



Human Cardiac Magnetic Resonance Spectroscopy

by

William T. Clarke

A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

Hilary Term 2016

University of Oxford

Oxford Centre for Clinical Magnetic Resonance Research

and

Worcester College

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Abstract

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The status of the myocardial “high energy phosphate” metabolism is a sensitive marker of the occurrence and progression of heart failure. Magnetic resonance spectroscopy enables non-invasive, direct and potentially quantitative measurements of the phosphate containing metabolites present in the human myocardium. This thesis is primarily concerned with the creation of measurement techniques for cardiac phosphorus magnetic resonance spectroscopy (^{31}P -MRS) at the 7 tesla field strength.

- Chapter 1 provides an overview of the physical basis of magnetic resonance spectroscopy, the myocardial high energy phosphate metabolism, and the clinical relevance of the technique.
- Chapter 2 describes the advantage of 7 tesla scanners over lower field strengths. The radio frequency coil hardware is characterised experimentally. The multi-voxel spectroscopy methods used throughout the thesis are described.
- Chapter 3 details the implementation of an open source spectroscopy fitting program. It is validated against previous closed-source implementations. The program’s use is demonstrated in several clinical studies of heart failure, and to improve a previously implemented ^1H spectroscopy coil combination method.
- In Chapter 4 the measurement of inorganic phosphate in the presence of overlapping peaks is attempted. Suppression of overlapping peaks, originating from the blood, is tried using B_0 gradients, then saturation transfer. The myocardial pH of hypertrophic cardiomyopathy patients is measured.
- Chapter 5 describes the effect of creatine kinase catalysed chemical exchange on the ^{31}P -MRS spectrum. A survey of methods suitable for measuring creatine kinase kinetics at 7 tesla is made. Multi-parametric fitting of variable repetition time saturation transfer data is explored in simulation and experiment.
- Chapter 6 describes the reimplementing and extension, for dynamic measurements, of the triple repetition time saturation transfer method for two clinical studies at 3 tesla. The creatine kinase forward rate constant is measured in heart failure and healthy cohorts, at rest, and during cardiac stress.
- In Chapter 7 a Bloch-Siegert B_1 mapping sequence is implemented for ^{31}P -MRS. An optimal Bloch-Siegert method for X-nuclear spectroscopy is calculated. B_1 maps are validated in skeletal muscle and collected in 5 volunteer’s hearts.
- Chapter 8 uses the Bloch-Siegert B_1 mapping sequence and the four angle saturation transfer method to implement creatine kinase rate measurement at 7 tesla. The first 3D localised creatine kinase rate measurements in the human myocardium are achieved in 10 volunteers.

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I dedicate this thesis to my late mother Dr. Susan Fiona Clarke.

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Chapter 1: Introduction

Phosphorus magnetic resonance spectroscopy (^{31}P -MRS) is a key weapon in the arsenal of scientists studying the energy metabolism of human and animal cells. ^{31}P -MRS enables direct and non-invasive measurements of the concentration and kinetics of in vivo metabolites central to core biological processes in cells ranging from neurons in the brain, to myocytes in the heart and skeletal muscle.¹ Applied in the human heart, ^{31}P -MRS unveils the state of metabolic energy supply required for the heart's continuous, energy-demanding, and essential workload. Over the last 30 years, ^{31}P -MRS has been found to be a sensitive marker of heart failure, one of the most prevalent diseases in the UK²; and it continues to give significant insights into the mechanism and causes of a range of cardiomyopathies.³

Despite its successes, ^{31}P -MRS is relatively signal-to-noise (SNR) limited; recently the advent of ultra-high field whole body magnetic resonance imaging (MRI) scanners have mitigated this limitation, with greater than linear SNR increases with field strength reported. The increased signal brought by these new scanners promises to bring a step change in the quality, resolution and speed of human ^{31}P -MRS.^{4,5}

This thesis describes the author's work to develop techniques for investigating the metabolic state of healthy and diseased human myocardium, primarily by ^{31}P -MRS using an ultra-high field 7T MRI scanner.

This chapter sets out the necessary background to understand the results presented in later chapters. In particular, it gives a brief introduction to: magnetic resonance, the physical basis of ^{31}P -MRS; the major applications of phosphorus magnetic resonance

spectroscopy in cardiovascular research; and finally the clinical motivation for this work, including a review of previous discoveries made using ^{31}P -MRS.

1.1 Magnetic resonance

“Nuclear magnetic resonance” (NMR), often simply called “magnetic resonance” (MR) in its human applications, arises from the interaction of the intrinsic “spin” of a nucleus with applied static and time-dependent magnetic fields.⁶

1.1.1 Basic Principals

This section gives a basic outline of the principles of NMR for MRS. The content of this chapter is drawn from a number of textbooks which may be referred to for further details.^{1,7,8}

A quantum mechanical description of the MR experiment is required to fully understand the microscopic (per-nucleus) process. However, analogies may be drawn to a classical angular momentum interpretation which are generally suitable to describe MRS experiments on a macroscopic collection of nuclei, such as those described in this thesis.

1.1.1.1 Origin of the signal

An object rotating around a fixed point, with an instantaneous position $\mathbf{r}$ relative to the fixed point, (linear) velocity $\mathbf{v}$ and mass m , has an angular momentum vector

$$\mathbf{L} = \mathbf{r} \times m\mathbf{v}. \quad [1.1]$$

A change in angular momentum must be precipitated by a torque

$$\mathbf{T} = \mathbf{r} \times \mathbf{F} = \frac{d\mathbf{L}}{dt}. \quad [1.2]$$

The magnetic dipole moment μ of a moving charge carrier characterises the magnetic field that it induces. The magnetic moment is given by the product of the current and the area swept out, i.e.

$$\mu = \frac{qv}{2\pi r} \pi r^2, \quad [1.3]$$

in which q is the charge on the object. Using Eq. [1.1], the relationship between angular momentum and the magnetic moment may be derived:

$$\mu = \frac{q}{2m} L = \gamma L, \quad [1.4]$$

where γ is the classical gyromagnetic ratio. Equation [1.4] is valid for any motion of elementary particles.

If a static external field B_0 is introduced, the object will experience a torque

$$T = \mu \times B_0. \quad [1.5]$$

Combining Equations [1.2], [1.4] and [1.5] gives:

$$\frac{d\mu}{dt} = \gamma \mu \times B_0, \quad [1.6]$$

which describes the change in orientation of the magnetic moment relative to B_0 , as the magnitude of μ is constant. The quantity γB_0 may be expressed as an angular velocity ω_0 or frequency ν_0 , leading to the Larmor equation:

$$\omega_0 = \gamma B_0, \quad [1.7]$$

and

$$\nu_0 = \frac{\omega_0}{2\pi} = \frac{\gamma}{2\pi} B_0, \quad [1.8]$$

where ν_0 is the Larmor precession frequency. The associated magnetic energy of this system is given by

$$E = -\boldsymbol{\mu} \cdot \mathbf{B}_0 = -\mu B_0 \cos \theta, \quad [1.9]$$

where θ is the angle between the magnetic moment and the static magnetic field. The energy will be minimised when the magnetic moment is parallel with $\mathbf{B}_0$ ($\theta = 0^\circ$), and maximised when it is anti-parallel ($\theta = 180^\circ$). In this classical description the object's angular momentum is continuous, i.e. it may take any value, and the associated energy may vary continuously between $+\mu B_0$ and $-\mu B_0$.

We now switch from this classical example of a single electric charge carrier to the quantum mechanical description of spin angular momentum for a single nucleus with a non-zero nuclear spin (considered here as an intrinsic property of a nucleus, and characterized by the spin quantum number). Quantum mechanics introduces the restriction that angular momentum is quantised. For a nucleus, the spin angular momentum is limited to $2I+1$ values, where I is the spin quantum number of the nucleus. In this framework

$$\langle \mathbf{L}^2 \rangle = \left(\frac{h}{2\pi} \right)^2 I(I+1), \quad [1.10]$$

and the projection of $\mathbf{L}$ along the z-axis (taken as the direction of the external magnetic field without loss of generality) is

$$L_z = \hbar m_z \quad [1.11]$$

where $m_z = I, I-1, I-2, \dots, -I$ and $\hbar = h/2\pi$. As L_z is quantised and $\boldsymbol{\mu} = \gamma \mathbf{L}$, μ_z is also quantised, the projection of $\boldsymbol{\mu}$ along the direction of $\mathbf{B}_0$ is

$$\mu_z = \gamma \hbar m_z. \quad [1.12]$$

By combining the classical description in Equation [1.9], with this quantised expression for the magnetic moment, the energy of the quantum mechanical system is

$$E = -\mu_z B_0 = -\gamma \hbar m_z B_0. \quad [1.13]$$

Therefore, for an $I = 1/2$ system, with possible values of m limited to $+1/2$ and $-1/2$ the energy difference between the two levels (Figure 1.1a) is,

$$\Delta E = \gamma \hbar B_0. \quad [1.14]$$

A transition between states of $m_z = +1/2$ and $-1/2$ (also known as “ α ” and “ β ”, or “spin up” and “spin down”) is possible in the presence of an oscillating magnetic field $\mathbf{B}_1$, perpendicular to μ_z , with frequency corresponding to the energy difference ΔE , i.e.

$$\nu_0 = \Delta E / h$$

$$\nu_0 = -\frac{\gamma B_0}{2\pi}. \quad [1.15]$$

The quantisation of energy means that θ , as defined from equation [1.9], must also take discrete values:

$$\cos \theta = \frac{m}{\sqrt{I(I+1)}}. \quad [1.16]$$

For a nucleus where $I = 1/2$, this restricts the values of θ to 54.74° and 125.26° relative to the z -axis. Thus, the picture we have described mathematically has a spin magnetic moment precessing at the Larmor frequency, around the z -axis at one of two fixed angles. The magnetic moment vector therefore describes the surface of one of two cones, corresponding to either the α or β state (Figure 1.1b).

The extension to a system with many spins, sees different Boltzmann populations for each spin state, with spins spread out randomly around the appropriate cone, in the ratio

$$\frac{n_{\alpha}}{n_{\beta}} = e^{\frac{\Delta E}{kT}} = e^{\frac{h\nu_0}{kT}}. \quad [1.17]$$

at thermal equilibrium. For a sample of ^1H nuclei in a 1.5 T field ($\nu_0 = 63.9 \text{ MHz}$), at 37°C (310.15 K) the ratio $n_{\alpha}/n_{\beta} = 1.0000098$, i.e. there are 5 spins per million more in the α state than the β state. At 7 T ($\nu_0 = 298 \text{ MHz}$) this number has increased to a difference of 23 spins per million. The MR signal is proportional to this population difference, thus an increase in static field strength leads to a proportional increase in the signal.

Total net magnetisation $\mathbf{M}$ of the system is given by the vector sum of the magnetic dipole moments of all the spins in the system. The longitudinal magnetisation at equilibrium is

$$\mathbf{M}_0 = \hat{\mathbf{k}} \sum_{i=1}^n \mu_i = (n_{\alpha}\mu_z - n_{\beta}\mu_z) \hat{\mathbf{k}}, \quad [1.18]$$

where $\hat{\mathbf{k}}$ is the unit vector along the direction of the B_0 field, or z-axis. At thermal equilibrium the spins are distributed at random angles around the two cones, there is no ‘phase coherence’, as a result there is no net magnetisation perpendicular to the z-axis in the xy- or ‘transverse’ plane. The small population difference results in a small net magnetisation vector parallel to $\mathbf{B}_0$, along the positive z-axis at equilibrium, the magnitude of which is denoted M_0 .

1.1.1.2 Excitation and precession – the B_1^+ and B_1^- fields

Rotation of the magnetisation vector into the transverse plane is achieved by introducing a second magnetic field, oscillating at the Larmor frequency, perpendicular to the z-axis. The Larmor frequency for common nuclei (e.g. ^1H , ^{31}P) is in the MHz or radio frequency (RF) range of the electromagnetic spectrum in human sized MRI scanners.

In the “laboratory frame”, i.e. with x-, y- and z-axis fixed relative to the MR scanner, the linearly polarised magnetic field component of an RF-field is

$$\mathbf{B}_1(t) = 2B_{1,\max} \cos(\omega t + \phi) \hat{\mathbf{i}}, \quad [1.19]$$

where $B_{1,\max}$ is the maximum amplitude of the field, ω is the angular frequency, ϕ is the phase, and $\hat{\mathbf{i}}$ is the unit vector along the x-axis. This linearly polarised field can notionally be decomposed into two circularly polarised components, rotating in opposite directions around the z-axis (one in the direction of the Larmor precession and the other counter-rotating):

$$\mathbf{B}_1(t) = B_{1,\max} (\cos(\omega t) \hat{\mathbf{i}} + \sin(\omega t) \hat{\mathbf{j}}) + B_{1,\max} (\cos(\omega t) \hat{\mathbf{i}} - \sin(\omega t) \hat{\mathbf{j}}). \quad [1.20]$$

Because the counter-rotating field influences the spins dependent on the ratio $(B_1/2B_0)^2$, the rotating wave approximation is made, and the counter-rotating term is neglected. Thus the effects of the linearly polarised field are approximated by a single circularly polarised component:

$$\mathbf{B}_1^+(t) = B_{1,\max} (\cos(\omega t) \hat{\mathbf{i}} - \sin(\omega t) \hat{\mathbf{j}}). \quad [1.21]$$

I.e. the complex components of the $\mathbf{B}_1^+$ field along the x- and y-axes, $B_{1,x}$ and $B_{1,y}$ are $B_{1,\max} \cos \omega t$ and $B_{1,\max} \sin \omega t$ respectively, which in the 'rotating frame' (see §1.1.1.5) may also be expressed as a time-independent complex value,⁹

$$\mathbf{B}_1^+ = \frac{B_{1,x} + iB_{1,y}}{2}. \quad [1.22]$$

At the microscopic level, the $\mathbf{B}_1^+$ field causes phase coherence between the spins, resulting in a net transverse magnetisation (Figure 1.1c). This can be described quantitatively using the density matrix or product operator formalism of magnetic resonance. However, for our purposes it is sufficient to consider that $\mathbf{M}$ experiences a torque, resulting in it 'nutating' around the vector sum of $\mathbf{B}_0$ and $\mathbf{B}_1^+$ simultaneously. Viewed in a stationary frame of reference the magnetisation vector traces a spiral path towards the transverse plane. In the rotating frame, defined as a frame of reference rotating clockwise at the Larmor frequency around the z-axis, $\mathbf{B}_1^+$ appears as a pseudo-static field along an axis in the transverse plane; thus the magnetisation only appears to precess around $\mathbf{B}_1^+$. For example a B_1 -field whose phase places it along the rotating frame x-axis will result in a rotation of spins from the z-axis towards the y-axis. The angle of rotation θ away from the z-axis depends on the amplitude of the RF pulse, the duration of the pulse T and the gyromagnetic ratio of the nucleus:

$$\theta = \gamma \int_0^T B_1^+(t) dt. \quad [1.23]$$

An RF pulse which rotates the magnetisation from the longitudinal (+z) direction towards the transverse plane is known as an excitation pulse.

At thermal equilibrium there is no phase coherence, and therefore no component of the magnetisation in the transverse plane. In this situation we are unable to detect the

static longitudinal magnetisation vector using magnetic resonance: the static vector doesn't create a changing field and thus does not induce an electrical signal in any detection hardware. If there is a component of $\mathbf{M}$ in the transverse plane M_{xy} , it precesses around $\mathbf{B}_0$ at the Larmor frequency. The precession of M_{xy} produces an oscillating magnetic field $\mathbf{B}_1^-$ which induces an electromotive force (EMF) in a receive coil placed near the sample.⁹ This EMF is the MR signal

$$s \propto (B_1^-)^* M_{xy}, \quad [1.24]$$

where,

$$(B_1^-)^* = \frac{B_{1,x} - iB_{1,y}}{2}. \quad [1.25]$$

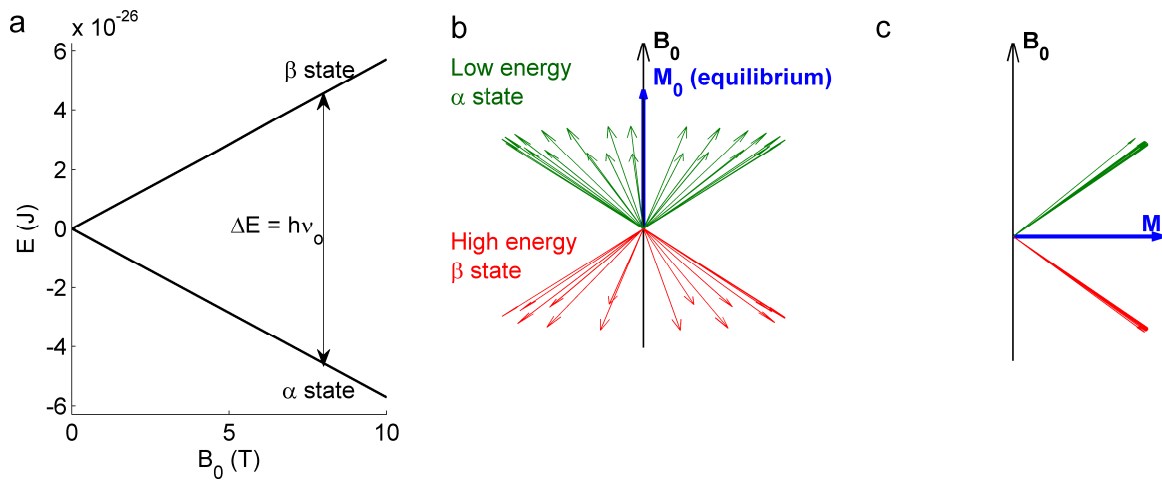


Figure 1.1 **a:** Energy difference between alpha and beta states of an isolated ^{31}P nucleus as a function of the magnitude of the B_0 field strength. **b:** Schematic representation of a collection of nuclear magnetic moments at thermal equilibrium. There are more nuclei in the low energy α spin state compared to the β state. Therefore, there are more spin magnetic moments distributed, at random intervals (with no overall coherence) around the cone with internal angle 54.74° . The macroscopic magnetisation vector (blue) is the sum of all the individual (red and green) magnetic moments, and lies along the positive z-axis, parallel with $\mathbf{B}_0$. **c:** State of a collection of spin magnetic moments instantaneously after a 90° excitation pulse. The populations of the α and β states are equal and there is no longitudinal magnetisation. But there is phase coherence between the spin magnetic moments and hence a transverse component of the magnetisation vector is seen.

1.1.1.3 Relaxation

Non-equilibrium magnetization undergoes two modes of “relaxation” resulting in a return of the spin system to the thermal equilibrium state.

Spin-lattice relaxation

Spin-lattice relaxation is the process that drives the longitudinal magnetisation back to equilibrium. It is the process by which the spins give up energy to their surroundings, “the lattice”, comprising nearby molecules e.g. water. At a microscopic scale this equates to spins in the higher energy state relaxing back to the lower energy state, re-establishing the equilibrium population. The process is characterised by an exponential time constant T_1 . The longitudinal component of magnetization M_z recovers according to:

$$\frac{dM_z}{dt} = -\frac{M_z(t) - M_0}{T_1}, \quad [1.26]$$

which may be solved to form

$$M_z(t) = M_0 - (M_0 - M_z(0))e^{-t/T_1}, \quad [1.27]$$

where t is the time (in s) and T_1 is the spin-lattice relaxation time constant (in s).

The relaxation rate, $R_1 = 1/T_1$, is dependent on the degree of coupling caused by the dominant through-space dipole-dipole interaction between spins, which in turn is dependent on the rate of tumbling and rotation of the molecules.¹⁰

Spin-spin relaxation

Spin-spin, or T_2 , relaxation is an “entropy-process” in which spins exchange energy, resulting in a loss of phase coherence. The rate at which the phase coherence is lost, and hence transverse magnetisation decays is:

$$\frac{dM_x}{dt} = -\frac{M_x(t)}{T_2}, \quad [1.28]$$

$$\frac{dM_y}{dt} = -\frac{M_y(t)}{T_2}. \quad [1.29]$$

The magnitude of transverse magnetisation $M_{xy} = M_x + iM_y$ decays according to:

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2}. \quad [1.30]$$

Relaxation time constants

The relaxation time constants T_1 and T_2 may be estimated for a pure substance using the Bloembergen-Purcell-Pound theory (BPP theory).¹¹ The theory takes into account the rotation of molecules within the substance and the effect on local (microscopic) field fluctuations (Figure 1.2).

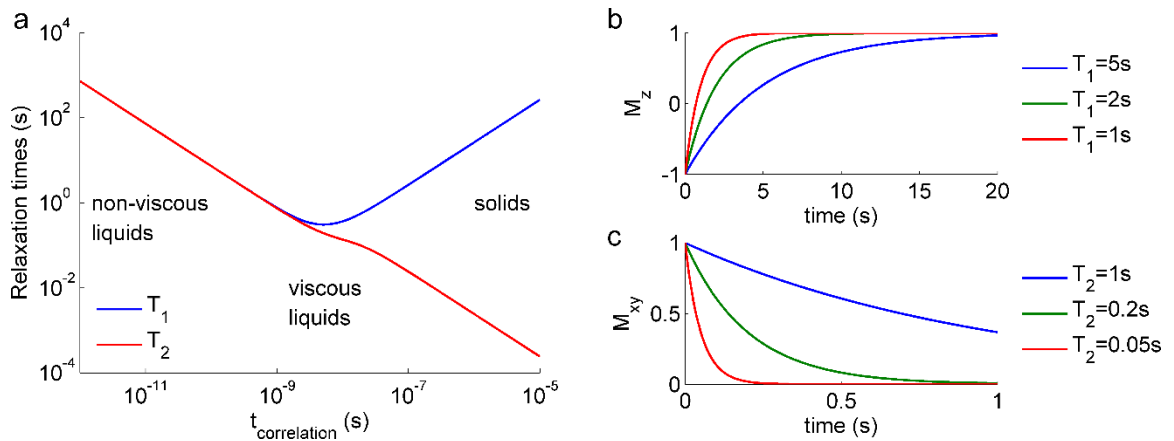


Figure 1.2 **a:** Relationship between ^{31}P T_1 and T_2 relaxation times and the rotational correlation time of constituent molecules in a substance according to BPP theory.¹¹ Solids, which have significantly stronger bonding between molecules, have a much longer correlation time than liquids. The minimum in T_1 occurs when $1/\tau_c \approx \nu_0$. **b&c:** Longitudinal (b) and transverse (c) components of magnetisation undergoing relaxation with different T_1 and T_2 relaxation constants.

The rate of rotation of molecules is characterized by their rotational correlation time τ_c , the average time taken for a molecule to rotate by one radian. The correlation time strongly depends on the substance's intermolecular bonding, for example solids which have strong intermolecular bonds have a long correlation time (milli- or micro-seconds), while pure liquid water has a correlation time measured in picoseconds.

1.1.1.4 Chemical shift

A nucleus with a non-zero spin quantum number has a specific gyromagnetic ratio γ , which may be treated as an intrinsic property for that isotope. Therefore, according to Eq. [1.15], in a homogenous static magnetic field B_0 , that isotope will have a fixed resonant frequency ν_0 and appear in a single location in a frequency domain spectrum formed in an MR experiment.

However, the chemical environment surrounding a nucleus results in a slight perturbation of the magnetic field actually experienced at the nucleus. A nucleus in an atom or molecule is 'shielded' by the electrons present around it, which circulate in their atomic or molecular orbitals. The external field stirs up a circulating motion of the electrons, which in turn generates a small magnetic field, which opposes the main B_0 field. Therefore, the B-field experienced by the nucleus is less than B_0 . Other shielding terms, such as the paramagnetic term, can create an increase in B_0 , and are more prevalent in nuclei heavier than ^1H . The field difference is described by the shielding constant σ : $B = B_0(1 - \sigma)$, and thus Eq. [1.15] may be rewritten as

$$\nu_0 = -\frac{\gamma B_0}{2\pi}(1 - \sigma). \quad [1.31]$$

σ is dependent on both local and molecule wide electron distributions as well as inter-molecular interactions.¹² The result is that the same nucleus, in a different chemical environment, will appear at different positions in a frequency domain spectrum.

Frequencies are typically recorded relative to a reference frequency, described by the chemical shift, a dimensionless parameter

$$\delta = 10^6 \left(\frac{\nu_0 - \nu_{0,\text{ref}}}{\nu_{0,\text{ref}}} \right). \quad [1.32]$$

Values are typically quoted in parts per million (ppm). The chemical shift is independent of B_0 .

1.1.1.5 Rotating frame and the Bloch Equations

Bloch Equations in the static (Laboratory) frame of reference

In a magnetic field, each magnetic moment, and therefore by Eq. [1.18], the magnetisation vector, will experience a torque (Eqs. [1.2] & [1.5]). Therefore, the equation of motion for the magnetisation vector in the static or laboratory frame is:

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t). \quad [1.33]$$

This equation describes fully the motion of the macroscopic magnetisation vector as a function of time; simultaneously including in the $\mathbf{B}(t)$ term the time varying external RF fields and the $\mathbf{B}_0$ field; the processes of relaxation; and any frequency offsets in the system (Figure 1.3a). In the presence of the static $\mathbf{B}_0$ and oscillating $\mathbf{B}_1$ field $\gamma \mathbf{B}(t) = \gamma [\mathbf{B}_0 + \mathbf{B}_1(t)]$, and relaxation, the previous equation may be broken down into Cartesian components and written:

$$\begin{aligned} \frac{dM_x(t)}{dt} &= \gamma [M_y(t)B_0 - M_z(t)B_{1y}] - \frac{M_x(t)}{T_2}, \\ \frac{dM_y(t)}{dt} &= \gamma [M_z(t)B_{1x} - M_x(t)B_0] - \frac{M_y(t)}{T_2}, \\ \frac{dM_z(t)}{dt} &= \gamma [M_x(t)B_{1y} - M_y(t)B_{1x}] - \frac{(M_z(t) - M_0)}{T_1}. \end{aligned} \quad [1.34]$$

Eqs. [1.34] are the Bloch equations in the laboratory or 'static' frame of reference.¹

Rotating frame

It is useful to describe the motion of the magnetisation vector in the 'rotating frame': a frame of reference with orthogonal unit vectors $\mathbf{x}', \mathbf{y}', \mathbf{z}'$, rotating around the $\mathbf{z}$ -axis at the frequency of the RF reference frequency, as defined by the vector rotation ω_{RF} . The equation of motion in the rotating frame is:

$$\frac{d\mathbf{M}'(t)}{dt} = \frac{d\mathbf{M}(t)}{dt} - (\omega_{\text{RF}} \times \mathbf{M}(t)), \quad [1.35]$$

which may be combined with Eq. [1.33],

$$\frac{d\mathbf{M}'(t)}{dt} = [\mathbf{M}(t) \times \gamma(\mathbf{B}_0 + \mathbf{B}_1(t))] + (\mathbf{M}(t) \times \omega_{\text{RF}}), \quad [1.36]$$

and rearranged to be written in the form of a time-dependent oscillating component and a fictitious field:

$$\frac{d\mathbf{M}'(t)}{dt} = \gamma \mathbf{M}(t) \times \left(\frac{\omega_{\text{RF}} - \omega_0}{\gamma} + \mathbf{B}_1(t) \right). \quad [1.37]$$

The individual components of the magnetisation in the rotating frame ($M'_i = M_i$) are

then given by the following equations:

$$\begin{aligned} \frac{dM'_x(t)}{dt} &= -(\omega_0 - \omega_{\text{RF}})M'_y(t) - \gamma B_{1y}M'_z(t) - \frac{M'_x(t)}{T_2}, \\ \frac{dM'_y(t)}{dt} &= -(\omega_0 - \omega_{\text{RF}})M'_x(t) - \gamma B_{1x}M'_z(t) - \frac{M'_y(t)}{T_2}, \\ \frac{dM'_z(t)}{dt} &= \gamma B_{1y}M'_x(t) - \gamma B_{1x}M'_y(t) - \frac{M'_z(t) - M_0}{T_1}. \end{aligned} \quad [1.38]$$

In the rotating frame, an RF pulse at a frequency different from that of the Larmor frequency may be understood to be the vector sum of $\mathbf{B}_1$ and the fictitious field,

defined as $(\omega_0 - \omega_{\text{RF}})/\gamma$, which forms an effective B_1 -field B_{eff} , around which the magnetisation rotates, in a right-handed manner (Figure 1.3b&c).

