}$ for white matter were close to the default values for white matter in the LCModel. Thus, this shows that even without any prior knowledge of what tissue was measured, an accurate value for the scaling factors was calculated. This indicates that the method gives an accurate estimate of the scaling factors. The repeated measurements showed high repeatability, and all variation could be explained by the uncertainty of the fit of the data. Thus our conclusion is that the precision of the method is satisfactory.

Furthermore, The difference in $R_1$, $R_2$, and $C_{H2O}$ between controls and MRI$_{pos}$ in Paper II propagated into significant difference in the scaling factor $F_{ABS}$ and a trend of different for the scaling factor $F_{AQ}$ in white matter. This show that pathology can have a significant impact on the water scaled concentrations.
Compartmentalisation

Basically there are two problems with MRS measurements on heterogeneous tissue when the water signal is used as internal reference. First, the relaxation function will be more complex for heterogeneous tissue. However, since the scaling factors were calculated from qMRI data which have high resolution, the scaling factors should be correct also in heterogeneous tissue. Second, the distribution of metabolite concentrations are probably also heterogeneous. E.g. essentially no metabolites (except glucose and lactate) are dissolved in the CSF, thus partial volume of CSF will reduce the measured concentration. However, by quantifying the amount of CSF it is possible to correct for the partial volume of CSF. This correction originally proposed by Ernst et al. (Ernst 1993) was implemented in Paper II. Thus the implemented method should be robust for measurements of heterogeneous tissues.

5.2. Absolute Uncertainty

The relative uncertainty (SD%) presented in the LCModel analysis was shown to be strongly dependent on the absolute metabolite concentrations. Unfortunately, SD% is sometimes used as an exclusion criterion in group analysis, thus there is a significant risk that subjects that have a low concentration of a metabolite will be excluded and therefore the mean concentration overestimated. The absolute uncertainty estimate (SD_ABS) calculated from the SD% was shown to be better explained by the factors that are likely to affect the signal quality, e.g., volume, coil and echo time. Moreover, no correlation of metabolite concentrations of intermediate SNR metabolites e.g. mIns and Glx was observed. In contrast, a positive correlation of metabolites with high SNR was observed. This was probably due to a quantisation error since the SD% was only given in integer percentage units.
5.3. Application of qMRS on Multiple Sclerosis

Glutamate in MS

In both Papers II and III, Glx in white matter was elevated for the MS groups compared to healthy controls, and it is also noteworthy that there was no difference in Glx in white matter between the MRI\textsubscript{neg} and the MRI\textsubscript{pos} groups in paper I, indicating that elevated levels of Glx are associated with intrinsic MS pathology.

![Diagram of glutamate signaling pathways](image)

Figure 5.1. The complex signalling cascades triggered by extracellular glutamate leading to glia injury. Selective activation of AMPA receptors (AMPA-R) and kainate receptors (Kai-R) leads to Na\(^+\) and Ca\(^{2+}\) influx through the receptor channel complex. Subsequent depolarisation activates voltage-gated Ca\(^{2+}\) channels (VGCC) and reverses operation of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX), which contributes to [Ca\(^{2+}\)]\(_i\) increase. Ca\(^{2+}\) overload rapid uptake by mitochondria, which results in attenuation of mitochondrial potential and an increase in the production of reactive oxygen species (ROS). The latter can also be generated by reversal of the glutamate-cystine exchanges, which results in cystine (CySS) efflux, and thereby glutathione (GSH) depletion. Cytochrome c (Cyt c) is released from depolarised mitochondria, interacts with apoptotic protease activating factor 1 (Apaf-1) and activates caspases. Moreover, in oligodendrocyte apoptotic, insults channeled through Kai-R activated caspases 9 and 3, whereas those activating AMPA-R induce apoptosis by recruiting caspase 8, which truncates Bid, caspase 3, and PARP-1, or cause necrosis. (re-printed with permission from (Matute et al. 2006))
regardless of whether the patients have developed lesions or not. Moreover, the positive correlation of Glx in white matter with MSSS in Paper II indicates that Glx is associated with the disease progression of MS. Therefore, there is a possibility that Glx could be used as a marker of severity of MS even in patients where no white matter lesions are found. Furthermore, Glx$_{\text{ABS}}$ in white matter was negatively correlated with BPF, indicating that in patients that do develop lesions the rate of atrophy is associated with Glx$_{\text{ABS}}$.

