Detection of $J$-Coupled Metabolites in Magnetic Resonance Spectroscopy

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Summary

During the past twenty years, magnetic resonance spectroscopy (MRS) has emerged as a valuable method for the investigation of physiological processes in the human body. It provides useful metabolic information complementing the insight delivered by magnetic resonance imaging (MRI) in numerous applications. While the detection and quantification of uncoupled metabolites is relatively straightforward, $J$-coupled metabolites still present a major challenge for spectroscopists working in the medical MR field, because their resonances are usually split into multiplets and can show a phase and amplitude modulation depending on the sequence timing. In this thesis, problems arising from scalar coupling are discussed and several solutions proposed.

Localization techniques such as the double spin-echo method called point-resolved spectroscopy (PRESS), which apply radio-frequency (RF) pulses in combination with field gradients for volume selection, suffer from chemical shift displacement artifacts, i.e., different volumes are selected for spins with different Larmor frequencies. For weakly coupled spin systems, this gives rise to a considerable signal intensity loss in echo sequences for certain echo times, an effect called anomalous $J$-modulation. In the first contribution to this thesis, this effect was investigated analytically and experimentally for lactate, a weakly coupled metabolite which plays a pivotal role in many brain disorders. In vivo and in vitro spectra were acquired with human imaging systems from different vendors at 1.5 T and 3 T to demonstrate the signal loss. Furthermore, the signal loss was quantified using a modified spectroscopic imaging (SI) sequence and compared with theoretical predictions. Finally, several strategies for avoiding or at least mitigating the signal cancellation are discussed.

The detection and quantification of coupled metabolites is usually im-
paired through a strong spectral overlap in one-dimensional (1D) spectra. A viable remedy are two-dimensional (2D) spectroscopy methods, which enable a better separation and resolution of multiplet resonances, hence facilitating the extraction of coupling information. A widely used 2D technique, which spreads the coupling information into a second dimension, is $J$-resolved spectroscopy. It can easily be implemented by acquiring PRESS spectra with an incremental echo time ($T_E$) variation encoding the indirect spectral dimension ($J$PRESS). In the second part of this thesis, an improved $J$PRESS method, which tackles problems arising at human MRI scanners, is presented and validated for brain spectroscopy. Through the implementation of a maximum-echo sampling scheme, the sensitivity is increased and the spectral degradation due to imperfect water suppression is alleviated. Besides, adverse eddy current effects are investigated and possible correction schemes proposed.

While $J$PRESS enables the separation of chemical shift and scalar coupling information, 2D S-PRESS is introduced as a novel approach for the detection of strongly coupled spin systems. Instead of varying $T_E$ for encoding all scalar coupling information in the indirect spectral dimension, the echo time is kept constant, but the two partial echo times $T_E_1$ and $T_E_2$ are varied simultaneously and $T_E_1$ is encoded in the indirect dimension. In the resulting 2D spectra, only the resonances of strongly coupled spin systems are spread into the second dimension, leading to more clearly arranged spectra. The method is particularly useful for the detection of citrate representing a strongly coupled AB spin system and was therefore analytically optimized for prostate spectroscopy at 3 T. Two-dimensional S-PRESS spectra were compared with $J$PRESS spectra in vitro as well as in vivo and the spectral parameters of citrate (coupling constant $J$ and chemical shift difference $\delta$) could be determined in vivo with reasonable accuracy from 2D S-PRESS spectra acquired in healthy subjects.

In the final part of this thesis, two-dimensional prior-knowledge fitting (ProFit) is used for the quantification of $J$PRESS spectra acquired at a field strength of 3 T from the human prostate in vivo. Since ProFit is based on the linear combination of two-dimensional basis spectra, it yields metabolite concentration ratios which are independent of sequence and echo time. Furthermore, ProFit turns out to be superior to one-dimensional fitting methods (e.g. LCModel) for the quantification of coupled metabolites like citrate, spermine and myo-inositol. A study carried out on ten healthy subjects shows that the prostate metabolites creatine, choline, cit-
rate, spermine, myo-inositol and scyllo-inositol can be reliably detected in vivo. Among these especially choline and citrate have proven to be useful markers for the detection of prostate cancer.
Zusammenfassung


werden mehrere Strategien zur Vermeidung oder Reduzierung des Signalverlusts diskutiert.


Während $J$PRESS die Trennung von Chemical-Shift- und Kopplungsinformation ermöglicht, wird 2D S-PRESS als neuer Ansatz für die Detektion von stark gekoppelten Spinsystemen präsentiert. Anstatt mit der Variation der Echozeit ($TE$) die gesamte Kopplungsinformation in der indirekten Dimension zu kodieren, wird $TE$ konstant gehalten, aber die partiellen Echozeiten $TE_1$ und $TE_2$ werden gleichzeitig variiert und mit $TE_1$ die indirekte Dimension kodiert. In den resultierenden 2D-Spektren erfahren nur stark gekoppelte Spinsysteme eine Aufspaltung in der indirekten Dimension, was zu einer übersichtlicheren Anordnung der Resonanzen führt. Die Methode ist vor allem für die Detektion von Citrat geeignet, das ein stark gekoppeltes AB-Spinsystem besitzt, und wurde deshalb analytisch für Prostata-Spektroskopie bei 3 T optimiert. 2D-S-PRESS-Spektren wurden mit $J$PRESS-Spektren in vitro und in vivo verglichen, und die spektralen Parameter von Citrat (Kopplungskonstante $J$ und Chemical-Shift-Abstand $\delta$) konnten mit relativ hoher Genauigkeit aus 2D S-PRESS-Spektren bestimmt werden, die in der Prostata von gesunden Probanden aufgenommen wurden.

Im letzten Teil dieser Doktorarbeit wird 2D Prior-Knowledge-Fitting (ProFit) für die Quantifizierung von $J$PRESS-Spektren verwendet, die bei
Introduction and Objectives

Since its invention in the early 1970s, magnetic resonance imaging (MRI) has been developed into an indispensable diagnostic tool for medical examinations. MRI exploits the fact that the body is transparent for radio-frequency (RF) waves and gives rise to an excellent soft tissue contrast. Its advantages over other imaging methods such as X-ray based computer tomography (CT) and positron emission tomography (PET) are its non-invasiveness and the lack of nocuous ionizing radiation. In addition to the morphological information provided by MRI, magnetic resonance spectroscopy (MRS) allows for the investigation of metabolic processes in the body and has therefore proved to be an invaluable method for the examination of tumors and many neuro-pathological and mitochondrial disorders.

The most abundant isotope in the human body that gives rise to an MR signal is $^1$H, which can be found in nearly all organic substances. In vivo $^1$H spectroscopy focuses on the detection of carbon-bound hydrogen nuclei, since protons in highly polar bindings to oxygen or nitrogen usually dissociate very fast compared to the spectral timescale and hence lose coherence. Due to the relatively low sensitivity of human MR systems, only those metabolites that occur in concentrations on the order of 1 mM can be detected in the human body. In vivo $^1$H spectra exhibit a relatively small chemical shift dispersion and rather broad linewidths, which leads to a considerable overlap of detectable resonances.

Furthermore, the detection and quantification of most metabolites are hampered by scalar coupling (or $J$ coupling), which gives rise to a line splitting and a more or less complicated phase and amplitude evolution of the observable resonances. In MRS one distinguishes two coupling regimes
depending on the coupling strength $J$ and the chemical shift difference $\delta$ of two coupled spins. When $J$ is small compared to $\delta$, the scalar coupling can be treated as a perturbation (weak coupling limit) and the $J$ evolution is periodic. In contrast, when this approximation is not valid, the spin system is said to be strongly coupled and its resonance undergoes a more complicated phase and amplitude modulation.\textsuperscript{1}

A popular approach for disentangling overcrowded spectra are so-called spectral editing techniques, which filter out certain $J$-coupled metabolites on the basis of their specific coherence evolution. One distinguishes difference editing techniques, where two spectra with oppositely phased resonances of interest are subtracted from each other, and coherence filters, which discriminate coupled and uncoupled resonances via the generation and filtering of certain coherence orders.\textsuperscript{2,3} However, spectral editing methods have to be tuned for specific metabolites or coupling networks and are not appropriate for the simultaneous detection and quantification of different $J$-coupled metabolites in the same spectral region.

A more versatile method for the detection of $J$-coupled metabolites is two-dimensional (2D) spectroscopy.\textsuperscript{4} Originally used for molecular structure investigations in the NMR field, 2D techniques have gained popularity for in vivo applications over the past decade. Spreading the spectral information into a second dimension helps to decrease the peak overlap and increase the distinguishability of multiplet resonances.

Since the signal-to-noise ratio (SNR) and the chemical shift scale with the external magnetic field ($B_0$), most spectroscopy techniques have gained sensitivity as well as specificity with the advent of higher field strengths ($B_0 \geq 3$ T). On the downside, problems such as localisation artifacts and field inhomogeneities are exacerbated through a higher $B_0$ and often need to be tackled with new concepts.

The overall objective of this thesis is the optimisation of localized spectroscopy for coupled metabolites in terms of SNR, resolution, artifact reduction and hence the improvement of peak assignment and quantification. Various problems encountered in the detection of weakly and strongly coupled spin systems are addressed in this work and specific solutions proposed. This includes the optimisation of existing and the design of new sequences as well as their validation by experimental and analytical means. Furthermore, new postprocessing and quantification strategies are presented and investigated.

The basic principles of $J$-coupling are introduced in Chapter 1, along
with a brief description of techniques applied in this thesis, such as PRESS localisation, spectroscopic imaging and two dimensional spectroscopy methods.

Chapter 2 deals with an effect called anomalous $J$-modulation, which leads to a signal loss for coupled resonances at certain echo times. This effect is quantitatively investigated for the weakly coupled spin system of lactate with single voxel as well as spectroscopic imaging (SI) experiments, carried out at human MR systems from different vendors and at different field strengths.

An improved $J$PRESS method is proposed in Chapter 3 and validated for brain spectroscopy at 3T. It focuses on the optimization of SNR and the mitigation of artifacts in 2D spectroscopy.

In Chapter 4 a novel 2D sequence for the detection of strongly coupled spin systems dubbed 2D S-PRESS is presented. The method is optimised for citrate detection in the human prostate and validated in vitro as well as in vivo.

Finally, Chapter 5 covers the quantification of 2D spectra. The 2D fitting method ProFit, originally proposed for the quantification of brain spectra, is adapted and optimised for metabolite quantification in the human prostate.
Chapter 1

Basics of Magnetic Resonance Spectroscopy

1.1 The Chemical Shift

The spectral dispersion in MRS is based on a phenomenon commonly referred to as chemical shift, a small change in the Larmor frequencies of individual nuclei, depending on their chemical environment. When a molecule is placed in a magnetic field, its electronic eigenstates are slightly modified. This gives rise to small additional magnetic fields at the nuclei, which oppose the externally applied field. The electron density around each nucleus in a molecule varies according to the types of nuclei and bonds in the molecule. The opposing field and therefore the effective field at each nucleus will vary with the chemical environment.

The chemical shift is proportional to the exterior magnetic field strength. It is usually defined as the difference between the measured resonance frequency \( \nu \) and the Larmor frequency \( \nu_{\text{ref}} \) of nuclei of a reference substance, normalized with the resonance frequency of unbound atoms \( \nu_0 \). It is given the symbol \( \delta \) and measured in ppm:

\[
\delta = \frac{\nu - \nu_{\text{ref}}}{\nu_0} \times 10^6
\]  

(1.1)

This definition makes the chemical shift field-independent and therefore facilitates the comparison of spectra acquired at different field strengths.
The most widely used spin species observed in MRS experiments is $^1$H, which has a favorable signal yield due to its high natural abundance and gyro-magnetic ratio. The chemical shift range of in vivo $^1$H spectra is about 5 ppm and tetramethylsilane (TMS) is used as a reference substance. Up to twenty metabolites can be detected in vivo with currently used clinical MR systems, operating at field strengths of 1.5 T or 3 T. Most of them give rise to multiple resonances stemming from different $^1$H nuclei in the molecule.

### 1.2 Scalar Coupling

Every metabolite gives rise to a characteristic pattern of resonances. However, their detection and quantification is complicated by scalar coupling, a magnetic spin-spin interaction which is mediated via Fermi contacts, i.e. the binding electrons within the molecules (Fig. 1.1).

#### 1.2.1 Classical Picture

Consider a molecule with two coupled spins A and B: Depending on the orientation of spin B with respect to the external magnetic field, the magnetic field experienced by spin A and therewith also its Larmor frequency...
1.2. SCALAR COUPLING

Figure 1.2: Energy level diagram for two spins A and B in the uncoupled (left) and weakly coupled (right) case, with four allowed transitions (transition frequencies: $\nu'_A$, $\nu''_A$, $\nu'_B$, $\nu''_B$. Under the influence of weak scalar coupling, the energy levels are shifted by $|\Delta| = |\pm J_{AB}|/4$ (with $J_{AB}$ being the coupling constant).

are slightly different. This gives rise to a small shift of the energy levels and hence also changes the frequencies of the transitions, which are observed in an MRS experiment (Fig. 1.2). While in the uncoupled case the frequencies of the two allowed transitions for spin A as well as spin B are equal ($\nu'_A = \nu''_A$, $\nu'_B = \nu''_B$), giving rise to two singlets, the two resonances are split into doublets in the coupled case (Fig. 1.3). Coupling networks with more than two coupled spins cause a splitting into triplets, quartets and so on. In addition to the line splitting, scalar coupling gives rise to a phase evolution of the coupled resonances as a function of echo time ($TE$). This is illustrated in Fig. 1.4: For echo times of $TE = 1/(2J)$ and $TE = 3/(2J)$ the two peaks of a doublet are in anti-phase with respect to each other, while at $TE = 1/J$ the resonance appears as an inverted in-phase doublet. After an echo time of $TE = 2/J$ the coupled resonance has experienced a phase evolution of $2\pi$ and appears as a positive in-phase doublet again.

Basic rules for spectra of $J$-coupled nuclei:
Figure 1.3: Resulting spectra in the uncoupled (left) and coupled (right) case. Instead of a singlet A the resonance frequency $\nu_A$ of the uncoupled proton, one observes a doublet at resonances $\nu_A' = \nu_A + J_{AB}/2$ and $\nu_A'' = \nu_A - J_{AB}/2$

- $J$ does not depend on the main field strength.
- The coupling strength decreases with increasing number of bonds relating coupled spins.
- Coupling and therefore splitting is mutual: $J_{AB} = J_{BA}$.
- Spin-spin coupling between magnetically equivalent nuclei produces no observable splitting.
- A nucleus coupled to $n_I$ equivalent nuclei of spin $I$ gives rise to $2n_I + 1$ lines.
- Coupling to different spin groups is additive.
- The line intensities in a weakly coupled resonance are distributed according to a binomial pattern.
- Strong coupling gives rise to more complicated spectra.

Spin echoes in MRS sequences are usually created with refocusing pulses. Those invert the spin dispersion from a previous evolution period and hence cancel the Zeeman interaction, when placed in the middle between excitation and acquisition. In contrast to that, a non-selective
Figure 1.4: Signal modulation in dependence of echo time $T_E$. During the evolution period the transverse magnetisation is converted from in-phase to anti-phase magnetisation and vice versa.
180° pulse does not refocus the effects of $J$-coupling, which results in a phase modulation in a spin echo experiment. However, when instead a spin-selective refocusing pulse is applied, i.e., only one of the two coupled spins is inverted, the $J$-evolution is refocused and the resonance appears as a positive in-phase doublet regardless of echo time. This phase modulation is exploited in spectral editing techniques.\(^2\)

### 1.2.2 Quantum-Mechanical Picture

In liquid phase NMR, the unperturbed nuclear spin Hamiltonian consists of two parts, a Zeeman term $H_Z$ and a coupling term $H_J$:

$$H = H_Z + H_J, \quad (1.2)$$

with

$$H_Z = \sum_{k=1}^{N} \omega_0 k I_{kz}, \quad H_J = 2\pi \sum_{k=1,l<k}^{N} \vec{I}_k J_{kl} \vec{I}_l. \quad (1.3)$$

$\vec{I}_k$ denotes the angular momentum spin operator and $\omega_0 k$ the Larmor frequency of spin $k$, $J_{kl}$ denotes the coupling strength between spin $k$ and spin $l$.

One distinguishes two different coupling regimes depending on the coupling strength $J$ and the difference in Larmor frequencies $\Delta \omega$ of two coupled spins: In the weak coupling limit ($2\pi J \ll \Delta \omega$) the non-secular part of $H_J$ can be neglected:

$$H_{J}^{wc} = 2\pi \sum_{k=1,l<k}^{N} J_{kl} I_{kz} I_{lz}. \quad (1.4)$$

In this case, coupling and Zeeman Hamiltonian commute: $[H_{J}^{wc}, H_Z] = 0$. As a consequence, chemical shift and coupling evolution are independent of each other and can be calculated separately, using the product operator formalism.\(^5\) If the secular approximation ($2\pi J \ll \Delta \omega$) is not valid, as for most homonuclear spin systems at typical field strengths of 1.5 or 3 T, the full coupling Hamiltonian has to be considered and the spin system is said to be strongly coupled. Since $H_{J}^{sc}$ and $H_Z$ do not commute, the product operator formalism cannot be applied any more and the analytical calculation of the spin evolution becomes more complicated.
1.3 Spatial Localisation

For spectroscopy application in the human body, where specific organs and their sub-structures are to be investigated, it is desirable to obtain spatially resolved spectral information. Especially for $^1$H spectroscopy, localisation is usually based on RF pulses in combination with linear field gradients. In general, RF pulses have a fixed finite bandwidth of typically a few hundred to a few thousand Hz. If a linear field gradient is applied to the sample, the Larmor frequencies of the spins show a linear dependence on the spatial position. When an RF pulse is irradiated at the same time, it affects only those spins, whose Larmor frequency lies inside the frequency band of the pulse. Applying this method consecutively with gradients along three orthogonal directions, a volume of interest (VOI) can be defined, from which the MR signal is acquired.