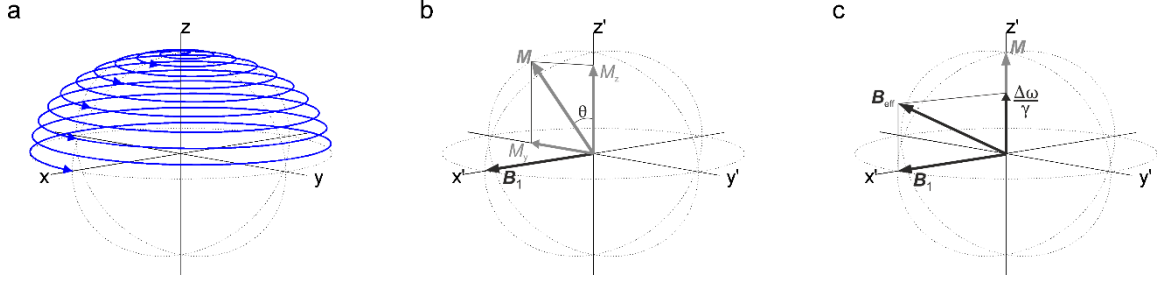


Figure 1.3 **a:** Path traced by a magnetisation vector during a 90° excitation pulse seen in the stationary laboratory frame of reference. **b:** In the rotating frame, which rotates around the z -axis at the frequency of the B_1 field, the magnetisation vector rotates around the B_1 field vector which now appears fixed along the x' axis. The final angle between the z -axis and the magnetisation vector (θ) is the flip-angle. **c:** An off-resonance RF field (one where $\omega_0 - \omega_{\text{RF}} = \Delta\omega \neq 0$) may be viewed as the vector sum of the B_1 field and the fictitious field, forming an effective B_{eff} vector about which the magnetisation rotates.

1.1.1.6 Fourier transform MR

Following an RF excitation pulse, the magnetisation precesses about B_0 at the Larmor frequency and can induce an EMF in a receiving coil. T_2 relaxation will cause the decay of the magnitude of the transverse magnetisation and the resulting EMF over time.

The detected time-dependent signal is known as the free induction decay (FID).

Quadrature (phase-sensitive) detection means that the FID effectively has independent real and imaginary components, i.e. each FID point is complex-valued.

The FID is commonly described as a projection onto the xt -plane and the yt -plane:

$$M_x = M_0 \cos[(\omega_0 - \omega)t + \phi] e^{-t/T_2}, \quad [1.39]$$

$$M_y = M_0 \sin[(\omega_0 - \omega)t + \phi] e^{-t/T_2}, \quad [1.40]$$

where ϕ is the phase of the magnetisation at $t = 0$ (Figure 1.4).

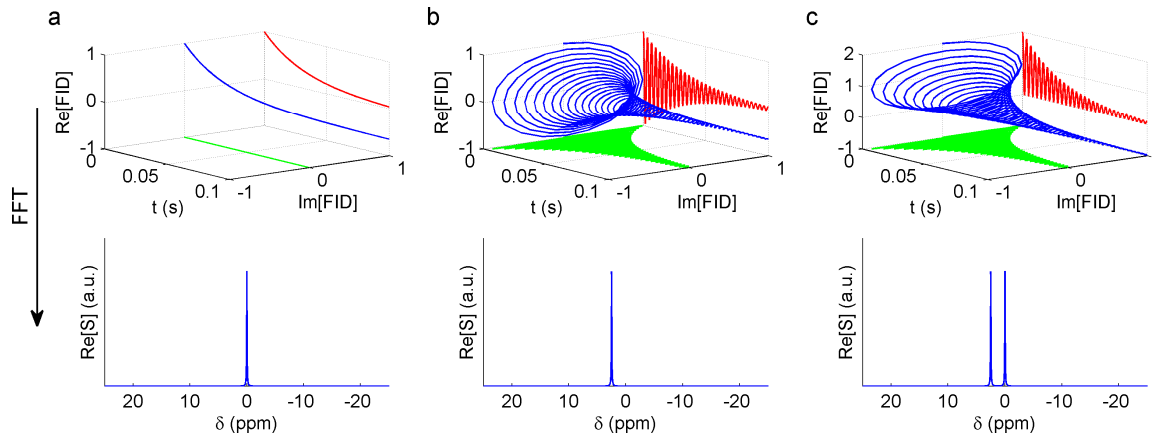


Figure 1.4 Three free induction decays (FIDs) and the corresponding real part of the frequency-domain spectra after the Fast Fourier transform (FFT) has been applied to the signal. The projections of the complex valued FID onto the xt - and yt -planes are shown as red and green lines. The FIDs contain signal from (a) a single on-resonance species, (b) a single resonance at a chemical shift of 2.5 ppm, and (c) the sum of both signals.

For display, and human interpretation, the FID is converted to the frequency domain using the Fast Fourier transform (FFT) algorithm. The signal in the frequency-domain is known as the “spectrum”, which may be converted back to the time-domain FID using the inverse FFT. Quadrature detection, enables the discrimination of positive and negative frequencies, which would otherwise be impossible with just a single component, as $\cos \omega = \cos(-\omega)$, but more significantly gives a $\sqrt{2}$ x SNR improvement.

The real and imaginary components of the spectrum $F(\omega)$ are,

$$\text{Re}[F(\omega)] = A(\omega) \cos \phi + D(\omega) \sin \phi, \quad [1.41]$$

$$\text{Im}[F(\omega)] = A(\omega) \sin \phi + D(\omega) \cos \phi; \quad [1.42]$$

where

$$A(\omega) = \frac{M_0 T_2}{1 + (\omega_0 - \omega)^2 T_2^2}, \quad [1.43]$$

$$D(\omega) = \frac{M_0 (\omega_0 - \omega) T_2}{1 + (\omega_0 - \omega)^2 T_2^2}, \quad [1.44]$$

describe the absorption and dispersion components of a Lorentzian line-shape.

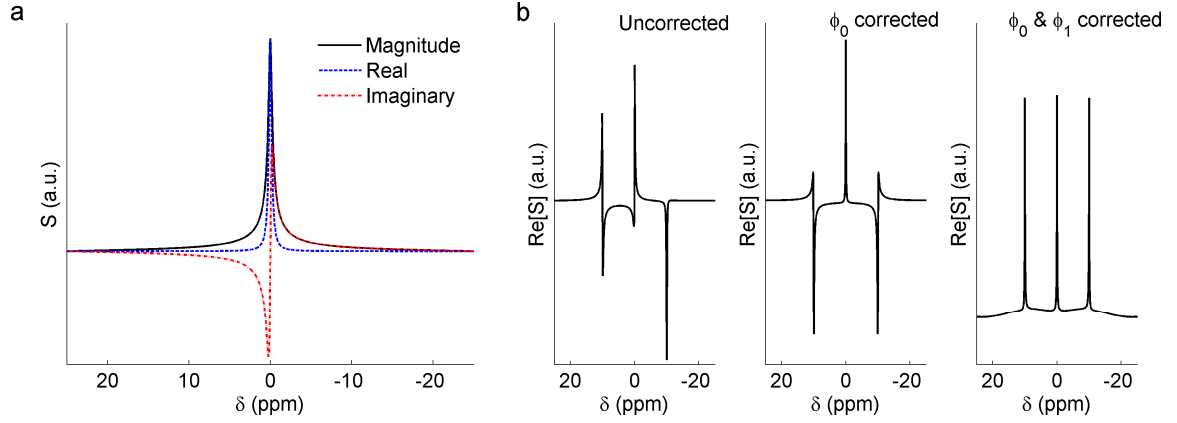


Figure 1.5 **a:** Absorption (real), dispersion (imaginary) and magnitude Lorentzian line-shapes of a single frequency-domain signal ('peak'). **b:** A spectrum of three peaks which have both zero-order and first-order phase before correction, after zero-order correction and after zero- and first-order correction. Note that first-order phase correction introduces some residual baseline artefacts, so data are typically recorded with minimum receiver dead-time experimentally.

Phase correction of spectra

The full width at half maximum (FWHM or 'damping') of the Lorentzian absorption

component is $(\pi T_2)^{-1}$, while the dispersive component is wider and has an integral of

zero (Figure 1.5a). Therefore, optimal resolution of a frequency-domain signal is

achieved with a purely absorptive spectrum, i.e. $\phi = 0$. Signal where $\phi = 0$ is unusual, as

excitation pulses or receive hardware usually introduce a constant "zero-order" phase

offset, and time delays between excitation and the start of reception (e.g. for coil ring-

down) result in a frequency dependent "first-order" phase. The resultant mix of

absorptive and dispersive line-shapes may be corrected, to give an approximately

absorptive spectrum, as follows:

$$A(\omega) = \text{Re}[F(\omega)] \cos \phi_c + \text{Im}[F(\omega)] \sin \phi_c, \quad [1.45]$$

where $\phi_c = \phi_0 + (\omega_0 - \omega)\phi_1$ in which ϕ_0 is the zero-order and ϕ_1 is the first order

phase correction term (Figure 1.5b).

Alternatively, a magnitude spectrum may be used if phase correction is not possible, or

not required:

$$|F(\omega)| = \sqrt{\text{Re}[F(\omega)]^2 + \text{Im}[F(\omega)]^2}. \quad [1.46]$$

Quantified peak parameters

Peaks in a spectrum are quantified using dedicated fitting software (Chapter 3), according to their amplitude, linewidth, chemical shift and phase. Throughout this work the amplitude is taken to mean the integral of the purely absorptive Lorentzian peak, i.e. after appropriate phase correction. Similarly the linewidth is the full width at half maximum, and the chemical shift the centre of the purely absorptive peak shape. Phase is the relative phase after first-order correction.

1.1.1.7 Spin-spin coupling

In high-resolution NMR spectroscopy it is possible to resolve the splitting of resonances from a single nuclear environment into several spectral peaks. This is often caused by “scalar” coupling, also known as “J” coupling. The effect arises due to the interaction of other MR active nuclei chemically bonded to the observed nucleus, via the electrons forming the chemical bond.

The interaction of a spin $\frac{1}{2}$ nucleus with another spin $\frac{1}{2}$ nucleus in a different chemical environment e.g. $^{13}\text{C}-^1\text{H}$ (observing ^{13}C split by ^1H), results in the splitting of the singlet peak into a symmetric doublet i.e. two peaks of equal intensity. The peak separation, defined as the coupling constant J and measured in Hz, is determined by the strength of this interaction and generally decreases with the number of intermediate atoms along the bonds linking the coupled nuclei. If the original observed nucleus couples with a second nucleus in the same chemical environment as the first coupling nucleus e.g. CH_2 , a triplet is formed with a ratio of 1:2:1 between the peaks. This pattern continues, forming a Fibonacci sequence of peak intensities with equal spacing between the peaks

i.e. $\text{CH}_3 = 1:3:3:1$, $\text{CH}_4 = 1:4:6:4:1$. The collection of peaks corresponding to a single nuclear environment is known as a multiplet. The sum of each peak's amplitude in a multiplet is equal to the amplitude of the theoretical un-split singlet.

Splitting of a resonance by multiple different nuclei in different environments is also possible, each coupling often has a different J value, so the distance between the splitting caused by each different environment will be different. For example in $^{13}\text{C}^1\text{H}^{19}\text{F}$ the peak corresponding to carbon will be split into a doublet by both the proton and the fluorine nucleus, resulting in a "doublet of doublets".

For a nucleus with a short T_2 , the linewidths might be wider than the coupling constant and hence the peak separation. Each peak in the multiplet will overlap and it might not be possible to resolve the individual components. In this case the multiplet will appear as a broadened singlet with non-Lorentzian line shape.

1.1.1.8 Signal-to-Noise ratio and the steady state

Signal-to-noise ratio is the main limiting factor in the majority of in vivo MR studies.

Noise primarily arises from thermal noise generated by random currents due to fluctuating motion of ions in the sample; noise in the coil and other electronics is negligible for human-sized biological samples. The thermal noise is white (Gaussian) with independent real and imaginary parts.¹³ Due to Faraday's law, the noise voltage induced in an RF coil is proportional to the Larmor frequency (and hence to B_0).¹⁴

Maximising SNR is critical for accurate interpretation of the MR signal. The SNR may be increased by increasing the intrinsic spin population difference (§1.1.1.1), by averaging multiple experiments, by maximising the magnitude of the transverse magnetisation, or collecting signal from a larger sample.

The signal detected in a receive coil is approximately proportional to B_0^2 because the $|B_1^-| \propto B_0$ (§1.1.1.1) and the EMF induced in the receive coil by that B_1^- field is then also proportional to the Larmor frequency (γB_0). Therefore, approximately $SNR \propto B_0^2/B_0 = B_0$. Thus, it will be advantageous to use an MR scanner operating at the highest available B_0 field strength from a signal-to-noise perspective. This has indeed been borne out in the history of magnetic resonance.⁵

However, increasing the B_0 field brings major technical challenges and cost, in construction of the main magnet and RF hardware (§1.1.2), and can require experiment redesign.

Multiple averages of the same experiment improves the SNR. The signal increases linearly with the number of summed averages n , whilst only the power of the random Gaussian noise increases linearly with n . As a result, the noise voltage, proportional to the square-root of the power, only increases by $\sqrt{n}$. SNR therefore increases by $n/\sqrt{n} = \sqrt{n}$. Repeated averaging brings diminishing returns: increasing the SNR by 2 times only requires 4 averages, whilst a factor of 10 increase in SNR requires 100 averages – i.e. 100 times as long for the experiment! In MR a trade-off must be made between SNR and experiment duration.

Fast MR and the steady-state

Increasing the B_0 -field increases the magnitude of the equilibrium magnetisation vector, however it is the component of that vector in the transverse plane which gives rise to the signal. Whilst a 90° excitation pulse will place all the magnetisation into the transverse plane, assuming the initial condition is that of equilibrium, the finite T_1 time

of the system necessitates a delay of $\sim 5T_1$, before the same experiment could be repeated.

Ernst and Anderson established that the maximum SNR efficiency is not achieved using 90° pulses and waiting for full longitudinal relaxation.¹⁵ Rather, higher SNR per unit time may be achieved with an experiment where excitation and reception is repeated at intervals, known as the repetition time T_R , less than the $5T_1$ time required for full longitudinal relaxation of the species being investigated.

A repeated experiment run under the conditions $\theta \leq 90^\circ$, $T_R < 5T_1$ and $T_R \gg 5T_2$ will rapidly approach a steady state, where the recovery of the longitudinal magnetisation during T_R is exactly balanced by the transfer of some of the longitudinal magnetisation to the transverse plane by the excitation pulse (Figure 1.6b). The signal acquired from such an experiment is said to be “partially saturated”, and M_0 may be calculated from the partially saturated signal using “saturation correction”. The longitudinal magnetisation M_z before each repeated pulse at steady state is

$$M_z(\theta, T_R, T_1) = \frac{M_0(1 - e^{-T_R/T_1})}{1 - \cos(\theta)e^{-T_R/T_1}}, \quad [1.47]$$

and the signal (Figure 1.6c) of such an experiment is given by

$$S(\theta, T_R, T_1) = M_z \sin \theta. \quad [1.48]$$

The flip angle which gives the maximum signal for a set repetition time and T_1 is known as the Ernst angle (Figure 1.6a)

$$\theta_E = \arccos(e^{-T_R/T_1}). \quad [1.49]$$

The SNR (Figure 1.6d) of a steady-state experiment is proportional to

$$SNR \propto \sqrt{\frac{1}{T_R}} \frac{\sin(\theta) M_0 (1 - e^{-T_R/T_1})}{1 - \cos(\theta) e^{-T_R/T_1}}. \quad [1.50]$$

From Eq. [1.50] it can be seen that the conditions for optimal SNR are dependent on the T_1 of the observed species, and therefore must be calculated for a specific system. With multiple species, which have different values of T_1 , there is no single Ernst angle and the experiment must be optimised considering the desired SNR of all peaks.

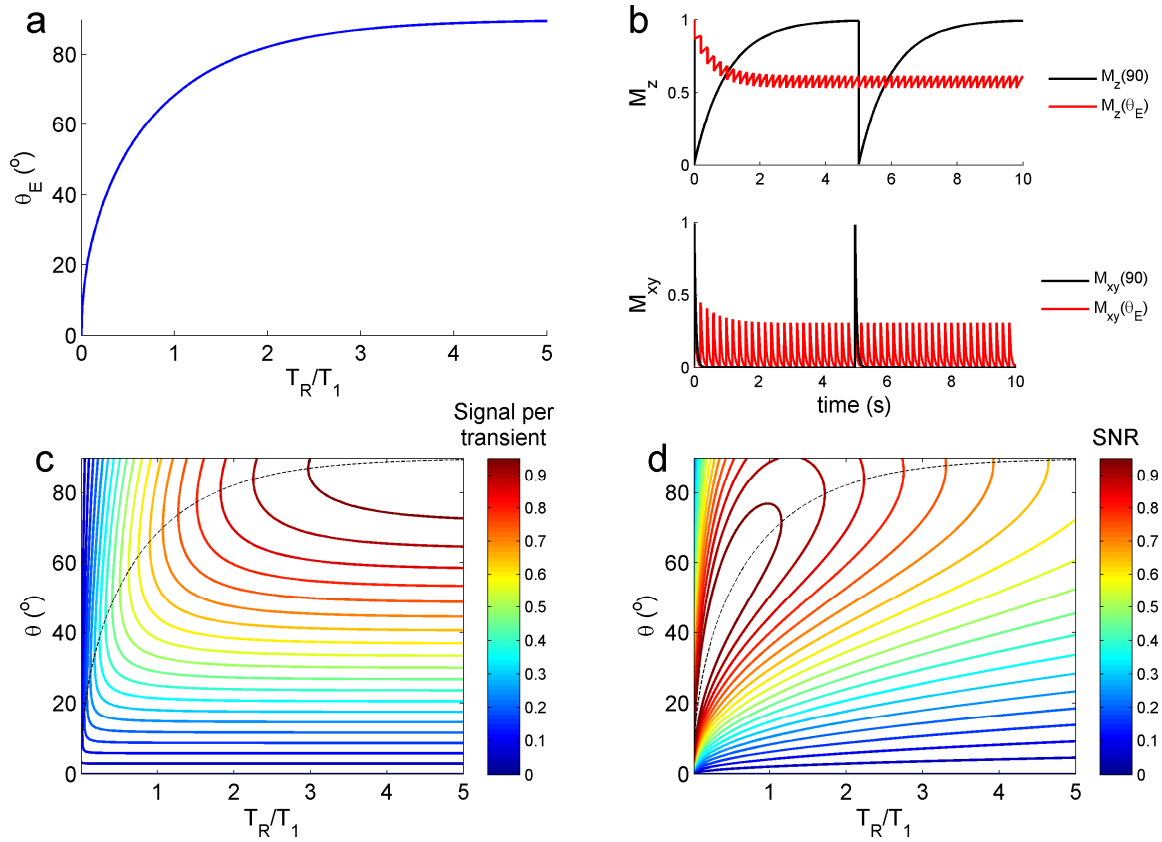


Figure 1.6 **a:** Ernst angle (flip-angle for maximum signal) as a function of T_R/T_1 (Eq. [1.49]). **b:** Contrasting magnetisation components (longitudinal $[M_z]$ = top, transverse $[M_{xy}]$ = bottom) of a ‘fully relaxed’ experiment (black) versus a steady state experiment (red). **c:** Signal per transient (repetition) as a function of T_R/T_1 and flip-angle (Eq. [1.48]). The dashed black line marks the Ernst angle and the maximum at each value of T_R/T_1 . **d:** SNR per unit time calculated using equation [1.50], normalised to the maximum.

1.1.2 Human MR Hardware

The hardware required to acquire MRS data from a human subject comprises four main components: the magnet, gradient coils, RF transmit and receive electronics, and

control and processing computers. An overview of each component is given in this section.

1.1.2.1 The magnet

The majority of human MR scanners use superconducting magnets due to their ability to create much stronger stable static fields than resistive or permanent magnets. The current highest field superconducting magnets for human MR are 10.5 T (CMRR, University of Minnesota) and 11.7 T (NIMH, Bethesda, MD, USA). Typically magnets for clinical MR are in the range of 1 T – 3 T, while ultra-high field scanners at 7 T are becoming more prevalent for research.

The superconducting magnet of an MR scanner is typically wound from NiTi alloy wires encapsulated in Cu wire for mechanical strength and high temperature conductivity. The NiTi core must be cooled to <9 K to become superconducting. Once the magnet is energised (“ramped up”), the magnetic field produced is very stable over time.

The homogeneity of the field is an important requirement for MRS. Ferromagnetic blocks of material (passive shims), placed at the time of installation, make the field as homogenous as possible, achieving variation of <5 ppm across a central spherical volume of diameter 45 cm, < 1 ppm in a 25 cm sphere, and <0.05 ppm in a 12 cm sphere, in a Siemens 7T Magnetom scanner. Additional shimming, homogenisation of the main static field, may be achieved using the gradient coils, for 1st order spherical harmonic corrections, or additional shim coils, for 2nd (and sometimes higher order) spherical harmonic corrections, to compensate for per-subject magnetic susceptibility differences.

The magnet is usually a horizontal hollow solenoid, with an internal “warm bore” of sufficient diameter to fit the gradient coils (and on a 1.5T or 3T scanner the birdcage RF transmit body coil which is not present on a Magnetom 7T), shim coils and, patient table mechanism whilst leaving room for the patient (~60 cm diameter), and surface RF coils.

1.1.2.2 Gradient coils

Gradient coils enable the scanner operator to produce a specified linear variation in the main field along an arbitrary direction on a microsecond timescale. Changing the main field in this fashion is one of the foundations of signal localisation in MRI and MRS.

The magnitude of the component along the z-axis (the direction of the field) of the static field $B_{0,z}$ may be varied independently along the z-, x-, and y-axes (away from, left to right, and bottom to top respectively if looking along the main field into the bore) using resistive electromagnetic coils.

$$\begin{aligned} G_x &= \frac{dB_{0,z}}{dx}, \\ G_y &= \frac{dB_{0,z}}{dy}, \\ G_z &= \frac{dB_{0,z}}{dz}. \end{aligned} \tag{1.51}$$

Modern gradient coils are optimized computationally, but originally were based on Maxwell pairs for the z-axis and sets of Golay (saddle) coils for the x- and y-axes.⁸ If a gradient is required in an axis not parallel to x, y or z, this is achieved by energising the G_x , G_y and G_z gradients with currents in suitable proportions, e.g. if a gradient of amplitude G is required at angle θ to the x-axis, then a gradient $G\cos\theta$ is applied in the x direction and $G\sin\theta$ in the y direction.

Gradients are optimised for peak amplitude, measured in tens of mT/m, for rapid switching, both in direction and amplitude – up to hundreds of mT/m/s, minimal RF interference, and linear behaviour. The rapidity of the switching is both hardware limited by the power required to generate the large currents required (~500 A), but also physiologically (§1.1.2.5).

1.1.2.3 RF transmission and reception

RF radiation is transmitted and received by inductively coupled ‘RF coils’. The type of RF coil chosen for a particular experiment depends on the desired magnitude and profile (shape and homogeneity) of the coil’s transmit B_1^+ and receive B_1^- fields. Note that while the coil never actually generates a B_1^- field, by the principle of reciprocity,^{9,16} the induced EMF in a coil (i.e. the coil’s sensitivity) caused by the magnetic field arising from the precession of transverse magnetisation, B_1^- , is proportional to the RF field generated at that point in a hypothetical experiment where the receive coil is imagined being used as a transmitter. Coils come in a wide array of designs, but may be roughly split into two broad categories: surface and volume coils.

Surface coils rest on the surface of the subject or object being scanned. One simple form of surface coil is a coil of wire broken at one point with a capacitor inserted in series. The inductance of the wire and the capacitor form a resonant circuit, which may be tuned, using the capacitor, to the resonant frequency of the nuclei being scanned. The impedance of the coil is matched to the impedance of the RF amplifier (which has an impedance of 50 Ω) using a second, matching capacitor, in series with the 50 Ω transmission line leading to the preamplifier (Figure 1.7a).

The usable field produced by a surface coil is small, with the field produced dependent on the size and shape of the coil element, but as a rule of thumb surface coils are sensitive up to a depth equal to the coil diameter. The transmit and receive fields are both inhomogeneous, decreasing rapidly with distance from the elements. The SNR of the coil is very high for spins proximal to the coil. Small surface coils may therefore be used to localise the MR signal in some experiments, the total signal received is dominated only by signal from the high SNR regions close to the coil.

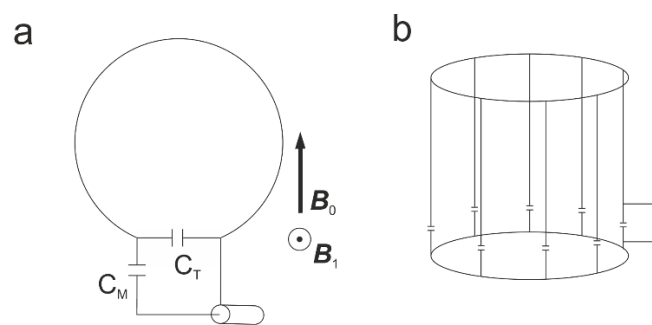


Figure 1.7 **a:** Diagram of a simple surface coil with two variable capacitors. Normally most of the capacitance of the coil (C_T) is distributed around the loop in fixed capacitors, with a single variable capacitor for tuning. **b:** Diagram of a low-pass birdcage volume coil. The object being imaged, which may be the whole body, is placed at the centre of the cage.

Volume coils are large enough to enclose either the whole body, or an extremity, such as the head or a limb. They produce B_1 -fields which are comparatively homogenous over the volume they enclose, though the magnitude of the field is lower than the fields produced close to a surface coil.

The most common volume coil design is a 'birdcage coil', consisting of conductors, arranged around a cylindrical former, running in the z direction, which are arranged to produce a cosine current variation around the coil and rings (Figure 1.7b). Birdcage coils are normally driven at two points 90° apart, generating circularly polarised B_1^+ directly.

Coils may be used for just transmission or reception, or both. If a single transmit-receive (Tx/Rx) coil is used the receiver electronics must be blanked when the coil is transmitting, using a transmit-receive (T/R) switch. The transmit EMF is on the order of kilovolts, whilst the receive preamplifier is designed to detect microvolt signals.

The RF transmit signal is created by a “local oscillator” operating at a frequency close to the Larmor frequency, and shaped into pulses by a mixer and waveform generator. The signal is amplified to thousands of watts by an RF power amplifier (RFPA). Off-resonance frequency shifts are achieved by the scanner’s “modulator” which phase shifts the signal on a ns timescale using a numerically controlled oscillator.

The small receive signal from each receive channel is preamplified typically within the coil housing, and then mixed with the local oscillator signal, and digitized using MHz analog to digital converters before decimation, digital filtering and down-sampling.

1.1.2.4 Scanner control

On the Siemens platform, the scanner hardware components (RF, gradients, active shimming) are controlled by a number of real-time computers controlled in turn by a console computer in the control room. The real-time elements ensure the accurate timing (locked to a 10MHz clock) between the gradient and RF waveforms required for high-resolution MRI.

The digitized MR signals are processed by an ‘image reconstruction’ computer.

1.1.2.5 Limitations and safety considerations

MRI and MRS are used to image human subjects. To guarantee the subject’s safety, scanners impose limits on the use of the hardware.

The most obvious risk for subjects undergoing an MR examination and operators is that of ferromagnetic objects being drawn into the scanner by the main static field. This safety concern is mitigated by careful control of access to the scanner room and screening of subjects.

Heating caused by RF fields

The high power RF fields used for excitation of the spins induces eddy currents in the subject due to the conductivity of tissue. Resistance to these currents then causes heating of the tissue. Power deposition, in the form of heating of the subject's body, is measured by the specific absorption rate (SAR), defined by the IEC as the power delivered to a 10g volume of tissue in W kg^{-1} .¹⁷

SAR for electromagnetic energy may be calculated from the electric field in the subject:

$$SAR = \frac{\sigma |E|^2}{2\rho}, \quad [1.52]$$

where σ is the sample electrical conductivity, E is the electric field, and ρ is the sample density. It has been demonstrated that the SAR is approximately proportional to the square of the frequency of the RF field; therefore more heating is produced at higher static field strengths.¹⁸

In the UK, the International Electrotechnical Commission (IEC) impose maximum doses as a proxy for regulating the allowable energy deposition in an MR exam. The limits are imposed as maximum average values over 6 minute and 10 s intervals. There are different limits for whole-body SAR and for local SAR, and depending on anatomical area (head, trunk or extremity).