Due to the overlapping spectra of Glu and Gln, it was only the sum Glx that was analysed here (using data obtained at 1.5 T); hence, these data do not show if it was Glu, Gln or both that were elevated. Srinivasan et al. (Srinivasan 2005) used TE varied PRESS to investigate Glu in NAWM and lesional white matter of MS patients and found a significant increase in the Glu concentration in NAWM and contrast enhancing lesions, while the Gln concentration was not significantly increased compared to healthy controls. Thus we conclude that the elevated Glx in our data is due to an increase in Glu.

Additionally, it has been demonstrated that elevated Glu leads to the destruction of oligodendrocytes (Matute et al. 2006; Fu et al. 2009), see Fig. 27. This suggests that there is a direct linkage between the elevated Glu concentrations and disease progression. One might speculate that our results with elevated Glx levels in the MRI$_{\text{neg}}$ group as well as in the MRI$_{\text{pos}}$ group might be associated with the same diffuse neurodegenerative process in MS.

**Diffuse Neurodegeneration in the White Matter**

Previous research has shown lower tNA in MS patients, based on concentration measures, not concentration ratios. However, a recent report has also shown on a normal tNA (Caramanos et al. 2005; Vrenken et al. 2005). Our results showed that the MRI$_{\text{pos}}$ and the Natalizumab-treated MS patients had lower tNA compared to the controls. Interestingly tNA was not lower for the MRI$_{\text{neg}}$ MS patients compared to the controls. Moreover, no significant correlation of tNA to age, MSSS, EDSS, disease duration, or BPF was observed in any of those in the MS group in Paper II. This indicated that the difference in tNA was due to the white matter lesions, but was not associated with the severity or the progression of the disease.

NAA is neuronal specific and can be used as a neuronal marker (Bjartmar et al. 2002), and NAAG is a neurotransmitter found in both astrocytes and neurones.
Since the singlet resonance of NAA and NAAG are partially overlapping at 1.5 T, tNA was analysed in Papers II and III instead of using separate NAA and NAAG quantification. Thus, the result of lower tNA in the MRI\textsubscript{pos} and the Natalizumab-treated MS patients did not show if was due to lower NAA or NAAG. However, tNA primarily reflects the concentration of NAA, and only to a lesser extent NAAG (Caramanos et al. 2005). Thus, it is likely that the lower tNA was a reflection of lower neuronal concentrations. This would indicate that typical MS patients that do develop lesions also have a lower concentration of neurones in the NAWM.

In contrast, \textit{m}Ins was elevated in both the MRI\textsubscript{pos} and the Natalizumab-treated MS patients had lower tNA compared to the controls, which has also been reported, and \textit{m}Ins in white matter was positively correlated with age, disease duration and inversely with BPF. Since these processes are correlated with each other, it is possible that the correlations are due to the same process. In contrast, the \textit{m}Ins in white matter was not correlated with MSSS or EDSS, indicating that the process that increases the \textit{m}Ins is not associated with the severity of the disease, at least not strongly, nor was the change in \textit{m}Ins during one year of treatment with Natalizumab correlated with levels of inflammatory marker in the CSF.