The most widely used technique based on this localisation approach is point-resolved spectroscopy (PRESS), which uses one $90^\circ$ excitation pulse and two $180^\circ$ refocusing pulses for localisation and echo formation at the same time (Fig. 1.5). The acquisition of the echo can be started either immediately after the second refocusing pulse (maximum echo) or only at the echo top (half echo). The excitation pulse and the two refocusing pulses become slice-selective by field gradients in the three spatial directions. The final echo signal therefore originates only from the volume element defined by the intersection of the three slice planes.

It is often desirable to obtain spectroscopic information with a spatial resolution. This can be achieved with spectroscopic imaging (SI), which is typically applied along two spatial dimensions. Other than in Fourier imaging where the signal is phase-encoded in one k-space direction and frequency-encoded in the other during acquisition, both spatial dimensions within an SI slice, which is typically selected with the excitation pulse, are phase-encoded (Fig. 1.6). This typically amounts to a scan time of several minutes, because in conventional SI experiments one excitation is required for each point in k-space that is to be read out. However, SI sequences can be accelerated by acquiring several echo signals per excitation. This method is called turbo spectroscopic imaging (TSI).

The localised spectra are reconstructed through a Fourier transformation along the temporal and the two spatial dimensions. The spatial distribution of a certain metabolite can then be determined by integrating all spectra within a specified frequency interval around the resonance fre-
Figure 1.5: Schematic representation of a PRESS sequence. Volume localisation is achieved with one excitation and two refocusing pulses in combination with selection gradients in three different directions.
Figure 1.6: K-space sampling in 2D SI (b) versus Fourier imaging (a). In conventional Fourier Imaging one spatial dimension is frequency-encoded during the acquisition ($x$) and the other phase-encoded ($y$). In 2D SI both spatial dimensions have to be phase-encoded, since the spectral information is read during the acquisition.
Figure 1.7: Creation of metabolite maps from SI data through peak integration.
quency (Fig. 1.7). With a Fourier interpolation of the calculated matrix of integration values, smoothed metabolite maps can be obtained and superimposed with an anatomical image for the clinical interpretation of the results.

For signal-to-noise considerations, the voxel size has to be significantly larger than in imaging sequences, typical minimal values are $1 \, \text{cm}^3$ for $^1\text{H}$ spectroscopy. It is important to suppress signal from fatty tissue, which often surrounds the region of interest (e.g. in the brain). Therefore, in basic applications, the SI method is combined with PRESS localisation and outer volume suppression (OVS).

### 1.4 Two-Dimensional Spectroscopy

In one-dimensional (1D) MRS the signal is recorded as a function of one time variable and then Fourier transformed to give a spectrum which is a function of one frequency variable. In two-dimensional (2D) MRS the signal is recorded as a function of two time variables, $t_1$ and $t_2$. The resulting data is Fourier-transformed along both dimensions to yield a spectrum which is a function of two frequency variables $f_1$ and $f_2$. The general scheme for 2D spectroscopy is illustrated in Fig. 1.8.

In the first period, called the preparation interval, the spins are excited by one or more pulses. The resulting transverse magnetization, which can consist of single and multiple quantum coherences, is allowed to evolve for the first time period $t_1$. Then another period follows, called the mixing interval, which can contain further pulse/gradient elements. After the mixing period the signal is recorded as a function of the second time variable $t_2$. The sequence components applied in the preparation and mixing periods (RF pulses, gradients, etc.) determine the information found in the spectrum and its spectral representation. A 2D experiment consists of a row of measurements with incrementally changed $t_1$, which are stored separately. Thus recording a 2D data set involves repeating a pulse se-

![Figure 1.8: General scheme of a 2D pulse sequence.](image-url)
quence for increasing values of $t_1$ and acquiring a signal as a function of $t_2$ for each value of $t_1$.

In the NMR field, two-dimensional spectroscopy methods play a pivotal role for molecular structure investigation, since they help to identify coupling networks and measure coupling constants as well as intra-molecular distances. In medical applications, which usually suffer from low SNR and limited spectral resolution, 2D sequences are used to enhance the distinguishability of resonances by spreading them into a second dimension. One of the most popular and robust 2D method is $J$PRESS, a 2D $J$-resolved technique, which is based on PRESS localisation. For a $J$PRESS experiment the echo time is varied and encoded in the indirect dimension $t_1$. The indirect frequency domain $f_1$ contains only coupling, but no chemical shift information as the direct dimension $f_2$. 
Chapter 2

Pitfalls in Lactate Measurements at 3 T

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2.1 Introduction

Lactate is a metabolite that plays a pivotal role in many brain pathologies such as tumors, stroke, cerebral ischemia, hypoxia and several mitochondrial disorders. The lactate concentration in healthy brain under normal conditions is about 1 mM. However, when oxygen availability is low due to metabolic stress, glucose molecules are no longer oxidized completely and pyruvate is produced. The pyruvate is converted to lactate, which can rise to concentrations above 10 mM. Lactate is therefore an important marker of anaerobic glycolysis taking place in the previously mentioned brain disorders.

The lactate molecule has two weakly coupled resonances in $^1$H magnetic resonance spectroscopy (MRS): a doublet (split by coupling to the methine (CH) proton) at 1.33 ppm arising from three magnetically equivalent methyl (CH$_3$) protons and a quartet (split by coupling to the protons of the methyl group) at 4.11 ppm, arising from the methine proton, which is usually not visible in vivo. The scalar coupling gives rise to a phase evolution of the methyl doublet, which depends on the echo time ($TE$). For $TE = 144$ ms, the resonance shows a phase of 180° leading to a negative
in-phase doublet, whereas an echo time of 288 ms gives rise to a positive in-phase doublet. Since only in-phase resonances can be quantified, echo times of 144 ms and 288 ms are preferable for lactate detection and assignment. The coupling evolution can also be exploited for difference editing techniques increasing the sensitivity of lactate detection.\textsuperscript{2}

Localization techniques such as the double spin echo method PRESS, which is the standard localization technique used on clinical MRI systems, suffer from chemical shift displacement artifacts. Importantly, this can give rise to signal misregistration for almost all metabolites, as only the signal from one specific frequency, usually the NAA frequency, originates from the selected volume of interest. Signal from protons with different chemical shifts, e.g. from other metabolites, stem from spatially shifted volumes. Furthermore, for weakly coupled resonances, there is an additional signal cancelation due to anomalous $J$-modulation.\textsuperscript{8,9} This additional artifact and its effect on the interpretation of lactate levels in clinical spectra are discussed in this work. As the chemical shift displacement roughly scales with the square of the field strength (due in part to reduced radio frequency (RF) pulse bandwidths, as well as increased chemical shift frequency separation), a severe underestimation of lactate occurs at 3 T, when PRESS localization is used with an echo time of 144 ms. A detailed explanation of the origin of anomalous $J$-modulation can be found in the appendix.

This work shows in vitro and in vivo examples of signal cancellation for several MRI systems. A strategy is presented to quantify the lactate signal loss and thus validate the underlying theory. Finally, suggestions for parameter choices on clinical systems are given to avoid or at least diminish the problem of lactate underestimation at 3 T.

\section*{2.2 Description of the Technique and Results}

\subsection*{2.2.1 Patient Measurements}

Two patients with high-grade gliomas underwent MRS both with echo times of 144 and 288 ms, performed on Philips Intera whole-body systems (Philips Medical Systems, Best, The Netherlands) by using a send-receive head coil. One patient was measured at a field strength of 1.5 T, while the other was measured at 3 T.

Another patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) was examined on 1.5 T and 3 T systems from
2.2. DESCRIPTION OF THE TECHNIQUE AND RESULTS

A second vendor (GE Healthcare, Milwaukee, WI). Two-dimensional multi-voxel spectra were acquired at both field strengths consecutively the same day, repeated with both $TE = 144$ ms and $TE = 288$ ms. Standard PRESS localization was used with the region of interest centered over an acute occipital lesion. On this system very selective saturation (VSS) bands\(^{10}\) are placed around the volume of interest by default. Some of the data presented here were acquired on a clinical basis, but all patients gave their written informed consent prior to participating in research experiments.

In the high-grade tumor in Fig. 2.1 the spectra acquired at 1.5 T both show a prominent lactate doublet, inverted for $TE = 144$ ms and in phase with NAA for $TE = 288$ ms. The lactate peak, clearly visible as inverted doublet at $TE = 144$ ms, is of greater area and amplitude at $TE = 144$ ms than at $TE = 288$ ms. This signal change reflects the known $T_2$ related signal attenuation with increasing echo times.

Comparable experiments were carried out on a 3 T system, scanning another patient with a glioma (grade III). While for $TE = 288$ ms a large lactate peak is observable at 1.33 ppm, the lactate resonance has completely vanished for $TE = 144$ ms due to anomalous $J$-modulation (Fig. 2.2).

The third patient example (Fig. 2.3), carried out on different systems, shows lactate peaks of similar relative amplitude with $TE = 144$ ms and $TE = 288$ ms at 1.5 T. The lactate signal loss, which is expected with $TE = 288$ ms compared to $TE = 144$ ms due to $T_2$ relaxation, is approximately compensated by the signal loss due to anomalous $J$-modulation with $TE = 144$ ms. At 3 T, however, anomalous $J$-modulation gives rise to a complete disappearance of the lactate peak.

2.2.2 Phantom Measurements

A standard brain metabolite phantom containing 5 mM lactate was measured on three 3T MRI scanners from three different vendors (GE Healthcare, Philips Medical Systems and Siemens Medical Solutions). Single-voxel MRS (volume of interest (VOI) size = $2 \times 2 \times 2$ cm\(^3\), PRESS localization) was acquired from the same volume once with $TE = 144$ ms and once with $TE = 288$ ms. The lactate peak seen at 1.33 ppm for $TE = 288$ ms would be expected to be smaller than the inverted doublet for $TE = 144$ ms due to $T_2$ relaxation. However, at 3 T, spectra from all three MR systems show significantly larger lactate resonances at $TE = 288$ ms than at $TE = 144$ ms. For one system the lactate peak has even
Figure 2.1: Single-voxel spectra acquired at 1.5 T from the brain of a patient (a) with a high-grade glioma by using PRESS localization: b) TE = 144 ms, c) TE = 288 ms. Cho indicates choline; Lac, lactate; NAA, N-acetylaspartate.
2.2. DESCRIPTION OF THE TECHNIQUE AND RESULTS

Figure 2.2: Single-voxel spectra acquired at 3 T from the brain of a patient (a) with a grade III glioma by using PRESS localization: b) $TE = 144$ ms, c) $TE = 288$ ms. Cr indicates creatine.
Figure 2.3: Multi-voxel spectra acquired one hour apart at 1.5 T and 3 T from the same region in the brain of a patient with MELAS, by using standard PRESS localization with $TE = 144$ ms and $TE = 288$ ms. An inverted lactate doublet is clearly visible at 1.5 T, but not at 3 T (arrows). Upright lactate peaks at $TE = 288$ ms are seen equally well at both field strengths (arrows).
Figure 2.4: Single-voxel proton spectra acquired from a standard brain metabolite phantom containing 5 mM lactate. The measurements were performed on three 3 T MR scanners from three different vendors with $TE = 288$ ms (upper row) and $TE = 144$ ms (lower row). RF pulse bandwidths for the selective refocusing pulses vary between vendors in the range 874-2300 Hz.

disappeared completely. This demonstrates that a significant amount of the lactate signal is lost (not visible) at $TE = 144$ ms (Fig. 2.4).

To quantify the signal loss due to anomalous $J$-modulation on the basis of the phantom spectra acquired for $TE = 144$ ms and $TE = 288$ ms, one would have to determine the $T_2$ relaxation constant of lactate first. Since the relaxation constant of lactate for the used brain metabolite phantom was unknown and difficult to determine with high precision, a different approach was chosen: Spectroscopic Imaging (SI) offers the possibility to dispense with spatially selective refocusing pulses, since the SI slice can be selected only by the excitation pulse and the spatial encoding within
the slice is achieved with phase encoding only. However, in a standard SI measurement protocol without PRESS localization, both the excitation pulse and the refocusing pulse, which is needed for the echo formation, are slice-selective on some scanners, leading again to some signal loss. To estimate the amount of signal loss due to anomalous J-modulation, SI data sets were acquired once with (“standard” SI sequence) and once without the slice selection gradient during the refocusing pulse. Theory predicts that the overall signal loss at $TE = 144$ ms is the same for a standard SI sequence as for a single-voxel protocol using PRESS (Appendix). It can also be calculated that no signal loss should occur for lactate in a standard SI measurement with $TE = 288$ ms. Therefore, to validate the theoretical predictions experimentally, SI data sets were acquired for these two echo times. Postprocessing of the spectra included exponential filtering of the time domain signal, cosine filtering in k-space and $B_0$ correction.

Fig. 2.5 shows the results of the SI measurements without PRESS. For echo times of 144 ms and 288 ms one representative SI voxel is shown (acquired once with (Figs 2.5a, 2.5c) and once without a refocusing pulse gradient (Figs 2.5b, 2.5d), respectively). Integrating the modulus spectra and comparing the results with and without anomalous J-modulation yielded a signal loss of 72.2 % for $TE = 144$ ms, whereas the signal intensity was approximately the same for $TE = 288$ ms. Theoretical calculations taking into account the chemical shift difference of the coupled nuclei and the bandwidth of the used refocusing pulses yield a relative signal loss of 81.2 % for $TE = 144$ ms, whereas for $TE = 288$ ms no signal loss due to anomalous J-modulation is predicted (Appendix). Thus the measured signal loss is consistent with the theoretically expected value. It should be noted that, in contrast to SI without PRESS, theory also predicts a small signal loss of 6.8 % at $TE = 288$ ms when using PRESS localization for single-voxel experiments. But this loss only needs to be taken into account when high precision quantification is desired.

2.3 Discussion

Detection of lactate using MRS plays an important clinical role in the assessment of a number of brain abnormalities, including tumor, stroke and mitochondrial disorders. The presence of lactate in the context of a tumor can be considered diagnostic for glioblastoma multiforme. Lactate is also elevated as a consequence of mitochondrial abnormalities in neurodegen-
Figure 2.5: Spectra from an SI dataset acquired at 3 T from a phantom containing 10 mM NAA and 20 mM lactate without PRESS localization: a) $TE = 144$ ms, with refocusing pulse gradient, b) $TE = 144$ ms, without refocusing pulse gradient, c) $TE = 288$ ms, with refocusing pulse gradient, d) $TE = 288$ ms, without refocusing pulse gradient.
Pitfalls in Lactate Measurements at 3 T

operative disorders, such as Huntington’s disease. Since for very short echo times there are often residual fat peaks visible in the spectral region of the lactate doublet, one has to resort to echo times near multiples of $1/J$ ($J$ being the coupling constant) for proper lactate detection and quantification. As an inverted doublet can be discriminated more easily against other resonances like lipids, PRESS with $TE = 144$ ms is often considered the most appropriate method for unambiguous lactate detection at 1.5 T. Although the effect of “anomalous J-modulation” and the potential signal loss for lactate at $TE = 144$ ms has been discussed in the literature,\textsuperscript{8,9} little attention has been given to this phenomenon in clinical routine so far. Neglecting this effect can potentially lead to a severe misinterpretation in clinical diagnosis as shown in our examples. The extent of the signal loss due to anomalous $J$-modulation can vary considerably depending on the field strength, the used coil and the sequence parameters. A practical recommendation for clinical MRS at 3 T is to perform a phantom study at $TE = 144$ ms and $TE = 288$ ms; if the lactate signal at $TE = 144$ ms is less than that seen at $TE = 288$ ms, it is recommended not to use $TE = 144$ ms, but rather only $TE = 288$ ms in clinical examinations, although in general the sensitivity decreases with longer echo times due to $T_2$ relaxation. However it should be noted that the quantitative influence of anomalous $J$-modulation can be different in specific in vivo examples, where inhomogeneous metabolite distributions can either aggravate or attenuate the effect compared to in vitro experiments.

Strategies to prevent or alleviate the signal loss due to anomalous $J$-modulation are discussed elsewhere in detail,\textsuperscript{8,9,11} but usually require changes in the scanner software. An approach implemented on several clinical scanners is to saturate the region of spin-selective refocusing with outer volume suppression (OVS) pulses prior to excitation. For example, on some scanners, quadratic phase suppression pulses have been implemented for this purpose,\textsuperscript{10} but they suppress the effect only partially, since they are not specifically tuned for lactate detection as can be seen both from in vivo and phantom examples in this work. However, at higher field strengths than 3 T, which have now become available for human studies as well, the chemical shift displacement will be so pronounced that this work-around will be less effective.

Another approach for high-field systems is using pulses with much larger bandwidths such as adiabatic pulses to alleviate the chemical shift displacement. However, since ordinary adiabatic pulses are usually not
spatially selective, they cannot be used in PRESS sequences. Localization by adiabatic selective refocusing (LASER)\textsuperscript{12} or single-shot adiabatic localized volume excitation (SADLOVE)\textsuperscript{13} applies pairs of large-bandwidth adiabatic full passage pulses for volume selection and echo formation at the same time and will probably be the method of choice for localization in high-field spectroscopy applications in the future. A further popular localization technique is STimulated Echo Acquisition Mode (STEAM), which uses three 90° pulses giving rise to a stimulated echo. Since 90° pulses have much larger bandwidths than 180° pulses, the chemical shift displacement is far less severe. However, for STEAM the signal intensity of coupled resonances not only varies with $T_E$, but also shows a strong modulation governed by the mixing time between the second and third pulse.\textsuperscript{7} Furthermore, SI offers the possibility to dispense with spatially selective refocusing pulses and, therefore, prevent the signal cancellation. However, SI protocols without PRESS localization are usually not implemented on purely clinical scanners and very good OVS is required for this approach to prevent the spectra from being impaired by subcutaneous fat signal.