The scanner predicts a sequence's SAR based on the power of the sequence RF, time delays, the subject's bodyweight and a calibrated coil scaling factor (the "k-factor"). RF pulses, and sequence repetition times, must be designed to work within the relevant SAR limits. Particular care must be taken if conductive items (internal implants or external items e.g. ECG leads) are in contact with the subject. Inductive heating of these items can cause serious burns if they happen to resonate at the transmitted RF frequency.

SAR Limit	Whole-Body Average	Head Average	Head, Trunk Local	Extremities Local
Normal	2 W/kg (0.5°C)	3.2 W/kg	10 W/kg	20 W/kg
First level	4 W/kg (1°C)	3.2 W/kg	10 W/kg	20 W/kg
Second level	4 W/kg (>1°C)	>3.2 W/kg	>10 W/kg	>20 W/kg
<i>Localized heating limit</i>	<i>39°C in 10 g</i>	<i>38°C in 10 g</i>		<i>40°C in 10 g</i>

Table 1.1 IEC guidelines on 6 minute SAR and heating in human MRI studies.¹⁹

Gradient magnetic fields

Rapid switching of the field gradients may induce voltages in tissue by Faraday's law.

The current induced in a loop of tissue is dependent on the rate of change of the magnetic field ($\frac{dB}{dt}$), the conductivity and the size of the loop. Currents can cause stimulation of the peripheral nerves which cause involuntary twitching and pain for the subject. Limits of 20 T s^{-1} (>120 μs ramp) or $2400/t_{\text{ramp}} \text{ T s}^{-1}$ (<120 μs ramp) on gradient switching are imposed by the IEC.

B_0 inhomogeneity and T_2^*

While macroscopic B_0 -inhomogeneity may be somewhat addressed by active shimming of the field on a per-subject basis, some inhomogeneity occurs at a microscopic, sub-

voxel level, and is impossible to correct by B_0 -shimming. Inhomogeneity may be caused by local magnetic susceptibility changes across a range of tissue-tissue or air-tissue interfaces as well as contrast agents or implants.

The inhomogeneity causes spins with a range of precession frequencies to be present within a single acquisition volume, which in turn causes a dephasing of transverse magnetisation, in addition to spin-spin T_2 relaxation. The empirical transverse magnetisation decay is characterised by a second T_2' time constant, which when combined with the intrinsic T_2 relaxation, produces a single, approximately mono-exponential decay T_2^* :

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'}. \quad [1.53]$$

1.2 In vivo cardiac phosphorus magnetic resonance spectroscopy

All naturally occurring phosphorus is the ^{31}P isotope, which has spin $I = \frac{1}{2}$ and is therefore MR-active. The gyromagnetic ratio of ^{31}P is $\gamma_{^{31}\text{P}} = 108.291 \times 10^6 \text{ rad s}^{-1} \text{ T}^{-1}$,

$\gamma_{^{31}\text{P}}/2\pi = 17.235 \text{ MHz T}^{-1}$, which is 2.47 times lower than ^1H (42.76 MHz T^{-1}). The

Larmor precession frequency of an unshielded ^{31}P nucleus is 51.705 MHz at 3.00 T and 120.645 MHz at 7.00 T.

Phosphorus magnetic resonance spectroscopy is used to investigate the metabolism of a number of different organs in the human (and mammalian) body. These include: brain, skeletal muscle, liver and heart. This work focuses on the investigation of myocardial metabolism, though the very similar metabolism of skeletal muscle, located

more superficially in the human body, is often used to explore and validate techniques before applying them in the heart.

1.2.1 Cardiac anatomy

The human heart is sited inside the chest between the lungs in a roughly central (left to right) position, with its centre of mass approximately at a depth of 8 cm from the skin of the anterior chest wall in lean males. The heart must pump blood around the circulatory system continuously, for the entire lifespan of the person, supplying oxygen and nutrients to all of the cells in the body.

The healthy human heart, shown in Figure 1.8, comprises four chambers: the left and right atria (LA & RA) and the left and right ventricles (LV & RV). The chambers are enclosed by the myocardium, the muscle of the heart. Deoxygenated blood initially enters the heart through the inferior and superior Vena Cava (from the body), flowing into the right atrium during diastole. The blood then flows through the tricuspid valve into the right ventricle. Simultaneously, oxygenated blood enters the left atrium from the pulmonary vein (from the lungs), and then flows into the left ventricle through the mitral valve. During systole, the myocardium contracts, and the blood in both the right and left ventricles is ejected from the heart into the pulmonary artery (to the lungs) and the aorta (to the body) respectively. This process, the cardiac cycle, then starts again.

Note that since the left ventricle must pump the blood around the entire body rather than just the lungs the LV wall myocardium is much thicker (6-10 mm) than the RV wall (3-5 mm).

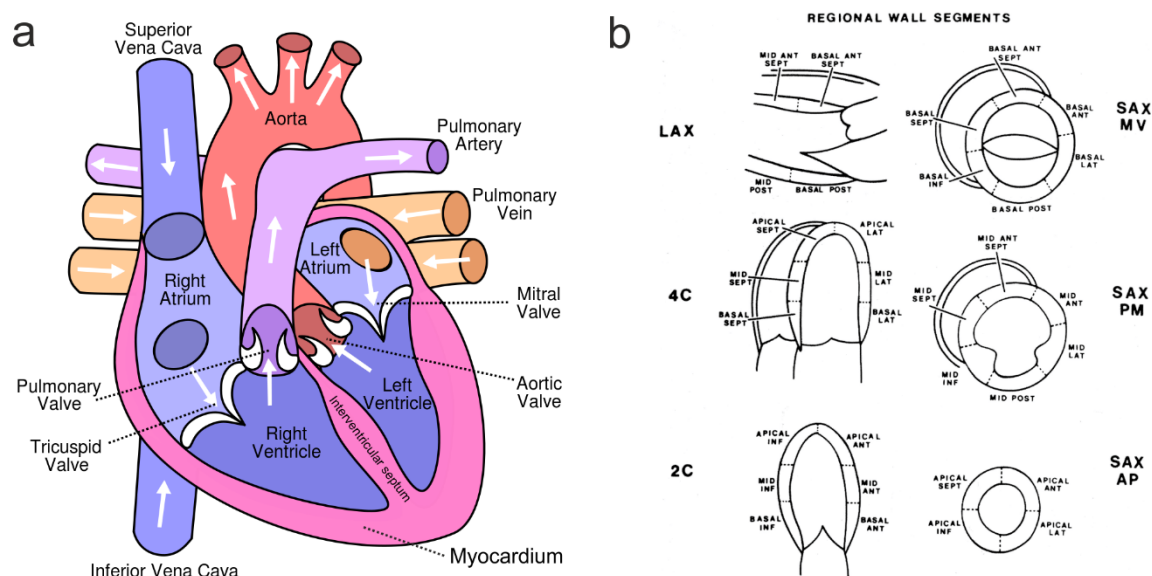


Figure 1.8 **a:** Cutaway diagram of the human heart. The myocardium, the muscle surrounding the two ventricles, pumps the blood out of the heart. ^{31}P -MRS predominantly detects signal arising from the myocardium. The interventricular septum, part of the myocardium which separates the two ventricles is the usual volume of interest for three dimensionally localised ^{31}P -MRS, as it is surrounded only by blood, remote from contaminating skeletal muscle, and experiences a relatively homogenous B_0 field.

b: Nomenclature and locations of myocardial regions of the human heart, as defined by the American Heart Association 17 segment model.²⁰ The diagrammatic views mirror those views collected as ^1H -MRI localisers at the start of a spectroscopy study. Acronyms: LAX indicates long axis; 4C, 4-chamber; 2C, 2-chamber; SAX MV, short-axis mitral valve; SAX PM, short-axis papillary muscle; SAX AP, short-axis apex; ANT, anterior; LAT, lateral; SEPT, septum; POST, posterior; INF, inferior.

a is modified from [https://commons.wikimedia.org/wiki/File:Diagram_of_the_human_heart_\(cropped\).svg](https://commons.wikimedia.org/wiki/File:Diagram_of_the_human_heart_(cropped).svg), and used under the CC BY-SA 3.0 licence. The original image has been modified by adding additional labels, and may be freely used under the terms of the same licence. **b** is reused with permission; from reference 20. Copyright American Heart Association 2002.

The complex motion of the cardiac cycle, with the atria entering systole before the ventricles, is controlled spontaneously by an independent nervous system within the heart governed by the sino-atrial node. The sino-atrial node alters the heart rate, which averages 40 – 100 beats per minute at rest, in response to signals from the involuntary nervous system and circulating hormones.²¹

The anatomy of the myocardium can be described using the American Heart Association 17 segment model.²⁰ Along the 'long axis' (LA), which runs from the apex to the centre of the outflow tracts, the myocardium is divided into apical, mid, and basal portions. Within each of these portions, when viewed in the short axis (SA) – which defines a

plane perpendicular to the long axis, the heart is further sub-divided. The myocardium that separates the two ventricles is called the interventricular septum. Localised spectroscopy of the heart usually targets the mid interventricular septum, as this is representative of cardiac tissue, is in a region of uniform magnetic field, and is remote from other tissues.

1.2.2 Myocardial “high-energy phosphate” metabolism

The myocardium, is made up of striated myocytes (muscle cells). The involuntary contraction of this muscle pumps blood around the body. To enable the constant and critical workload of the myocardium, the myofibrils (organelles containing actin and myosin filaments which actually perform the contractile work within the myocytes) must be constantly supplied with energy, in the form of the metabolite adenosine triphosphate (ATP). The heart requires a throughput of 6 kg of ATP per day, which is higher than any other organ.²²

While cardiac metabolism is complex, the process by which ATP is produced at the myofibrils may be broken down into three distinct stages:²³

1. *Substrate utilization.* Substrates, free fatty acids, glucose and ketone bodies, originating from food and present in the blood, are taken up by the cell and broken down by beta-oxidation and glycolysis. The resultant intermediates enter the Krebs cycle, in the mitochondria, as acetyl coenzyme A. The Krebs cycle produces the reduced form of nicotinamide adenine dinucleotide (NADH).
2. *Oxidative phosphorylation.* NADH is used to produce a proton electrochemical gradient across a membrane which drives the oxidative addition of inorganic

phosphate (P_i) to adenosine diphosphate (ADP) yielding ATP. This

phosphorylation step also takes place in the mitochondrion and is catalysed by the enzyme ATP synthase.

3. *ATP transfer and consumption.* Oxidative phosphorylation produces the required ATP in the mitochondria, which are distributed throughout the myocytes. ATP is required throughout the bundles of myofibrils and passive diffusion is not sufficient to achieve the rate of transfer required. Therefore the enzyme catalysed creatine-kinase energy shuttle is used to both transport ATP to the myofibrils and buffer the ATP concentration at the site of use, via conversion to phosphocreatine (PCr).²⁴ The ATP is subsequently used for the actuation of the myosin elements, enabling muscle contraction.

Recent work using high-resolution microscopy has found that the mitochondria themselves are involved in the distribution as well as the generation of energy.²⁵

Mitochondria, in striated myocytes, were found to create extensive conduction pathways, with energy distribution primarily achieved with conductance rather than diffusion of ATP or oxygen. The authors conclude that the highly optimised creatine-kinase shuttle step brings a further, small but significant, advantage in muscle performance.

1.2.2.1 The creatine-kinase energy shuttle

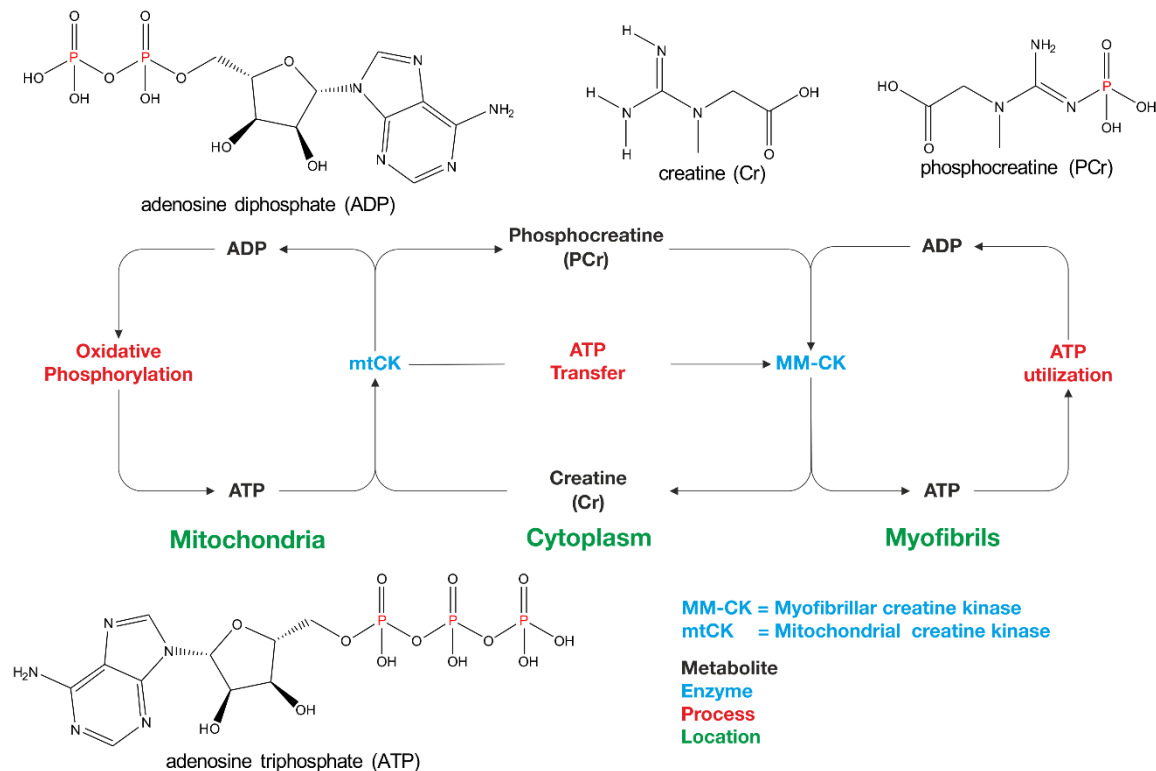


Figure 1.9 Diagram of the “creatine kinase energy shuttle”. The shuttle takes place in two distinct locations: the first part, the initial generation of PCr from ATP takes place in the mitochondria of the myocyte; the second part, the conversion of PCr back to ATP happens throughout the myofibrils close the point of use. Different isoenzymes are responsible for the conversion at each site. The skeletal chemical structures of the metabolites are shown with phosphorus nuclei highlighted in red.

The creatine kinase (CK) energy shuttle plays a key role in transporting ATP to the myofibrils and in buffering the resultant ATP concentration against increased demand.

At physiological pH the ATP molecule exists as a dibasic ion coordinated with a single magnesium (II) ion, the charge and size of the complex slows its rate of diffusion.

Therefore, in the mitochondria, the enzyme creatine kinase (CK_{mito}) catalyses the transfer of a phosphate group from ATP to creatine (Cr) to produce PCr and ADP.

Creatine is produced in the liver and is actively taken up by the cell, across the plasma membrane against a large concentration gradient.²² PCr then diffuses from the mitochondria to the myofibrils.²⁶ At the site of use, within the myofibrils, myofibrillar creatine kinase (CK_{MM}) catalyses the reverse reaction to produce ATP and Cr from ADP

and PCr. The equilibrium constants of the system are such that approximately two thirds of “total creatine” is present as PCr, forming a reserve buffer at the myofibrils, and such that PCr is preferentially depleted over ATP, keeping the concentration of ATP stable in the myofibrils.²⁷

1.2.3 The ^{31}P -MRS spectrum

The human cardiac ^{31}P spectrum comprises seven generally observable peaks and a number of unresolved regions (Figure 1.10). The most prominent peak, which also acts as an internal chemical shift reference with an assigned chemical shift of 0.00 ppm, is the singlet arising from the single phosphate group in PCr.

Upfield of the PCr peak, and in order of decreasing chemical shift, are the multiplet peaks for the γ - (terminal), α - and β -phosphate groups of ATP; located at -2.48 ppm, -7.52 ppm and -16.26 ppm respectively. The γ - and α -groups are split into doublets (two equal amplitude peaks) by the single adjacent β -phosphorus nucleus. In turn the β -phosphate peak is split by the two others into a triplet (1:2:1 amplitude peaks). The homonuclear scalar couplings are $^2J_{\alpha\beta} = 15.8 \text{ Hz}$ and $^2J_{\beta\gamma} = 16.0 \text{ Hz}$. The chemical shifts and scalar couplings of the ATP peaks are sensitive to the magnesium ion (Mg^{2+}) concentration.¹ The splitting of the ATP peaks is generally only observed in-vivo at 1.5T, while at higher fields B_0 inhomogeneity broadens the peaks, causing coalescence of the multiplets.

Downfield of PCr the phosphodiester (PDE) glycerol 3-phosphorylethanolamine and glycerol 3-phosphorylcholine create, typically broad and poorly resolved, peaks at 2.67 ppm and 3.20 ppm. The region from 5-7ppm contains three groups of peaks: the two phosphate groups of 2,3-diphosphoglycerate (2,3-DPG), arising from the blood

pool, dominate in cardiac spectra, obscuring the phosphomonoesters (PME) [6-7 ppm] and the two, intra- and extra-cellular, inorganic phosphate (P_i) peaks [~ 5 ppm]. Though in other tissues, in the absence of a large quantity of blood and therefore 2,3-DPG, the PME and P_i peaks are readily resolvable.

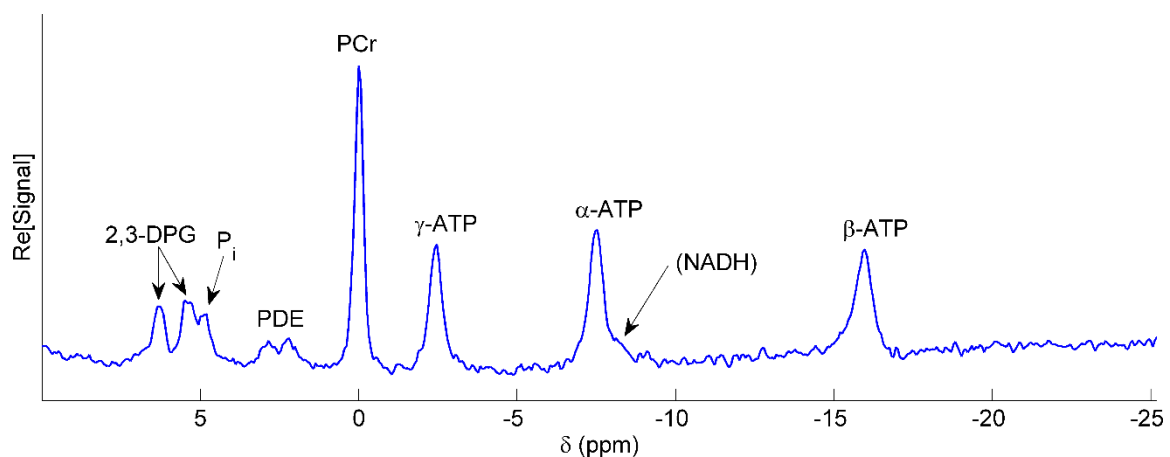


Figure 1.10 Cardiac ^{31}P spectrum acquired from the myocardium of a healthy volunteer at 7T.

Additional peaks are known to be present in the spectrum but are typically unobservable: ADP has a low concentration in the healthy myocardium and the α -ADP and β -ADP peaks lie beneath the larger γ - and α - peaks of ATP; NADH is observed upfield of the α -ATP peak in high SNR acquisitions as a small broad peak.

1.2.4 Typical measurements

^{31}P -MRS gives the researcher access to a number of directly measurable quantities and derived measures of myocardial metabolism. The method is completely non-invasive, may be localised to specific tissue volumes and has a temporal resolution on the order of minutes. The spectrum predominantly contains signals from metabolites present in the creatine kinase energy shuttle (§1.2.2.1), thus research has focused on the measurement of the PCr energy buffer created by the shuttle and the throughput of the CK shuttle.

1.2.4.1 PCr/ATP ratio

A commonly reported parameter is the ratio of the PCr peak amplitude over the ATP peak amplitudes, known as the PCr/ATP ratio. The ratio may be calculated using the amplitude from just one, or the average of two or three ATP peak amplitudes.

If the spectrum is acquired with $T_R \geq 5T_{1,\max}$, i.e. allowing full longitudinal relaxation of the metabolites, for all the metabolites used, the PCr/ATP ratio is equivalent to the ratio of the metabolite concentrations. However, if the spectrum is acquired with a $T_R < 5T_{1,\max}$ the peak amplitudes must be corrected for partial saturation of the signals (§1.1.1.8, Eq.[1.48]), which requires knowledge of the flip angle achieved at the chemical shift of each metabolite, and each metabolite's T_1 , to yield a “saturation-corrected PCr/ATP” which is equivalent to a ratio of metabolite concentrations. In the absence of this information, PCr/ATP ratios may still be compared across scans and subjects providing consistent acquisition parameters (flip-angle and T_R) and minimal changes in metabolite T_1 . Saturation correction does not generally account for the effect of chemical exchange of PCr and γ -ATP (see Chapter 5).²⁸

Bounds on the accuracy of the measured ratio may be readily calculated (see Chapter 3). The accuracy is typically improved by the inclusion of more than one ATP peak, but care must be taken to ensure each ATP has been saturation corrected independently using the appropriate (different) T_1 values, so as not to introduce a measurement bias.

Typical values of the saturation-corrected PCr/ATP ratio reported in the literature are summarised in Table 1.2.

Ref	1 st Author, Year	Field strength (T)	Number of subjects	Corrected* PCr/ATP	Inter-subject SD
29	Bottomley, 1991	1.5	19	1.76	0.22
30	Deroos, 1992	1.5	9	1.65	0.26
31	Smith, 2006	1.5	14	1.90	0.30
32	Tyler, 2008	1.5	6	1.76	0.38
33	Bottomley, 2009	1.5	15	1.87	0.45
32	Tyler, 2008	3	6	1.62	0.22
34	Tyler, 2009	3	20	2.11	0.38
4	Rodgers, 2014	3	9	1.70	0.50
35	Bashir, 2014	3	22	1.94	0.15
36	Levelt, 2016	3	20	2.05	0.34
37	Menon, 1992	4	6	1.80	0.10
38	Hetherington, 1995	4	10	1.80	0.32
4	Rodgers, 2014	7	9	2.10	0.30
Average[†]				1.85±0.16	0.30±0.11

Table 1.2 Selection of reported human PCr/ATP ratios at various field strengths. * PCr/ATP values are saturation corrected (if required) and blood contamination corrected if provided by the study.

[†]Average values are the inter-study mean (± standard deviation).

1.2.4.2 Absolute quantification of metabolite concentrations

The PCr/ATP ratio is typically used under the assumption that the PCr concentration

changes whilst the ATP concentration is constant.³⁹⁻⁴¹ Whilst the assumption is

reasonable – as the workload rises in a healthy heart PCr concentration decreases, ATP

concentration is steady and free ADP rises – at high workloads the ATP concentrations

decrease and using the PCr/ATP ratio reveals no information about baseline ATP

concentrations in different patient groups.²² Measuring the ‘absolute concentration’

(i.e. the concentration calibrated to recognised units e.g. mol L⁻¹ or mol kg wet weight⁻¹)

of the metabolites overcomes these limitations. Using the MR measured peak

amplitude and additional information about the system it is possible in principle to

calculate the concentration of the metabolite within the target volume. Absolute

concentration measurements enable independent and simultaneous measurements in

changes of both PCr and ATP concentrations, and are directly comparable across different patient groups and acquisition protocols.⁴²

However, the challenges associated with concentration measurements are significant. Flip-angles must be calculated or measured and T_1 values known to calculate M_0 after appropriate saturation correction. Then the receive sensitivity (B_1^- , preamp gain factor, etc.) of the receive coil for the volume that the signal came from must be known. The resulting corrected concentrations are then calibrated against a primary standard of known concentration, for which the same parameters must also be determined. Additional corrections for sequence dead times and the size of the localised volume may be made. The full equation as given in reference 43 for the concentration of species p , as calculated from an external reference r , is

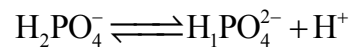
$$[p] = \frac{S_p \cdot [r] \cdot V_r \cdot \psi_r \cdot \phi(B_r) \cdot F(T_{1,r}, \theta_r, T_R) \cdot E(T_{2,r}^*, T_d)}{S_r \cdot V_p \cdot \psi_p \cdot \phi(B_p) \cdot F(T_{1,p}, \theta_p, T_R) \cdot E(T_{2,p}^*, T_d)}, \quad [1.54]$$

in which S is the integrated MR signal, V is the localised volume contributing to the spectrum, ψ is the receive coil sensitivities in the contributing volume, ϕ accounts for signal loss due to phase variation in the volume, F is the saturation factor and E accounts for the loss of signal due to the dephasing of the transverse magnetisation between excitation and signal reception.

1.2.4.3 pH measurement

³¹P-MRS is able to provide a measurement of the intracellular pH. Under extreme workload or in the presence of pathological ischemia, the pH of myocardium drops from its normal value (~ 7.1), in a process known as acidosis.⁴⁴ This is the result of lactic acid being produced in the cell by anaerobic respiration.

The chemical shift of the free inorganic phosphate species is pH dependent.⁴⁵ The sensitivity to pH arises as the alcohol groups (OH) are protonated and deprotonated at low and high pH respectively. Phosphoric acid H_3PO_4 and each of its possible bases: H_2PO_4^- , $\text{H}_1\text{PO}_4^{2-}$, PO_4^{3-} predominately exist at a certain pH, and have distinct chemical shifts. With the addition, or loss, of protons from the molecule the shielding constant changes, in turn changing the chemical shift. For example, the equilibrium that dominates at physiological pH (~ 7) is the one between the mono and dibasic phosphate salts:



in which $\delta_A = 5.7$ ppm, $\delta_{\text{HA}} = 3.23$ ppm. The exchange between the two forms is fast on the MR time scale, therefore coalescence to a single resonance is observed, with its chemical shift dependent on the relative concentrations of the protonated and deprotonated species. pH may subsequently be calculated using a modified Henderson-Hasselbach relationship:

$$pH = pK_A + \log_{10} \left(\frac{\delta - \delta_{\text{HA}}}{\delta_A - \delta} \right), \quad [1.55]$$

where δ is the observed chemical shift of the coalesced phosphate peak and pK_A is the logarithm of the acid-base equilibrium constant.⁴⁶ All chemical shifts must be referenced to a peak at a fixed chemical shift e.g. PCr. In principle, therefore, ^{31}P -MRS can provide a non-invasive measurement of pH in vivo.

Whilst the P_i peak is clearly visible in skeletal muscle, brain and liver spectra, in the heart the 2,3-DPG peaks, which arise from the blood in the left and right ventricular blood pools overlap the P_i peak and obscure its position.

1.2.4.4 Creatine kinase forward rate constant and total ATP flux

The pseudo first-order rate constants of the creatine kinase energy shuttle may be determined using magnetisation transfer techniques. Measurement of both the forward rate constant of the shuttle and the PCr concentration enable the calculation of the ATP flux, i.e. the rate of creation of ATP, at the myofibrils. Chapters 5, 6 and 8 contain an extensive discussion on the measurement of these parameters and their uses in clinical studies.

1.2.5 Signal contamination

Acquisition without localizing to a specific volume, or the imperfections in MR localization methods (Chapter 2), may lead to spectra that comprise a sum of signals from more than one anatomical region or tissue type. This is a particularly pressing consideration in the heart, because it lies close to a number of other tissues which give similar, but different, ^{31}P -spectra.

Skeletal muscle is found in the chest wall superficial to the heart. A skeletal muscle spectrum contains PCr, ATP, PDE and P_i peaks. However the concentrations of these metabolites, and hence PCr/ATP ratio is different to cardiac spectra. The normal PCr/ATP ratio is 4–5 in skeletal muscle.⁴⁷ Without any distinguishing peaks, low or medium level contamination of a cardiac spectrum by skeletal muscle signal is hard to detect, and is prone to introduce a bias to any measured PCr/ATP ratio. Skeletal muscle is typically closer to the receive coil than the heart, which raises the sensitivity of the experiment to signal from the skeletal muscle compared to the myocardium. RF “saturation bands”, or additional hardware is often used to suppress these signals arising from skeletal muscle.⁴⁸

The liver is inferior and to the right of the heart and is further from the heart than chest wall skeletal muscle. The liver is generally more remote from surface transmit and receive coils, reducing the chance of significant contamination. However, if volume excitation or reception is used, then signal from the liver might become a significant source of contamination. There are more peaks present in a liver ^{31}P -spectrum, particularly in the PDE and PME regions of the spectrum. The three peaks of ATP and P_i are apparent, whilst PCr is absent.⁴⁹ Contaminating signal from the liver introduces a bias in the opposite direction to that introduced by skeletal muscle i.e. lowering the ratio measured.