Furthermore, a number of studies have shown an increased tCr concentration (Caramanos et al. 2005; Vrenken et al. 2005). In Papers II and III, tCr was not significantly increased for any of those in the MS group. However, the confidence intervals showed a trend for increased tCr in the MRI\textsubscript{pos} and Natalizumab-treated MS groups, while the MRI\textsubscript{neg} group did not show any indication of different tCr compared to the controls. Moreover, tCr, like \textit{m}Ins, was positively correlated with age and disease duration, and inversely correlated with BPF. In contrast to \textit{m}Ins, tCr\textsubscript{ABS} was also positively correlated with MSSS and tCr\textsubscript{AQ} showed a trend of positive correlation to MSSS, indicating that at least a part of the process of increased tCr was associated with the severity of the MS-pathology. Moreover, the change in tCr in the Natalizumab-treated MS patients showed a positive correlation with inflammatory markers in the CSF.

Both \textit{m}Ins and tCr can be used as glia markers, thereby indicating the increased \textit{m}Ins or trend of increased tCr could be due to increased glia cell concentrations. However, we also observed a difference between the process of increased \textit{m}Ins and tCr, where tCr was associated with levels of inflammatory marker in the CSF and
with severity of the disease. This indicated that the increases of $m$Ins and tCr are not entirely connected to the same process.

To conclude, for both MS patients and controls a positive correlation for tCr and $m$Ins with age was observed. Moreover the MS patients also showed a correlation for tCr and $m$Ins with BPF. In contrast tNA did not show a dependence on age or PBF. Since, tCr and $m$Ins are glia markers and tNA neuronal specific, one possible model for explaining the results could be that the atrophy is caused by a reduction in both myelin amount and neuronal loss. However, the density of neurones is constant due to compression of the tissue. Which would imply that decreased BPF and constant local neuronal concentration results in decreased total neuronal volume. Thus BPF is a potential measure of neuronal volume. The increase in glia cell density could be caused both by proliferation of the astrocytes (Bjartmar et al. 2001), or alternatively it could be a simple consequence of the compression of the tissue. This model of atrophy agrees with the observation by Laule et al. (Laule et al. 2004), who concluded that increased $R_2$ rate in MS NAWM was due to diffuse demyelination, and with Vrenken et al. (Vrenken et al. 2005) who concluded that the glia density was increased in MS NAWM.

In the MS patient it is possible that the disintegration of the neurones in the NAWM were caused by the distal lesion through ‘Wallerian degeneration’. Wallerian degeneration has been observed in NAWM where the transected axon in a lesion is depleted with a few weeks, while in contrast, the myelin in the oligodendrocyte remain intact for years (Bjartmar et al. 2001). Moreover, by combining quantitative $R_1$ mapping and magnetisation transfer ratio (MTR) mapping Vrenken et al. concluded that the damage s found in NAWM mainly arise as a secondary results of visible lesions and the $R_1$ changed gradually closer to the lesion while the MTR were only effected in the lesion. It would be interesting to repeat that experiment and add qMRS for specific neuronal detection.

**Discrepancy in tCr and tNA results**

In a meta-analytic review by Caramanos et al. (Caramanos et al. 2005) it was concluded that the results from measurements of NAWM vary, with several reports of higher tCr and normal tNA concentration in NAWM of MS patients compared to the controls, in contrast to other reports in which lower tNA and normal tCr concentration were observed. A number of factors could explain these variations. First, as we have reported here, tCr concentration depended on age, and therefore
age should be taken into account when analysing the data in detail, either as a factor in an ANOVA, or in a mixed linear model (MLM), or simply by age matching the groups. Second, the data also showed that there was a correlation between tCr concentration and MSSS, thus the tCr concentration depends on the specific MSSS level of the included patients. Alternatively, methodological differences between different studies such as repetition time, echo time, MRS voxel size, quantification method (e.g., LCModel version, basis set used), or MR-system, could be the cause of the discrepancy.