2.4 Appendix

The signal cancellation by anomalous $J$-modulation for coupled resonances arises from the chemical shift displacement artifact. The relative voxel displacement for two protons with chemical shifts $\delta_1$ and $\delta_2$ equals the ratio of the chemical shift difference ($\Delta \omega_{CS} = \delta_1 - \delta_2$) and the bandwidth of the RF pulse ($\Delta \omega_{RF}$) used for volume selection. For the signal of the methyl (CH$_3$) resonance of lactate at 1.33 ppm, the signal loss can be understood as follows: Due to this chemical shift displacement, the volume selected by one single refocusing pulse decomposes into a region where both the methyl and the methine (CH) protons of the lactate molecule are affected by the pulse (“non-selective” pulse) and a region where only the methyl spin is inverted (“selective” pulse) and therefore the coupling evolution is refocused. This leads to a superposition of signal with different phases giving rise to signal cancellation in the spectrum. The volume selected by a PRESS sequence, which uses two refocusing pulses, consists of four partial volumes with different phase evolutions, depending on whether none, one or both of the two refocusing pulses are spin-selective (Fig. 2.6). In a PRESS sequence the signal loss is also determined by the sequence timing,
Figure 2.6: Partial volumes and their coupling evolution for a single-voxel PRESS experiment: The 90° excitation pulse is applied with a gradient in the z direction, whereas the two refocusing pulses are applied with gradients in the x and y direction, respectively. The size of the partial volumes is determined by the chemical shift displacement. The signal phase of the magnetization is determined by $T_E$, the scalar coupling constant ($J$) of lactate and the time interval ($t_1$) between the excitation pulse and the first refocusing pulse. Since the spins are inverted twice by the two spatially selective refocusing pulses, by adding the signal terms for the four partial volumes, the resulting signal loss can be calculated. This effect termed “anomalous $J$-modulation” is particularly pronounced for $TE = 144$ ms, when the two superimposed signals from the partial volumes $V_1$ and $V_4$ have a phase difference of 180° and therefore the cancellation is most effective, whereas for $TE = 288$ ms almost no cancellation occurs (Fig. 2.6).

According to theory, the relative signal loss for lactate due to anomalous $J$-modulation at $TE = 144$ ms amounts to

$$\frac{\Delta S}{S} = 2\frac{\Delta \omega_{CS}}{\Delta \omega_{RF}},$$

(2.1)
where $\Delta \omega_{CS}$ is the chemical shift difference of the coupled nuclei and $\Delta \omega_{RF}$ is the bandwidth of the refocusing pulse. At 3 T the chemical shift difference between the methyl and the methine proton is 355 Hz, and the refocusing pulse used in this example had a bandwidth of 874 Hz. This predicts a relative signal loss of 81.2 % due to anomalous $J$-modulation for $TE = 144$ ms. The theoretical signal loss for single-voxel PRESS measurements at $TE = 288$ ms depends on the position of the two refocusing pulses within the sequence. Usually the PRESS sequence is rendered as asymmetric as possible with the first refocusing pulse being irradiated as soon as possible (at time $t_1$) after the excitation pulse. For the standard PRESS sequence implemented on a 3T Philips Intera Scanner theory predicts the following signal loss due to anomalous $J$-modulation at $TE = 288$ ms:

$$\frac{\Delta S}{S} = 2 \frac{\Delta \omega_{CS}}{\Delta \omega_{RF}} \cdot \left(1 - \frac{\Delta \omega_{CS}}{\Delta \omega_{RF}}\right) \cdot (1 - \cos (\pi \cdot J \cdot 2t_1)) = 6.8\%.$$  \hspace{1cm} (2.2)

For an SI sequence without PRESS localization, but with a slice-selective refocusing pulse for the echo formation, there are only two interfering partial volumes (corresponding to $V_1$ and $V_4$ in Fig. 2.6). For $TE = 144$ ms, this leads to the same relative signal loss as for a single-voxel PRESS sequence, whereas no signal loss at all occurs for $TE = 288$ ms.

To understand the basic behavior of the lactate resonances in a PRESS sequence, it is sufficient to approximate the used pulses as ideal hard pulses. However, pulse imperfections give rise to a far more complicated evolution behavior, which was investigated analytically, simulated numerically and discussed in detail in several publications.\textsuperscript{15–19} Since the chemical shift is proportional to $B_0$ and, due to $B_1$ limitations, the pulse bandwidths are approximately inversely proportional to $B_0$, the chemical shift displacement and therewith the lactate signal loss due to anomalous $J$-modulation at $TE = 144$ ms roughly scale with the square of the field strength.
Chapter 3

Improved Two-Dimensional J-Resolved Spectroscopy

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3.1 Introduction

The main objective of in vivo proton MRS is the determination of individual metabolite concentrations. This can be a difficult task owing to generally complex and overcrowded spectra. A powerful and versatile approach to increase specificity is multi-dimensional spectroscopy, where indirect dimensions are encoded by varying the lengths of evolution periods. These sequences greatly dominate the in vitro NMR field, yielding a wealth of information such as connectivities [COSY21] or molecular distances [NOESY22]. However, the application of multi-dimensional spectroscopy in vivo is unpopular for several reasons. Time, hardware and other constraints only permit the application of the most basic multi-dimensional sequences such as J-resolved spectroscopy23,24 or COSY.25 Additionally, post-processing techniques are not as advanced as regular 1D spectroscopy, that is, no appropriate fitting procedures exist for extracting the maximum information from the 2D spectra.
Two-dimensional $J$-resolved spectroscopy\textsuperscript{26} is one of the simplest 2D sequences and well suited for in vivo applications. It consists of a series of spin-echo experiments with different echo times encoding the indirect $t_1$ dimension. The equivalent in vivo MRS sequence is dubbed $J$PRESS\textsuperscript{24} since it employs point-resolved spectroscopy (PRESS)\textsuperscript{27} for volume localisation. $J$PRESS provides an additional encoding of the $J$ coupling, hence improving specificity in detecting $J$-coupled metabolites and alleviating a major limitation of 1D MRS, the spectral overlap of resonances.

However, $J$PRESS has been applied for in vivo MRS in only a few studies. A recent review of the application to small animals is given by Méric et al.\textsuperscript{28} $J$PRESS has been applied to the human brain\textsuperscript{24,29–36} muscle\textsuperscript{23,37} prostate\textsuperscript{38,39} and breast.\textsuperscript{40} The method is highly robust to large water and lipid signals (e.g. in the breast), hence permitting spectroscopy without water suppression.\textsuperscript{31,38} Interesting variants of $J$PRESS include a weighted accumulation scheme for filtering the indirect dimension already during acquisition,\textsuperscript{41} the combination with chemical shift imaging and spiral sampling\textsuperscript{42} and the constant-time version, termed CT-PRESS\textsuperscript{43} or CSSF,\textsuperscript{44} where the chemical shift (CS) is encoded in the indirect dimension. The sensitivity and robustness of traditional $J$-resolved spectroscopy can be improved by a maximum-echo sampling scheme,\textsuperscript{45} which was applied in the human brain at 1.5 T.\textsuperscript{41} In maximum-echo sampling, the acquisition starts immediately after the final crusher gradient of the last refocusing pulse. This leads to both an increase in sensitivity and a tilt of the peak tails in the spectra. The experiment with the maximum-echo sampling scheme was further modified to acquire multiple echoes, all sampled as long as possible, in rat brain at 7 T.\textsuperscript{46}

$J$PRESS spectra are typically quantified in two different ways. The first one is the spectral integration of metabolite peaks directly in two dimensions, which is the most common method in in vitro NMR. As the line-shapes of 2D peaks in the real spectrum are phase-twisted, the magnitude spectrum is usually preferred for integration. This results in a considerable loss of information and in an increased overlap due to broad dispersion tails of magnitude peaks.\textsuperscript{4} The other possibility is the application of 1D MRS techniques to cross-sections through the 2D spectra. However, this excludes information from the spectral analysis. In the cross-section at $t_1 = 0$ Hz, also called $TE$-averaged PRESS,\textsuperscript{35} most of the signal from $J$-coupled metabolites is lost. When taking another cross-section at the corresponding $J$ frequency,\textsuperscript{32,33,36} one loses the signal from other peaks.
of the same metabolite. This can be seen, for instance, in γ-aminobutyric acid (GABA), which has 11 peaks (i.e. two triplets and one quintet). By taking the cross-section, only one peak remains for quantification, hence losing a large amount of information. Furthermore, the overlap with the predominant singlets can still be considerable, as in case of GABA with creatine at 3 ppm, leading to poor experimental reproducibility.

In this work, several reconstruction issues are discussed, along with strategies for post-processing. The specific benefits of the maximum-echo sampling scheme for JPRESS\textsuperscript{45} are elucidated, which are mainly an increase in sensitivity and a decrease in spectral overlap. The sensitivity of JPRESS is analytically compared with that of PRESS. The qualitative behaviour of eddy currents occurring in this type of experiment is assessed and an efficient 2D eddy current correction scheme based on the 1D phase-deconvolution method\textsuperscript{48} is proposed. Furthermore, it is possible to devise a realistic model for two-dimensional fitting\textsuperscript{49} with the knowledge of the qualitative behaviour of eddy currents.

3.2 Theory and Methods

Localised two-dimensional $J$-resolved spectroscopy (JPRESS) is a simple spin-echo experiment with different echo times encoding the $J$ coupling in the indirect $t_1$ dimension. The echo top is used as reference point for the reconstruction along $t_1$ so that no chemical shift (CS) evolution is present in that dimension. The $J$ evolution is not influenced by the $180^\circ$ pulses and is hence resolved along $t_1$. The acquisition is encoded along the direct $t_2$ dimension, which contains both CS and $J$ evolution. After a Fourier transformation of the data in two dimensions, a spectrum is obtained with its resonances aligned on the horizontal (0 Hz) axis. $J$-coupled spin systems are split up into multiplets tilted by $45^\circ$, as depicted for GABA in Fig. 3.1. The preceding considerations hold strictly only in the weak coupling limit. Strongly coupled spin systems give rise to additional resonances at the mean chemical shift frequency.\textsuperscript{4,24}

The JPRESS implementation used for this work is derived from the regular, asymmetric PRESS sequence\textsuperscript{27} (Fig. 3.2). The first echo time ($TE_1$) is always chosen as short as possible and only the second refocusing pulse is shifted for encoding $t_1$. The sampling of the echo signals starts immediately after the final crusher gradient of the last $180^\circ$ pulse,\textsuperscript{45} henceforth called maximum-echo sampling. This acquisition scheme has several
Figure 3.1: In vitro spectrum of GABA plotted in magnitude mode (top) and phased complex mode (bottom). Two triplets are visible at 3.01 and 2.28 ppm and one quintet at 1.89 ppm. The CS evolution is only effective in the direct $f_2$ dimension. One multiplet is therefore located at one CS frequency along $f_2$. J coupling is effective in both $f_1$ and $f_2$, hence the resonances are split up into multiplets tilted by 45$^\circ$. The minimum echo time was 31 ms, hence the multiplets already exhibit some anti-phase evolution (bottom). Almost all structure is lost in the magnitude spectrum (top) due to the broad dispersion tails. (100 mM GABA; 2 Hz Gaussian line broadening in both $t_1$ and $t_2$)
advantages over the traditional half-echo sampling, where the acquisition starts at the echo top. Highly beneficial, as described later, is the resulting tilt of the peak tails. Furthermore, the sensitivity is increased by acquiring more signal. This gain in sensitivity is calculated analytically in the Appendix by integrating over the damping curves in the time domain, which corresponds to the peak height in the frequency domain. The noise is the same for experiments of the same duration and repetition time. The sensitivity can be compared by calculating the ratio of peak heights. This is analytically derived in the Appendix for shortest echo-time PRESS and JPRESS with two different sampling schemes (i.e. maximum and half echo).

The integral of a resonance line is proportional to the first point of the FID. This applies also to the two-dimensional case; the point in the echo signal responsible for the integral under the spectrum is the centre of the reference, as seen in the middle of Fig. 3.3(a) and (b). This point is not affected by the sampling scheme and therefore the areas underneath the resonances remain constant. The reconstruction of JPRESS spectra from the maximum-echo data is straightforward. Its reference points are the minimum echo time $TE_{\text{min}}$ and the echo top of the $t_1$ and $t_2$ dimension, respectively. $TE_{\text{min}}$ is identical with the minimum echo time of the
Figure 3.3: Traditional half-echo sampling (a and c) in comparison with maximum-echo sampling (b and d). The acquisition of the echo starts either at the echo top (a) or as soon as possible (b). The tilt of the truncation line for the acquisition begin (b) translates directly into a tilt of the peak tails away from $f_2$-axis (d). In the traditional sampling, both tails are rectangular to each other. The reference point of the 2D Fourier transformation is shifted to the middle in both domains. The echo signals are additionally zero-padded to twice the number of rows in $t_1$. 
corresponding half echo PRESS sequence and governed mainly by the duration of the RF pulses and gradients. For every time increment $\Delta TE$, the maximum-echo sampling starts the acquisition $\Delta TE/2$ earlier with respect to the echo top. Therefore, the different rows have to be time-shifted to the same reference point (i.e. the echo top) in the time domain. This can be performed flexibly in the frequency domain by multiplication with the linear phase

$$\tau(t_1, f_2) = \frac{1}{2} t_1 f_2 \quad (3.1)$$

where $t_1 = TE - TE_{\text{min}}$ is the time in the indirect dimension (Fig. 3.3) and $f_2$ is the frequency in the direct dimension. For this time shift, the data is Fourier transformed only along the direct $t_2$ dimension. A $J$PRESS spectrum is obtained after Fourier-transforming the time-shifted signal also along the indirect $t_1$ dimension.

In the shifted $J$PRESS time domain data, the echo truncation line is therefore tilted by $\arctan(1/2) \approx 26.6^\circ$ against the vertical $t_1$-axis (Fig. 3.3). This truncation line directly translates into the frequency domain, where the peak tails along $f_2$ are tilted by the same angle against the horizontal $f_2$-axis (Fig. 3.3). The angle is independent of the sampling frequencies in $f_1$ and $f_2$. However, the $f_1$ dimension should be oversampled sufficiently in order to prevent folding of the residual water peak tail back into the spectral region of interest. These additional acquisitions are usually free of charge, because oversampling amounts to averaging; and a main limitation for the detection of $J$-coupled metabolites is the sensitivity.

Nuisance gradients, caused mainly by eddy currents, lead to a lineshape distortion. Knowledge of the qualitative behaviour of these gradients is important for devising correction strategies. Eddy currents are space and time dependent and induced by switching the gradients of the MR system. They decay in a multi-exponential fashion, leading to additional multi-exponential gradients. A specific voxel at a single echo time always shows the same decay, as long as the experimental settings are not altered by any intermediate preparations. To correct PRESS spectra, it therefore suffices to measure the reference signal with an identical experiment, but omitting the water suppression. The phase of the echo in the time domain is ideally constant (for water being on-resonant) and any deviation reflects the additional nuisance gradient. Symmetric and undistorted lineshapes can be restored by subtracting the phase of the reference signal from the
data under analysis, which amounts to a phase deconvolution.\textsuperscript{48}

The eddy currents in a 2D JPRESS experiment are induced mainly by the last pair of crusher gradients. Acquisition in maximum-echo sampling always starts at the same relative time after these gradients. Hence its decay behaviour is similar for all echo times, despite the shifted echo top. This important finding was verified by phantom measurements shown in Fig. 3.4 and can be used to devise an eddy current correction scheme. A single 1D spectrum, typically from the shortest echo time, has to be acquired without water suppression. The phase of this 1D reference signal can be subtracted from the phase of each echo of the actual experiment in the time domain. Alternatively, this qualitative behaviour may serve as a physically realistic model of the gradient distortion in fitting routines. The most feasible model for the phase distortions in JPRESS was a biexponential decay with fixed decay times.\textsuperscript{49}

Phase-sensitive JPRESS spectra exhibit phase-twisted 2D lineshapes arising from the product of two complex signals. Each domain contains absorption (A) and dispersion (D) components. The real part of the (phased) spectrum is given by

\[
Re\{(A_1 + iD_1)(A_2 + iD_2)\} = A_1A_2 - D_1D_2. \quad (3.2)
\]

Phase-twisted lineshapes are inherent to this kind of experiment owing to phase modulation in \(t_1\).\textsuperscript{4,7} However, the cross-section through the centre of the peak still exhibits a pure (1D) absorption lineshape and can be processed in complex mode. The 2D lineshape is further complicated by the tilt of the peak tails due to the maximum-echo sampling scheme (Fig. 3.3), resulting in a reduced peak tail of the Voigt lineshape. Moreover, the \(t_1\) dimension starts at the minimum echo time and hence some \(J\) evolution has already taken place. The 2D peaks of \(J\)-coupled metabolites therefore exhibit a small anti-phase component (Fig. 3.1, bottom). Nevertheless, these effects are deterministic and do not hamper a quantification by fitting. The advantages of quantifying the real part of the spectrum (i.e. less overlap) greatly exceed its disadvantages (i.e. distorted lineshapes with negative components).