1.2.5.1 Blood Correction

The proximity of the blood in the left and right ventricular pools to the septum, the usual volume of interest, results in non-negligible contamination of cardiac spectra with ^{31}P signal arising from blood borne metabolites.

Erythrocytes (red blood cells) contain 2,3-DPG, ATP and P_i , whilst the serum contains P_i .⁵⁰⁻⁵² The 2,3-DPG is resolvable from most other peaks in the spectrum and thus offers a quantifiable measure of contamination. The ATP peaks of the blood lie directly under those arising from the myocardium and are therefore indistinguishable.

“Cardiac” spectra contaminated by signal from blood will appear to have a lower PCr/ATP ratio than the true value in the myocardium. Through dedicated ex vivo spectroscopy of blood, it has been established that in human blood the ratio

$M_0^{\text{ATP}} / M_0^{\text{DPG}} \approx 0.30$.^{50,51} Therefore, when processing spectra in vivo, 30% of the

corrected DPG amplitude (the mean of both DPG peaks) is subtracted from the ATP amplitudes to remove this effect.

However, due to the flowing nature of blood, it is likely that blood does not experience the same steady-state conditions as the myocardium, e.g. the flip-angle is different in the right and left ventricles and the majority of blood refreshes in the healthy heart every heartbeat, which suggests that the same saturation correction may not apply to the DPG and the portion of ATP arising from blood. This is an unresolved problem in cardiac ^{31}P -MRS.^{53,54}

1.3 Heart failure and ^{31}P -MRS

Heart failure (HF) is characterised by a complex collection of symptoms that indicate the heart is impaired in its ability to circulate blood in the body. The causes of heart failure are varied and may be structural or functional.⁵⁵ Heart failure is common; 1% of the UK population and 2% of the USA population are diagnosed with heart failure.^{2,56} In the UK, the 5-year survival rate of patients with heart failure was about 60%.⁵⁷ The incidence of heart failure is expected to increase as more patients survive an initial acute myocardial insult, and as a result of an ageing population.²²

Heart failure progresses by gradual myocardial remodelling. Although this is initially beneficial, in the long term it results in a less efficient pumping process. Remodelling has a positive feedback loop: a less efficient heart results in more extensive remodelling, which in turn decreases the efficiency further.⁵⁸

1.3.1 Metabolic derangement as a model for heart failure

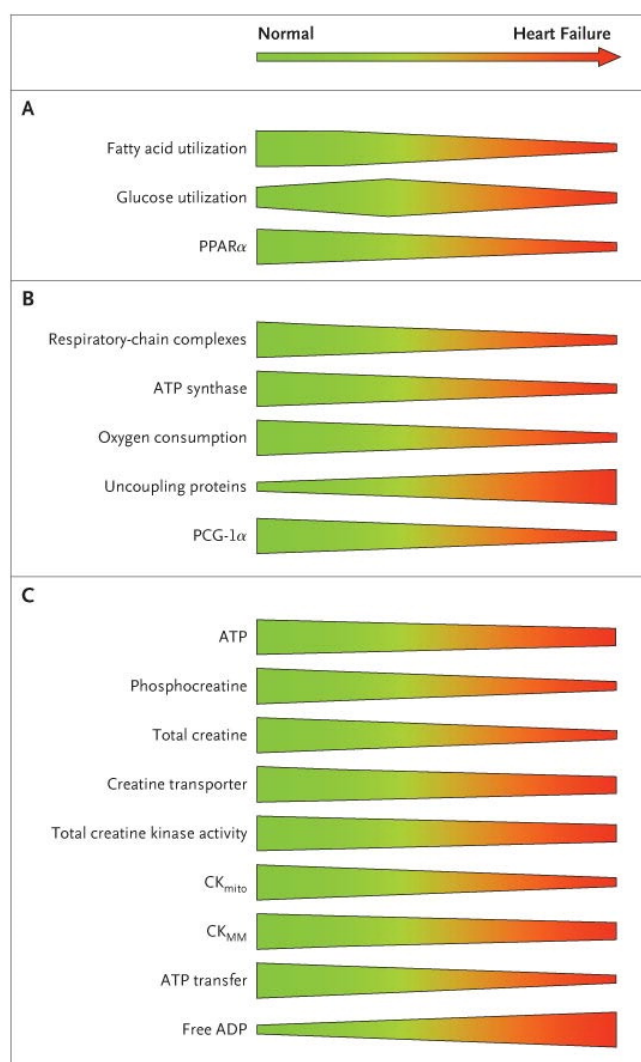


Figure 1.11 Changes in substrate utilization (A), oxidative phosphorylation (B), and high-energy phosphate metabolism (C) in patients with heart failure. ^{31}P -MRS is sensitive to the ATP, phosphocreatine, creatine kinase activity and ATP transfer levels in C. The decrease in PCr/ATP ratio seen in heart failure is a result of the PCr levels falling more rapidly than the ATP levels. However as is evident in this figure absolute concentration measurements are more sensitive to the dynamic levels of both metabolites. Measurement of ATP transfer and CK activity are described in detail in Chapter 5. Acronyms: PPAR α = peroxisome proliferator-activated receptor α , PCG-1 α = PPAR α coactivator 1 α , CK_{mito} = mitochondrial creatine kinase isoenzyme, and CK_{MM} = myofibrillar creatine kinase isoenzyme. *Reproduced with permission from 22, Copyright Massachusetts Medical Society.*

The idea that heart failure is caused by ineffective cardiac metabolism has been prevalent in the literature for decades.^{27,59} The “energy starvation hypothesis” is a key area of current research. In short, this hypothesis states that “the failing heart is an

engine out of fuel” and therefore suggests that supporting or supplementing the energy supply to a failing heart may allow it to recover to some extent.²² This theory is supported by the observation that “energy-sparing” treatments, such as ACE inhibitors and beta-blockers, improve patient prognosis.⁶⁰

³¹P-MRS enables the non-invasive investigation of whether metabolism causes heart failure.³ The only alternative is biopsy, which is painful and damaging to the patient, and potentially inaccurate due to the rapid continuing metabolism of ATP and PCr in excised tissue.

1.3.2 Types of heart failure

The study of heart failure in humans using ³¹P-MRS has predominately focused on diffuse cardiomyopathy, specifically hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). However, it is becoming clear that metabolic derangement is a major contributor to many other types of heart failure, such as that secondary to type II diabetes mellitus (T2DM) or atrial fibrillation (AF).

1.3.2.1 Dilated cardiomyopathy

Dilated cardiomyopathy is characterised by: a dilation of the ventricles and thinning of the myocardium (Figure 1.12a), impaired cardiac function (reduced ejection fraction), myocardial fibrosis, with a derangement of the energy metabolism a key etiology. DCM is thought to be a broad categorisation covering symptoms common to a group of non-ischaemic metabolic and genetic pathologies.^{22,61}

³¹P-MRS studies of human subjects with DCM have shown that the PCr/ATP ratio is a better predictor of mortality than the New York Heart Association class.⁶² A PCr/ATP ratio of less than 1.6 was found to correlate with a significant rise in mortality as a result

of DCM. The PCr/ATP ratio has consistently been found to be decreased in DCM patients, both with and without preserved cardiac function, compared to control subjects.⁶³ Three studies have also demonstrated that the decreased PCr/ATP ratio observed in DCM patients can recover after treatment.⁶⁴⁻⁶⁶

1.3.2.2 Hypertrophic cardiomyopathy

HCM is a common inherited heart disease. The disease has a diverse range of phenotypes, and clinical presentations, including “patients” who are genotype-positive but phenotype-negative. The defining, severe, late-stage complication of HCM is hypertrophy (i.e. thickening) of a region of the myocardium, which can obstruct the ventricular outflow tract (Figure 1.12c).

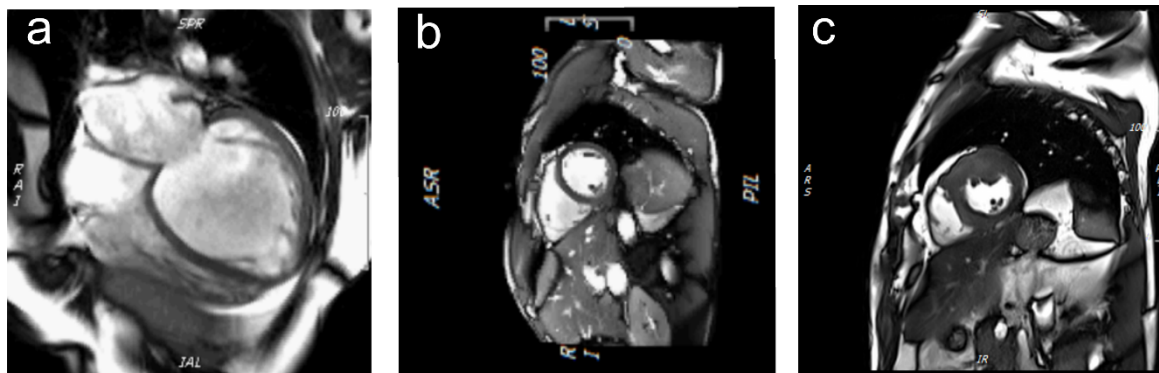


Figure 1.12 **a:** Horizontal long axis, or 4 chamber, view of the heart of a patient with DCM. The left ventricular myocardium is thinned and the LV is more spherical than in the healthy heart. **b:** Mid short axis view of a healthy heart. **c:** Short axis view of a patient with HCM. Thickening of the left ventricular myocardium is seen in all segments, though thickening is greatest in the mid-anterior and mid-lateral segments. The annotations indicate the orientation of the image in the subject: *A/P* = anterior/posterior, *S/I* = superior/inferior, *R/L* = right/left.

³¹P-MRS has shown reduced PCr/ATP ratios in patients with HCM, including patients who show no morphological changes and even in phenotype-negative, genotype-positive relatives.^{67,68}

1.3.2.3 Diabetes mellitus

Patients with diabetes mellitus (type 1 and type 2) have reduced PCr/ATP ratios.^{69,70}

Type 2 diabetes mellitus (T2DM) is the result of insulin resistance. The primary causes of T2DM are lifestyle factors (obesity, poor diet and lack of physical exercise), and genetic predisposition.

Remodelling of the left ventricle and an associated loss of cardiac function is often observed in T2DM patients, though the causal link is still unclear. It is thought to be related to cardiac steatosis (accumulation of fat) and impaired energy metabolism.³⁶

1.3.2.4 Ischaemic cardiomyopathy

Ischaemic cardiomyopathy and heart failure arises as a result of decreased blood flow, and therefore oxygen supply, to the myocardium. The most common cause of heart failure is coronary artery disease, a group of diseases including atherosclerosis (the narrowing of the coronary artery lumen by fatty deposits) and acute myocardial infarction.

Patients with previous myocardial infarction have been shown to have PCr and ATP concentrations about 40% lower, and reduced ATP flux in the myocardium.³³ Increased workload also results in a decrease in PCr/ATP ratio in patients with significantly occluded coronary arteries.⁷¹ In the Weiss *et al.* study, resting values were normal but decreased to 1.5-0.9 with a 30-35% increase in workload using hand-grip exercise, and did not recover to normal levels after cessation of the exercise. The PCr/ATP ratio became more normal after interventional revascularisation.

1.3.3 Monitoring of acute local ischaemia in animal models

The low spatial resolution, low SNR in the anterior myocardium (with surface coils), and long duration of cardiac ^{31}P -MRS has meant that monitoring of the recovery of tissue subject to acute myocardial infarction has not yet been possible with ^{31}P -MRS in humans.

However, surgically-prepared porcine and canine animal models have been used to monitor the cardiac energy metabolism during and immediately after artificial occlusion of the coronary arteries.⁷²⁻⁷⁴ These experiments typically use small surface coils sutured directly to the myocardium, enabling resolution of the epi- and endocardium and avoid significant signal contamination by other tissue (skeletal muscle, liver, or LV blood pool).

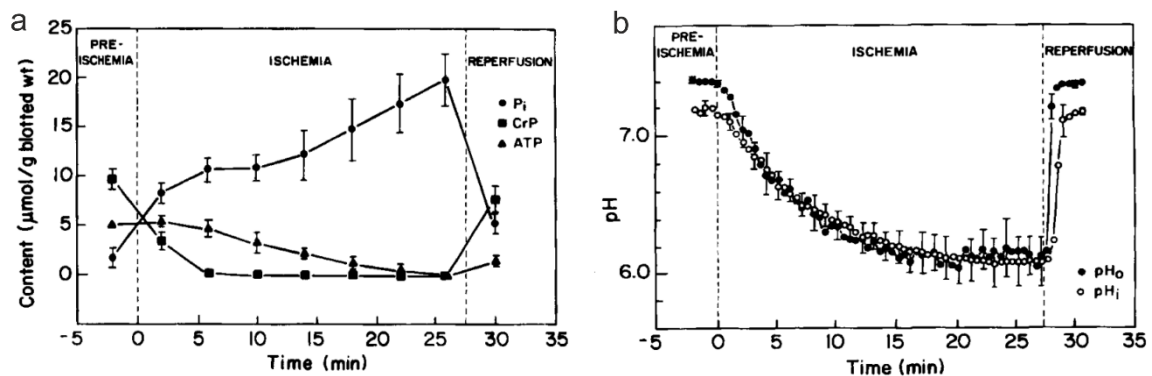


Figure 1.13 **a:** Changes in myocardial PCr (CrP), ATP and P_i levels during 28 min global ischemia followed by reperfusion in a perfused rat heart. **b:** Change in myocardial intracellular pH (pH_i) and extracellular pH (pH_o) during 28 min global ischemia followed by reperfusion in a perfused rat heart. Figures reused with permission from 40, copyright John Wiley & Sons, Ltd 1993.

Zhang *et al.* showed in canines, moderate occlusion of the coronary arteries led to a decrease in the PCr and ATP concentration, which worsened with duration of occlusion; after 240 min of occlusion PCr was at 53% and ATP at 77% of the baseline level. ADP levels initially increased but returned to baseline after 240 minutes. The P_i/PCr ratio

increased significantly immediately after occlusion.⁷⁴ In another study the PCr/ATP ratio was found to increase above baseline levels immediately after reperfusion after 15 minutes of occlusion, whilst CK activity stayed suppressed.⁷²

1.4 Aims

It is clear from the literature that the ³¹P-MRS spectrum is a sensitive measure for the presence and severity of heart failure. To-date the PCr/ATP has been used as a reliable but fundamentally flawed measurement of the myocardial energy metabolism;

Hudsmith and Neubauer⁷⁵ conclude:

“However, in heart failure, both PCr and ATP decrease in parallel, so PCr-to-ATP ratios cannot detect the full extent of these changes. Using absolute quantification ... dilated cardiomyopathy patients showed a 51% reduction in absolute PCr levels, a 35% reduction in absolute ATP, and a 25% decrease in PCr-to-ATP ratio (35). Therefore, PCr-to-ATP ratios underestimate the true extent of metabolic derangement in heart failure.”

Kinetic measurements of the creatine kinase energy shuttle, combined with absolute quantification of metabolite concentrations represent the new generation of more sensitive measurements.

Whole body 7T scanners have brought a step change in the SNR available to the researcher using ³¹P-MRS. Whilst the SNR allows greater precision measurements of the PCr/ATP ratio, it also opens up the ability to make the multiple measurements required for CK kinetic measurements in a single scan session.

Therefore, the overarching aim of the rest of this thesis is to develop new methods, and adapt old measurements, for the 7T field strength, with the specific aim of making measurements of the creatine kinase rate constants in the healthy and diseased human myocardium.

1.4.1 Thesis outline

After Chapter 2 this thesis is organised in approximately chronological order. Chapters five through eight present a coherent progression of work on the measurement of the creatine kinase mechanism. The most significant output of this thesis is contained in Chapter 7 (a Bloch-Siegert B_1^+ -mapping technique for ^{31}P -MRS) and Chapter 8 (the first 3D resolved k_f^{CK} measurements in human myocardium).

Chapter 1 has provided an overview of the physical basis of magnetic resonance, the uses of phosphorus magnetic resonance spectroscopy in the understanding of cardiac metabolism and the clinical relevance of the technique.

Chapter 2 profiles the standard multi-voxel ^{31}P -MRS experiment at Oxford, with the aim to inform the reader of the techniques and hardware available to the author for the work contained in the thesis. The results of a recent study comparing ^{31}P -MRS at 7 tesla to 3 tesla are summarised, the RF coil hardware is characterised experimentally, the 3D localisation scheme detailed, and a method to estimate the in vivo flip-angles based on the Biot-Savart law is described and validated.

Chapter 3 details the implementation of an open-source toolkit for automatic fitting and post-processing in vivo spectroscopy data. The tool kit enables the identical analysis of large numbers of datasets, computation of error estimates for derived parameters (e.g. PCr/ATP), and allows the user full control and insight of the processing routine. The toolkit, including a spectral fitting algorithm, was written in Matlab. The fitting program is validated against existing algorithms and analytical equations. The toolkit is demonstrated for the analysis of ^{31}P -MRS study data and for array combination of ^1H -MRS data.

Chapter 4 details attempts to resolve the inorganic phosphate peak in cardiac ^{31}P -MRS, which is obscured by peaks originating in the blood. Resolution is attempted in healthy subjects using B_0 gradients and saturation transfer difference spectroscopy to suppress the DPG peak signal obscuring the P_i peak. Using the increased SNR at 7T, high resolution 3D-CSI is used to measure the P_i peak and the pH of the myocardium of three hypertrophic cardiomyopathy patients.

The effect of the creatine kinase catalysed exchange of phosphate on the appearance of the cardiac ^{31}P -spectrum is described in Chapter 5. The exchange, especially at short repetition times, significantly biases the measured PCr/ATP ratio. Aims to measure 3D localised myocardial creatine kinase rate constants at 7T are set out. The suitability of previously published methods for use at 7T are assessed. A multi-parametric saturation transfer fitting approach is analysed in simulation and in experiment.

The creatine kinase measurement has previously been identified as a more sensitive marker of heart failure than the PCr/ATP ratio. In Chapter 6 this is exploited for two patient studies, investigating the “obesity paradox” and hypertrophic cardiomyopathy. The TRiST⁷⁶ measurement technique is implemented on a Siemens scanner at 3T for the first time, and extended for rapid consecutive measurements. The preliminary results of each patient study are presented, with differences in measured values from the original implementation analysed in detail.

Chapter 7 describes the creation of a Bloch-Siegert, ^{31}P -MRS based B_1^+ mapping sequence. In Chapter 5 it is established that for accurate saturation transfer CK rate measurements that the excitation flip-angle must be known. The optimum implementation of a Bloch-Siegert B_1 -mapping method for X-nuclear spectroscopy is

determined. A pulse sequence is validated against multi-TR methods in phantom and skeletal muscle, before being demonstrated in the hearts of 5 healthy subjects. This work develops the first viable B_1^+ mapping technique for cardiac ^{31}P -MRS.

In Chapter 8, the four angle saturation transfer method⁷⁷ is combined with the Bloch-Siebert measurement of Chapter 7, to enable the first 3D resolved cardiac creatine kinase forward rate constant measurements. The acquisition parameters of the proposed sequence are optimised and sources of errors explored. Measurements, localised to voxels with a nominal volume of 5.6 ml are made in ten healthy subjects. The technique brings a step-change in the localisation performance of cardiac k_f^{CK} measurements.

The overall conclusion, with a view to proposed future work, is given in Chapter 9.

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Chapter 2: ^{31}P -MRS methods

2.1 Introduction

Chapter 2 gives an overview of the hardware, pulse sequences and analysis techniques employed for ultra-high field cardiac ^{31}P -MRS at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR).

The chapter begins with a brief overview of the benefits of 7 tesla MR for ^{31}P cardiac spectroscopy over lower field strengths. The results of a comparison of the technique at 3T and 7T are summarised.¹

The second section describes the available RF coil hardware. The transmit and receive fields of 3T and 7T coils are characterised in a phantom.

Subsequently, the chemical shift imaging (CSI) spectroscopy sequence that is the basis for most of the spectroscopy experiments in later chapters is described, including its phase encoding scheme for non-uniform k-space sampling (known as “acquisition weighting”). The section concludes with a short overview of typical excitation pulses.

Finally, the estimation of flip-angles, for saturation correction, by scaled and co-registered Biot-Savart field maps is described. The validity of the estimated values is discussed with reference to coil loading experiments.

2.2 Ultra-high field ^{31}P -MRS

2.2.1 Scanner systems

^{31}P -MRS studies at OCMR are carried out at two field strengths: 2.89 T (on a “3T Trio”, Siemens, Germany) and 6.98 T (on a “Magnetom 7T”, Siemens, Germany). Both are 60cm horizontal bore scanners. The Trio has gradients of a maximum amplitude of

40 mT m⁻¹ (26 mT m⁻¹ on any axis) and a maximum slew rate of 170 mT m⁻¹ s⁻¹, and 2nd order shims. The Magnetom 7T has gradients of maximum amplitude 70 mT m⁻¹ (40 mT m⁻¹ on any axis) and a maximum slew rate of 200 mT m⁻¹ s⁻¹, and 2nd order shims. The magnet is actively shielded, resulting in a long (approx. 3m) bore length. It is not equipped with a ¹H RF “body coil”, therefore ¹H imaging for spectroscopic localisation, B_0 shimming etc. must be accomplished with local ¹H RF coils.

2.2.2 ³¹P-MRS at 7T

Theory predicts that the signal-to-noise ratio in unit time ($\text{SNR}/\sqrt{t}$) of ³¹P-MRS data increases approximately in proportion to a scanner's B_0 field.² The actual net increase in quality is dependent on how much the increase in ³¹P Boltzmann equilibrium magnetization out-weighs increases in B_0 -inhomogeneity, RF-induced heating and the increased RF power needed to excite the same chemical shift range by the same flip-angle at higher field, particularly for cardiac ¹H imaging at 7T where dielectric effects in the subject increase B_1 inhomogeneity.³ The relaxation times T_1 and T_2 for ³¹P-containing metabolites also depend on B_0 .⁴

An increase in SNR and fitting precision with the increase from 1.5T to 3T has been shown at Oxford⁵, and more recently the benefit of moving from 3T to 7T has been demonstrated.¹ Rodgers *et al.*¹ reported that the cardiac ³¹P-metabolite T_1 values were significantly shorter at 7T than those measured at lower field strengths ($T_1^{\text{PCr}} = 5.3$ s [3T] vs 3.09 s [7T]), agreeing with earlier work on skeletal muscle.⁶ This contrasts with increases in water T_1 values at 7T, and reflects the major contribution of chemical shift anisotropy to T_1 relaxation of ³¹P-containing metabolites.^{7,8} From 3T to 7T, the ³¹P SNR

was found to increase by 2.8x and the metabolite amplitude fitting accuracy by 5x in normal volunteers. No significant difference in measured PCr/ATP ratios was found between the two field strengths. Recently, similar increases in SNR have been demonstrated in larger cohorts, including patients with DCM and T2DM.⁹

The increase in spectral SNR with a corresponding protocol at 7T yields higher precision measurements of derived parameters (e.g. PCr/ATP ratio). Alternatively, one can increase spatial resolution or decrease total scan duration, whilst maintaining the original lower field SNR. For example, a 6 min acquisition at 7T can give SNR greater than a 30min acquisition at 3T.¹

2.3 RF coils

RF coils must be tuned to the Larmor frequency, i.e. for a particular nucleus being studied, at a set scanner field strength. Therefore the 3T and 7T scanners each have a range of dedicated, but not interchangeable, ³¹P coils (Figures 2.1 & 2.2).

2.3.1 3T RF coils

10 cm loop (Figure 2.1a)

A ³¹P only transmit/receive coil with a single circular 10 cm diameter loop element, inductively coupled to the RF feed, with mechanical adjustment of the 10cm loop – drive loop overlap for matching. Manufactured by PulseTeq Ltd (Chobham, UK). The coil must be matched, to each subject, using an RF sweep box (Morris instruments, Canada).

8-channel array (Figure 2.1b)

A two piece coil: the anterior piece of the array contains a 30 × 29 cm² transmit loop and four 6 × 20 cm² receive elements and is positioned on the subjects chest; the

posterior piece contains four $6 \times 20 \text{ cm}^2$ receive elements and sits under the subject in the patient table.¹⁰ Manufactured by Rapid Biomedical GmbH (Rimpar, Germany).

'Heart-liver' coil (Figure 2.1c)

A three element coil which is positioned in the plane of the patient table, with the subject lying prone on top of the coil. A large rectangular ($28 \times 26 \text{ cm}^2$) element is dual-tuned to both the ^1H frequency (127.7MHz) and the ^{31}P frequency (49.8MHz). This element acts as a local transmit/receive ^1H coil and the ^{31}P transmit coil. A "loop-butterfly" quadrature pair of elements with a hardware combiner for ^{31}P receive complete the coil. Originally manufactured by Siemens (Erlangen, Germany) for use at 1.5T and retuned to 3T in-house.

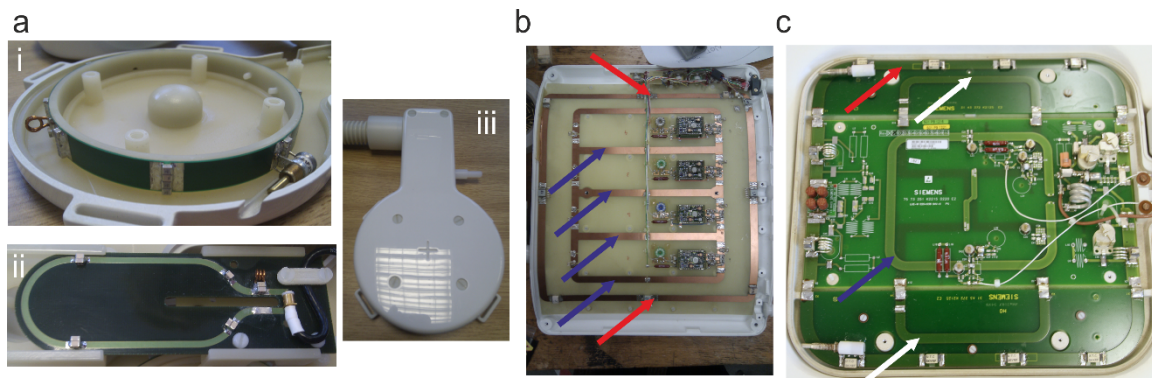


Figure 2.1 3T coils. **a:** ^{31}P 10cm transmit/receive loop coil. The coil comprises two resonant circuits. The main 10 cm loop (i) is inductively driven by a second (ii) which is electrically connected to the T/R switch. Both elements are held in a single housing (iii) which allows the relative position of the two elements to be varied for coil matching. **b:** Anterior element of the 8-channel array. The transmit element (red arrows) is located around the edge of the 4 anterior receive elements (blue arrows). 4 further receive elements are located in the posterior segment of the coil (not shown) in a similar arrangement. **c:** 'Heart-Liver' coil. The transmit/receive ^1H coil and the ^{31}P transmit coil (red arrow) is located around the edge of the coil. The quadrature pair receive elements are made up of 'loop' (blue arrow) and 'butterfly' (white arrows) elements.

2.3.2 7T Coils

10 cm loop (Figure 2.2a)

A ^{31}P only transmit/receive coil with a single circular 10 cm diameter loop element. The coil was manufactured “in-house” (the coil was made by Carl Snyder from CMRR, and the casing was made by the author) and connected to a separate T/R switch and preamp box manufactured by Virtumed LLC (Minneapolis, MN, USA). The coil is manually tuned and matched, by adjusting two variable capacitors, on a per-subject basis using an RF sweep box.

16-channel array (Figure 2.2c)

A flexible 16-channel receive array coil with a rigid 26 x 28 cm² rectangular ^{31}P transmit element. The flexible array contains 16 overlapping 4 cm diameter circular elements in a 4-by-4 grid. The ^{31}P elements are actively detuned, allowing transmission at the ^1H -frequency RF whilst the coil is in-situ. However the thickness of the coil precludes the use of a 10cm ^1H transmit/receive loop placed above the coil for localisation and B_0 -shimming due to the low image SNR in the heart and liver.