A common characteristic of the careful studies that reported a normal tNA and higher tCr seems to be that they used a long repetition time (6 s) and a short echo time (<35 ms) (Caramanos et al. 2005). Thus, if $R_1$ (and $R_2$) of tNA and tCr were affected by the MS pathology, NAWM studies with shorter repetition time would be influenced by a difference in degree of saturation of the metabolite signals between MS patients and the controls. However, if the lower tNA and normal tCr concentrations were caused by saturation effects, as is reported in this study, then there must be a difference in $R_1$ for tNA and tCr between the MS patients and the controls. Furthermore, since NAA is neuronal specific and tCr is more abundant in glia cells (Brand et al. 1993), the mobility of the metabolites in both glia cells and neurones needs to be affected simultaneously to change the relaxation times of both tNA and tCr. For this reason, further investigation of how MS pathology affects metabolite relaxation times is clearly justified.

5.4. Application of qMRS on Normal Pressure Hydrocephalus

Low concentrations of the neuronal marker NAA in thalamus of the iNPH indicate that the basal ganglia-thalamic-subcortical frontal circuits are damage or at least strongly modulated in the thalamus. To further explore the hypothesis of a disturbance in the circuits it would be interesting to investigate the neuronal concentrations in basal ganglia.

A particularly interesting result from the absolute quantification point of view, was the NAA/tCr ratio of FDWM that was negatively correlated with the motor scores. If the NAA/tCr-ratio had been used for interpreting the result one possible interpretation could have been that the negative correlation between NAA/tCr and
motor score showed that the increase disturbance of motor function in iNPH patients was related to a decrease in neuronal density. However, our results show that the negative correlation of NAA/tCr was due to a positive correlation between tCr and motor score, and that the NAA in FDWM did not show any correlation to motor score.

5.5. Application of qMRS on Klein-Levine Syndrome

The correlation between strong activation in left thalamus during a working memory test with the neuronal marker NAA indicate that the KLS patients that have low neuronal concentration also needed to utilise the working memory circuitry more heavily in order to perform the task compared to healthy subjects; indicating that KLS patients have a pathological disturbance somewhere in the neuronal network responsible for working memory-performance.

5.6. Statistical Methods

In clinical studies there are often a number of factors that potentially can influence the measurements e.g. age, sex, education, treatment, disease type, etc., preferably the effects are limited by good stratification of the included subjects. However, it is difficult to stratify for all possible effects and it is difficult in advance to know which effects that significantly affect the data. Then the mixed linear model (MLM) can be used and the effects can be included in the model. Another advantage is that since the MLMs can include both nominal effects (such as disease type, examination, sex, etc.) and continuous factors such as age, BPF etc. they can be simultaneously analysed on the same data. E.g. the MLM used in Paper II showed that the mIns concentration differed between controls and MRIpos MS patients and also that mIns was positively correlated with the age for both MS patients and controls.

5.7. Limitations

The major limitation for the validation of the method was that no ‘golden standard’ method was used as reference, because no practicable implementation of
a gold standard method was available. It would be interesting to implement the ERETIC method and use as reference.

The spatial chemical shift between different metabolites and water could induce errors when using the water as reference. For the MRS sequence used in this thesis (PRESS TE 25-30 ms, 1.5 T) the spatial chemical shift between water and NAA was approximately 1 mm which is about the same as the resolution of the qMRI data. Thus the error in registration of the MRS VOI should not be significant. However, if the same MRS sequence is used on a 3 T system the spatial shift will be approximately 4 mm between NAA and water. Consequently, this should be included when calculating the registration of the MRS VOI in the qMRI data. Alternatively other acquisition sequences such as semiLASER could be used for minimising the influence of this selection problem (see below).

5.8. Further Developments

It would be interesting to investigate the effect of metabolite relaxation on the measured concentrations. There are methods available that have included a calibration for metabolite relaxation (Brief et al. 2009). However, the work by Brief et al. was based on tabulated values of metabolite relaxation rates. Thus, if the metabolite relaxation was affected by the pathology this calibration would not help. Therefore a calibration for the effect of metabolite relaxation should be measured for each individual MRS voxel. However, measuring metabolite relaxation rates is difficult although some methods have been proposed (Brief et al. 2003; Brief et al. 2005; Kreis et al. 2005; Edden et al. 2012).