All experiments were performed on a Philips Intera 3T whole-body scanner (Philips Medical Systems, Best, The Netherlands) equipped with a transmit/receive head coil. The echo times ranged from 31 to 229 ms in steps of \(\Delta TE = 2\) ms and the bandwidths in \(f_1\) and \(f_2\) were 0.5 and 2 kHz with 100 and 2048 sampling points, respectively. Four-step phase cycling
3.2. THEORY AND METHODS

Figure 3.4: Effect of eddy currents on the echo signal, depicted here by plotting all echo times in lines with different colours/shades. The upper, middle and bottom rows show the magnitude, the original phase and the corrected phase of the echoes, respectively. The echo is acquired in a phantom (15 mM Cr) without water suppression and the original phase should be ideally zero. Any phase distortion is due to experimental imperfections, attributed to mainly eddy currents. Different echo times always give rise to the same phase distortion (middle) despite the shifted echo top (upper plot). Therefore, it is sufficient to acquire a reference spectrum for a single echo time and subtract its phase from the phase of all signals acquired with other echo times (bottom).
for each echo and a repetition time of $TR = 2.5\ \text{s}$ amount to a total scan duration of 17 min. Water was suppressed by selective excitation in conjunction with gradient spoiling. Twenty-seven healthy volunteers (age $35.4 \pm 7.5$ years; 20 female, 7 male), who provided written informed consent, were scanned in the parietal lobe. The voxel was $2.5\ \text{cm} \times 2.5\ \text{cm}\times 2.5\ \text{cm}$ in size, consisting mainly of grey matter, but some contamination with white matter was unavoidable. Quantification was performed with the two-dimensional fitting procedure ProFit. Another exemplary $J$PRESS spectrum was acquired in a transplanted kidney (age 39 years; female) with the same parameters as used for the brain.

### 3.3 Results and Discussion

The maximum-echo sampling scheme has several advantages. Highly beneficial for the robustness of the experiment is the tilt of the peak tails, hence improving robustness of the standard PRESS sequence, while retaining its ease of application. A typical $J$PRESS spectrum of a healthy volunteer scanned in the parietal lobe is shown in Fig. 3.5. The contamination from poorly suppressed water is greatly reduced. In the traditional half-echo sampling, the spectra are sometimes contaminated, hence impairing quantification. However, the maximum-echo sampled spectrum often allows accurate quantification. This effect can be also utilised for spectra containing a large amount of lipids, as shown in a transplanted kidney in Fig. 3.6. The glutamate and glutamine resonance is separated from lipids, hence greatly reducing contamination.

In maximum-echo sampling, most of the signal is acquired, hence increasing sensitivity. An analytical sensitivity comparison is given in the Appendix. Some actual examples for 1D PRESS and 2D $J$PRESS with typical values are given in Table 3.1. The maximum-echo sampling generally leads to a considerable gain in sensitivity as compared with half-echo $J$PRESS. For longer $T_2$ relaxation times, the sensitivity between PRESS and maximum-echo sampled $J$PRESS is approximately the same. It is important, however, to restrict the sampling time along $t_1$ for metabolites with short $T_2$s. The $J$ evolution has to be sufficiently resolved, hence the maximum echo time of 229 ms is a reasonable choice. The sensitivity calculations (Appendix) considered only non-coupled spins. Although $J$ coupling can be included, this is not particularly helpful and intuitive as it must be done individually for each metabolite. Most brain metabolites
3.3. RESULTS AND DISCUSSION

Figure 3.5: Typical in vivo JPRESS spectrum plotted with contour lines on logarithmic colour scale. Only the real part of the phased spectrum is shown, hence also giving rise to negative components. The data were zero-filled to 512 samples in the indirect dimension and apodised with a Gaussian filter with 2 Hz width in both $t_1$ and $t_2$. Directly visible are the predominant singlets from N-acetylaspartate (NAA), total creatine (Cr) and choline-containing compounds (Cho), but also some of the J-coupled metabolites, namely glutathione (GSH). More information is still present in this spectrum, which can be extracted with advanced post-processing methods such as two-dimensional fitting.
Figure 3.6: J PRESS spectrum of a transplanted kidney. The assignment of resonances is based on Dixon and Frahm. The lipids and the partially suppressed water are tilted away from the spectral region of interest, hence contamination is greatly reduced. The short $T_2$ relaxation time is visible by broad linewidths in both $f_1$ and $f_2$.

<table>
<thead>
<tr>
<th>$T_2$ (ms)</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-TE PRESS</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Maximum-echo J PRESS</td>
<td>62</td>
<td>82</td>
<td>95</td>
<td>104</td>
</tr>
<tr>
<td>Half-echo J PRESS</td>
<td>43</td>
<td>55</td>
<td>63</td>
<td>69</td>
</tr>
</tbody>
</table>

Table 3.1: Comparison of sensitivity (%) between 1D PRESS and 2D J PRESS with both maximum-echo and half-echo sampling for $T_2^* = 50$ ms (corresponding to a line-width of 6.4 Hz) and the actual $t_1$ sampling range of $T_{S1} = 200$ ms. The sensitivity of the PRESS experiment is scaled to 100 % for each $T_2$ time. The maximum-echo sampling scheme is highly beneficial for gaining sensitivity in 2D experiments.
Figure 3.7: Zero-Hertz cross-section through JPRESS spectrum of water with (dashed) and without (solid) eddy current correction. Symmetry is increased, hence increasing also the peak height.
are strongly coupled at typical field strengths of $B \leq 3$ T, hence impeding analytical calculations. However, comparing the experiments provides some insight. The $J$ evolution depends in both PRESS and JPRESS on the minimal echo time. A PRESS spectrum can be reconstructed from JPRESS by projection onto the $f_2$ axis. This can lead to signal loss when anti-phase terms are summed. Regular 1D PRESS always suffers from these destructive interferences and therefore performing a 2D experiment, where the $J$ evolution is encoded, is advantageous. The minimum echo time of 31 ms already leads to anti-phase components. However, such a long minimum $TE$ is state of the art on whole-body 3 T scanners, owing to low transmitting RF field strengths and hence long pulses.

The scan duration is closely related to the sensitivity. The relatively long scan time of 17 min was mainly chosen to increase sensitivity and can be readily shortened by decreasing the phase-cycling steps or the amount of oversampling in the indirect dimension. Another favourable option for the sensitivity would be the reception with surface coils. However, this was not considered here, because of the reduced bandwidths of the RF pulses when transmitting with the body coil instead of the transmit/receive head coil. As a consequence, the chemical shift displacement is large, giving rise to localisation errors and anomalous $J$ evolution.\textsuperscript{9}

The effect of the eddy current correction is clearly visible in the in vitro experiments without water suppression (Figs. 3.4 and 3.7). The strength of the last pair of crusher gradients is increased from 12 to 20 mT/m in order to provoke eddy currents. The reference signal should be measured in the same scan without intermediate preparations. However, this was impossible in the current implementation and the separately measured reference scan showed slightly different eddy current effects compared with the actual measurement. In general, it is difficult to see the spectral distortions from eddy currents in the in vivo spectra, because of linewidths of typically 6-9 Hz. Moreover, these distortions are small owing to highly optimised gradient systems.

The acquired data can also be reconstructed differently, for instance as CSSF.\textsuperscript{44} Other options are JPRESS with different $J$ evolutions, which can be achieved by moving the reference of the 2D Fourier transformation along $t_1$. For example, the medium echo time often implies more in-phase evolution for some metabolites at the cost of greatly increased truncation wiggles. Shifting the data in either domain does not alter its information content. For a subsequent fit of the spectra, the reconstruction choice is
mainly governed by ease of implementation, thus favouring JPRESS over CSSF.

### 3.4 Conclusions

The maximum-echo sampling scheme increases sensitivity, while at the same time further improving the specificity of the JPRESS experiment. In addition to the description of the qualitative behaviour of the eddy currents in 2D JPRESS, this is the basis for a successful and accurate quantification of the data using the 2D fitting procedure ProFit.\(^{49}\)

### 3.5 Appendix: Sensitivity Comparison

A common definition of the sensitivity is the signal-to-noise (SNR) ratio per unit time.\(^4\) For identical experimental durations and a constant acquisition time window, the noise will remain the same and it is sufficient to compare the signal. The signal is commonly defined as the peak height in the frequency domain, which is equivalent to the integral of the time domain signal. For singlets, it suffices to integrate over the exponential damping curve of the echo along the times \(t_1\) and \(t_2\). The digitisation does not fundamentally change the signal and therefore it suffices to take the continuous integral. The 1D PRESS experiment can be compared to 2D experiments simply by integrating over the indirect \(t_1\) axis with always the same intensity. The signal from PRESS is therefore given by

\[
\int_0^{T_{S1}} \int_0^\infty \exp \left( -\frac{t_2}{T_2^*} \right) \, dt_2 \, dt_1 = T_2^* T_{S1}, \quad (3.3)
\]

where \(T_{S1}\) is the sampling time along \(t_1\). The PRESS sequence with the shortest possible echo time is used for an equitable comparison. The echo time of this PRESS sequence is also equivalent to the shortest echo time of JPRESS.

The signal of JPRESS can be split up into two halves. The damping curves in the traditional half-echo sampling is equivalent to the right half and given by

\[
\int_0^{T_{S1}} \int_0^\infty \exp \left( -\frac{t_2}{T_2^*} \right) \cdot \exp \left( -\frac{t_1}{T_2} \right) \, dt_2 \, dt_1 = T_2^* T_2 \left( 1 - \exp \left( -\frac{T_{S1}}{T_2} \right) \right). \quad (3.4)
\]
The left half of the maximum-echo signal can be calculated by

\[ \int_{-t_1/2}^{0} \int_{0}^{T_S} \exp \left( \frac{t_2}{T_2^*} \right) \cdot \exp \left( -\frac{t_1}{T_2} \right) dt_2 dt_1 = T_2^* T_2 \left( 1 - \exp \left( -\frac{T_{S1}}{T_2} \right) \right) - T_2^* t_x \left( 1 - \exp \left( -\frac{T_{S1}}{t_x} \right) \right), \quad (3.5) \]

where \( t_x = \frac{2T_2 T_2^*}{T_2 + 2T_2^*} \) is introduced for notational convenience. Adding both halves together, yields the total signal of the maximum-echo sampled JPRESS

\[ 2T_2^* T_2 \left( 1 - \exp \left( -\frac{T_{S1}}{T_2} \right) \right) - T_2^* t_x \left( 1 - \exp \left( -\frac{T_{S1}}{t_x} \right) \right). \quad (3.6) \]

These formulae can now be used to compare the various experiments with each other.

The ratio maximum-echo JPRESS (Eqn. 3.6) to PRESS (Eqn. 3.3) is given by

\[ \frac{2T_2}{T_{S1}} \left( 1 - \exp \left( -\frac{T_{S1}}{T_2} \right) \right) - \frac{t_x}{T_{S1}} \left( 1 - \exp \left( -\frac{T_{S1}}{t_x} \right) \right), \quad (3.7) \]

and half-echo JPRESS (Eqn. 3.4) to PRESS (Eqn. 3.3) by

\[ \frac{T_2}{T_{S1}} \left( 1 - \exp \left( -\frac{T_{S1}}{T_2} \right) \right). \quad (3.8) \]

Examples with typical values are given in Table 3.1.
Chapter 4

Prostate Spectroscopy at 3 Tesla Using Two-Dimensional S-PRESS

(*published in: Magnetic Resonance in Medicine*\textsuperscript{51})*

4.1 Introduction

Because of its non-invasive nature, magnetic resonance imaging (MRI) has proven to be an indispensable method for diagnosing many pathologies. However, the morphological and relaxometric information provided by MRI is not sufficient for unequivocally diagnosing certain tumor types. In such cases MR spectroscopy (MRS) can provide valuable complementary metabolic information.\textsuperscript{52} For the diagnosis of prostate cancer, the metabolites choline (Cho) and citrate (Cit) are of particular interest.\textsuperscript{52,53} Cit is secreted in relatively large amounts by the epithelial cells of the prostate. High levels of zinc inhibit the oxidation of Cit in the Krebs cycle.\textsuperscript{54} However, in cancerous epithelial tissue the zinc concentration is considerably reduced, resulting in lower net Cit levels. On the other hand, the Cho concentration is increased due to the rapid cell turnover in
tumorous tissue. Therefore, the Cho-to-Cit concentration ratio can help to discriminate between malignant adenocarcinoma and benign hyperplasia.\textsuperscript{53,55,56}

The preferred method for localized prostate \textsuperscript{1}H MRS is the double spin-echo sequence called point-resolved spectroscopy (PRESS).\textsuperscript{27} Because of its high signal yield (e.g., compared to stimulated-echo acquisition mode (STEAM)\textsuperscript{57}), it is the method of choice, especially when surface coils are used for signal reception.

While the detection and quantification of the uncoupled Cho resonance is straightforward, Cit represents a strongly coupled AB spin system at typical field strengths available for in vivo MRS ($B_0 \leq 7$ T) and is therefore a challenge for spectroscopists. The strong coupling gives rise to a complicated $J$-modulation of the Cit resonance that critically depends on the sequence timing. Several groups have investigated the signal behavior of Cit and optimized the PRESS sequence to yield maximum signal intensity and a line-shape appropriate for Cit quantification.\textsuperscript{1,58–63}

Expanding PRESS to a second dimension has been shown to be useful for disentangling overcrowded spectra. A powerful method that enables the separation of chemical shift and coupling information for $J$-coupled metabolites is 2D $J$-resolved PRESS ($J$PRESS).\textsuperscript{24} PRESS spectra are acquired with varying echo times, which encode the indirect $t_1$ dimension. The application of $J$PRESS in prostate examinations enables the spectral separation of creatine (Cr) and Cho from spermine (Spm), which has a similar resonance frequency.\textsuperscript{38,39} Kim et al.\textsuperscript{64} combined $J$PRESS with spectroscopic imaging (SI) to identify the region of abnormal metabolism due to prostate cancer more accurately. The line-widths in the indirect dimension of $J$PRESS spectra are governed only by the $T_2$ decay and not by field inhomogeneities as in 1D spectra, where $T_2^*$ determines the line-widths. Thus a better spectral resolution of coupled resonances is achieved. The possibility of determining $T_2$ values from 2D $J$-resolved spectra is an additional benefit.

However, strong scalar coupling leads to additional peaks in 2D spectra, which can complicate peak assignment and quantification.\textsuperscript{65} While weakly coupled two-spin systems give rise to four peaks in a $J$PRESS experiment, the Cit molecule shows a spectral pattern with eight peaks. The additional peaks stem from the coherence transfer between the coupled spins. Thrippleton et al.\textsuperscript{65} suggested several methods for suppressing these strong coupling peaks in $J$PRESS experiments. One approach is a
multi-scan technique that employs an incremental simultaneous displacement of the two refocusing pulses within the sequence, leaving the total echo time \((TE)\) unchanged. Gambarota et al.\(^{66,67}\) proposed a difference editing method based on the strong-coupling point-resolved spectroscopy (S-PRESS) sequence. It uses PRESS localization \((90^\circ_x - [TE_1/2] - 180^\circ_y - [TE_1/2 + TE_2/2] - 180^\circ_y - [TE_2/2] - Acq)\) and consists of two measurements with the same overall \(TE\) \((TE = TE_1 + TE_2 = \text{const})\), but with different \(TE_1\) and \(TE_2\). When the two resulting spectra are subtracted from each other, uncoupled and weakly coupled resonances are suppressed, while the strong coupling peaks remain. At a field strength of 3 T, density matrix simulations predicted a maximum \(J\)-modulation of Cit for a \(TE\) of 280 ms and helped to determine the optimal \(TE_1\) values for difference editing.

In this work we combine the strengths of 2D spin-echo spectroscopy and the S-PRESS approach by implementing S-PRESS as a 2D technique. The method was analytically optimized for prostate MRS and validated in vitro as well as in vivo. 2D S-PRESS was used to determine the spectral parameters of Cit \((J\) and \(\delta)\) in vivo with relatively high precision.

## 4.2 Theory

The \(^1\)H MR signal of Cit arises from two magnetically equivalent methylene groups (CH\(_2\)) that constitute strongly coupled AB spin systems at currently available field strengths for in vivo MRS \((B_0 \leq 7\, \text{T})\). In 1D PRESS the Cit resonance consists of four lines (Fig. 4.1) centered around 2.6 ppm. While the separation between the two leftmost and the two rightmost lines equals the field-independent coupling constant \((J = 16.1\, \text{Hz})\), the distance between the two inner lines depends on the magnetic field strength, due to the linear field dependence of the chemical shift difference between the A and B spins \((\delta = 0.149\, \text{ppm})\).\(^{68}\) As a characteristic feature of strongly coupled spin systems, the two inner lines show higher signal intensity than the two outer lines. Furthermore, the strong coupling causes a complicated amplitude and phase modulation of the Cit resonance as a function of \(TE\). This behavior cannot be easily understood in the classic sense, and requires a quantum mechanical description. The Hamiltonian for an AB spin system can be written as

\[
H = (\omega_A A_z + \omega_B B_z) + 2\pi J(A_x B_x + A_y B_y + A_z B_z) \quad (4.1)
\]
Figure 4.1: Plot of the citrate signal as obtained in a pulse-acquire experiment at 3 T. J denotes the strength of the scalar coupling in Hz and \( \Lambda = \sqrt{\delta^2 + J^2} \) the strong coupling frequency, where \( \delta \) is the difference in Larmor frequencies between the two coupled protons. The total intensity is normalized to one.

where \( A_{x,y,z} \) and \( B_{x,y,z} \) are the angular momentum operators in Cartesian coordinates, \( \omega_A \) and \( \omega_B \) are the chemical shifts, and \( J \) is the coupling constant of the two coupled spins A and B. The nonsecular contribution \( A_x B_x + A_y B_y \) is characteristic of strongly coupled spin systems and is usually neglected in the limit of weak scalar coupling. Writing this expression in terms of raising and lowering operators yields \( \frac{1}{2} \cdot (A_+ B_+ + A_- B_-) \), indicating that there is a coherence transfer between spins A and B due to strong coupling. By analytically calculating the density matrix evolution with this Hamiltonian or applying the Kay-Mc-Clung formalism, the signal resulting from a PRESS sequence can be expressed in terms of four groups of Cartesian single-quantum coherence product operators \{\( (A_y + B_y) \), \( (2A_x B_z + 2A_z B_x) \), \( (A_x - B_x) \), \( (2A_y B_z - 2A_z B_y) \}\).\(^1\) The amplitudes of the detectable in-phase components \( M_y \) and \( M_x \) are:

\[
\langle M_y \rangle = \langle A_y \rangle = \langle B_y \rangle = -\cos(\pi J \cdot TE) \cdot [(\delta/\Lambda)^2 + (J/\Lambda)^2 \cos(\pi \Lambda \cdot TE)] \\
- \sin(\pi J \cdot TE) \cdot [(J/\Lambda)^3 \sin(\pi \Lambda \cdot TE)] \\
+ (2J\delta^2/\Lambda^3) \sin(\frac{\pi}{2} \Lambda \cdot TE) \cos(\frac{\pi}{2} \Lambda \cdot (TE_1 - TE_2)) \quad (4.2)
\]
\[
\langle M_x \rangle = \langle A_x \rangle = -\langle B_x \rangle \\
= -\cos(\pi J \cdot TE) \cdot \left[ (J^2\delta/\Lambda^3)^2 (2\sin(\frac{\pi}{2}\Lambda \cdot TE) \\
\cdot \cos(\frac{\pi}{2}\Lambda \cdot (TE_1 - TE_2)) - \sin(\pi\Lambda \cdot TE)) \right] \\
- \sin(\pi J \cdot TE) \cdot \left[ (2J\delta/\Lambda^2) \sin(\frac{\pi}{2}\Lambda \cdot TE) \\
\cdot \sin(\frac{\pi}{2}\Lambda \cdot (TE_1 - TE_2)) \right]
\] (4.3)

Most amplitude terms only show a dependence on the total \(TE\), but are independent of the particular choice of \(TE_1\) and \(TE_2\). However, the amplitude expressions also contain summands depending on the difference \(TE_1 - TE_2\). These terms are characteristic for strongly coupled spin systems and vanish in the weak-coupling limit.