The design of the flexible receive elements is modelled on multi-channel ^1H cardiac arrays available from a number of original equipment manufacturers, for example: the “SENSE Torso/Cardiac Coil 32 elements” (Philips, Amsterdam, The Netherlands) and “32-channel-body-coil” (Siemens, Erlangen, Germany). The coil was manufactured by Rapid Biomedical GmbH (Rimpar, Germany).

Quadrature coil (Figure 2.2b)

A single piece, in-house designed and built coil. The coil contains two ^{31}P transmit-receive elements below a single 9 cm circular ^1H transmit-receive loop. The ^{31}P elements

are two overlapping 15 cm diameter circular loops. The ^{31}P loops are driven in quadrature i.e. with a 90° phase offset, and are de-coupled from the ^1H frequency by their geometric arrangement and by the inclusion of an LCC trap, resonant at the ^1H frequency, in each loop.¹¹

^1H loop

With the exception of the quadrature coil, all ^1H measurements at 7T were made with a transmit-receive coil comprising a single 10cm circular element. Manufactured by Rapid Biomedical GmbH (Rimpar, Germany) and supplied with the scanner.

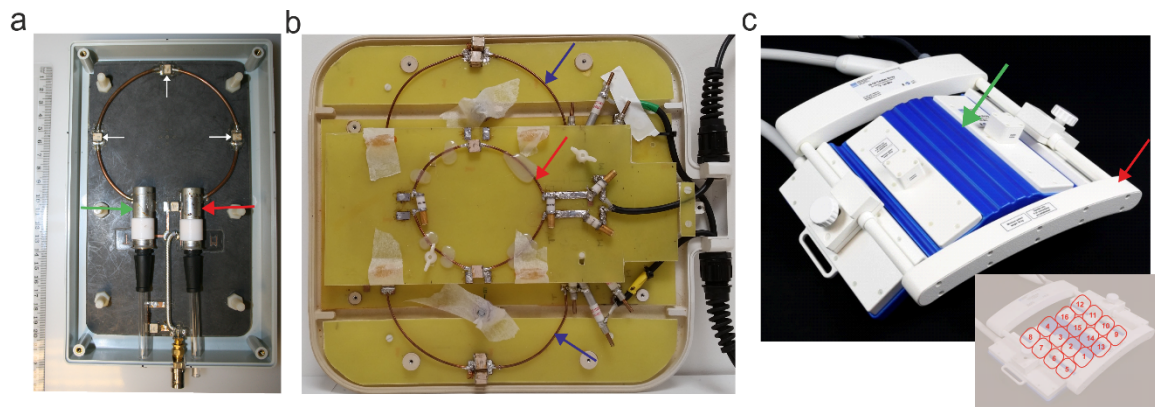


Figure 2.2 7T coils. **a:** ^{31}P 10cm transmit/receive loop coil. The adjustable tune (green arrow) and matching (red arrow) capacitors are seen below the single element which contains further distributed fixed capacitance (white arrows). **b:** Quadrature coil. The ^1H element (red arrow) is located above the ^{31}P quadrature pair elements (blue arrows). **c:** 16 channel array coil. The inset shows the arrangement of the receive elements. The white outer ring contains the transmit element (red arrow) while the flexible blue section (green arrow) contains the flexible receive array.

2.3.3 B_1^+ and B_1^- performance of available coils

The transmit and receive performance of a particular coil may be characterized on a specific experimental setup. Using each coil in turn, the “reference voltage” was measured (this is a Siemens-specific term defined as the RMS voltage at the coil plug on the patient table required to achieve a 180° flip angle with a 1 ms hard pulse in the target volume). A series of fully relaxed (i.e. $T_R > 5T_1$) non-localised FIDs were acquired

with a range of transmit voltages, on a single voxel phantom designed to mimic the conductivity of skeletal muscle. By varying the depth of the cube, a 1D profile of a coil's B_1 performance may be obtained.

The single voxel phantom (Figure 2.3) comprises a $2 \times 2 \times 2 \text{ cm}^3$ cube of $\text{KH}_2\text{PO}_4(\text{aq})$, suspended in 18 L physiological saline (73 mM $\text{NaCl}(\text{aq})$). Two thumb screws allow adjustment of the distance between the top of the phantom (and RF coil) and the KH_2PO_4 cube in situ in the scanner without moving the coil or phantom. The KH_2PO_4 in the cube has a $T_1 = 2.00 \text{ s}$ at 3T and $T_1 = 1.81 \text{ s}$ at 7T. The phantom was designed by the author, fabricated by k2 Associates Ltd (Bishops Cleeve, UK), and adapted by the workshops of the Physical and Theoretical Chemistry Laboratory, University of Oxford.

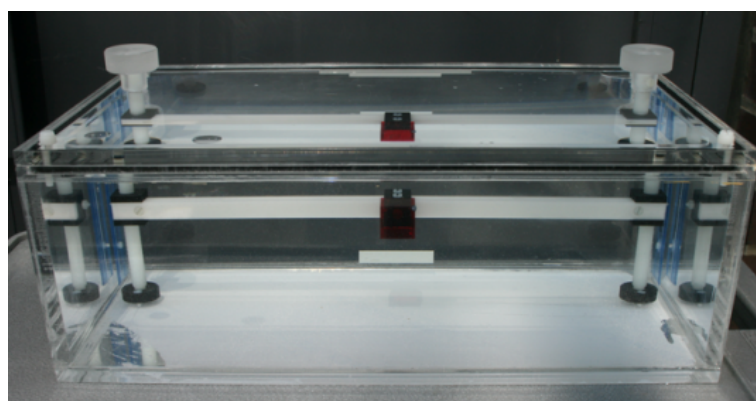


Figure 2.3 Single voxel phantom. The red $2 \times 2 \times 2 \text{ cm}^3$ cube at the centre contains $\text{KH}_2\text{PO}_4(\text{aq})$. The cube is surrounded by physiological saline (73 mM $\text{NaCl}(\text{aq})$). The winding gear, on the left and right of the photo allows the vertical position of the cube to be adjusted.

2.3.3.2 Coil transmit performance

The amplitudes of the single phosphate peak, recorded at increasing transmit voltages of a hard pulse, were least-squares fitted with a sinusoidal function. The first peak value, the point of maximum signal, corresponds to a flip angle of 90° , whilst the first null point corresponds to inversion, a flip angle of 180° . This allows the reference

voltage (V_{ref}) and the B_1^+ at the maximum transmit voltage (V_{max}) to be calculated for each coil at each measured depth:

$$B_1^+ = \frac{500}{\gamma} \frac{V_{\text{max}}}{V_{\text{ref}}} \quad [2.1]$$

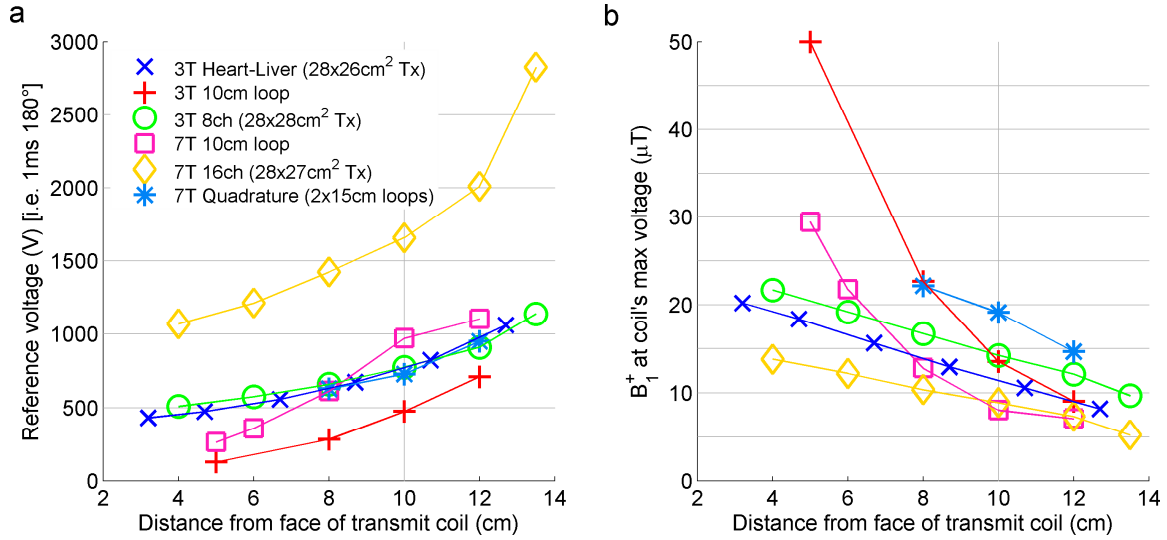


Figure 2.4 Coil transmit performance described as (a) reference voltage and (b) B_1^+ at the maximum transmit voltage allowed by the hardware. The x-axis gives the distance between the phosphate containing cube and the closest face of the transmitting coil.

Lower reference voltages correspond to a higher B_1^+ . 3T coils outperform similar 7T coils by a factor approximately the ratio of the Larmor frequencies ($\sim 7/3 \approx 2.3$), because of the frequency term in the reactance of an inductor. Small loops produce higher fields close to the coil but decrease more steeply than larger loops with depth. In an in vivo cardiac experiment, the most posterior parts of the myocardium are approximately 10 cm from a surface coil in lean normal volunteers. At this depth, with the exception of the quadrature transmit coil, the maximum B_1^+ of the 7T coils is limited to $< 10 \mu\text{T}$.

2.3.3.3 Coil receive performance

The matched-filtered SNR of the 90° acquisition acts as a surrogate measurement of the coil's receive sensitivity or B_1^- .

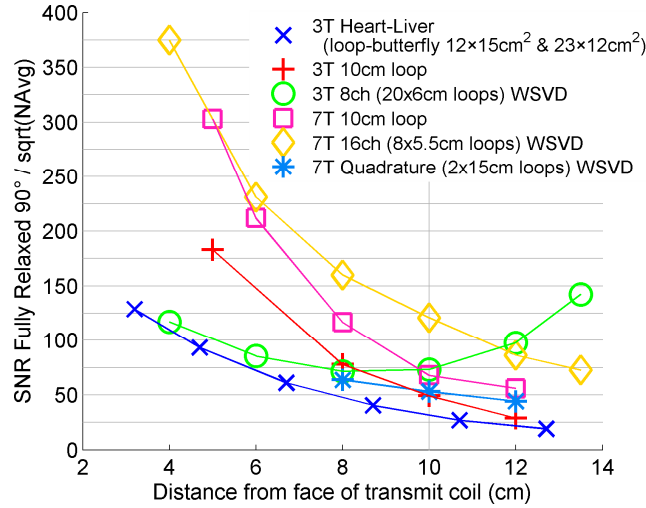


Figure 2.5 Coil receive performance estimated as the matched-filtered SNR of the 90° acquisition. The SNR was normalised for the number of acquired averages. The increased SNR of the 7T coils relative to the 3T coils is due in part to the increased equilibrium magnetisation of the sample at 7T.

The highest SNR is achieved by the 16 channel receive coil at 7T when combined using the whitened singular value decomposition (WSVD) method.¹² Small loop coils once again show high SNR near the coil but this rapidly decreases with depth. The effect of the posterior elements of the 3T 8ch array coil can be seen as the SNR rises from its minimum at 8 cm as the depth is increased further.¹⁰

2.3.3.4 In vivo B_1 -mapping

Recently, we have developed a ^{31}P -MRS B_1 -mapping protocol using a Bloch-Siegert method (Chapter7).¹³ This enables the rapid mapping of the full three dimensional transmit RF field within the sample at the resolution of a CSI spectroscopy experiment (Figure 2.6). Initial in vivo data suggests that the measured B_1^+ is higher than that

estimated in the 1D profiles measured in the single voxel phantom in the previous subsections (Figure 2.7). Further detail can be found in Chapter 7.

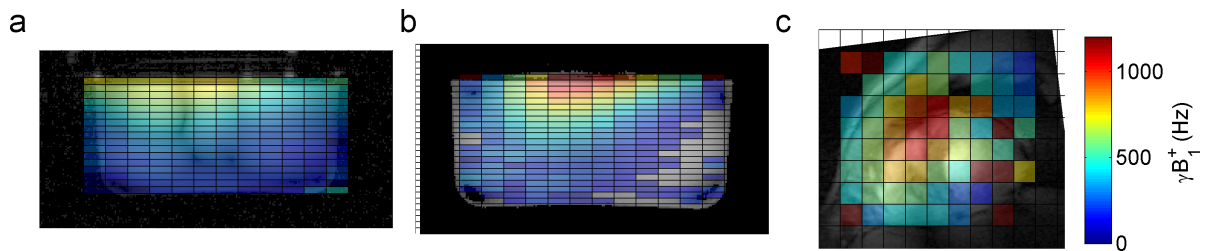


Figure 2.6 ^{31}P -CSI based γB_1^+ maps of uniform phosphate phantoms using (a) the quadrature coil and (b) the 7T 10 cm loop coil; and (c) measured in the short-axis plane of a healthy volunteers, using the 10 cm loop coil.

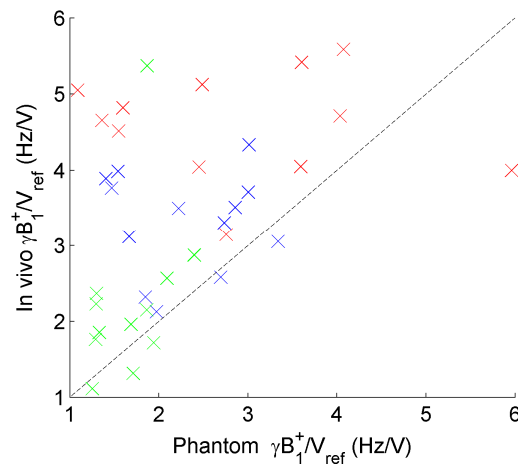


Figure 2.7 Comparison of direct γB_1^+ measurements, scaled by the reference voltage of a coil fiducial, from septal voxels in 13 healthy subjects, acquired with the 10 cm loop coil, with γB_1^+ estimated from the phantom measurements described in § 2.3.3.2. These values should be independent of the per-subject coil loading factor. This is an extract of Figure 7.10.

2.4 Base spectroscopy sequence

The base spectroscopy sequence used at OCMR has four parts: i) the excitation pulse, ii) phase encoding, iii) the read-out (or ADC event), iv) gradient spoiling, and v) dead-time for the “ T_R fill”. Additional sensitising RF pulses or gradients may be introduced between the excitation pulse and the read out (or ADC). Localisation may be excluded to create a FID sequence, if desired. Before excitation, optional BISTRO saturation bands for

suppression of skeletal muscle signal can be added.¹⁴ A minimum time gap of approximately 10 μ s between the end of an RF pulse and the ADC is enforced by the scanner to enable the coil hardware to switch from transmit to receive mode.

2.4.1 Chemical Shift Imaging

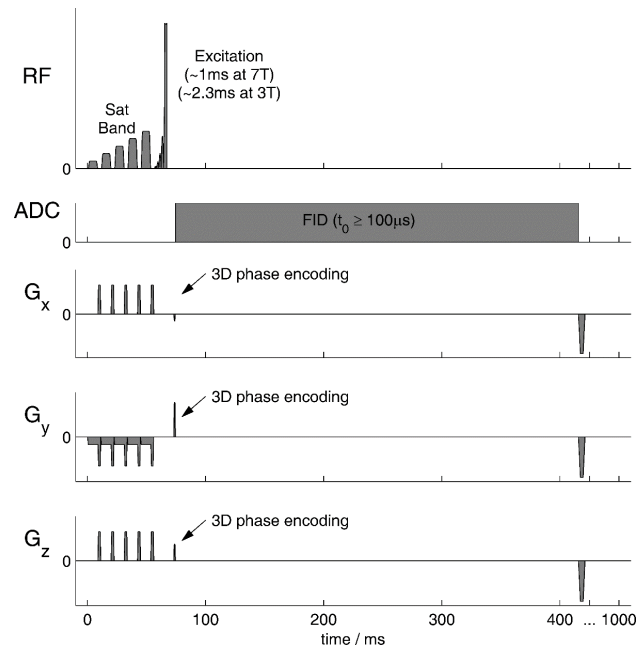


Figure 2.8 Pulse sequence diagram of the UTE-CSI pulse sequence used to record ^{31}P spectra. The plot shows a single T_R , which is repeated approximately 1700 times during a typical scan.

Localisation of the MR signal is achieved by chemical shift imaging (CSI). This requires the introduction of trapezoidal phase encoding gradients on one, two or three logical gradient axes, for 1D, 2D or 3D localisation respectively.

During a phase encode gradient pulse G_{phase} (where “phase” may be substituted for any orthogonal direction x, y and z, or any combination for oblique CSI), the precession frequency of the spins will be modified from the central frequency ω_0 according to

$$\omega = \mathbf{r} \cdot \mathbf{G} = \mathbf{r} \cdot d\mathbf{B}_{0,z}, \quad [2.2]$$

or specifically for the x-axis

$$\omega(x) = \gamma x G_x. \quad [2.3]$$

For a constant-amplitude gradient pulse of duration t , Eq. [2.3] is equivalent to a spatially dependent phase shift ϕ given by:

$$\phi(x) = \gamma x G_x t. \quad [2.4]$$

After the gradient, transverse magnetisation throughout the sample will be spatially phase encoded, and will remain so until the transverse magnetisation decays to zero due to transverse relaxation. The spectrum acquired from each point in space (imagining this to be individually observable) after G_x is

$$\text{Re}[F(\omega, x)] = A(\omega) \cos \phi(x) + D(\omega) \sin \phi(x), \quad [2.5]$$

$$\text{Im}[F(\omega, x)] = A(\omega) \sin \phi(x) + D(\omega) \cos \phi(x); \quad [2.6]$$

i.e. a modification of Eqs. [1.44] and [1.45]. The total time-domain signal observed after G_x is:

$$S(t) = \int_0^{+\infty} s(x, t) dx. \quad [2.7]$$

The total frequency spectrum, without phase encode gradients (or with $G_{\text{phase}} = 0$),

$F(\omega)$ is given by the Fourier transformation of $S(t)$, which in turn is equivalent to the spatial integral of spectra from small volume elements $f(\omega, x)$ of the sample

$$F(\omega) = \int_{-\infty}^{+\infty} S(t) e^{-i\omega t} dt = \int_{-\infty}^{+\infty} f(x, \omega) dx. \quad [2.8]$$

The phase effect of a single G_{phase} , as described by Equation [2.4], on a spectrum arising from a single volume element may be written as a linear, position dependent, phase ramp across the all volume elements,

$$f'(x, \omega) = f(x, \omega) e^{i\gamma x G_x t}. \quad [2.9]$$

Combining Equations [2.8] and [2.9] gives

$$F(G_x, \omega) = \int_{-\infty}^{+\infty} f(x, \omega) e^{i\gamma x G_x t} dx, \quad [2.10]$$

We substitute $k_x = \gamma G_x t$, to convert Equation [2.10] to

$$F(k_x, \omega) = \int_{-\infty}^{+\infty} f(x, \omega) e^{ik_x x} dx, \quad [2.11]$$

where k_x is the amount of k-space (frequency space or reciprocal space) transversed, along the x direction, during gradient pulse G_x . Taking the inverse Fourier transform of the phase modulated spectrum $F(k_x, \omega)$, built up by repeated acquisitions with different G_x values, will give the spectrum in each volume element:

$$f(x, \omega) = \int_{-\infty}^{+\infty} F(k_x, \omega) e^{-ik_x x} dk_x, \quad [2.12]$$

Eq. [2.12] may be extended to the three Cartesian spatial dimensions:

$$f(x, y, z, \omega) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} F(k_x, k_y, k_z, \omega) e^{-i(k_x x + k_y y + k_z z)} dk_x dk_y dk_z. \quad [2.13]$$

2.4.1.2 The point spread function and acquisition weighting

Equation [2.13] implies that signal is sampled in infinitely small steps and infinitely widely in k-space, however this would necessitate an infinitely long experiment. Rather, k-space is sampled at N discrete points in k-space. These are usually taken to be equally spaced between $-\Delta k N/2$ and $\Delta k(N/2 - 1)$, where the k-space steps Δk are the product of the gyromagnetic ratio, gradient pulse amplitude steps ΔG and the gradient duration t :

$$\Delta k = \frac{\gamma \Delta G t}{2\pi}. \quad [2.14]$$

The digitisation is subject to the Nyquist sampling criterion, where the maximum phase shift difference over the whole field-of-view (FOV) for an increment of G_x is 2π ,

$$\text{i.e. } 2\pi = \gamma FOV \Delta G t, \text{ or } FOV = \frac{2\pi}{\gamma \Delta G t} = \frac{1}{\Delta k}. \quad [2.15]$$

The nominal real-space voxel size Δx , may be found by dividing Equation [2.15] by N :

$$\Delta x = \frac{FOV_x}{N} = \frac{2\pi}{N \Delta k_x}. \quad [2.16]$$

As a result of the discretization and truncation of the k-space sampling, the Fourier transform must be substituted for the discrete Fourier transform (DFT), i.e. Equation [2.12] is re-written as

$$f(\Delta x, \omega) = \sum_{q=-N_x/2}^{N_x/2-1} F(q \Delta k_x, \omega) e^{-iq \Delta k_x \frac{x}{N_x}}. \quad [2.17]$$

A consequence of the discrete sampling of k-space is to introduce aliasing to the resultant real-space image. For a non-truncated (infinitely wide) but discretely sampled k-space, represented by a Dirac comb function, the DFT produces a real-space Dirac comb with spacing of $1/\Delta k = FOV$ between the delta functions, i.e. the image will be repeated or aliased once every FOV. The aliasing manifests as the inability to distinguish signal arising from the sample outside the FOV from signal at the corresponding position inside the FOV.

Truncation of the sampled k-space corresponds to the multiplication of the k-space Dirac sampling comb with a rectangular filter function, or equivalently the convolution of the Fourier transform of the filter function (in this case a “sinc” or $\sin x/x$ function) with the real-space Dirac comb. The result is a repeating sinc function which ‘blurs’ the image, and is known as the point spread function (PSF). The PSF tells us which points in

space contribute to the signal in a voxel, or equivalently which voxels contain signal from a particular point in space.

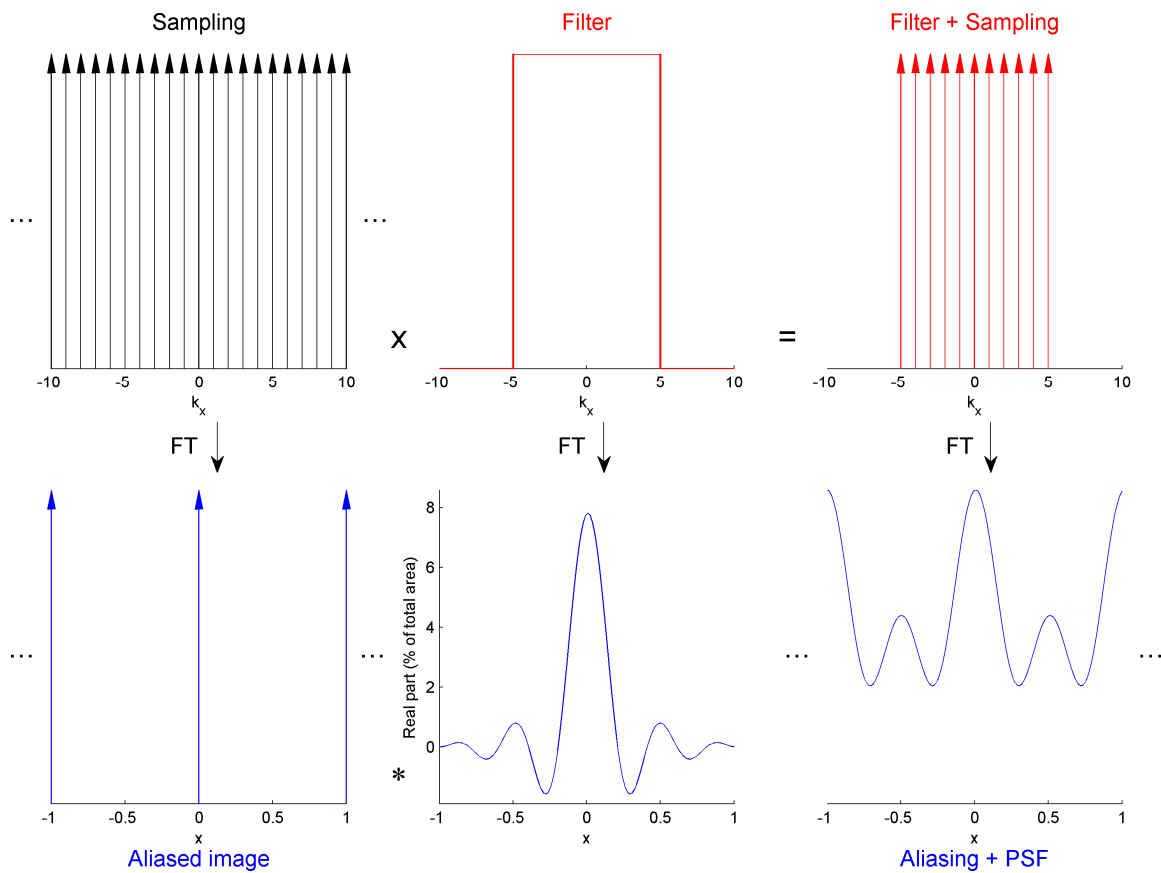


Figure 2.9 Effect of discrete and truncated sampling of k-space on the real-space signal. Sampling, equivalent to multiplication by a Dirac comb, and shown here by arrows, introduces aliasing of the real-space image after application of the FT. Truncation, or multiplication by a filter, introduces broadening characterized by the point spread function (PSF). The multiplication of the discrete sampling function by the filter function in k-space is equivalent to the convolution of the aliased image with the PSF in real-space. The PSF, or the real part of the Fourier transform of the filter, indicates the proportion of the total signal in a voxel arising from a point in real space. Both negative and positive signal contributions come from areas outside the nominal voxel.

The sinc-shaped PSF has a non-uniform central lobe and decaying positive and negative side lobes, leading to a complex relationship between spatial origin of the MR signal observed and the voxel it is observed in. The positive and negative side lobes of the PSF can be suppressed but at the cost of a broader central lobe using a generalized Hamming window function

$$W(k) = 0.5 + 0.5 \cos\left(\pi \frac{k}{k_{\max}}\right), \quad [2.18]$$

where $k_{\max}$ is the maximum sampled position in k-space.¹⁵

The shaped window function may be implemented either in the reconstruction stage, by multiplying the uniformly sampled k-space by the windowing function, or at run-time by using multiple averages of different point in k-space to approximate the shape of the window function (Figure 2.10a).¹⁶ In ³¹P-MRS multiple averages are required for sufficient spectral SNR thus the latter option is possible, and does not have the SNR penalty associated with the former approach. But approximation of the function by only a few averages leads to degradation of the target PSF back towards the un-windowed function (Figure 2.10b). Therefore a combination of run-time averages and post processing weighting to bring the integer number of averages to the proper (usually non-integer) Hamming filter weights is used.¹⁷

Finally, when extended to two (or three) dimensional phase encoding it is advantageous to sample k-space in an elliptic (or ellipsoidal) pattern, i.e. not to sample at all any points at the corners of k-space where the Hamming filter weight would be <1 average. This gives a considerable saving in acquisition time for a minimal cost in terms of SNR and PSF width (Figure 2.10d).¹⁷

This specific combination of hamming windowing, at both run-time and in the reconstructions stage, and ellipsoidal sampling is known as “acquisition weighting” throughout the rest of this work. Whilst reducing PSF ripple, for a typical 16x16x8 CSI grid the FWHM of the central lobe is 116% larger than in the uniformly sampled case and in 3D the integrated volume to 50% of peak PSF is 156% larger than the nominal

voxel size (assuming each voxel is a cuboid). For the same CSI grid acquisition weighting will also reduce the $SNR / \sqrt{T_A}$ by >15% if fewer than 5 averages are used to describe the windowing function (Figure 2.10c).