In the qMRS method proposed by Brief et al. (Brief et al. 2009) the water signal was calibrated using a CPMG sequence with 32 echoes. Thus it was possible to segment the tissue into grey matter, white matter and CSF, and to quantify the amount of myelin in the tissue (Whittall et al. 1989). The signal measured using the QRAPMASTER sequence will appear monoexponential due to the fast relaxation of the myelin signal and exchange between the myelin compartment and the cellular compartment. However, the FID signal is typically sampled with a bandwidth of 1000 Hz, thus the time resolution of the FID allows separation of signal components with much smaller relaxation difference than the qMRI data. The FID signal relaxes with $R_2^*$. but if combined with the QRAPMASTER data it
might be possible to both separate the signals from myelin water and cellular water (using the FID signal) and quantify the water concentrations (using the QRAPMASTER data). Then the correlation between tCr and mIns with BPF and age of the MS patients could be put in relation to the myelin amount.

Furthermore, the PRESS sequence has severe limitations, e.g., large spatial chemical shift at higher field strength (e.g., at 7 T) difficulties to separate overlapping multiplets, and difficulty of detecting metabolites with complex spectra. Thus it would be interesting to implement the quantification method for other MRS sequences. E.g., semiLASER which features reduced spatial chemical shift, TE varied PRESS for separate detection of Glu and Gln, or spectral editing such as MEGA-PRESS for GABA detection (Puts et al. 2012). Moreover, it should be fairly straightforward to extend the method developed here for other MRS-sequences, as long as the water signal can be measured using the specific MRS sequence.

Finally, if the method implemented here is going to be used by other research groups and also have a significant clinical impact, the entire quantification method needs to be conveniently available and implemented on MR systems from all manufacturers. Preferably, the complete procedure should also be implemented seamlessly on the scanners such that no raw data needs to be exported in order to calculate absolute concentrations as a post processing procedure. This would also included the implementation of LCModel.
6. Conclusions

It is important to emphasise that the implemented qMRS-method was entirely based on standard and available MR-scanner protocols, thus making the method straightforward for application in a demanding clinical setting. Moreover, all analysis steps were automatic and user-independent, and most importantly, no prior information was needed. Thus, the method is suitable for clinical applications which is a very important result. In addition, it was possible to calculate an uncertainty estimate in absolute physical concentration units that were not biased due to low concentrations.

The correlation between the glia cell markers tCr and mIns with age and BPF, and the lack of correlation between the neuronal marker tNA with age and BPF, indicated that the process of atrophy in MS patients was associated with locally increased glia tissue concentration, and constant neuronal cell density which could be due to compression of tissue. This showed that the global atrophy was correlated with neuronal loss. Moreover, high levels of intrathecal inflammation were associated with increases in tCr and tCho concentrations.

The observed elevated levels of Glx concentrations in all categories of MS subjects could be due to increased Glu concentration. Thus one might speculate that apoptosis due to high levels of Glu is central in the pathogenesis of MS.

The low concentration of the neuronal marker NAA in the thalamus of the iNPH indicated that the basal ganglia-thalamic-subcortical frontal circuits were damaged or at least strongly modulated in the thalamus. To further explore the hypothesis of a disturbance in the circuits it would be interesting to investigate the neuronal concentrations in the basal ganglia.

The correlation between strong activation in the left thalamus during a working memory test with the neuronal marker NAA indicated that the KLS patients that had low neuronal concentration also needed to utilise the working memory circuitry more heavily in order to perform the task as well as healthy subjects. This indicates that KLS patients have a pathological disturbance somewhere in the neuronal network responsible for working memory performance.
7. Bibliography


Bloch (1946). "Nuclear Induction."


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