A 2D S-PRESS experiment consists of multiple PRESS measurements with a constant \(TE\) but a simultaneous incremental variation of \(TE_1\) and \(TE_2\). When \(TE_1\) is encoded in the indirect dimension \((t_1)\) and a 2D Fourier transformation is applied to the data, the resulting 2D spectrum displays the strong coupling information resolved in the indirect spectral dimension \((f_1)\). Strongly coupled spin systems reveal a line-splitting in \(f_1\), whereas uncoupled and weakly coupled metabolites resonate only at \(f_1 = 0\) Hz. For AB spin systems, these strong coupling peaks arise from the terms depending on \(TE_1 - TE_2\) in Eqs. (4.2, 4.3) and appear at frequencies \(f_1 = \pm\Lambda/2\), where \(\Lambda = \sqrt{\delta^2 + J^2}\). Since all acquired echo signals have undergone the same \(T_2\) decay and are aligned along their echo tops in \(t_1\), the spectral resolution in the indirect dimension of a 2D S-PRESS spectrum is limited only by the \(TE_1\) encoding range, and not by field inhomogeneities or \(T_2\) relaxation. The signal intensity of the strong coupling S-PRESS peaks at \(f_1 = \pm\Lambda/2\) shows a modulation with \(TE\). It can be calculated as a function of \(TE\) from the amplitude terms given in Eqs. (4.2, 4.3), as shown in the Appendix. The strong coupling peaks even vanish for certain \(TEs\) \((TE = \{n \cdot 2/\Lambda, n\epsilon N\})\). This has to be taken into account when setting up a 2D S-PRESS experiment.

While the trigonometric factors in the amplitude terms (Eqs. (4.2, 4.3)) can be adjusted and optimized via \(TE\), the polynomial factors depend only on the coupling strength \(\kappa = J/\delta\) of the AB spin system, which can be
shown with basic substitutions:

\[ P_{1,x} = 2J^2\delta/\Lambda^3 = \frac{2 \cdot \kappa^2}{(1 + \kappa^2)^{1.5}} \quad (4.4) \]

\[ P_{2,x} = 2J\delta/\Lambda^2 = \frac{2 \cdot \kappa}{1 + \kappa^2} \quad (4.5) \]

\[ P_{1,y} = 2J\delta^2/\Lambda^3 = \frac{2 \cdot \kappa^2}{(1 + \kappa^2)^{1.5}} \quad (4.6) \]

Plotting these polynomial factors (Fig. 4.2) shows that they reach maxima for \( \kappa_{\text{max}} = \sqrt{2} \), \( \kappa_{\text{max}} = 1 \) and \( \kappa_{\text{max}} = 1/\sqrt{2} \), respectively. This means that the highest signal yield for S-PRESS experiments is achieved for spin systems with \( \kappa_{\text{max}} = J/\delta \approx 1 \), which is the case for Cit at a field strength of 3 T.

The spectral parameters of Cit (\( J \) and \( \delta \)) sensitively depend on the chemical environment. Van der Graaf et al.\(^{68}\) showed in phantom experiments that \( J \) and \( \delta \) increase with the concentration of divalent cations, such as \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \), and \( \text{Zn}^{2+} \). They also reported a strong dependence on the pH value. This raises the question as to whether there is a detectable difference in \( J \) and \( \delta \) between cancerous and healthy tissues. The \( \text{Zn}^{2+} \) concentration plays a particularly important role in the pathophysiology of adenocarcinoma. It is largely reduced in cancerous tissue and may therefore be useful as a marker for prostate cancer. Prostate spectroscopy usually exploits the fact that zinc ions prevent Cit from being oxidized. A reduced \( \text{Zn}^{2+} \) concentration is accompanied by a diminished Cit level, which is detected in MRS experiments. Therefore, we carried out a phantom experiment to investigate whether this zinc decrease could be detected on the basis of changes in \( J \) and \( \delta \).

### 4.3 Materials and Methods

All experiments were performed on a Philips Achieva 3T system (Philips Medical Systems, Best, The Netherlands). Phantom experiments were carried out with a transmit/receive head coil. All in vivo spectra were acquired with a two-element ellipsoidal surface coil (semi-major axis = 17 cm, semi-minor axis = 14 cm), and the body coil was used for transmission.
$P_{1,x}(\kappa) = \frac{2 \cdot \kappa^2}{(1 + \kappa^2)^{1.5}}$

$\kappa_{\text{max}} = \sqrt{2}$

$P_{2,x}(\kappa) = \frac{2 \cdot \kappa}{1 + \kappa^2}$

$\kappa_{\text{max}} = 1$

$P_{1,y}(\kappa) = \frac{2 \cdot \kappa}{(1 + \kappa^2)^{1.5}}$

$\kappa_{\text{max}} = \frac{1}{\sqrt{2}}$

Figure 4.2: Polynomial amplitude factors of the strong coupling terms in Eqs. (4.2, 4.3) as functions of the coupling strength $\kappa = J/\delta$: a) $P_{1,x} = 2J^2\delta/\Lambda^3$, b) $P_{2,x} = 2J\delta/\Lambda^2$, c) $P_{1,y} = 2J^2/\Lambda^3$. 
Both 2D S-PRESS and JPRESS measurements are based on the PRESS sequence:

\[ 90^\circ_x - [TE_1/2] - 180^\circ_y - [TE_1/2 + TE_2/2] - 180^\circ_y - [TE_2/2] - Acq \] (4.7)

The acquisition employed a maximum-echo sampling scheme with 1024 sampling points covering a bandwidth of 2000 Hz. For both 2D S-PRESS and JPRESS experiments the measurement started with the manufacturer’s default PRESS implementation, in which \( TE_1 \) is as short as possible. In the 2D S-PRESS experiments \( TE_1 \) and \( TE_2 \) were varied simultaneously to keep the total \( TE \) constant. \( TE_1 \) was encoded in the indirect \( t_1 \) dimension. For the JPRESS experiments, starting from the shortest possible \( TE \), only \( TE_2 \) and hence the total \( TE \) were varied and encoded in the indirect \( t_1 \) dimension.

The water signal was suppressed with selective excitation in conjunction with gradient spoiling prior to the PRESS sequence. Band-selective inversion with gradient dephasing (BASING) further improved the water suppression for the 2D S-PRESS measurements. The combination of these two techniques suppressed the water resonance almost completely, and thus prevented folding artifacts from large water peak tails. Fat suppression was omitted because careful voxel selection ensured that no periprostatic fat was included. The \( TE \)s for the 2D S-PRESS measurements were longer than 200 ms and therefore were much longer than typical \( T_2 \) values of macromolecules.

In vitro measurements were carried out on a phantom solution containing 15 mM Cho, 30 mM lactate (Lac, weakly coupled), and 50 mM Cit (strongly coupled) to demonstrate the different behavior of weakly and strongly coupled spin systems. A 2D S-PRESS spectrum was acquired at \( TE = 438 \) ms using 20 \( TE_1 \) encoding steps with a spacing of \( \Delta TE_1 = 20.86 \) ms, yielding a nominal spectral resolution \( \Delta f_1 = 2.4 \) Hz in the indirect dimension. For comparison a JPRESS spectrum was acquired with the same nominal resolution in both \( f_1 \) and \( f_2 \).

In vivo spectra were acquired from the prostates of healthy subjects, who provided written informed consent prior to participating in the study. The voxel (14 mm × 27 mm × 14 mm = 5.3 ml) was positioned in the center of the prostate. A repetition time (\( TR \)) of 1.2 s was chosen because the \( T_1 \) relaxation time of Cit is quite short (≈ 470 ms) and a high sensitivity for Cit detection was desired. Forty equidistant \( TE_1 \) encoding steps in the range of 32.54 to (\( TE - 21.12 \)) ms with 16 spectral averages each
and a 16-step phase-cycling scheme were used for the 2D S-PRESS experiments, amounting to an overall scan time of 13 min. Two-dimensional S-PRESS spectra were acquired for TEs of 235 ms, 282 ms, 313 ms, and 353 ms with nominal spectral resolutions of 5.4 Hz, 4.3 Hz, 3.8 Hz, and 3.2 Hz, respectively, in the indirect dimension. These TEs were chosen on the basis of a pilot measurement that suggested intensity maxima (TE = 282 ms and TE = 353 ms) and complete cancellation (TE = 235 ms and TE = 313 ms) of the strong coupling peaks for certain TEs. For comparison a JPRESS spectrum was acquired using 80 encoding steps (TE range: 53.67 - 282 ms), eight spectral averages, and eight phase cycles. This amounts to the same overall scan time. The nominal spectral resolution in the indirect dimension (4.3 Hz) was chosen to be the same as for the S-PRESS experiment with TE = 282 ms. For the JPRESS acquisition, BASING water suppression was omitted to avoid a restriction to long TEs and an associated loss of sensitivity. Considerable oversampling in the indirect dimension was necessary to prevent impairment of the 2D spectra by back-folded peak tails of the residual water signal. Three in vivo 2D S-PRESS spectra from the same subject, acquired with different TEs and in different scan sessions, were used to optimize the measurement protocol with respect to the TE. The coupling constant J and the chemical shift difference δ of the two coupled protons were determined from the Cit peak positions in the four spectra and averaged. Using these values, we optimized the 2D S-PRESS sequence for Cit detection by calculating the peak intensity dependence on the TE under in vivo conditions (see Appendix). Furthermore, the inter-subject variability of J and δ was determined by 2D S-PRESS experiments (TE = 280 ms) on 11 healthy subjects (21-45 years old).

To investigate whether a zinc decrease in the prostate due to prostate cancer could be detected on the basis of changes in J and δ, we prepared two phantom solutions with approximate in vivo metabolite concentrations as found in literature (Table 4.1), with and without zinc ions. In two 2D S-PRESS experiments, the spectral parameters J and δ were determined for both phantoms.

Postprocessing of all acquired data was performed with in-house-written MATLAB code (The MathWorks, Inc.) and included zero-filling to 512 samples in t_1 and 2048 samples in t_2, a 2D Fourier transformation of the acquired data, and 1.5 Hz Gaussian filtering in both f_1 and f_2. All spectra in this paper are presented in magnitude mode as logarithmically scaled.
Table 4.1: Literature values for metabolite concentrations in the healthy prostate.\(^{68,70,73}\) A solution with these concentrations was used to investigate the influence of the Zn\(^{2+}\) concentration on the spectral parameters of citrate under in vivo conditions.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration [mM]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit</td>
<td>90</td>
<td>68</td>
</tr>
<tr>
<td>Cho</td>
<td>9</td>
<td>73</td>
</tr>
<tr>
<td>Cr</td>
<td>12</td>
<td>73</td>
</tr>
<tr>
<td>Lac</td>
<td>10.3</td>
<td>70</td>
</tr>
<tr>
<td>Spm</td>
<td>18</td>
<td>73</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>145</td>
<td>68</td>
</tr>
<tr>
<td>K(^+)</td>
<td>61</td>
<td>68</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>18</td>
<td>68</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>14.7</td>
<td>68</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>8.5</td>
<td>68</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>47.4</td>
<td>68</td>
</tr>
</tbody>
</table>

4.4 Results

The results of the in vitro experiments are presented in Fig. 4.3. The JPRESS spectrum (a) shows a simple splitting of the Lac resonance (two peaks separated by \(J \approx 7\) Hz in \(f_1\)) and the typical multiplet of eight peaks for Cit. Substituting \(TE_2\) by \(TE - TE_1\) in the analytical terms of Eqs. (4.2, 4.3), expanding the trigonometric expressions and collecting contributions with a \(TE\) dependence yields resonances at \(f_1 = \{\pm J/2, \pm \Lambda/2 \pm J/2, \pm \Lambda/2 \mp J/2\}\) and thus confirms the results from numerical simulations.\(^{39}\) In the 2D S-PRESS spectrum (b), the Lac resonance does not show a line-splitting along \(f_1\). The Cit signal consists of the typical quartet at \(f_1 = 0\) Hz, which is also observed in 1D PRESS, and two doublets at \(f_1 = \pm \Lambda/2\) (henceforth called “strong coupling peaks”). The resonance frequencies of these peaks in \(f_2\) correspond to those observed in 1D PRESS spectra. The spectral parameters of Cit were determined from the positions of the strong coupling peaks in \(f_1\) and \(f_2\): \(J = 15.13\) Hz,
Figure 4.3: JPRESS (a) and 2D S-PRESS (b) magnitude spectra (acquired at 3 T from a phantom solution containing 15 mM choline (Cho), 30 mM lactate (Lac) and 50 mM citrate (Cit)). The JPRESS experiment contained 80 $\text{TE}$ encoding steps in the range $[41.58 \text{ ms}, 438 \text{ ms}]$. The 2D-SPRESS experiment ($\text{TE} = 438 \text{ ms}$) contained 20 $\text{TE}_1$ encoding steps in the range $[25.96 \text{ ms}, 422.40 \text{ ms}]$. The citrate multiplets with peak assignments are shown in zoomed sections (right graphs).
Figure 4.4: JPRESS (a) and 2D S-PRESS (b) magnitude spectra acquired at 3 T from the prostate of a healthy subject. The JPRESS experiment contained 80 TE encoding steps in the range [53.67 ms, 282 ms]. The 2D-SPRESS experiment (TE = 282 ms) contained 40 $T_E$ encoding steps in the range [32.54 ms, 260.36 ms]. Detected metabolites are: choline (Cho), citrate (Cit), creatine (Cr), lipids (Lip), spermine (Spm).

$\delta = 14.34$ Hz, $\Lambda = 20.84$ Hz.

Figure 4.4 shows an in vivo JPRESS spectrum (a) with $TE$ ranging from 53.67 to 282 ms and a 2D S-PRESS spectrum (b) with $TE = 282$ ms. Both spectra have a nominal spectral resolution of 4.3 Hz in the $f_1$ dimension. In both spectra, Cho, Cr, and Cit resonances are visible. Additionally, the JPRESS spectrum shows a Spm multiplet around 3.1 ppm and lipid (Lip) resonances around 1.3 ppm. Because of their short $T_2$ relaxation time, those metabolites are not visible in the 2D S-PRESS spectrum, which does not contain short-$TE$ data. The multiplet pattern of Cit is similar to the in vitro case (Fig. 4.3). Only the relative signal intensities of the JPRESS peaks are different. The following in vivo Cit parameters were determined as averaged values from this and two other 2D S-PRESS spectra acquired from the same subject: $J = (15.77 \pm 0.20)$ Hz, $\delta = (19.94 \pm 0.40)$ Hz, $\Lambda = (25.42 \pm 0.66)$ Hz. Figure 4.5 shows two cross sections through the 2D S-PRESS spectrum at the frequencies $f_1 = 0$ Hz and $f_1 = -12.7$ Hz. The cross section at $f_1 = 0$ Hz shows four reso-
4.4. RESULTS

Figure 4.5: Cross section through the 2D S-PRESS magnitude spectrum in Fig. 4.4b at $f_1 = 0$ Hz (a) and $f_1 = -12.7$ Hz (b).

nance lines and looks similar to a usual PRESS spectrum acquired with the same $TE$. In the cross section at $f_1 = -\Lambda/2 = -12.7$ Hz, only a Cit doublet is visible at the two leftmost Cit resonance frequencies in $f_2$. These two peaks are separated by $\Delta f_2 = J$. The intensity modulation of the strong coupling peaks was determined according to Eq. (4.14) with the measured in vivo parameters $J$ and $\delta$. The intensity graph (Fig. 4.6) shows zero-crossings for $TE = \{n \cdot 2\pi/\Lambda, n\in\mathbb{N}\}$. We verified the accuracy of the determined spectral parameters by acquiring additional 2D S-PRESS spectra with $TE$s of 235 ms, 313 ms, and 353 ms (Fig. 4.7). For $TE = 235$ ms and $TE = 313$ ms, the strong coupling peaks of Cit are expected to have zero intensity, and indeed they vanished at these specific $TE$s. The maxima of the intensity graph in Fig. 4.6 are preferable $TE$s for 2D S-PRESS in the human prostate. The $TE$ should be large enough to yield the required spectral resolution in $f_1$ and short enough to avoid too much signal loss due to $T_2$ relaxation. Therefore, $TE = 278$ ms and $TE = 353$ ms are most appropriate for S-PRESS prostate spectroscopy.