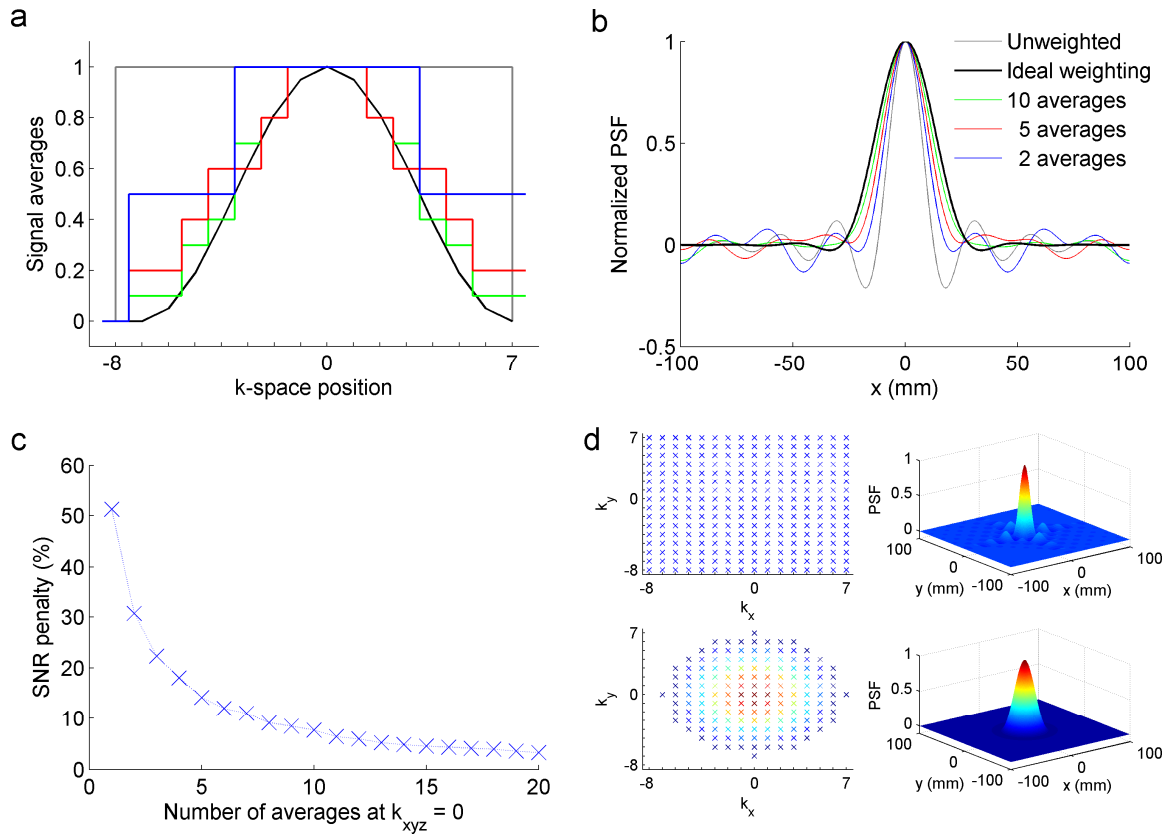


Figure 2.10 Acquisition weighting. **a:** Approximation of a Hamming window using integer numbers of k-space averages. **b:** The PSF for uniformly sampled, ideally weighted and three integer average approximations to the weighting function. **c:** SNR reduction due to the numerical weighting of k-space after initial approximation using an integer number averages at each k-space point for fixed total scan time. **d:** 2D PSF of uniform square k-space sampling and elliptical acquisition weighted k-space.

2.4.1.3 UTE-CSI

The T_2 values of skeletal muscle ^{31}P metabolites, which range from 29 ms to 314 ms at 7T^6 , and are similar to some myocardial values¹, are much longer than the CSI deadtime ($\sim 100\ \mu\text{s}$), but T_2^* , the time constant of transverse relaxation due to both intrinsic spin-spin relaxation and intra-voxel B_0 inhomogeneity, may be as short as 6.5 ms in the heart at 7T^1 . Therefore it is important to minimise the duration of the phase encoding

gradients, without introducing unwanted eddy-current effects by using very high gradient amplitude or slew rates.

Using the ultrashort echo time (UTE) CSI method¹⁸, the duration of each phase encoding gradient is minimised, whilst keeping the phase encoding gradients within the limits of 20 mT/m and 100 mT/m/s. The minimisation is calculated for each k-space location, resulting in a different T_E value across k-space. A minimum T_E of 100 μ s is set to allow time for coil hardware switching.

The shortest T_E values are obtained at the centre of k-space where $k_{x,y,z} = 0$. The variable T_E means that it is impossible to calculate a precise first-order phase correction, however typically the residual phase roll after phase correction based on an average T_E is less than $\pm 5^\circ$ across a 6000 Hz bandwidth spectrum for a 240x240x200 mm³ FOV.

2.4.2 Excitation pulses

There is no restriction on the amplitude or phase profile of an excitation RF pulse. The design or choice of pulse must take into account both experimental targets such as excitation bandwidth, desired flip-angle and phase profile, and limitations such as peak B_1^+ , intra-pulse T_2 relaxation, the SAR limit and maximum pulse duration.

Standard pulses used for cardiac ³¹P-MRS at OCMR and in the literature are detailed in the following subsections.

2.4.2.1 Hard pulses

A “hard” or rectangular amplitude modulation function, of duration T_p and with an amplitude B_1 , creates a flip-angle at the pulse central frequency of:

$$\theta = 360 \cdot \gamma B_1 \int_{t=0}^{T_p} dt. \quad [2.19]$$

The frequency response of the pulse is given by a sinc function, the Fourier transform of a rectangular function.

2.4.2.2 Shaped pulses

It is desirable to uniformly excite the whole chemical shift range (25 ppm or approximately 3000 Hz at 7T) that metabolites appear across in ^{31}P cardiac spectra.

Though short ($\leq 50 \mu\text{s}$) rectangular excitation pulses will accomplish a flip-angle variation of $<1\%$ across this bandwidth, peak B_1^+ and SAR limits will restrict the maximum flip-angle to angles under 20° , for all coils, in the myocardium.

Sinc pulses provide uniform excitation, but with an unacceptably long dead-time.

Therefore asymmetric amplitude modulated pulses, known as “MDR7T” and “MDR3T”, were created, using a “projection onto convex states” method, to provide uniform flip-angle (and phase) excitation. Figure 2.11a shows the amplitude modulation of the 1.015 ms MDR7T pulse. The excitation phase varies by less than 2° and the flip-angle varies by less than 0.5% over the whole ^{31}P cardiac spectrum at 7T using this pulse when centred -586 Hz from PCr (Figure 2.11b). The MDR3T pulse has the same modulation but is stretched to 2.4 ms, and is suitable for whole spectrum excitation at 3T. Alternatively the MDR3T pulse may be used at 7T to increase the flip-angle compared to MDR7T, but at a penalty of only uniformly exciting 2,3-DPG, PDE, PCr and γ -ATP when placed 266 Hz from PCr.

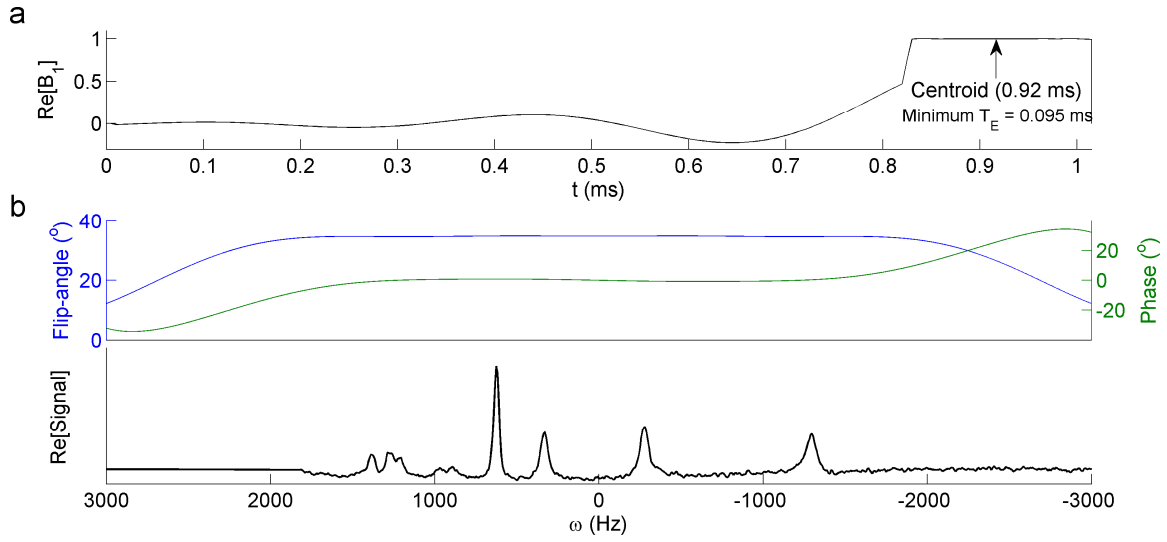


Figure 2.11 “MDR7T” pulse. **a:** Normalized amplitude modulation of the pulse. Negative values are implemented using a 180° phase shift. **b:** Flip-angle, and phase, frequency response of the pulse shown above a spectrum acquired using the pulse. Both the phase line and the spectrum have been first-order phase corrected for the delay between the centroid of the pulse and the acquisition event.

2.4.2.3 B_1 -insensitive adiabatic pulses

Adiabatic frequency and amplitude modulated pulses can provide B_1 -insensitive excitation of spins.¹⁹ Adiabatic pulses such as the B_1 -insensitive rotation (BIR-4)²⁰, adiabatic half passage (AHP)²¹ and hyper secant (HS^n)²² family of pulses produce a constant excitation flip-angle, across a calibrated bandwidth of frequencies, at values of B_1^+ exceeding the “adiabatic threshold”. The adiabatic threshold is given by the condition

$$|\gamma B_{\text{eff}}| \gg \left| \frac{d\alpha}{dt} \right|, \quad [2.20]$$

where $d\alpha/dt$ is the rate in change of the orientation of the B_{eff} vector,

$$\alpha(t) = \arctan \left[\frac{\gamma B_1(t)}{\Delta\omega(t)} \right], \quad [2.21]$$

in which $\Delta\omega$ is the difference between the instantaneous RF frequency and the Larmor frequency.

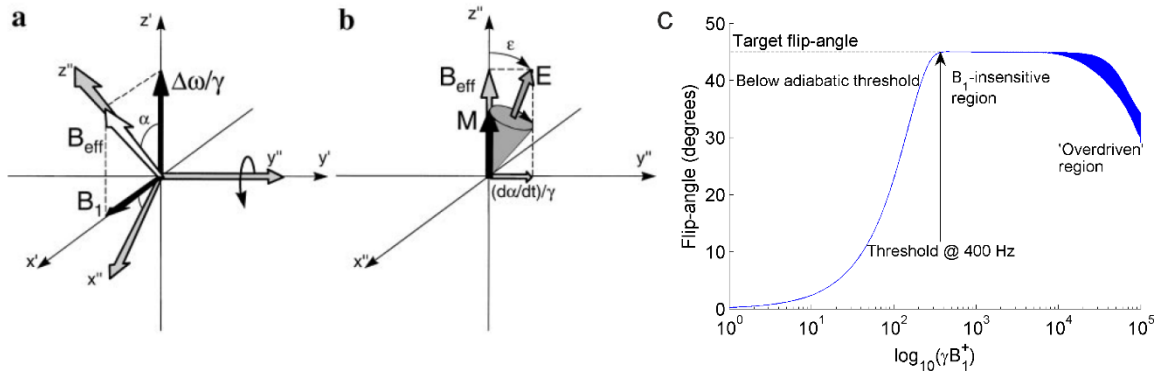


Figure 2.12 Adiabatic pulse frequency modulation gives rise to two further frames of reference, the frequency modulated frame **(a)** $[z', x', y']$ and the B_{eff} frame **(b)** $[z'', x'', y'']$. In the former, rotating at the instantaneous pulse frequency around the scanner z -axis, the B_{eff} vector can be calculated as the vector sum of the B_1 vector and the fictitious or longitudinal field $\Delta\omega/\gamma$. In an adiabatic pulse $\Delta\omega$ is time dependent (Eq. [2.21]), thus the B_{eff} frame, in which the z'' axis is collinear with the B_{eff} vector, rotates at a rate $d\alpha/dt$. In the B_{eff} frame of reference, the rotation results in a second magnetic field component $(d\alpha/dt)/\gamma$, with a resultant magnetic field E . The magnetisation M precesses around E , and thus is never at an angle more than 2ϵ from B_{eff} . When the adiabatic condition is fulfilled ϵ is small and the magnetisation is 'spin-locked' to B_{eff} in all frames of reference. Panel **c** demonstrates the adiabatic threshold for an on resonance spin after a 10 ms BIR-4 pulse. The flip-angle is insensitive to B_1 over a large range (400-10000 Hz). Panel **a** and **b** are reused with permission from ref. 19. Copyright John Wiley & Sons, Ltd 1997.

For ^{31}P -MRS at 1.5 T, BIR-4 pulses offer the ability to produce an arbitrary excitation flip-angle across several hundred Hz bandwidth, however at higher field strengths, RF coils tend to have a maximum B_1^+ too low to exceed the BIR-4 adiabatic threshold throughout the myocardium.²³ Lengthening adiabatic pulses, which already have multiple milliseconds durations, only reduces the threshold slightly. The lengthened pulses produce undesirably high levels of RF heating (SAR), and T_2 dephasing of the spin-locked magnetisation during the pulse.

AHP pulses have a relatively low adiabatic threshold, approximately 200 Hz for a 7.5 ms pulse, therefore have been used at 3T^{23,25} and recently at 7T for B_1 insensitive excitation.²⁴ Furthermore, recently acquired in vivo B_1 maps (see §2.3.3.4) suggest that B_1^+ values achieved in the interventricular septum with our quadrature coil at 7T might even pass the adiabatic threshold for optimised BIR-4 pulses.

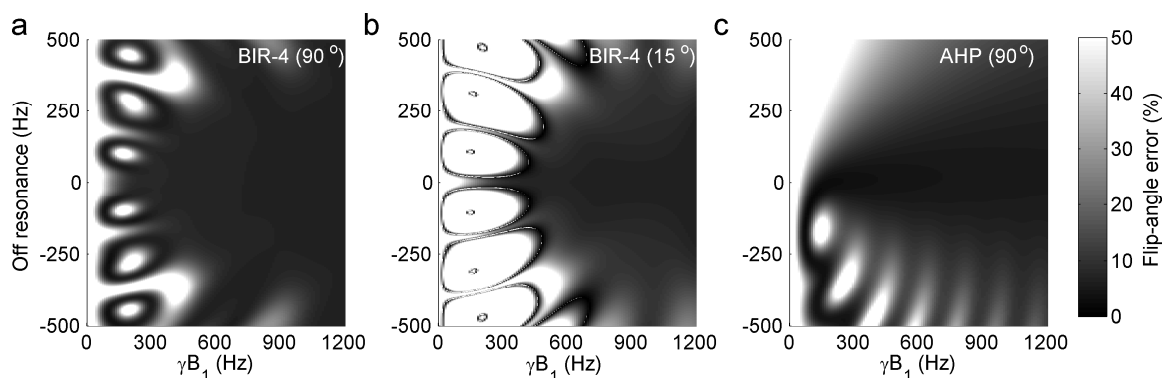


Figure 2.13 Example B_1 /frequency-offset plots of adiabatic pulses. The darker regions show the area where the pulses achieve the target flip-angle most accurately. **a**: 10 ms 90° BIR-4 pulse, **b**: 10 ms 15° BIR-4 pulse, **c**: 7.5 ms 90° AHP pulse. The AHP pulse has the lowest adiabatic onset, so it is used at $3T^{23}$ and now at $7T$.²⁴

2.5 Post-processing of spectra

Time-domain free induction decay (FID) k-space data is Fourier transformed in all spatial dimensions to render a spatially localised CSI grid of FIDs online by the scanner's image reconstruction system. Data is exported from the scanner in the DICOM spectroscopy format. Spectra from coils with multiple receive elements are combined using the WSVD method.^{10,12} Regions of the spectra containing signal from exogenous reference fiducials are zeroed before WSVD combination.

Data in the DICOM format is loaded into a custom Matlab spectroscopy handling package. Reference localiser images are automatically associated and oriented on-screen with the CSI grid.

The implementation and use of prior-knowledge constrained fitting of spectra by the AMARES algorithm in Matlab is described in detail in Chapter 3.²⁶ Fitted peak amplitudes, linewidths, chemical shifts and phases may then be used to further calculate derived parameters. The process by which metabolite amplitudes are corrected for the effects of partial saturation and signal contamination, including the estimation of excitation flip-angles is described in the following subsections.

2.5.1 Saturation correction

For metabolites experiencing longitudinal relaxation at different rates and a sequence that is not fully-relaxed ($T_R > 5T_1$), the effect of differing partial saturation (Eq. [1.48]) must be taken into account before meaningful metabolite concentrations or concentration ratios are calculated.²⁷ For this correction, the sequence repetition time, excitation flip-angle and the metabolite T_1 values must be known. The repetition time is trivially read from the DICOM headers, though in a gated acquisition an average time will be recorded. Due to time constraints, T_1 values are typically taken from the literature, but since the T_1 value is a function of chemical exchange, which may differ in diseased subjects (see Chapter 5), they perhaps ought to be measured per-subject. The flip-angle changes significantly between scans due to coil placement, subject anatomy and loading of the RF coil. Therefore, the flip-angle must either be measured directly (ref. 28 and Chapter 7) or must be calculated by scaling an estimated coil transmit field (as detailed in the following subsections).

2.5.1.1 Biot-Savart field maps

The “Biot-Savart” flip-angle calculation process starts with the creation of a Biot-Savart-law-derived field map for each transmit and receive coil. The Biot-Savart law describes the magnetic field generated by a non-varying electric current. The equation for the magnetic field $\mathbf{B}$ at position $\mathbf{r}$ is

$$\mathbf{B}(\mathbf{r}) = \frac{\mu_0}{4\pi} \int_C \frac{I d\mathbf{l} \times \mathbf{r}'}{|\mathbf{r}'|^3}, \quad [2.22]$$

which describes the path integral over all elements of the coil element's wire $\mathbf{l}$ at a displacement from position $\mathbf{r}$, $\mathbf{r}'$, and carrying current I . μ_0 is the permeability of free space.

The Biot-Savart law is limited by two approximations: firstly that the current is D.C. rather than high frequency (e.g. 120.3 MHz) alternating current, secondly that the fields permeate through free space rather than materials with varying dielectric properties (e.g. permittivity, permeability, conductivity, etc.). Providing the frequency is low enough, the “quasi-static” formalism of electromagnetism allows the Biot-Savart law to be applied to oscillating fields.²⁹

2.5.1.2 Coil spatial localisation

Once the Biot-Savart field map has been generated it must be co-registered to the coil and spectroscopy volume of interest on a per-subject basis. The coil position is recorded at the start of each protocol using MRI. At 3T, ^1H images record the position of cod liver oil capsules fixed to the coil. At 7T, since there is no ^1H body coil in a 7T scanner, ^{31}P FLASH imaging is used to record three orthogonal projection images of the location of three or more markers containing concentrated phenylphosphonic acid solution, doped with chromium (III) acetylacetonate for short T_1 .¹

The known coil geometry, and hence the Biot-Savart calculated field map, may then be coregistered to the position of the markers. The position of the markers are recorded in the same DICOM coordinate frame as any spectroscopy data acquired later in the scan session, thus by coregistering the field map with these images, it is also coregistered to the spectroscopy volume.

Flexible coils, such as the receive elements of the 7T 16-channel array coil, require additional processing. Five coil markers are attached to different areas of the flexible array. Using the single-element FLASH images from receive elements nearest to each marker, the positions of the 5 markers are unambiguously determined from the 3 orthogonal FLASH images. Matlab software, using calibrated offsets between the five fiducials, is then used to compute the out-of-plane bending of the coil, which determines the three dimensional shape of the coil elements, and allows computation of the field-maps.

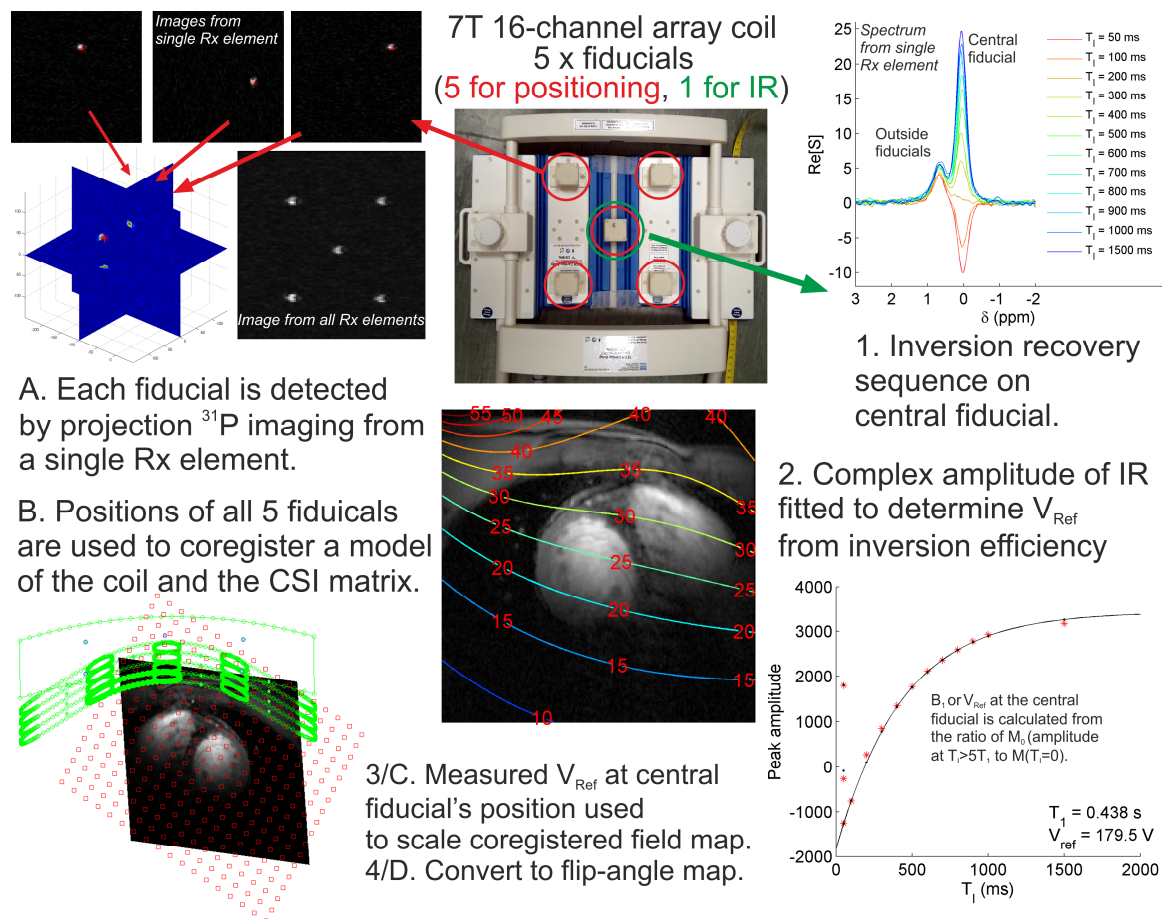


Figure 2.14 Process of estimating the in vivo flip-angle using coregistered and scaled Biot-Savart calculated field maps.

2.5.1.3 Scaled Biot-Savart estimation of the B_1 field

At 7T one of the same markers (the “primary fiducial”), and at 3T a separate ^{31}P fiducial, is used to scale the registered Biot-Savart field maps on a subject by subject basis.

A two parameter least-squares fit of an inversion recovery experiment on the fiducial enables the calculation of the reference voltage at the location of the fiducial. The field maps are then scaled to this value at the spatial location of the fiducial.

Finally a description of the excitation pulse is used to calculate the flip-angle at an arbitrary location, using the scaled B_1^+ field/reference voltage map.

2.5.1.4 B_1^+ in the fiducial as a measure of coil loading

Different samples (phantom vs. human, chest vs. extremities) have varying impedances, and therefore “load” a surface coil to a different degree. The coil must be impedance matched (§2.3) to each sample. The relative loading of a coil also changes the “quality factor” Q of the coil

$$Q = \frac{\omega L}{R}, \quad [2.23]$$

where ω is the angular frequency, L is the inductance of the coil and R is the resistance. I.e. it is the ratio of the reactance to the resistance. The filling factor is the ratio of the magnetic energy stored inside a sample loading the coil to the magnetic energy in the coil

$$\eta_f = \frac{\int_{V_{\text{load}}} |B|^2 dV}{\int_V |B|^2 dV} \approx \frac{B_1^2}{QP}, \quad [2.24]$$

where B_1 is the magnetic field in a particular point in space and P is the input power.

For a fixed power B_1 is approximately proportional to $\sqrt{Q}$, across a wide range of loads.³⁰

Scaling the Biot-Savart field maps using the B_1^+ measured at the fiducial ($B_1^+ \propto 1/V_{\text{Ref}}$) is intended to account for the variable loading of the coil by different samples, and therefore the change in B_1^+ due to the loading. To confirm the relationship between the B_1^+ measured in the fiducial and the degree of coil loading, the fiducial B_1^+ was measured, as described above, and the coil quality factor was measured, using a vector network analyser in reflection mode,³¹ with a range of sample loads. The experiment was completed on the single voxel phantom (18 litres of saline) and a single thigh, using the 7T 10 cm loop coil. The loading was varied for each sample by spacing the coil from the sample in increments of 1 cm.

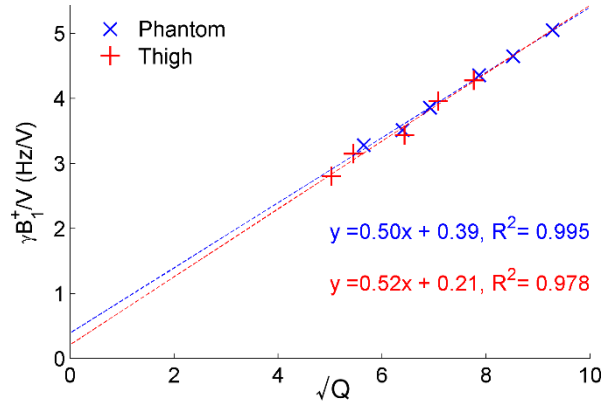


Figure 2.15 Relationship between the degree of loading of the 10 cm loop coil (quality factor, Q) and the B_1^+ measured using inversion recovery in the coil fiducial ($\gamma B_1^+ / V$).

The superimposed straight-line relationships measured (Figure 2.15) indicate the B_1^+ measured in the fiducial correctly scales the field map at the fiducial to account for coil loading.

2.5.1.5 Comparison of scaled Biot-Savart estimates with measured field maps

The same Bloch-Siegert field maps used in §2.3.3.4 were compared with scaled Biot-Savart estimated voxel field values. Though the Biot-Savart estimated values were found to be of the same order of magnitude, comparison with over 500 per-voxel Bloch-Siegert measurements showed low correlation (Figure 2.16). A full description and discussion can be found in Chapter 7.

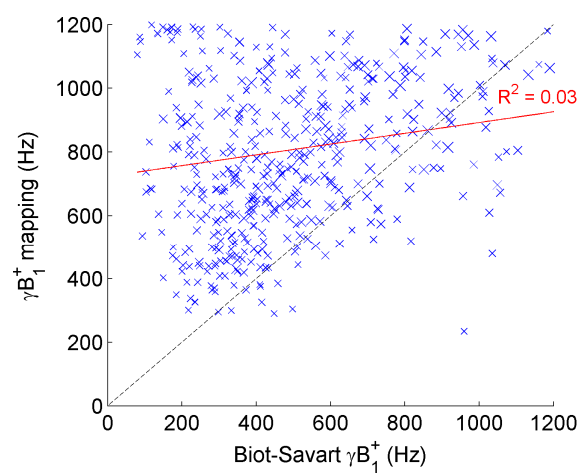


Figure 2.16 Extract from Figure 7.11. Correlation plot comparing the γB_1^+ values estimated by the scaled Biot-Savart method and a Bloch-Siegert B_1 -mapping method (Chapter 7).

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Chapter 3: Automatic fitting and post-processing of MRS data

3.1 Introduction

This chapter details the implementation of an open-source toolkit for automatic fitting and post-processing of in vivo spectroscopy data. The motivation for this work was a desire to:

1. Process large numbers of data sets using precisely the same analysis pipeline;
2. Select and fit arbitrary selections of voxels in multi-voxel spectroscopy;
3. Permit computation of error estimates of derived parameters, such as saturation and blood corrected PCr/ATP ratios;
4. To facilitate a study on array coil combination;¹
5. Enable the user to control all aspects of the fitting routine;
6. Allow rapid re-analysis of a whole study's data in the event of changes to the analysis pipeline.

To achieve these aims the fitting and processing pipeline was consolidated into a single Matlab (The Mathworks Inc., Natick, Ma, USA) “toolkit” which was designed to be able to replicate our existing analysis pipeline based on jMRUI, Matlab scripts and Excel spreadsheets.²

The first part of the chapter describes the MRS analysis toolkit, including the fitting algorithm and pre- and post-processing steps. The estimation process used to calculate the confidence intervals of fitted parameters is described in detail.

Section 3.3 contains the validation of a Matlab based fitting program against the existing publicly available fitting package jMRUI, as well as validating the estimated confidence intervals against analytical expressions from the literature.³

Finally two applications of this new automatic fitting program and analysis pipeline are shown: fitting multi-voxel ^{31}P and single-voxel ^1H -MRS data collected in Oxford and at collaborators' sites.

3.2 The AMARES fitting algorithm

The core of the analysis pipeline is a fitting method based on the advanced method for accurate, robust, and efficient spectral fitting (AMARES) algorithm.⁴ AMARES is designed to “accurately and efficiently estimat[e] the parameters of noisy magnetic resonance spectroscopy (MRS) signals in the time domain”.

AMARES makes the following improvements over previous algorithms, such as VARPRO:⁵

1. Per-peak assignments of Lorentzian, Gaussian or Voigt (a convolution of Gaussian and Lorentzian) line shapes.
2. Nonlinear least squares algorithms, which allow the imposition of upper and lower bounds on fitted parameters.
3. A singlet approach for the imposition of prior knowledge, allowing a wider range of functional relationships between model parameters to be described.

Upper and lower bounds enable AMARES to restrict model parameters to physically reasonable values, e.g. positive linewidths and amplitudes, which previous methods which use simple (e.g. Levenberg-Marquardt) least squares algorithms cannot do.

AMARES fits the MRS time domain data, a free induction decay (FID), in a least-squares sense to the model function

$$y_n = y_n + e_n = \sum_{k=1}^K a_k e^{i\phi_k} e^{(-d_k(1-g_k+g_k t_n)t_n)} e^{i2\pi f_k t_n} + e_n, \quad [3.1]$$

where $i = \sqrt{-1}$, a_k is the amplitude, ϕ_k is the phase, d_k is the damping factor, and f_k is the frequency of the k^{th} sinusoid ($k = 1, \dots, K$), and e_n is complex white Gaussian noise. $t_n = n\Delta t + t_0$ where Δt is the dwell time (or sampling interval) and t_0 is the “begin time” which accounts for receiver dead-time and the finite length of the RF excitation pulse.

The AMARES algorithm performs similarly to LCModel, a method based on the linear combination of model spectra, commonly used in ^1H -MRS of the brain.⁶

3.2.1 Prior knowledge

“Prior knowledge” means a set of restrictions on the values of parameters in a model derived from physical insight into the system. The prior knowledge in AMARES fitting of MRS data may be split into three categories:

1. Initial values of model parameters e.g. known metabolite chemical shifts.
2. Upper and lower bounds on model parameters.
3. Algebraic relationships between model parameters. I.e. a second model parameter is estimated as a function of one or more fitted model parameter(s).

The first category ensures that the model converges rapidly to the true minimum, rather than converging to another local minimum, or requiring a (very) large number of steps to converge.

The second category ensures that model parameters, fitted and derived, remain within physically reasonable values when the data have low signal-to-noise. Or, in the case of closely approaching peaks, that the peaks remain separated and do not converge to fit a single, non-Lorentzian, peak.

The third category decreases the uncertainty in each related parameter's fit, increasing the precision of the overall measurement.⁷ The relationships are usually based on chemical knowledge of the system. Common examples of the relationships imposed in cardiac ³¹P-MRS are:

- Fixed phases of signals arising from the same tissue. Note that first-order phase and phase introduced by any RF pulse may still result in differing peak phases.
- Equal amplitudes between (near) equivalent groups in the same molecule. E.g. equal amplitudes of the two phosphate groups in 2,3-DPG.
- Fixed linewidths, phases, chemical shift displacements and amplitude ratios between individual peaks in a multiplet. E.g. γ - and α -ATP (1:1 doublets) and β -ATP (1:2:1 triplet).

3.2.2 Cramér-Rao estimation of the error

The Cramér-Rao Lower Bound (CRLB) gives a lower bound of the variance of unbiased estimators. In the context of fitting MRS data, the CRLB may be seen as the attainable precision of estimates of a parameter, given a set of observations.^{8,9}

The CRLB of a parameter p of peak l may be calculated as the square root of the corresponding element in the inverse of the Fisher information matrix $\mathbf{F}$:

$$\sigma_{p_l} \geq \text{CRLB}_{p_l} = \sqrt{\mathbf{F}_{ll}^{-1}}. \quad [3.2]$$

The Fisher information $\mathbf{F}$ is calculated as follows

$$\mathbf{F} = \frac{1}{\sigma^2} \Re(\mathbf{P}^T \mathbf{D}^H \mathbf{D} \mathbf{P}). \quad [3.3]$$

$\mathbf{D}$ is defined as

$$\mathbf{D}_{nl} = \frac{\partial \hat{x}_n}{\partial p_l}, \quad n = 0, 1, \dots, N-1, \quad l = 1, 2, \dots, N_p, \quad [3.4]$$

where $\hat{x}$ is the complex time domain model output (“estimator”), p_l is one of N_p real-valued model parameters. $\mathbf{P}$ is the prior knowledge matrix

$$\mathbf{P}_{lm} = \frac{\partial p_l}{\partial p'_m}; \quad l = 1, 2, \dots, 4K; \quad m = 1, 2, \dots, L; \quad [3.5]$$

in which $p' = (p'_1, \dots, p'_L)^T$ is the L unconstrained parameters p estimated.³ K is the number of damped sinusoids (or peaks); if $L = 4K$ (i.e. amplitude, phase, chemical shift and linewidth [4 parameters] for each peak K), then $\mathbf{P}$ is the identity matrix.

The prior knowledge matrix $\mathbf{P}$ converts between the full set of N_p parameters, which includes all the model parameters and also any derived parameters (e.g. PCr/ATP ratio), and the possibly smaller set of L fitted parameters. Imposing prior-knowledge results in a minimisation problem with fewer parameters, and a lower CRLB for each parameter i.e. a greater fit precision. Typically, the largest reductions in parameter CRLBs occur in cases where the peaks overlap to an extent that they can no longer be resolved, but where a single Lorentzian or Gaussian peak is insufficient to fit the experimental data.⁴

3.3 Implementation and validation of AMARES in Matlab

Previously the MRS analysis pipeline at Oxford required data to be manually transferred between a Matlab program, for loading and interpreting from “.RDA” format, TWIX format and DICOM format, with some pre-processing steps; jMRUI, for fitting; and Excel, for saturation-correction, collation of data across a study and statistical analysis.

It was decided to consolidate all parts of this process into a single programming environment, Matlab. To accomplish this, the AMARES algorithm had to be implemented into Matlab and subsequently validated against the existing implementation in jMRUI. Once achieved, the fitting process could directly interface with an extensive DICOM and spectroscopy data handling framework already written by our group, and the results could be automatically processed in Matlab.

The subsequent subsections detail the implementation of AMARES in Matlab and its validation against AMARES in jMRUI. The Matlab and jMRUI AMARES estimates of the CRLB were also checked against analytical expressions from the literature.

3.3.1 Methods

3.3.1.1 AMARES in Matlab

The AMARES algorithm was first tested in Matlab R2014a on Windows 7 64-bit computers. It has subsequently been used with Matlab R2012b to R2015b, running on Windows XP, 7, 8, 8.1, 10 (32-bit and 64-bit), Linux 64-bit and Mac OS/X platforms.

The AMARES algorithm fits the raw (not apodized) time-domain signal in a voxel, taking as inputs:

- The data in the form of the free induction decay (FID).
- Experimental acquisition parameters, e.g. the bandwidth and central frequency.
- A description of the prior-knowledge. The prior-knowledge contains:
 - Initial values for each fitting parameter.
 - Upper and lower bounds, if any, on each parameter.
 - Arbitrary constraints between the fitted parameters.

Data is provided to the core fitting routine on an individual voxel basis, though the driving code can handle an arbitrary number and selection of voxels to fit consecutively.

The output provides the values, and CRLB, of the chemical shift, amplitude, linewidth and phase for each peak described in the prior knowledge.

Pre-processing

Either the user supplies essential parameters such as the begin time and frequency offset to the fitting program or a driving function is used to compute or estimate these parameters automatically. The begin time is calculated on a per-subject basis, using sequence parameters (pulse duration, CSI matrix size and sequence dead-time) read from the DICOM file headers. The frequency offset, the distance from the 0 ppm point in the prior knowledge to the central frequency point in the data, is estimated by convolving the experimental magnitude spectrum with a synthetic spectrum constructed from the prior knowledge initial values.

Fitting

Before the primary non-linear least squares step, a linear least squares step is used to estimate the peak amplitudes and phases. The Matlab function “mldivide” (i.e. “\”) is

used obtain a solution to the linear equation $\mathbf{Ax} = \mathbf{B}$; where x is a complex vector used to scale, per-peak or group of peaks with the same phase or amplitude - as indicated in the prior knowledge supplied by the user, the initial phase and amplitude values to the input data. This linear step decreases the iteration count and decreases the chance of the solution converging to local minima in the main non-linear iterative step.

The main non-linear step uses the the Matlab optimisation toolbox function “lsqcurvefit” for the iterative least squares fitting. lsqcurvefit’s “trust-region-reflective” algorithm options permits the imposition of upper and lower bounds on any parameters.

Presentation of the fit

Matlab AMARES returns the amplitudes, chemical shifts, linewidths and phases of each individual peak, reporting multiplet components separately. The convergence of the fit is reported as detailed in the documentation of “lsqcurvefit”. An option controls whether the per-voxel fit is displayed (Figure 3.1).

Error estimation

Matlab AMARES outputs the Cramér-Rao Lower Bounds, a lower bound on the variance of the model estimators. The CRLBs are calculated as described in section 3.2.2 and references 3 and 10. The standard deviation of the noise is estimated from the baseline of part of the spectrum remote from any peaks, as defined by the user.

For Lorentzian peaks, the individual elements of the Jacobian of the fitting function are pre-computed for speed, without loss of generality.

The framework for calculating the CRLB allows the user to calculate arbitrary derived parameters and their corresponding CRLB e.g. metabolite ratios. This was not possible

with the jMRUI implementation of AMARES because it did not output the full covariance matrix, only the diagonal terms.

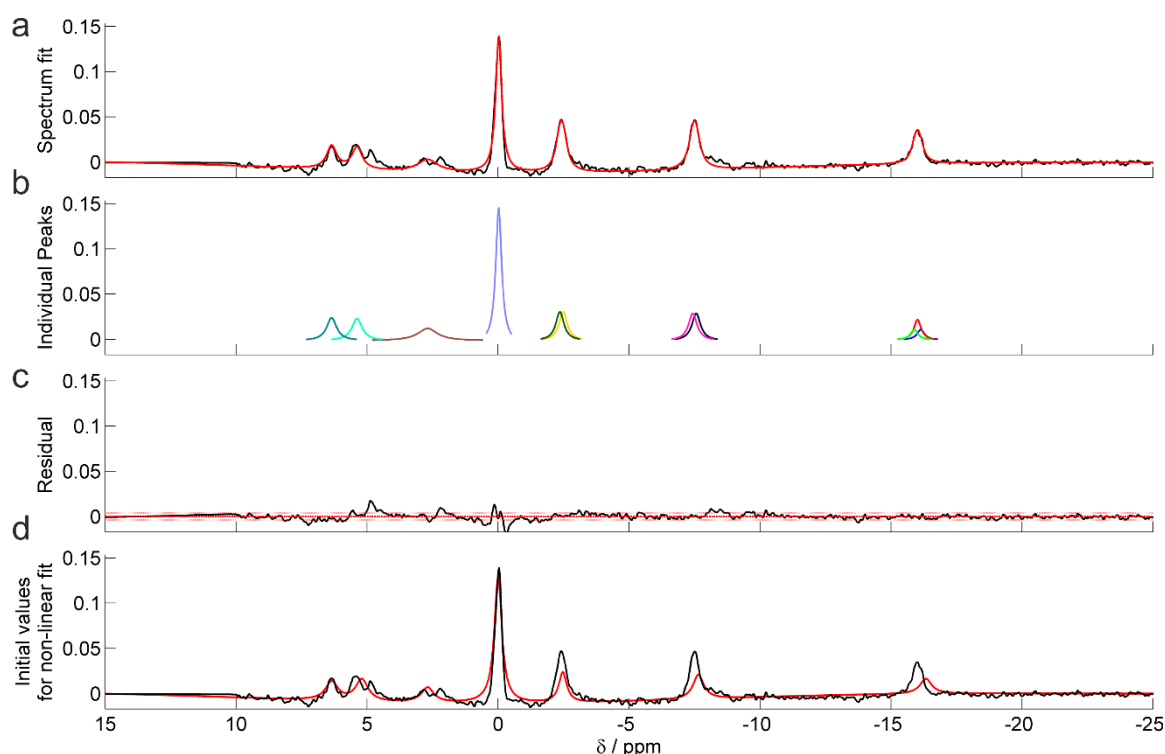


Figure 3.1 Sample graphical output from the Matlab AMARES fitting program. A 7T cardiac ^{31}P -MRS spectrum (black curve, **a&d**) from the mid interventricular septum of a normal volunteer has been fitted (red curve in **a**). Starting from the model described by the prior-knowledge with the linear least squares step (**d**, black=data, red=model). The fit comprises 7 individual metabolite peaks, comprising both singlet and multiplets (plotted separately in **b**). The phases of all the peaks are restricted to a single value and chemical prior knowledge has been provided for the (unresolved) multiplets. The residual (black line in **c**, red dashed lines mark ± 1 noise SD) makes it evident that there are peaks not-included in the prior knowledge which have not been fitted (at 5 ppm, 2 ppm and -8 ppm).

3.3.1.2 Automatic jMRUI implementation

To make a detailed comparison of the AMARES algorithm in Matlab and jMRUI feasible, software was created to allow jMRUI to be started and controlled from Matlab. A Java (Oracle, Redwood City, CA, USA) application was created to interact directly with the Java-based user interface of jMRUI. In turn, this Java application may be controlled by a Matlab program without user intervention.

The full process may be summarised as follows:

1. Data is loaded from DICOM files by our Matlab code.
2. Selected data, one or more voxels, is exported to text file in jMRUI-readable format.
3. A new instance of jMRUI is started by the Matlab/Java driving application and the data from the same text file loaded by jMRUI.
4. Any phasing, apodization and frequency offsets are applied to the data.
5. The AMARES quantitation module is opened, prior knowledge files loaded and the quantitation run. The prior knowledge may be updated dynamically if required (e.g. for different begin times) by Matlab code that alters the jMRUI prior-knowledge (.pk, .sv, or .op) binary files.
6. The jMRUI AMARES results are saved in text format.
7. These results are read in Matlab.

Version 4 (build 113) of jMRUI was used throughout the comparison.

3.3.1.3 One peak comparison

Fitting of one singlet peak with AMARES in Matlab was compared against the results of fitting the same data with AMARES in jMRUI. Synthetic time-domain signals, the inverse Fourier transform of a single on-resonance singlet peak with an amplitude of 10, a linewidth of 10 Hz and 0° phase, were generated with a variable amount of additive white complex Gaussian noise. The variance of the real and imaginary parts of the noise was stepped logarithmically in 8 steps from 10^{-4} to 10^3 . For each noise level, 500 spectra

were generated with different, but equal variance noise in a Monte Carlo fashion. Other simulated experimental parameters were bandwidth = 6000 Hz, 2048 samples, central frequency = 120 MHz and begin time = 0 s.

Each of the 4000 simulated spectra were fitted in both programs with single Lorentzian peaks. The fitted parameters: amplitudes, chemical shifts, linewidths and phases, and each parameter's respective CRLB were recorded. The total time taken to fit the 4000 spectra by each implementation of AMARES was recorded.

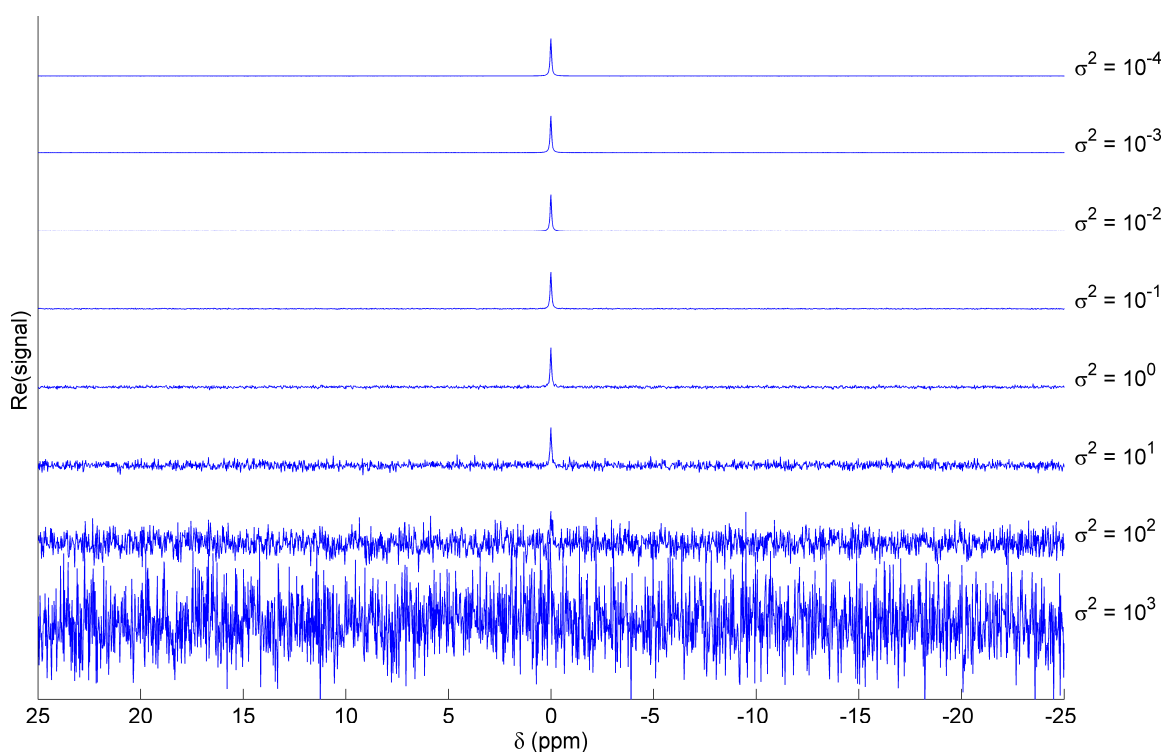


Figure 3.2 Example frequency-domain synthetic data of a singlet on-resonance peak. Each spectrum is a single example of the 500 spectra generated per noise level. The variance of the real and complex parts of the noise is denoted on the right of the figure.

The mean and standard deviation of each fitted parameter and CRLB was calculated for each noise level. The mean fitted parameters were compared either as percentage errors (amplitude and linewidth) or as the difference between the true and fitted values (chemical shift and phase). The mean CRLBs were compared against analytical

expressions for the CRLB of an isolated singlet peak,³ and the Monte Carlo standard deviation of the corresponding fitted model parameter.

3.3.1.4 Multiplet comparison

The Monte Carlo test was repeated for on resonance, symmetrical doublet and triplet multiplets. An additional test was carried out for the multiplets: the CRLBs were compared with analytical expressions for symmetric doublet and triplet peaks as a function of the scalar coupling (multiplet peak separation).

The multiplet comparison was carried out as described for single peaks. The doublet peaks were separated by 14 Hz; the triplet side peaks were separated by 14 Hz from the central peak. The peaks were fitted with prior-knowledge which enforced equal linewidth and phase, fixed chemical shift and suitable amplitude ratios (1:1 for doublets and 1:2:1 for triplets) between the multiplet peaks. The individual fitted multiplet components were restricted to Lorentzian lineshapes.

Cavassila *et al.*³ derived correction factors to be applied to the isolated singlet CRLB expressions when fitting multiplets. The correction factors are functions of an overlap factor, $R = (2\alpha) / \Delta\omega$, where α is the damping ($\alpha = \pi \times \text{FWHM linewidth}$) in Hz and $\Delta\omega$ is the separation of the component multiplet peaks in rad s^{-1} . The correction factors are high-order polynomials, unique for different prior-knowledge, and may be found in Tables 2 and 3 of reference 3.

Additional tests of the multiplet CRLB, in situations with different peak overlaps, were made on low noise data, $\sigma^2 = 10^{-3}$. The centre of the multiplet peaks was set to 0 ppm, the linewidths set to 10 Hz and all peaks had 0° phase. Total multiplet amplitude

was equal to 10. $\Delta\omega$ was varied so that the peaks were separated by between 0 and 480 Hz (0 to 4 ppm).

The CRLB were compared at each separation value using three different sets of prior knowledge:

1. Independent peaks. All fitted parameters for a peak were estimated simultaneously, but independent of the other constituent peaks in the multiplet.
2. Fixed absolute chemical shifts and fixed linewidths. No restrictions on relative amplitudes or phase.
3. Chemical prior knowledge. I.e. Equal linewidths and phases, fixed relative chemical shifts and appropriate amplitude ratios (1:1 for doublets and 1:2:1 for triplets).

3.3.1.5 Testing with experimental ^{31}P -data

The AMARES implementations in jMRUI and Matlab were compared using 45 cardiac ^{31}P -MRS datasets acquired at 3T and 7T as described previously.¹¹ For each subject, the spectrum from the mid-interventricular septal voxel was selected for comparison. In both programs the data was fitted using standard cardiac prior knowledge (Appendix B.1). Begin time and frequency offsets were calculated independently of either fitting method on a per-subject basis. The fitted parameters and CRLB from the two versions of AMARES were compared across three peaks in the spectrum (PCr, γ -ATP, PDE), representing high, medium and low relative SNR.

3.3.2 Results

3.3.2.1 Single peak

Both Matlab AMARES and jMRUI correctly estimated the parameters of the single peak for noise variances less than 10^3 . At the 10^3 level both methods demonstrated bias in fitting the amplitude and linewidth parameters (Figure 3.3).

Below the 10^3 noise level, the mean CRLB of each fitted parameter, estimated by the Matlab AMARES program, matched - within 1% - the standard deviation of the fitted parameters of both jMRUI and Matlab AMARES and the values predicted by Cavassila *et al.* for isolated singlets. At the 10^3 noise level, the standard deviation of the jMRUI and Matlab AMARES fits deviated (by $< 10\%$) from the CRLB estimates and the literature values (Figure 3.4).

The mean CRLB values estimated by jMRUI initially did not match the standard deviation of the fitted parameters as estimated by jMRUI or Matlab AMARES, or the predicted values of Cavassila *et al.* However, dividing the jMRUI computed CRLB values by the estimated noise corrected the jMRUI CRLBs to the expected values. This was later confirmed as being a bug in jMRUI in a private email communication with the developers of jMRUI. The bug is no longer present in v5 of jMRUI, but is thought to be present in versions prior to v4.

The time taken to fit all 4000 spectra, including all steps (1-7) from §3.3.1.2, was 91 s for the automatic jMRUI program and 80 s for the Matlab implementation.

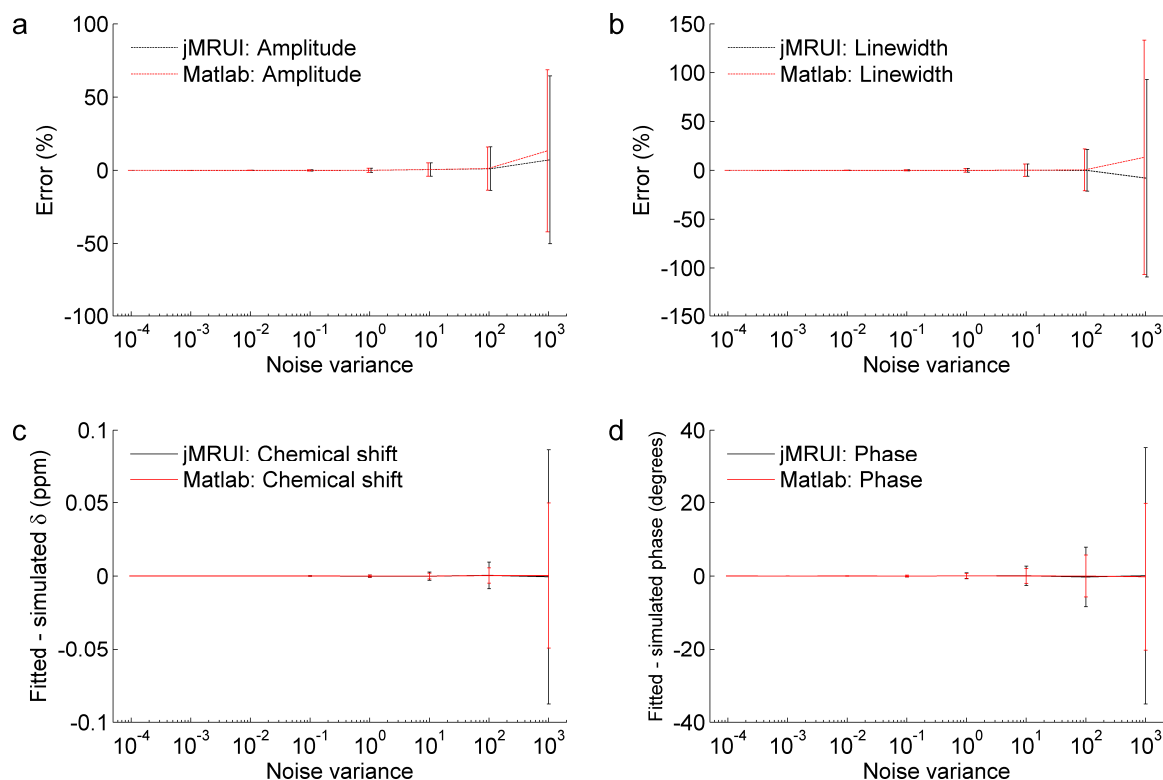


Figure 3.3 Monte Carlo comparison of the fitted parameters (**a**: amplitude, **b**: linewidths, **c**: chemical shifts and **d**: phase) of a single on-resonance singlet peak at varying noise levels. The values are plotted as the mean error (amplitude and linewidth) or difference (chemical shift and phase) between the fitted and simulation input values of 500 independently fitted spectra per noise level. The standard deviation of the values are plotted as error bars.

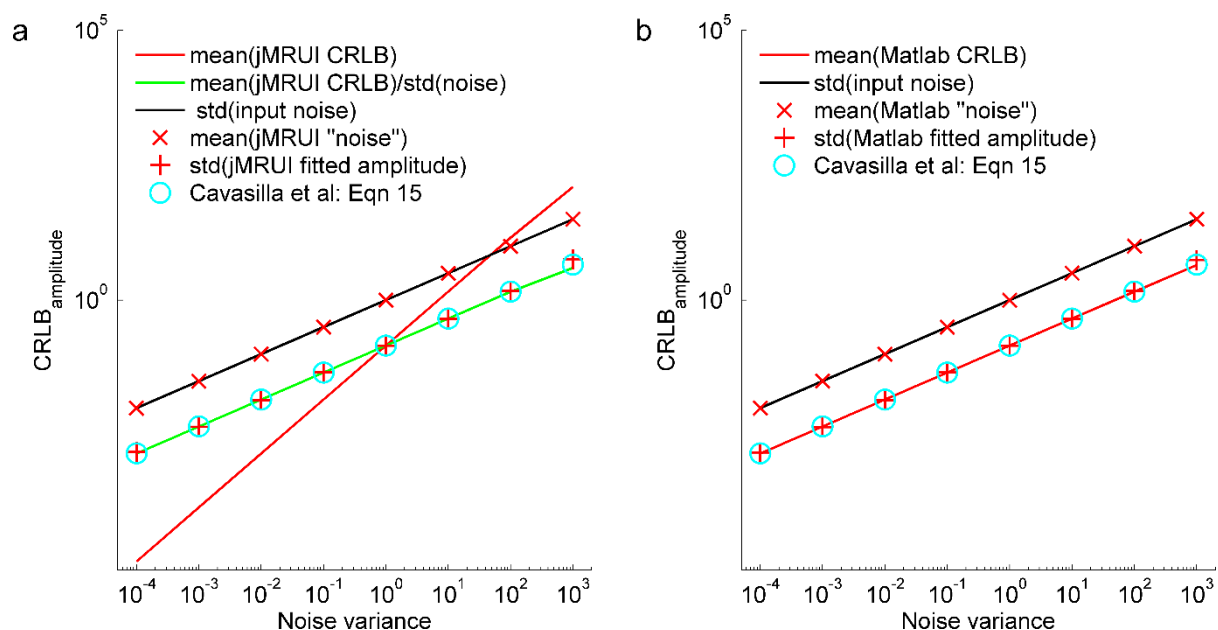


Figure 3.4 Comparison of the amplitude CRLB of a single isolated peak, estimated by jMRUI (**a**) and Matlab (**b**), with the standard deviation of the fitted parameters and the analytical expression given by Equation 15 in reference 3. The CRLB estimated by jMRUI was divided by the noise estimate to correct a confirmed bug. Additionally the standard deviation of the baseline noise estimated by both implementations is compared with the standard deviation of noise generated in the simulation.