The spectral parameters of Cit ($J$ and $\delta$) for 11 healthy subjects are listed in Table 4.2. They yield mean values of $J = (15.99 \pm 0.49)$ Hz and $\delta = (19.99 \pm 0.70)$ Hz. The inter-subject standard deviation (SD) is slightly larger than the SD due to different voxel positions and measurement imperfections only.

The 2D S-PRESS experiments with the two prostate phantom solutions yielded the following spectral parameters:
Figure 4.6: In-vivo signal intensity of the strong coupling S-PRESS peaks at $f_1 = \pm \Lambda/2\pi$ as a function of TE, neglecting $T_2$ relaxation. The echo times, for which 2D S-PRESS spectra were acquired, are indicated with dotted lines: $TE = 235\text{ ms}, 282\text{ ms}, 313\text{ ms}, 353\text{ ms}$ (see Figs. 4.4b and 4.7a-c).
Figure 4.7: 2D S-PRESS magnitude spectra acquired at 3 T from the prostate of a healthy subject for echo times a) 235 ms, b) 313 ms, c) 353 ms. The strong coupling peaks of citrate vanish for $TE = 235$ ms and $TE = 313$ ms.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>$J$ [Hz]</th>
<th>$\delta$ [Hz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>15.77</td>
<td>19.94</td>
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<td>11</td>
<td>44</td>
<td>15.90</td>
<td>20.58</td>
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mean: $27.7 \pm 8.6$, $15.99 \pm 0.49$, $19.99 \pm 0.70$

Table 4.2: Spectral parameters of citrate ($J$ and $\delta$) for 11 healthy subjects aged between 21 and 45, determined with 2D S-PRESS spectroscopy.

1. Phantom solution with zinc ions (8.5 mM, corresponding to healthy tissue): $J = 15.97$ Hz, $\delta = 18.62$ Hz.

2. Phantom solution without zinc ions (corresponding to cancerous tissue): $J = 15.84$ Hz, $\delta = 18.63$ Hz.

4.5 Discussion

Two-dimensional S-PRESS facilitates the unequivocal detection and quantification of strongly coupled metabolites. The combination of 2D spectroscopy techniques with PRESS localization is an obvious and promising approach since no additional pulses or gradients are required and the technique is fairly robust. Spreading the spectral information into a second dimension helps to disentangle overcrowded spectra and improve the resolution of coupled resonances. JPRESS has been established as a useful method for detecting $J$-coupled metabolites because it helps to distinguish coupled from uncoupled resonances. Furthermore, it provides better spectral resolution in the indirect dimension, where the line-widths are
governed only by $T_2$ decay and not by field inhomogeneities. The application of a maximum-echo sampling scheme\textsuperscript{20} tilts the peak tail of the residual water resonance away from the $f_2$ axis and prevents them from contaminating the spectral region of interest (ROI).

However, $J$PRESS spectra can be relatively complex, especially when strongly coupled spins are involved, giving rise to additional peaks in the spectrum. This also hampers the determination of exact peak positions in $f_1$ (Fig. 4.4a). For this reason Thrippleton et al.\textsuperscript{65} devised several strategies to suppress these strong coupling peaks in $J$PRESS and correlation spectroscopy (COSY) experiments. The 2D S-PRESS method represents a complementary approach that enables strongly coupled metabolites to be identified on the basis of their strong coupling peaks. In the case of an AB spin system, the 2D S-PRESS sequence gives rise to four strong coupling peaks of equal intensity at one characteristic frequency: $f_1 = \pm \Lambda/2$. Thus, strongly coupled metabolites can easily be distinguished from uncoupled and weakly coupled ones, which resonate only at $f_1 = 0$ Hz. A cross section through the 2D S-PRESS spectrum at $f_1 = \pm \Lambda/2$ can be used for visual inspection and quantification of the Cit resonance (Fig. 4.5b). This particular choice of a cross section is equivalent to the S-PRESS difference editing approach presented by Gambarota et al.\textsuperscript{66,67} A cross section at $f_1 = 0$ Hz, as presented in Fig. 4.5a, corresponds to the integral over the 2D time domain data along $t_1$ with a subsequent 1D Fourier transformation. This is analogous to a $TE$-averaged spectrum,\textsuperscript{35} which can be retrieved from a $J$PRESS spectrum as a cross section at $f_1 = 0$ Hz. Cross sections at $f_1 = \pm \Lambda/2$ can be reconstructed by multiplying the 2D time domain data with the linear phase factor $\exp(i\varphi)$ (with $\varphi = \pi \Lambda t_1$) prior to integration along $t_1$ and Fourier transformation. For S-PRESS difference editing, two spectra are acquired with $TE_1 = \tau_1$ and $TE_1 = \tau_2$, which are chosen such that the Cit resonances for these two particular choices of $TE_1$ have a phase difference $2\pi \cdot \Lambda/2 \cdot (\tau_1 - \tau_2) = \pi$ with respect to each other ($\Lambda/2$ being the modulation frequency).\textsuperscript{66,67} Applying the above-mentioned linear phase factor to the 2D S-PRESS data amounts to a phase difference $\Delta \varphi = \varphi_1 - \varphi_2 = \pi \Lambda (\tau_1 - \tau_2) = \pi$ for the two acquired spectra, which corresponds to a difference editing scheme when integrating over the indirect dimension (two-point Fourier transformation). Therefore, the S-PRESS spectrum shown by Gambarota et al.\textsuperscript{66,67} corresponds to a superposition of the two cross sections in the 2D S-PRESS spectrum at $f_1 = \pm \Lambda/2$.

As an additional benefit, the spectral resolution in the indirect dimen-
ision of a 2D S-PRESS spectrum is limited only by the $T_E$ encoding range, and not by field inhomogeneities or $T_2$ relaxation as in $J$PRESS. However, the truncation in the $t_1$ domain requires apodization ($\sim 1 - 2$ Hz) to prevent wiggles in the spectrum. For studies in which sensitivity is not a major concern, a sufficiently long $TE$ can be used to obtain the desired spectral resolution in $f_1$. To obtain an optimal SNR for the strong coupling peaks, the $TE$ has to be adjusted individually for every strongly coupled spin system. We found that $TE = 278$ ms and $TE = 353$ ms are preferable $TE$s for in vivo Cit detection. This result is in good agreement with Gambarota et al.,\textsuperscript{66,67} where an optimal $TE$ of 280 ms was suggested in the context of the original S-PRESS implementation as a difference editing approach.

As shown in this work, the spectral parameters $J$ and $\delta$ of strongly coupled metabolites can be determined with reasonable precision from a 2D S-PRESS spectrum. Prostate spectroscopy using a 1D PRESS sequence does not properly resolve the Cit multiplet. Even at 3 T, the two inner lines usually overlap so heavily that they appear as one peak. 2D S-PRESS enables complete and unequivocal resolution of the Cit resonances and thereby facilitates the determination of $J$ and $\delta$. The advantage of 2D S-PRESS over COSY,\textsuperscript{21} which is often used to determine in vivo coupling constants, is that spatial localization can be achieved at no extra cost. The determined spectral parameters ($J = 15.99$ Hz, $\delta = 0.157$ ppm) slightly deviate from the results obtained by van der Graaf et al.\textsuperscript{68} ($J = 16.1$ Hz, $\delta = 0.149$ ppm), who measured a phantom solution with approximate in vivo metabolite concentrations. It is particularly important to know the coupling parameters when they are susceptible to changes in the chemical environment of the molecule. A comparison of the measured in vitro and in vivo parameters of Cit revealed the following findings: In the Cit phantom, $J$ and $\delta$ were decreased by 5 % and 28 % compared to their respective in vivo values in healthy prostate tissue. Even in the prostate phantom that contained all major in vivo metabolites and ions, $\delta$ was significantly reduced compared to its value in healthy subjects. However, when the 2D S-PRESS spectra from the two prostate phantoms (with and without zinc ions) were compared, there was no significant difference in $\delta$ and only a marginal difference (0.13 Hz = 0.8 %) in $J$, which would not be detectable under in vivo conditions. The experiments carried out by van der Graaf et al.\textsuperscript{68} on a phantom solution containing 90 mM Cit and 8.5 mM zinc chloride yielded much larger differences (2.4 % in $J$ and
5.5 % in $\delta$), which may be detectable even under in vivo conditions considering the estimates for the intra-individual error margins found in this work (1.3 % for $J$ and 2.0 % for $\delta$). This discrepancy reflects the fact that large concentrations of magnesium (14.7 mM) and calcium (18 mM) ions reduce the influence of zinc ions. The spectral parameters appear to depend on the total number of divalent ions, which undergoes only a small relative change, even when the concentration of zinc ions drops dramatically. Therefore the coupling evolution of Cit does not change significantly under pathological conditions. This is an important point to note because a different coupling evolution in pathological and healthy tissue might impair quantitative studies in which the Cho-to-Cit ratio in different parts of the prostate is compared (e.g., in an SI experiment) and used for clinical diagnosis. $J$ and $\delta$ may also be influenced by the concentration of Spm molecules, which have opposite polarity with respect to the Cit molecules. It correlates with the Cit concentration and is therefore also decreased in cancerous tissue. Apart from that, $J$ and $\delta$ depend on the Cit concentration itself because a reduced Cit concentration enhances the influence of the divalent ions. However, the reduced Cit and Spm concentrations under pathological conditions were not taken into account for the prostate phantom. The pH dependence of the spectral parameters was not considered either, because it is primarily adjusted through the concentration of Cit and divalent ions. Knowledge of the spectral parameters and the exact quantum-mechanical evolution is of great importance for the quantification of coupled metabolites. LCModel has been established as the most objective and robust method for metabolite quantification in recent years. It models the measured spectrum as a linear combination of single metabolite basis spectra, which can either be acquired in phantom experiments or simulated. This makes LCModel particularly appropriate for fitting metabolites with complicated coupling networks, for which no compact analytical description of the coupling evolution under a PRESS sequence is available. It is difficult to exactly match in vivo conditions in a phantom experiment, and therefore the coupling evolution of metabolites, such as Cit, in a phantom solution can differ from the evolution in vivo. Since this results in quantification errors, simulating the basis spectra may be a better approach for coupled resonances, such as those of Cit. For a correct simulation, however, the exact spectral parameters $J$ and $\delta$ must be known. A 2D S-PRESS spectrum delivers these parameters at no extra cost. Instead of fitting and quantifying cross sections through the 2D
S-PRESS spectrum with LCModel, a 2D fitting method, such as ProFit, can be directly applied to the 2D S-PRESS spectrum to include all prior knowledge.

An alternative method for detecting strongly coupled spin systems was presented by Trabesinger et al., who suggested spectral editing with a single quantum coherence filter for taurine detection. This technique exploits the fact that detectable single quantum coherences are created through the coherence transfer between the strongly coupled spins, while for uncoupled and weakly coupled spin systems only even coherence orders are produced, which are not directly observable in the acquisition. However, spectral editing techniques based on coherence pathway selection usually have an inherent signal loss of 50%. On the other hand, 2D S-PRESS requires longer TE than common coherence filtering techniques and is therefore not the method of choice for large metabolites with very short $T_2$ relaxation times.

Since prostate cancer usually occurs in the peripheral zone of the prostate, single-voxel measurements, as performed in this work, are not appropriate for clinical diagnosis. However, one could increase the SNR considerably by using an endorectal coil for signal reception instead of surface coils. This might enable a combination of 2D S-PRESS with SI in order to obtain spatial distributions of metabolites over the whole prostate. Fat suppression, which is not an issue for the single-voxel approach, could be achieved with either broadband outer volume suppression (OVS) pulses or a dual-band BASING pulse, which inverts water and fat at the same time.

Analytical considerations presented in the Theory section show that Cit is an ideal metabolite for S-PRESS. Its coupling strength ensures a near-optimal signal yield at 3 T, and the comparably simple signal structure of an AB spin system is appropriate for analytical investigations, which can be used to optimize the sequence. However, 2D S-PRESS may also turn out to be useful for spectroscopy in the brain, where several metabolites of interest (e.g., glutathione, GABA, myo-inositol) are strongly coupled. In particular, the cysteinyI group of glutathione, which has a relatively simple coupling network (ABX), may be an interesting target metabolite. Gambbarota et al. recently investigated the signal modulation of glutathione with $TE_1$ and $TE$ applying numerical and experimental methods. However, a compact analytical description for the signal response of an ABX spin system to a PRESS sequence would be helpful for optimizing 2D
S-PRESS for glutathione detection.

4.6 Conclusions

We have shown that 2D S-PRESS is a viable alternative to the widely used JPRESS method for prostate spectroscopy. The technique may help to further establish MRS as a method for detecting metabolic changes associated with prostate cancer, and has the potential to be useful for spectroscopy in other anatomical regions as well.

4.7 Appendix

The overall signal intensity of the strong coupling peaks can be calculated as the integral over the 2D magnitude spectrum. An integration over a complex spectrum $f(\omega) = f_r(\omega) + i \cdot f_i(\omega)$ corresponds to the time domain signal $F(t) = F_r(t) + i \cdot F_i(t)$ at the reference point (zero point) of the FFT:

$$F(t) = \int f(\omega) \cdot \exp(i\omega t) \cdot d\omega \Rightarrow F(0) = \int f(\omega) \cdot d\omega. \quad (4.8)$$

However, this relation is no longer valid for the magnitude data, unless all resonances are in-phase with respect to each other. Only in this case all signal can be transferred to the real part $F_r(t)$ by rephasing the spectrum, which amounts to

$$|F(0)| = |F_r(0)| = \int |f_r(\omega)| \cdot d\omega = \int |f(\omega)| \cdot d\omega. \quad (4.9)$$

Only the strong coupling terms (depending on $TE_1 - TE_2$) in the in-phase magnetization expectation values (Eqs. (4.2, 4.3)) are taken into account for the integration ($\varphi = \pi/2 \cdot \Lambda \cdot (2TE_1 - TE)$):

$$\langle A_y^{sc}\rangle = \langle B_y^{sc}\rangle = -2J^2 \delta^2 / \Lambda^3 \cdot \sin (\pi J \cdot TE) \cdot \sin \left(\frac{\pi}{2} \Lambda \cdot TE\right) \cdot \cos (\varphi)$$

$$= F_{1,y}(TE) \cdot \cos (\varphi) \quad (4.10)$$

$$\langle A_x^{sc}\rangle = -\langle B_x^{sc}\rangle = -2J^2 \delta / \Lambda^3 \cdot \cos (\pi J \cdot TE) \cdot \sin \left(\frac{\pi}{2} \Lambda \cdot TE\right) \cdot \cos (\varphi)$$

$$-2J^2 \delta / \Lambda^2 \cdot \sin (\pi J \cdot TE) \cdot \sin \left(\frac{\pi}{2} \Lambda \cdot TE\right) \cdot \sin (\varphi)$$

$$= F_{1,x}(TE) \cdot \cos (\varphi) + F_{2,x}(TE) \cdot \sin (\varphi) \quad (4.11)$$
Because of $\langle A^sc \rangle = - \langle B^sc \rangle$ the signal intensities have to be calculated separately for the two spins. Due to the factor $\sin(\varphi)$ in the x-magnetization amplitude, the strong coupling peaks in the positive ($f_1 > 0$) and negative ($f_1 < 0$) half-plane are out of phase with respect to each other and need to be integrated separately. This requires splitting up the trigonometric factors $\cos(\varphi)$ and $\sin(\varphi)$ into exponential expressions:

$$\cos(\varphi) = \frac{1}{2} (\exp(i\varphi) + \exp(-i\varphi))$$  \hspace{1cm} (4.12)

$$\sin(\varphi) = \frac{1}{2i} (\exp(i\varphi) - \exp(-i\varphi))$$  \hspace{1cm} (4.13)

All terms with $\exp(i\varphi)$ give rise to resonances in the positive half-plane, while all terms with $\exp(-i\varphi)$ give rise to resonances in the negative half-plane. Assuming quadrature detection, the overall signal turns out to be the sum of four contributions:

$$I_{sc} = \left| \langle A^sc \rangle \big|_{f_1>0} + \langle A^sc \rangle \big|_{f_1<0} + \langle B^sc \rangle \big|_{f_1>0} + \langle B^sc \rangle \big|_{f_1<0} \right|$$

$$= \left| \frac{1}{2} (F_{1,x} - i(F_{2,x} + F_{1,y})) \cdot \exp(i\varphi) \right|$$

$$+ \left| \frac{1}{2} (F_{1,x} + i(F_{2,x} - F_{1,y})) \cdot \exp(-i\varphi) \right|$$

$$+ \left| \frac{1}{2} (-F_{1,x} + i(F_{2,x} - F_{1,y})) \cdot \exp(i\varphi) \right|$$

$$+ \left| \frac{1}{2} (-F_{1,x} - i(F_{2,x} + F_{1,y})) \cdot \exp(-i\varphi) \right|$$

$$= \sqrt{F_{1,x}^2 + (F_{2,x} + F_{1,y})^2} + \sqrt{F_{1,x}^2 + (F_{2,x} - F_{1,y})^2}. \hspace{1cm} (4.14)$$
Chapter 5

Quantitative $J$-Resolved Prostate Spectroscopy Using Two-Dimensional Prior-Knowledge Fitting

(Submitted to: Magnetic Resonance in Medicine

5.1 Introduction

Digital rectal examination, prostate-specific antigen (PSA) blood test and transrectal ultrasound (TRUS) guided biopsy are standard methods for the diagnosis and staging of prostate cancer. Over the past two decades magnetic resonance imaging (MRI) has been established as a supplementary non-invasive method, identifying pathological tissue on the basis of relaxometrical information. However, like the afore-mentioned tools, MRI lacks sensitivity as well as specificity in clinical diagnosis, especially when it comes to distinguishing prostate cancer from benign prostate hyperplasia (BPH), which is very common in older men. In particular, since the advent of endorectal receiver coils, magnetic resonance spectroscopy (MRS) has emerged as another promising method providing additional metabolic
information for a clinical diagnosis.\textsuperscript{52} Proton MR spectra of cancerous prostate tissue exhibit largely reduced citrate (Cit) levels and increased levels of choline-containing compounds (t-Cho) as compared to healthy tissue.\textsuperscript{52,53} Thus the Cit/t-Cho concentration ratio has been used as a marker for prostate cancer in a large number of clinical studies. Furthermore, ex vivo NMR studies have identified spermine (Spm) and myo-inositol (MI) as additional potential metabolic markers for prostate cancer, although their patho-physiological role is not yet well understood.\textsuperscript{38,55,73,74,80,81}

Two-dimensional (2D) spectroscopy methods at least partially resolve the spectral overlap usually observed in one-dimensional spectra and hence facilitate peak assignment and improve metabolite quantification. The feasibility of localized 2D $J$-resolved spectroscopy (JPRESS) in the human prostate with an endorectal/pelvic phased array coil system was shown in several works.\textsuperscript{38,39,64} Swanson \textit{et al.} demonstrated that five metabolites (Cit, creatine (Cr), choline (Cho), Spm and lactate (Lac)) can be observed in vivo with this method.\textsuperscript{38} Yue \textit{et al.} identified the characteristic 2D multiplet patterns of Cit and Spm in JPRESS spectra with a reasonable resolution.\textsuperscript{39} Kim \textit{et al.} combined the JPRESS method with spectroscopic imaging to identify the region of abnormal metabolism in patients with prostate cancer.\textsuperscript{64} Recently 2D S-PRESS was presented as an alternative two-dimensional method for prostate spectroscopy, particularly well suited for Cit detection.\textsuperscript{51} However, since relatively long echo times are required to obtain a reasonable spectral resolution in the indirect dimension, 2D S-PRESS is not the method of choice for the detection of metabolites with very short $T_2$ relaxation constants like Spm.

When there is a considerable overlap of resonances in a 1D spectrum, conventional quantification methods such as peak integration or simple line fitting fail to yield reliable results. Over the years, more advanced quantification methods have been developed, using time-domain as well as frequency-domain fitting procedures, which enable the inclusion of prior knowledge to fit spectra with a physically reasonable model.\textsuperscript{82,83} An objective and widely used frequency-domain fitting method is LCModel,\textsuperscript{76} which incorporates the entire prior knowledge by fitting spectra as linear combinations of basis spectra, which can be either simulated\textsuperscript{19} or acquired in phantom experiments. As an additional benefit, LCModel yields quality estimates for the determined concentration ratios in the form of Cramér-Rao lower bounds (CRLBs).\textsuperscript{84}

The vast majority of prostate MRS studies apply simple peak inte-
5.1. INTRODUCTION

gration to the postprocessed spectra for metabolite quantification. Unfortunately, there is a strong overlap of detectable metabolites in the chemical shift range between 3.0 and 3.3 ppm (Cr, t-Cho, MI, taurine (Tau) and polyamines (PA, mainly Spm)), which aggravates quantification by peak integration considerably. Therefore, the concentration ratios \(\text{Cit}/(\text{Cr}+\text{t-Cho})\) or even \(\text{Cit}/(\text{Cr}+\text{t-Cho}+\text{PA})\) were used as a patho-physiological marker for prostate cancer in many studies. Another difficulty for metabolite quantification in the prostate arises from the fact that resonances of strongly coupled metabolites (e.g. Cit, MI, Tau, PA) undergo a signal intensity modulation, which makes concentration ratios determined via peak integration echo-time dependent. This complicates the comparison of quantitative results obtained in MRS measurements at different field strengths and with different sequences. Peak fitting procedures can increase the accuracy and robustness of metabolite quantification in the prostate. Scheenen et al. applied a basic fitting algorithm assuming Gaussian line-shapes to the spectra prior to integration. Kurhanewicz et al. quantified prostate spectra by means of a modified PIQABLE algorithm, while García-Segura et al. used a fitting routine based on least-squares optimization. Alternatively spectra can be fitted in the time domain, e.g., using the AMARES algorithm. A detailed comparison of a frequency domain analysis based on peak integration and time domain fitting based on AMARES for prostate spectroscopy can be found elsewhere, suggesting that the time domain fitting method is more accurate. However, even with these 1D fitting methods metabolite quantification of in vivo prostate spectra is usually limited to Cit, Cr and t-Cho. High-resolution ex vivo studies reported a much larger number of detectable and quantifiable metabolites, also including Lac, alanine (Ala), glutamate (Glu), glutamine (Gln), scylo-inositol (Scy), MI, Tau and PA.

Since the extra information contained in 2D spectra promises greater robustness and higher quantification accuracy, several attempts have been undertaken to adapt established 1D fitting methods to two dimensions. De Beer et al. suggested a 2D Hankel singular value decomposition (HSVD) method, while Slotboom et al. developed a pseudo-2D technique based on constrained 1D fits. Recently Schulte et al. proposed a 2D fitting procedure dubbed ProFit (Prior-Knowledge Fitting), which fits JPRESS spectra as linear combinations of 2D basis spectra, thus incorporating the maximum prior knowledge available. Like LCModel, ProFit combines a
non-linear least-squares algorithm, which optimises the model parameters (chemical shifts, line widths, phases and line shapes), with a linear least-squares algorithm for the determination of the metabolite concentrations. The fitting method was validated for brain spectroscopy in the parietal and frontal lobe and proved to be more accurate than LCModel fitting of 1D spectra acquired from the same volume with equal scan time.

In this work we adapt the ProFit procedure for \textit{J}-PRESS spectroscopy in the human prostate and show that via 2D prior knowledge fitting six metabolites can be quantified with reasonable precision. The method is compared with LCModel fitting of a PRESS spectrum acquired from the same volume and validated in ten repetitive \textit{J}-PRESS measurements in the same subject as well as in a study on ten healthy subjects.

5.2 Materials and Methods

All prostate spectra were measured on a Philips Achieva 3T system (Philips Medical Systems, Best, The Netherlands) with a two-element ellipsoidal surface coil (semi-major axis = 17 cm, semi-minor axis = 14 cm), while the body coil was used for signal excitation. The 2D \textit{J}-resolved spectra were acquired with a PRESS sequence ($90^\circ \cdot [TE_1/2] - 180^\circ \cdot y - [TE_1/2 + TE_2/2] - 180^\circ \cdot y - [TE_2/2] - Acq$), where the first echo time $TE_1$ was held constant, while the second echo time $TE_2$ and therewith the total echo time $TE$ were varied for encoding the coupling information in the indirect dimension ($f_1$). The water signal was suppressed with selective excitation in conjunction with gradient spoiling prior to the PRESS sequence. A maximum-echo sampling scheme was applied, i.e., the acquisition started directly after the second refocusing pulse for each encoding step. Apart from the SNR gain, this acquisition scheme leads to a tilt of the truncation-induced peak tails in the 2D spectrum, which prevents the residual water peak from spoiling the spectral region with the metabolites of interest. $TE$ was varied in the range between 49 and 447 ms, covering 100 equidistant encoding steps with $\Delta TE = 4$ ms. This gives rise to a bandwidth of 250 Hz and a nominal spectral resolution of 2.5 Hz in $f_1$. In the direct dimension ($f_2$), 1024 samples were acquired covering a bandwidth of 2000 Hz. With an eight-step phase-cycling scheme and a repetition time $TR = 1.7$ s, this amounts to an overall scan duration of about 23 min. The voxel was placed in the middle of the prostate covering predominantly central gland tissue (Fig. 5.1). The voxel size had to be adjusted to the shape of the prostate
to exclude periprostatic lipid signal and was varied in the range from 5.3
to 5.8 ml. Postprocessing of the JPRESS spectra prior to fitting included
frequency shifts in $f_1$ and $f_2$ as well as zeroth-order phase correction.

The ProFit algorithm consists of a non-linear and a linear optimization
loop. The constrained non-linear algorithm optimizes the following global
parameters: zeroth-order phase $\varphi_0$, Gaussian line-broadening $\sigma_g$ in $f_2$, a
frequency shift $\Delta_1$ in $f_1$ and a bi-exponential phase decay $\theta(t_2)$ model-
ing line shape distortions due to eddy currents. Individual fit parameters
(specific for each metabolite $m$) in the non-linear optimization are: an
exponential line broadening $\sigma_{e,m}$ due to $T_2$ decay in $f_1$ and $f_2$ and a fre-
cquency shift $\Delta_{2,m}$ in $f_2$. The metabolite concentrations $c_m$ are determined
by linear least-squares optimization based on the Moore-Penrose pseudoin-
verse. The measured spectrum $S$ is updated with $\Delta_1$, $\varphi_0$ and $\theta(t_2)$ in each
iteration of the non-linear optimization, yielding $S_u$, while the metabolite
basis spectra $B_m$ are updated with $\Delta_{2,m}$, $\sigma_{e,m}$, $\sigma_g$, yielding $B^u_m$. For fur-
ther mathematical and algorithmical details the reader is referred to the
original ProFit publication.\textsuperscript{49} The cost function for the non-linear opti-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.1}
\caption{Experimental setup for the prostate experiments: A
single voxel (measuring approximately 16 mm $\times$ 23 mm $\times$ 16 mm)
was placed in the centre of the prostate, excluding periprostatic fat

tissue.}
\end{figure}
Quantitative \(J\)-Resolved Prostate Spectroscopy Using 2D Fitting

The cost function was optimised for the spectral region \(\{(f_1, f_2)| -28 \text{ Hz} < f_1 < 28 \text{ Hz} \wedge 2.2 \text{ ppm} < f_2 < 4.1 \text{ ppm} \wedge f_2 - 2 \cdot f_1 < 4.3 \text{ ppm}\}\), to exclude residual water and lipid signal and to reduce the numerical burden. Thus, the measured \(J\)PRESS spectrum and the basis spectra were cropped to a matrix size of \(64 \times 164\). Since the \(T_2\) relaxation constants of the individual prostate metabolites vary within one order of magnitude, the exponential line broadenings have to be regularized with metabolite-specific values \(\sigma_{e,m} = 1/\pi T_2,m\). This implies an extension to the original ProFit implementation where one single regularization constant for all metabolites was used.\(^{49}\)

The metabolite basis spectra can be either simulated or acquired from pure phantom solution. The advantage of measured basis spectra is that they account for most measurement imperfections in the spectrum under analysis. However, \(J\) coupling constants and relative chemical shifts in coupled metabolites depend on the chemical environment and can therefore be significantly different in vitro and in vivo, giving rise to a different spin evolution. Especially the coupling constant and chemical shift difference in the AB spin system of Cit exhibit a relatively strong dependence on the concentration of divalent ions.\(^{68}\) This can be particularly severe for long echo times as used in \(J\)PRESS experiments and impair quantitation with basis spectra considerably. Therefore, in this work, the basis spectra were simulated numerically with the GAMMA library,\(^ {95}\) using chemical shifts and coupling constants from literature.\(^ {51,96,97}\)

Literature suggests that the following metabolites can be detected and
quantified ex vivo from healthy prostatic tissue.\cite{52,73,90,91} Lac, Ala, Cit, Cr, PA, free choline (Cho), phosphorylcholine (PCh) + glycerophosphorylcholine (GPC), Scy, MI, Tau, Glu, Gln, leucine (Leu), acetic acid, N-acetyl neuraminic acid. However, for the sake of robustness, the fit should have a restricted number of degrees of freedom. Therefore the basis set should not be too large and contain too many similar metabolites. The resonances from acetic acid and N-acetyl neuraminic acid are too weak to be detected in vivo, while the Lac and Leu resonances lie outside the chosen fit region. Ala gives rise to a doublet at 1.48 ppm (outside the fit region) and a quartet at 3.77 ppm, which failed to yield a significant contribution in an initial ProFit test run. The PA signal contains contributions from several metabolites (mainly Spm, but also spermidine and putrescine), which give rise to very similar resonance patterns and frequencies.\cite{97} Therefore, in this work, the PA signal was only fitted with a basis spectrum from Spm. Since PCh and GPC have almost identical resonance frequencies in the spectral region around 3.2 ppm, only a PCh basis spectrum was included in the basis set. A set of ten basis metabolites turned out to be the most suitable choice for fitting JPRESS prostate spectra acquired in vivo: Cr, PCh, Cit, Spm, Cho, MI, Scy, Tau, Glu and Gln.

To enhance the efficiency and robustness of the non-linear fitting algorithm, it is iterated three times with increasing degrees of freedom. The initial iteration step only fits the dominant metabolites Cr, PCh, Cit and Spm. In the second iteration a line-shape distortion function is added, while in the final iteration the spectrum is fitted with all ten basis metabolites. The fit parameters of each iteration are initialized with the results from the previous iteration.

No absolute quantification was attempted, but metabolite concentrations were determined as ratios to Cr. Since relaxation effects were neglected in the simulation of the basis spectra the obtained metabolite concentration ratios had to be corrected for $T_1$ and $T_2$ decay:

$$
\left( \frac{c_{met}}{c_{Cr}} \right)_{corr} = \left( \frac{c_{met}}{c_{Cr}} \right)_{fit} \cdot \frac{1 - \exp \left( -TR/T_{1, Cr} \right)}{1 - \exp \left( -TR/T_{1, met} \right)} \cdot \frac{\exp \left( -TE_{min}/T_{2, Cr} \right)}{\exp \left( -TE_{min}/T_{2, met} \right)}.
$$

(5.2)

The $T_1$ and $T_2$ relaxation constants of prostate metabolites were obtained from literature.\cite{91} Since the relaxation constants for Glu and Gln have not been measured in prostate tissue yet, they were estimated to be: $T_1 =$
1200 ms, $T_2 = 600$ ms. The $T_2$ constants were also used for the regularization in the cost function (Eq. 5.1).

For comparison, measuring one subject, a short echo time PRESS spectrum was acquired from the same volume with the same total scan time and quantified using LCModel. The basis set used by LCModel contained the same metabolites as the one used by ProFit and the basis spectra were also simulated with the GAMMA library.

To investigate the reproducibility of the method, one subject was repetitively measured in different sessions over a period of several weeks. Some spectra had to be discarded due to extremely poor spectral quality, but ten data sets were fitted with ProFit and included in the statistical analysis. Furthermore, average metabolite concentration ratios in the human prostate were determined in a study on healthy subjects (between 20 and 46 years old), who gave written informed consent according to the rules of the Local Ethics Committee (EK: 09/2006 (ETH)). Again, ten data sets had a sufficient spectral quality for 2D fitting and quantification.

### 5.3 Results

Figure 5.2 shows a typical $J$PRESS prostate spectrum, the fitted spectrum and the fit residue. Besides the prominent Cit multiplet at 2.6 ppm, there is a large number of hardly distinguishable coupled and uncoupled resonances in the spectral region between 3.0 and 3.3 ppm. At 2.05 ppm, a broad resonance with a strong inter-subject amplitude fluctuation was observed. It was attributed to lipid signal from outside the prostate due to imperfect volume selection and therefore not included in the fit region. However, the human prostate is a very difficult anatomical region for spectroscopy experiments with surface coils. For some measured subjects, neither PRESS nor $J$PRESS spectra showed any metabolite peaks apart from water and fat and therefore had to be excluded from the study. This is mainly due to considerable $B_0$ and $B_1$ inhomogeneities in the prostate, which can give rise to bad shimming and compromised power optimisation. Apart from that, motion artifacts are far more severe than in other organs due to the small size of the prostate and can degrade the spectral quality considerably. The sensitivity of the method strongly depends on the build of the subject, but also the positioning of the receiver coils turned out to be extremely critical.

Figure 5.3 shows the PRESS spectrum acquired from the same volume
Figure 5.2: JPRESS prostate spectrum (top), its fit (middle) and the fit residue (bottom) as logarithmically scaled colour plots determined with ProFit. The white box defines the spectral region of interest where the cost function of the fit was minimised.
Figure 5.3: PRESS prostate spectrum fitted with LCModel. The same basis metabolites were used as for the ProFit analysis (Fig. 5.2). The bold red line indicates the fit, while the fit residue is shown above the fitted spectrum.
Table 5.1: Metabolite concentrations (as ratios to Cr) in the prostate of one healthy subject. Both JPRESS and PRESS spectra were acquired subsequently from the same volume with identical scan time and quantified with ProFit and LCModel, respectively. Fit quality measures for individual metabolites were determined in the form of CRLBs.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>JPRESS/ProFit</th>
<th>PRESS/LCModel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. [/Cr]</td>
<td>CRLB [%]</td>
</tr>
<tr>
<td>Cr</td>
<td>1.00</td>
<td>5.1</td>
</tr>
<tr>
<td>PCh</td>
<td>0.43</td>
<td>7.0</td>
</tr>
<tr>
<td>Cit</td>
<td>9.78</td>
<td>1.2</td>
</tr>
<tr>
<td>Spm</td>
<td>5.68</td>
<td>4.8</td>
</tr>
<tr>
<td>Cho</td>
<td>0.15</td>
<td>14.2</td>
</tr>
<tr>
<td>MI</td>
<td>1.94</td>
<td>8.0</td>
</tr>
<tr>
<td>Scy</td>
<td>0.16</td>
<td>14.6</td>
</tr>
<tr>
<td>Tau</td>
<td>0.56</td>
<td>24.9</td>
</tr>
<tr>
<td>Gln</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Glu</td>
<td>0.84</td>
<td>18.8</td>
</tr>
<tr>
<td>t-Cho</td>
<td>0.58</td>
<td>3.7</td>
</tr>
<tr>
<td>Glx</td>
<td>0.84</td>
<td>22.9</td>
</tr>
</tbody>
</table>

as the JPRESS spectrum (Fig. 5.2) and fitted with LCModel. Since the water suppression is poor, the baseline of the PRESS spectrum is heavily distorted, which is accounted for by LCModel through fitting with spline functions. In contrast to that, the spectral region of interest in the JPRESS spectrum is hardly affected by the poor water suppression, due to the maximum-echo sampling scheme. Table 5.1 shows the quantitative fit results obtained by ProFit and LCModel, fitting the JPRESS (Fig. 5.2) and the PRESS (Fig. 5.3) spectrum, respectively. Error margins were calculated as CRLBs from the Fisher information matrix.\(^{84}\) Due to their mutual overlap, Cho and PCh as well as Glu and Gln have large CRLBs and can therefore be more reliably quantified as sums \(\text{Glx} = \text{Glu} + \text{Gln}\) and \(\text{t-Cho} = \text{Cho} + \text{PCh}\).

The metabolite ratios turn out to be quite different (e.g. for Spm). Generally, ProFit yields much smaller CRLBs than LCModel, especially
Table 5.2: Metabolite concentrations in the prostate of one healthy subject (as ratios to Cr), determined with ProFit, as averages over ten JPRESS measurements in different sessions. Average error margins for the ratios to Cr were calculated from the average CRLBs via error propagation. The standard deviations of the metabolite ratios are shown in the last column.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Conc. [/Cr]</th>
<th>CRLB [%]</th>
<th>Error [%]</th>
<th>SD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>1.00</td>
<td>6.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t-Cho</td>
<td>0.58</td>
<td>5.4</td>
<td>8.5</td>
<td>20.4</td>
</tr>
<tr>
<td>Cit</td>
<td>10.48</td>
<td>1.7</td>
<td>6.7</td>
<td>21.8</td>
</tr>
<tr>
<td>Spm</td>
<td>5.98</td>
<td>6.3</td>
<td>9.1</td>
<td>21.2</td>
</tr>
<tr>
<td>MI</td>
<td>1.87</td>
<td>10.8</td>
<td>12.6</td>
<td>13.9</td>
</tr>
<tr>
<td>Scy</td>
<td>0.18</td>
<td>16.2</td>
<td>17.4</td>
<td>11.7</td>
</tr>
</tbody>
</table>

for coupled metabolites such as Cit and Spm. This is due to the increased information content and reduced peak overlap in the 2D spectrum. While the Spm resonance can hardly be recognized in the PRESS spectrum (Fig. 5.3), it appears as a characteristic multiplet pattern in the JPRESS spectrum (broad negative peaks at \( f_1 \approx \pm 6 \text{ Hz} \) in Fig. 5.2) and can thus be distinguished more easily from other uncoupled (Cr, Cho/PCh) and coupled (MI, Tau) resonances in the spectral region around 3.15 ppm. This also explains the large discrepancy between the Spm/Cr ratios determined by ProFit and LCModel. Furthermore, the consistently lower concentration ratios obtained by LCModel suggest an overestimation of the Cr concentration due to the overlap with other resonances.

Table 5.2 shows the average concentration ratios (with average CRLBs for individual metabolites) from ten JPRESS/ProFit experiments in the same subject. Confidence intervals for the ratios were calculated from the CRLBs via error propagation. In the last column, the standard deviations (SDs) of the ratios are listed, indicating the reproducibility of the method. Only quantitation results with CRLBs below 20% are commonly considered reliable. Therefore Cho, Tau, Gln, Glu and Glx with huge CRLBs in most fits, were excluded from the list. Since Cho and PCh can be more reliably quantified together, only the t-Cho concentration is listed and addressed in the further discussions. Six metabolites yielded average CRLBs
5.3. RESULTS

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Conc. [Cr]</th>
<th>CRLB [%]</th>
<th>Error [%]</th>
<th>SD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>1.00</td>
<td>6.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t-Cho</td>
<td>0.61</td>
<td>4.6</td>
<td>7.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Cit</td>
<td>12.49</td>
<td>1.4</td>
<td>6.4</td>
<td>38.9</td>
</tr>
<tr>
<td>Spm</td>
<td>6.88</td>
<td>5.1</td>
<td>8.2</td>
<td>37.4</td>
</tr>
<tr>
<td>MI</td>
<td>2.11</td>
<td>9.3</td>
<td>11.2</td>
<td>22.6</td>
</tr>
<tr>
<td>Scy</td>
<td>0.15</td>
<td>18.9</td>
<td>19.9</td>
<td>22.1</td>
</tr>
</tbody>
</table>

Table 5.3: Metabolite concentrations in healthy prostate tissue (as ratios to Cr) determined with ProFit and averaged over ten healthy subjects. Average error margins for the ratios to Cr were calculated from the average CRLBs via error propagation. The inter-subject standard deviations of the metabolite ratios are shown in the last column.

below 20 % (Cr, Cit, Spm, MI, Scy and t-Cho). For MI and Scy the SDs are comparable to the average error margins calculated from the CRLBs, whereas for Cit, Spm and t-Cho the SDs are considerably higher. This discrepancy suggests that the VOI placement might have been slightly different for the singular measurements and there might have been natural fluctuations in metabolite ratios between measurements. However, imperfections in the fitting model have to be taken into account as well.

Table 5.3 shows the determined metabolite concentration ratios averaged over ten subjects, with average CRLBs and average error margins. Additionally, the inter-subject SDs of the metabolite ratios are shown in the last column. For Cit and Spm, the inter-subject SD is many times larger than the error estimate calculated from the CRLBs, while for t-Cho, MI and Scy error margins and SDs only differ by a factor of up to 2. This can be partly explained with varying amounts of glandular and stromal tissue in the VOI. The Cit and Spm concentrations in glandular tissue are many times larger than those found in stromal tissue, while the concentrations of the other metabolites (Cr, t-Cho, MI, Scy) vary only slightly with the tissue type.\(^1\) The calculated error margins in Table 5.3 are very similar to those in Table 5.2, but the inter-subject SDs are about twice as large as the individual SDs. This corroborates the finding of considerable inter-subject variations in metabolite concentration ratios.
5.4 Discussion

Quantitative prostate studies based on peak integration as carried out by many groups over the past decade suffer from subjectiveness and a heavy overlap of metabolite peaks in the spectral region between 3.0 and 3.3 ppm, rendering the comparison of quantitative results obtained at different sites with different sequences difficult. Furthermore, strongly coupled resonances show a signal intensity modulation with echo time. This obviates the need for an objective, user-independent fitting method like LCModel, which includes prior knowledge in the form of metabolite basis spectra. However, especially for coupled metabolites (e.g. Cit, Spm and MI), the spectral information content can be considerably increased through 2D spectroscopy methods like JPRESS, which enhance the orthogonality of the basis spectra and thus the accuracy of quantitation results. This is demonstrated by comparing the quantitation results of JPRESS/ProFit with those obtained using PRESS/LCModel from the same volume in the same scan time. In particular for coupled metabolites, the CRLBs of the ProFit results are much smaller than those of the LCModel results.

It is not surprising that there is a large difference in metabolite concentrations determined in this work in comparison with other in vivo and in vitro prostate studies, where simple peak integration was applied for quantification.\textsuperscript{52, 55, 81, 86, 90–92, 99–102} Compared to results obtained by Swanson \textit{et al.}\textsuperscript{91} through quantification of 1D in vitro spectra, the proposed method yields similar concentration ratios for t-Cho/Cr (this work: 0.61, Swanson \textit{et al.}: 0.78) and Scy/Cr (this work: 0.15, Swanson \textit{et al.}: 0.2), whereas Cit/Cr (this work: 12.49, Swanson \textit{et al.}: 4.75), MI/Cr (this work: 2.11, Swanson \textit{et al.}: 3.77) and PA/Cr (this work: 6.88, Swanson \textit{et al.}: 2.03) are completely different. This demonstrates that for uncoupled resonances (Cr, t-Cho, Scy) quantification via simple line fitting and peak integration works reasonably well, whereas for coupled metabolites the results are strongly distorted by J modulation, which leads to considerably decreased signal intensity at certain echo times. J modulation is taken into account when the spectrum is fitted using metabolite basis spectra (as done in this work), but it is not considered when quantifying via simple constrained peak fitting, which renders quantification sequence and echo time dependent. Consequently, the Cit/t-Cho concentration ratio, which was found to be around 20 in healthy glandular tissue, is also much larger.

However, the regularisation parameters used in the cost function (Eq.
5.4. DISCUSSION

5.1), in particular $\rho_{\sigma_e}$, have a considerable influence on the fitting results. The regularization constants $\rho_{\sigma_e}$ and $\rho_{\Delta_2}$ were chosen such that the deviation of $T_2$ relaxation constants and chemical shifts from literature values did not become excessively large. Chemical shifts and coupling constants have been well investigated in brain, but not in prostate tissue. Therefore literature values determined in brain tissue\textsuperscript{96} have been taken for the simulation of the basis spectra except for Cit and Spm. For this reason, a divergence of a few Hz in the $\Delta_{2,m}$ values was tolerated in the fit. The exponential broadening factors $\sigma_{e,m}$ were regularised such that their deviation from $\sigma_{\rho_{e,m}}$ stayed approximately in the range of the error estimates in.\textsuperscript{91} However, the regularization underlies a certain subjectiveness. This can give rise to systematic quantification errors beyond the range of the CRLBs, which assume a perfect fitting model.

Since prostate cancer usually occurs in the peripheral zone of the prostate, single voxel measurements as performed in this work are not appropriate for clinical diagnosis. However, the signal-to-noise ratio can be raised considerably by using an endorectal/pelvic phased array coil system for signal reception,\textsuperscript{39} hence enabling a further increase in quantification accuracy. This should enable much smaller voxels or a combination of JPRESS with spectroscopic imaging\textsuperscript{64} in order to obtain spatial distributions of metabolites over the whole prostate.
Quantitative $J$-Resolved Prostate Spectroscopy Using 2D Fitting
Conclusions and Outlook

The objective of this thesis was to improve the detectability of $J$-coupled metabolites and to enhance the accuracy of quantitative measurements. Due to their complicated coupling evolution, strongly coupled spin systems are usually a greater challenge for spectroscopists than weakly coupled ones. However, anomalous $J$ modulation, discussed in Chapter 2, is most severe for weakly coupled protons with large chemical shift differences such as in the lactate molecule. The resulting signal loss approximately rises with $B_0^2$, becoming severe for $B_0 \geq 3$ T. Whole-body 3T MR systems are no longer limited to research facilities, but have also arrived in many hospitals where clinical studies are carried out with standard protocols and without access to more technical sequence parameters like selection gradients as well as pulse shapes and bandwidths. For lactate detection in clinical environments, the most obvious remedy is therefore the adaptation of the echo time, which should be either 288 ms or as short as possible.

Among the 2D spectroscopy methods used on human MR systems, $J$PRESS has proven to be most robust and versatile. Its applicability was demonstrated in Chapter 3 for brain spectroscopy and in Chapter 5 for prostate spectroscopy. The proposed improvements such as the maximum-echo sampling scheme, which increases the SNR and reduces baseline distortions through imperfect water suppression, are essential for spectroscopic applications in difficult anatomical regions like prostate, kidney or liver, where sensitivity and residual nuisance signal are a major concern. Such an optimised $J$PRESS sequence is also an excellent experimental basis for fitting and metabolite quantification, as has been demonstrated for brain spectroscopy\textsuperscript{49} and for prostate spectroscopy (Chapter 5). The additional information for $J$-coupled metabolites contained in $J$PRESS spectra can best be exploited by fitting and quantification with 2D basis spec-
tra incorporating the whole prior knowledge available. This enables the determination of objective metabolite concentration ratios in the human prostate where quantitative measurements are normally based on simple peak integration. Furthermore, the confidence intervals provided by ProFit in the form of CRLBs are an asset for the objective evaluation of quantitative studies. The introduction of such a method as a golden standard in clinical prostate studies could facilitate the comparison of results obtained at different sites and with different measurement parameters. The application of the ProFit algorithm for the quantification of other 2D spectra such as COSY or 2D S-PRESS is readily feasible with minor adjustments of the fitting model (e.g. the line-shape in the indirect dimension).

While JPRESS is the method of choice when the simultaneous detection and quantification of a large number of J-coupled metabolites is aspired, 2D S-PRESS has been designed for the detection and investigation of strongly coupled metabolites such as citrate. The more clearly arranged spectral pattern of citrate in 2D S-PRESS spectra as compared to JPRESS spectra can be exploited to determine the spectral parameters of citrate (chemical shift difference $\delta$ and coupling constant $J$) in vivo with reasonable accuracy (Chapter 4). But MRS not only reveals information about the concentrations of MR-active metabolites, but also about their chemical environment (e.g. the solvent or certain ion concentrations). Therefore the spectral evolution and appearance of the citrate multiplet can be quite different in vitro and in vivo, as was demonstrated with the 2D S-PRESS method in Chapter 4. Hence citrate basis spectra for metabolite quantification using LCModel or ProFit should be simulated rather than measured in phantom solution as usually recommended.

In most clinical applications of MRS, in particular for prostate examinations, the spatial distribution of certain metabolites is of interest, e.g. to identify cancerous or necrotic tissue in a tumor region. Therefore the combination of 2D spectroscopy with spectroscopic imaging methods might play a major role in future high-field applications. To keep scan time within acceptable limits, fast SI sequences such as parallel spectroscopic imaging, echo planar spectroscopic imaging (EPSI), spectroscopic GRASE and Spectroscopic RARE are potential methods of choice for such a combination.

The presented methods focused on $^1$H spectroscopy at a field strength of 3 T, which has become more and more available for clinical purposes over the past few years. Human MR systems operating at 7 T are cur-
rently being installed at many research sites. This opens up a wide range
of new applications benefiting from the increased SNR and spectral reso-
lution, but also exacerbates common problems encountered on human MR
systems such as $B_0$ and $B_1$ inhomogeneities, SAR limitations and chem-
ical shift displacement artifacts. The latter can become so severe that
the selected volumes for spins with Larmor frequencies which are further
apart in the spectrum hardly overlap any more in PRESS experiments.
Consequently, signal loss due to anomalous $J$-modulation, as discussed
in Chapter 2 for the lactate resonance, will result in considerable signal
misregistration for other weakly coupled metabolites with much smaller
chemical shift differences than observed in lactate. This also has to be
taken into account for 2D spectroscopy methods, which are commonly
based on PRESS localisation, e.g. $J$PRESS and 2D S-PRESS. Apart from
over-prescribed PRESS,$^{109}$ LASER$^{12}$ (or SADLOVE$^{13}$) and STEAM$^{37}$ ba-
sically turn out to be the most promising localisation methods at ultra-
high field strengths. In a LASER sequence, refocusing and slice selection
are achieved with two consecutive adiabatic full passage (AFP) pulses in-
stead of one non-adiabatic pulse. To achieve localisation with adiabatic
pulses only, a LASER sequence applies a spatially non-selective excita-
tion pulse and three pairs of slice-selective AFP pulses. Apart from the
fact that the signal is refocused three instead of two times, the coherence
pathway is identical to that of a PRESS sequence so that PRESS can
be readily replaced by LASER localisation in $J$PRESS and 2D S-PRESS
sequences. However, the six adiabatic inversion pulses entail a consid-
erable RF power deposition, which can necessitate longer repetition times.
In a STEAM sequence, consisting of three 90° pulses, a stimulated echo
is acquired, which has a different coherence history than ordinary spin
echoes.$^7$ For $J$-encoding the indirect spectral dimension, the second and
third pulse would have to be shifted simultaneously, therewith varying
$TE$, but keeping the mixing time ($TM$) constant. However, zero-quantum
cohereces during the mixing time, which are not filtered out by the selec-
tion gradients, can give rise to a strong $TM$-dependent signal modulation
for $J$-coupled metabolites, which would have to be eliminated by addi-
tional pulse/gradient elements during $TM$.$^7,110$ Constant-time PRESS
(CT-PRESS)$^{43,44}$ might also gain popularity at field strengths above 3 T.
It is based on the same sequence as $J$PRESS, but is reconstructed dif-
ferently. While in a $J$PRESS reconstruction the acquired echo signals are
aligned along the echo top in $t_1$ before Fourier transformation, a fixed time
point with respect to the excitation is used as reference for a CT-PRESS reconstruction. Thus the indirect dimension primarily displays the chemical shift instead of the coupling information. While the $J$-dispersion in the indirect dimension of a JPRESS spectrum is independent of $B_0$, CT-PRESS benefits from high field strengths through the increased chemical shift range of resonances in both spectral dimensions. It remains to be seen which of these prospective 2D high-field applications prevail.
References


REFERENCES


REFERENCES


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Curriculum Vitae

I was born on August 22nd 1974 in Krefeld (Germany) as the first child of Gisela and Friedrich-Georg Lange. In the same place, I attended the Arndt-Gymnasium (high school), from where I graduated in May 1994 (Abitur). After one year of mandatory military service, I started my studies in Physics at the technical university RWTH Aachen (Germany) in October 1995. Between 1997 and 1998, I spent one year as an exchange student at the University of Edinburgh (Scotland). In 2000, I worked for two months as a student trainee in the Corporate Research Department of Infineon Technologies (Munich). After my diploma thesis at the Institute of Solid State Research (Forschungszentrum Jülich, Germany), I graduated from the RWTH Aachen with a physics diploma in September 2001. In December 2001, I joined the bio-physics group of Prof. P. Bösiger at the Institute for Biomedical Engineering, University and ETH Zurich (Switzerland), as a PhD student and research assistant. My research was focused on various aspects of magnetic resonance spectroscopy at higher field strengths, in particular the detection of \( J \)-coupled metabolites in the human brain and prostate as well as two-dimensional spectroscopy techniques.