3.3.2.2 Multiplets

Both jMRUI and Matlab AMARES showed negligible errors in fitting peak parameters for doublets or triplets at the fixed separation of 14 Hz at noise levels below 10^2 (Figure 3.5a). At 10^2 a low (<1%) mean systematic error in amplitude and linewidth was observed in both jMRUI and Matlab, at higher noise levels larger errors were observed with both implementations for all estimated parameters.

The CRLB estimates calculated by Matlab AMARES were within 1% of the Monte Carlo standard deviation of the fitted parameters, and the values predicted by Cavasilla *et al.*, at all noise levels (Figure 3.5b). At noise levels above 10 some divergence from the standard deviation of the fitted parameters was observed, though this was low (<10%) until 10^3 where the values of the CRLB and the standard deviation diverged by up to 50%.

Total fitting times were 103 s and 95 s for jMRUI and Matlab AMARES respectively.

The CRLB of all fitted parameters matched, within 0.1%, those predicted by Cavassila *et al.* (Ref. 3 Table 2), for doublets with varying peak separation and different prior-knowledge constraints (Figure 3.6). The CRLB values for triplets also matched, within 0.1%, the values given by Cavassila *et al.* (Ref. 3, Table 3), for all separations and prior-knowledge sets (not shown).

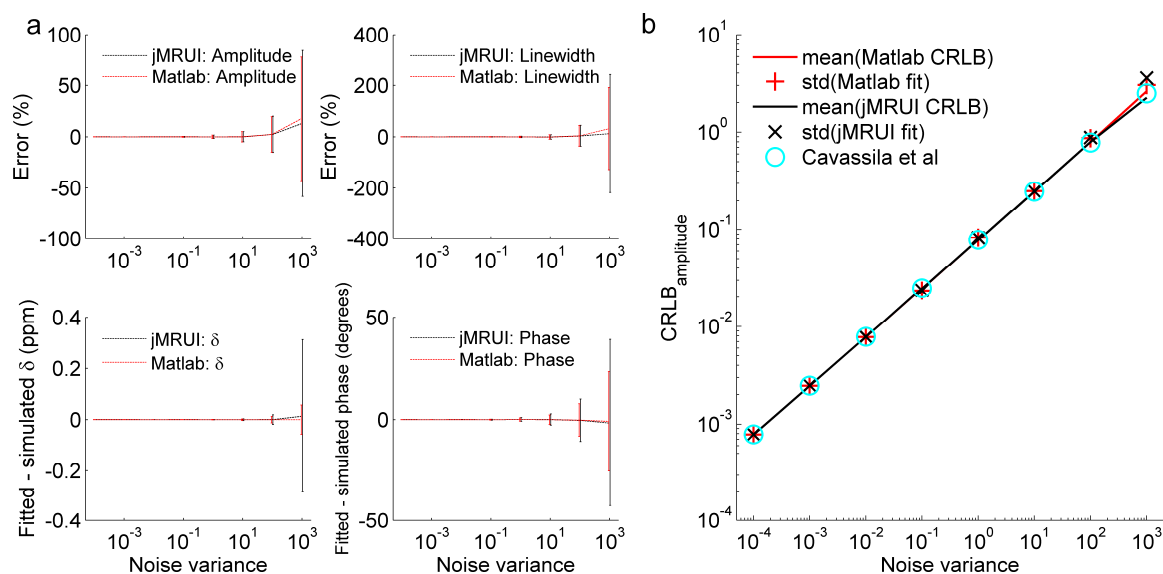


Figure 3.5 **a:** Monte Carlo comparison of the fitted parameters of one peak of an on-resonance doublet, separated by 14 Hz from the other peak, at varying noise levels. The values are plotted as the mean error (amplitude and linewidth) or difference (chemical shift and phase) between the fitted and simulation input values of 500 independently fitted spectra per noise level. The standard deviation of the values are plotted as error bars. **b:** Comparison of the amplitude CRLB of the doublet peak with the standard deviation of the 500 fitted values per noise level and the analytical values given in Table 2 of reference 3.

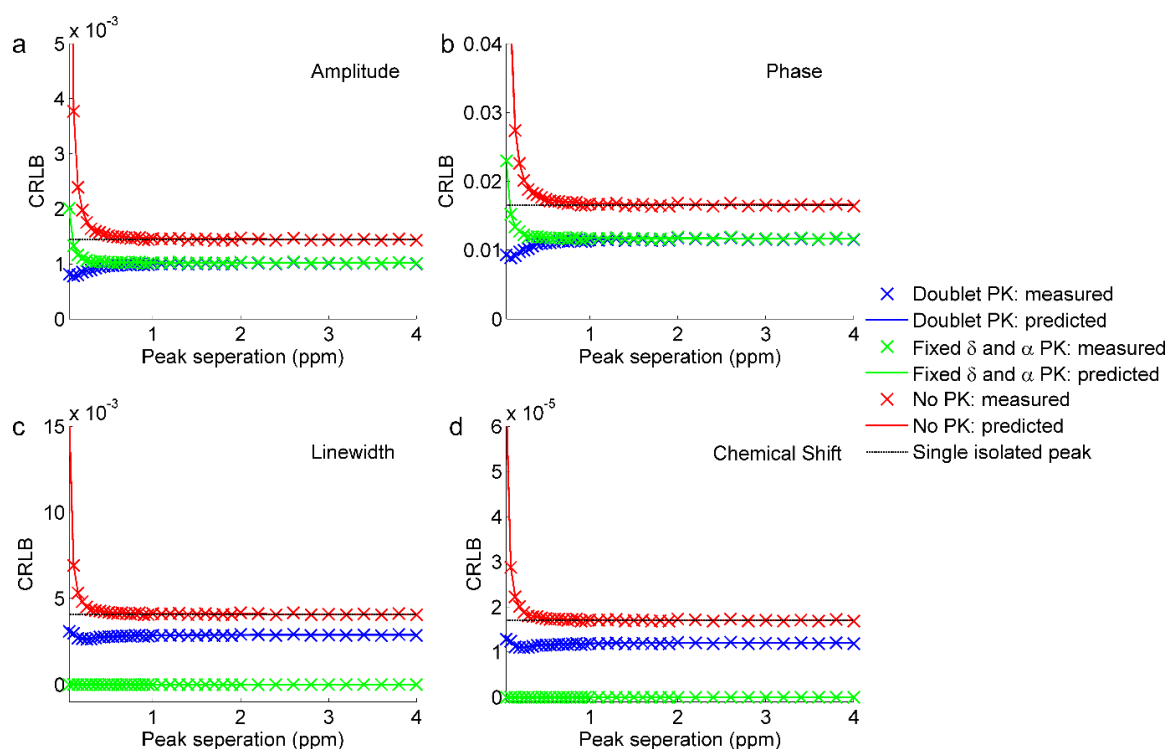


Figure 3.6 CRLB on the four fitted parameters (**a:** amplitude, **b:** phase, **c:** linewidth and **d:** chemical shifts) for one doublet peak at varying peak separation. “Measured” values are the values output by the Matlab AMARES implementation, “predicted” are the values calculated from Table 2 of reference 3. The different colours indicate the values calculated for different prior knowledge sets; as expected the most restrictive prior knowledge produces the smallest CRLBs.

3.3.2.3 Comparison using ^{31}P -MRS data

Excellent correlation (>0.99) was observed between the peak parameters of the PCr singlet and γ -ATP doublet fitted by jMRUI AMARES and Matlab AMARES (Figure 3.7). The low SNR PDE peak showed lower correlation, though always above 0.7. Very low, but statistically significant ($p < 0.05$) bias between the methods was observed for amplitude and linewidths for all peaks. No significant bias was seen for chemical shift and phase. Mean ($\pm$ SD) peak SNR was 23.0 (± 12.9) for PCr, 12.7 (± 6.6) for γ -ATP and 4.1 (± 1.5) for PDE.

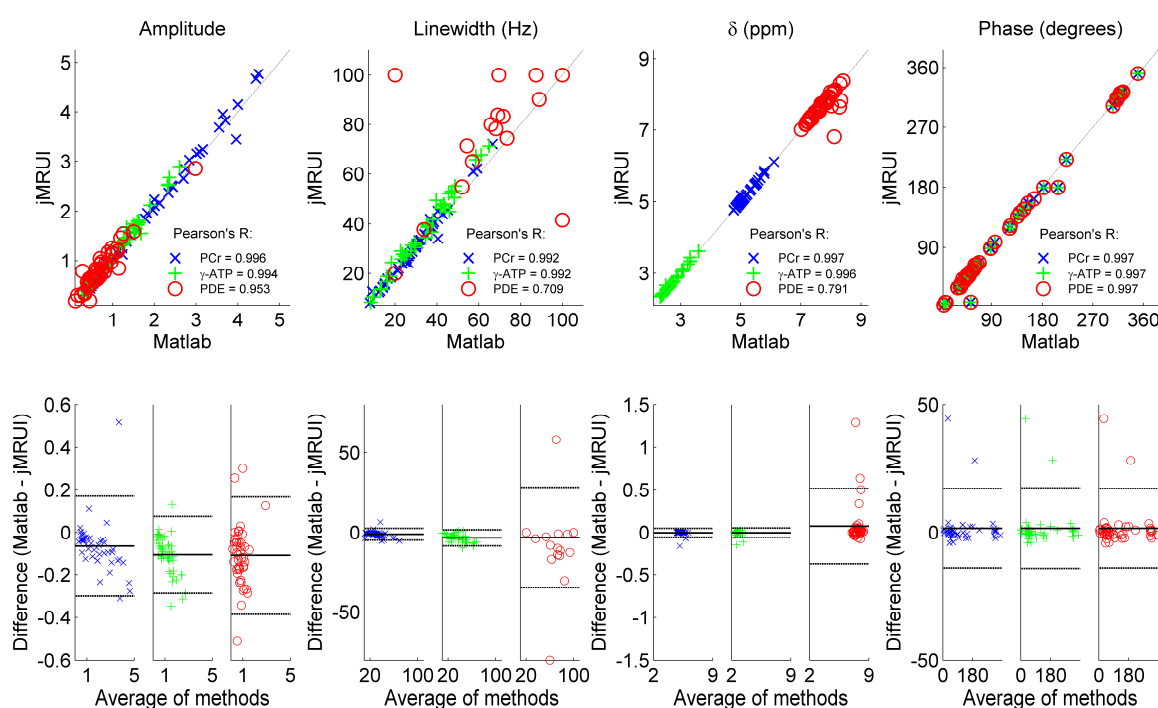


Figure 3.7 Correlation and Bland-Altman plots for each peak parameter fitted across high (PCr), medium (γ -ATP) and low (PDE) SNR peaks by jMRUI and Matlab AMARES for 45 cardiac ^{31}P spectra.

Strong correlation (>0.95) was also observed between the jMRUI and Matlab estimates of the CRLB peak parameters of the PCr and γ -ATP peaks (Figure 3.8). PDE showed correlations >0.7 . Very low, but significant ($P < 0.05$) bias was observed for all compared CRLBs except the phase and linewidth of PDE. This was found to be as a result of the

different noise estimation mechanism in each method. jMRUI estimates the noise from the whole residual, whilst the Matlab AMARES method estimates the noise from the baseline of part of the spectrum with no peaks.

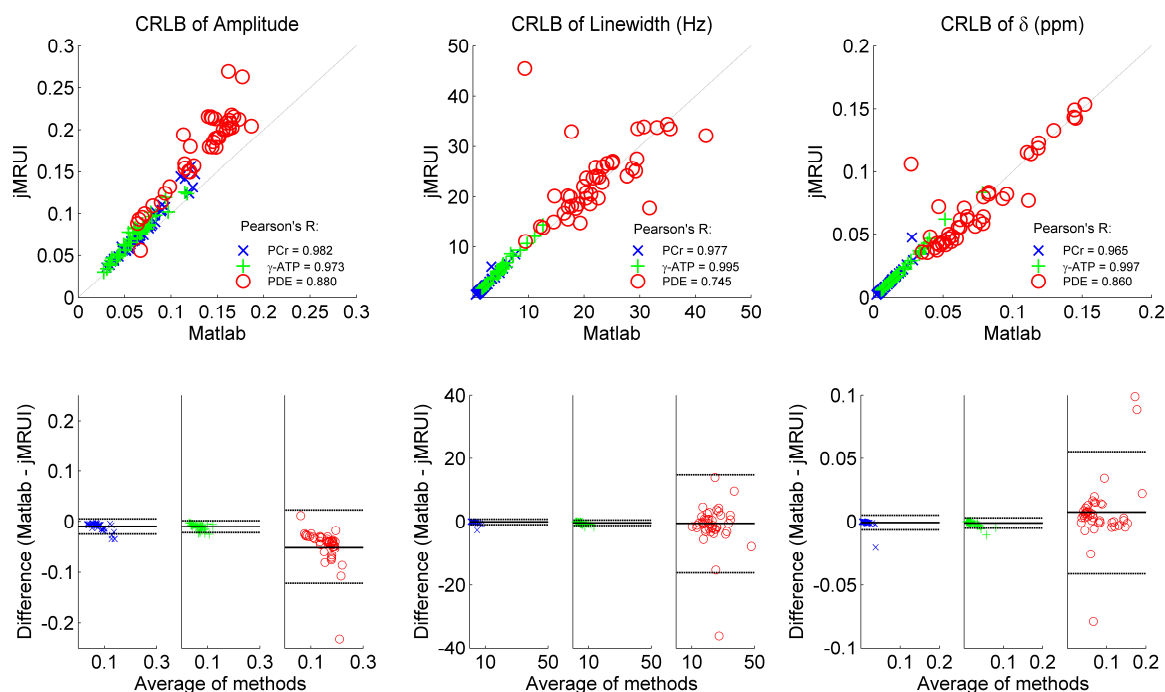


Figure 3.8 Correlation and Bland-Altman plots for each peak parameter CRLB across high (PCr), medium (γ -ATP) and low (PDE) SNR peaks. The CRLB of the phase was not output by jMRUI.

3.3.3 Conclusion

The Matlab implementation of the AMARES fitting algorithm has been demonstrated to perform equivalently to the closed-source binary implementation in the jMRUI software package. This was established using extensive tests on simulated and real world data. Additionally the confidence intervals of the fitted parameters have been tested against analytical expressions for singlet and multiplet peaks.

The open source Matlab implementation of AMARES enables the seamless and rapid fitting of large quantities of data as part of an integrated pipeline, whilst also allowing the user extensive customisability over all aspects of the fitting process. For example, it

would be trivial to impose spatial smoothness constraints between neighbouring voxels, or to jointly fit spectra in a rest-exercise-recovery protocol, or to jointly fit repeated measurements in a subject with some parameters fixed and others allowed to vary.

3.4 Applications

3.4.1 Automatic analysis of ^{31}P -MRS data

During my doctoral work, I have assisted with four clinical studies that used cardiac ^{31}P -MRS for investigating the metabolic effects of dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), type 2 diabetes mellitus (T2DM) and atrial fibrillation (AF). These studies, involving both recruited patient and healthy populations, are led by clinical doctoral students, who are not experienced in MR physics or in analysis of spectroscopy data. Therefore, there is a large quantity of acquired data, which requires a well-validated and simple-to-operate, analysis tool. The tool should be flexible enough to process data from all ongoing ^{31}P -MRS studies, i.e. those measuring a PCr/ATP ratio, in the department.

Overall, during this time, 297 patient and 98 healthy volunteer datasets have been acquired in the department's 3T and 7T scanners (see Table 3.1 for a breakdown of the numbers).

Based around the Matlab AMARES implementation and an extensive spectroscopy and DICOM data handling package, written by Dr Rodgers in Matlab, I created and validated a standalone analysis program. The program is capable of loading multi-voxel MRS data, acquired on Siemens scanners, from the DICOM format, automatically fitting the spectra, calculating the saturation corrected PCr/ATP ratio and finally exporting reports

summarising the data in both PDF and Excel formats. The tool may be compiled (using the Matlab compiler toolbox) for use on computers without a Matlab license.

Subject group	Field Strength	Number of datasets	References	Study fellow
Type 2 Diabetes Mellitus (T2DM)	3 T	66	12,13	EL
	7 T	11	†	
Dilated cardiomyopathy (DCM)	3 T	25	14	VS
	7 T	25	14	
Atrial Fibrillation (AF)	3 T	95	†	RW
Hypertrophic cardiomyopathy (HCM)	3 T	75	†	RA
Healthy volunteers	3 T	88	12,13, †	All
	7 T	10	14	

Table 3.1 Summary of patient and healthy volunteer cardiac ³¹P-MRS data acquired at 3T and 7T magnetic field strengths at Oxford between 2012 and 2015. †Denotes data from Oxford that is unpublished, or “in press”.

Analysis may be run at scan-time for immediate quality-control of the data, or it may be run on an arbitrary number of data sets for batch processing of data. To compare ease-of-use quantitatively, one clinical fellow fitted a set of 10 data sets using the Matlab tool and our previous jMRUI-based pipeline (applicable to 3T data only). The mean analysis time was 1:08 min per dataset with the Matlab tool, compared to 4:31 min using the original processing pipeline (in Matlab, jMRUI and Excel).

By offering an easy-to-use semi-automated analysis tool, where the researcher only has to provide basic experiment information (e.g. choice of prior knowledge based on organ and field strength), and choose the voxel to analyse, the chances of human error and/or

researcher bias should be decreased. At the same time, the new tool assists researchers in two ways:

- Enabling simple, but consistent, analysis and quality control in larger studies - some current studies will have 200 data sets when complete.
- Allowing the pooling of data for meta-analysis of several studies more easily. A unified analysis pipeline and the structured output format of the reports allows the extensive historical ^{31}P -MRS data archive held by Oxford (e.g. over 80 3T ^{31}P -MRS HCM datasets exist from before 2012) to be utilised to increase the statistical power of new investigations.

Finally it is hoped that both the dedicated analysis tool, and the underlying Matlab object-oriented toolkit for MRS data loading, processing and fitting, will become the basis of a more widely-distributed standard set of tools for ^{31}P -MRS. To that extent, the tool has been distributed to national and international collaborators at the University of Edinburgh, the University of Auburn, the Center for Magnetic Resonance Research (CMRR) in the University of Minnesota, the Medical University of Vienna, the Radboud University Medical Centre in Nijmegen, and the University Medical Center (UMC) in Utrecht.

The Matlab AMARES code and the compiled analysis tool are intended to be released as open source software once a manuscript describing the implementation is accepted for publication (recently submitted to NMR Biomed.).

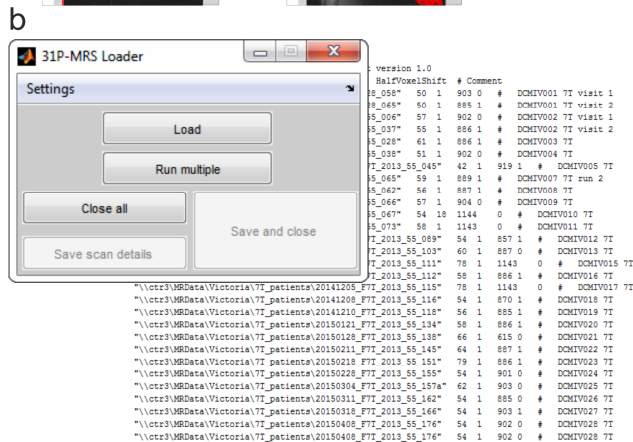


Figure 3.9 Automated Matlab AMARES fitting program and output. **a:** Main Matlab spectroscopy handling software GUI for the processing and analysis of single datasets. **b:** GUI and example text file for the automated analysis software. The text file specifies the file directory of the data along with the DICOM series, instance and voxel to use in the analysis. The prior knowledge can also be defined in this file or be chosen at runtime. **c:** Sample fitting output report in PDF format. The AMARES fit and localisers are displayed alongside the raw fitting data and the derived PCr/ATP ratios (and CRLB). The data is also exported to Microsoft Excel for easy collation of the data.

3.4.2 Automatic coil combination and fitting of cardiac ^1H spectroscopy data from a receive array

Excess accumulation of lipids in the myocardium (myocardial lipodosis) may contribute to the progression of heart failure, especially in the context of ageing and obesity.¹⁵

Myocardial lipid concentrations may be monitored using ^1H single voxel MRS. However, cardiac ^1H spectroscopy is challenging due to low metabolite concentrations, cardiac and respiratory motion, and the B_0 -inhomogeneity across the heart even after

shimming, which all result in comparatively low SNR, even at 3T. Nevertheless, previous work at Oxford established that cardiac ^1H -MRS for lipid quantification could be acquired in a single 10-second breath-hold.¹⁶ This was achieved by combining multiple Stimulated Echo Acquisition Mode (STEAM) averages, acquired in the single breath hold, from a 15-channel (Siemens) body receive array on a Trio 3T scanner (Siemens, Germany).

Over 200 datasets have been collected in the last 2 years at Oxford, in HCM, obese, and healthy populations, using an extended version of the method set out in reference 16 – multiple breath-held measurements are now made to increase SNR. Three averages without water suppression are acquired in a single breath hold, and five averages with water suppression are then acquired in each of five breath holds, resulting in a total of 25 water-suppressed spectra.

The analysis of this data is complicated: multiple array coil elements must be combined across multiple averages (within each breath hold) and across the repeated breath holds. The combination of elements using Roemer's formula,¹⁷ is achieved using separately measured unsuppressed water signal to calculate a per-element amplitude and phase complex weighting coefficient.¹⁸ However, between intra-breath hold averages, separated by a cardiac cycle, and inter-breath hold repetitions, separated by a number of breathing cycles, the element weightings, especially their phases, will change. Therefore a hybrid method is used, combining Roemer combination of elements, with an AMARES fitted phase to combine the inter-breath hold repetitions for maximum lipid SNR.^{16,19}

A Matlab AMARES based analysis pipeline was created to replace the original implementation of the hybrid combination process. The original pipeline had both Matlab and jMRUI steps, and required extensive manual intervention.

The two post-processing methods were compared on 15 data sets under control of an experienced clinical fellow. The average analysis time was 5.8 ± 0.3 min with the original method compared to 0.75 ± 0.08 min with the new method. In large part this is because the new pipeline requires no manual intervention. For a 100 subject study, batch processing reduced the analysis time from 9.7 hours to 1.3 hours in total, and the operator time from 9.7 hours to <30 min.

The new Matlab-only analysis program was validated against the original combined Matlab/jMRUI pipeline (Figure 3.10). The comparison was made using 85 cardiac datasets (10 HCM and 75 healthy volunteers). The unsuppressed water amplitudes had an excellent correlation ($r > 0.99$) and no significant bias. The fitted CH_2 amplitudes were strongly correlated ($r = 0.95$). The final lipid-to-water ratio was also strongly correlated ($r = 0.93$) and showed a small but significant ($P = 0.03$) bias, with the new analysis tool estimating a myocardial lipid fraction that was 0.25% higher than the previous method.

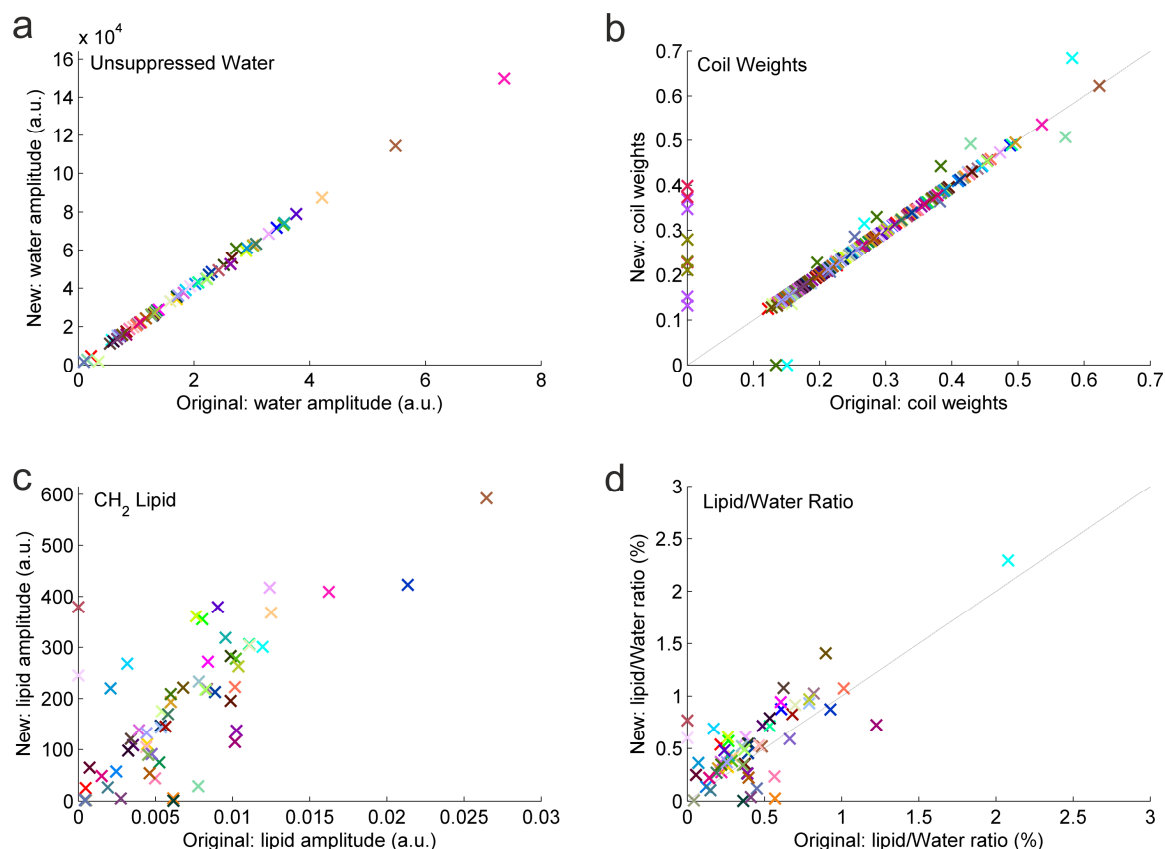


Figure 3.10 Comparison of results from “original” Matlab/jMRUI cardiac ^1H -MRS analysis pipeline and the results from the “new” Matlab-only analysis program. **a:** Fitted amplitude of the unsuppressed water peak. **b:** The coil amplitude weightings of coils contributing more than 10% of the signal, differences occurs when one program includes an additional coil above this arbitrary cut-off. **c:** Fitted amplitude of the CH_2 lipid peak in the presence of water suppression. **d:** CH_2 to unsuppressed water ratio.

3.4.2.1 WSVD combination of ^1H spectra

The move to a completely integrated Matlab analysis pipeline for cardiac ^1H -MRS makes it straightforward to also apply any of our previously validated coil combination algorithms to cardiac ^1H -MRS. We tested a range of coil combination algorithms to choose the best performing one for future studies.

The combination of unsuppressed water spectra is generally achieved using Roemer’s point source formalism, with coil weightings derived from direct measurement of the observed water peaks by each coil element in a separate acquisition. However in the presence of:

- 1) a suppressed water peak and no other high SNR peak,
 - 2) different breath hold position(s) during the collection of “suppressed water” data and “unsuppressed water” data, resulting in different coil phase weightings,
- applying the coil amplitude and phase weightings measured at a single time point may not give the optimum combination of coils and averages.

I instead applied the WSVD algorithm²⁰ to combine, simultaneously three non-water-suppressed spectra (by stacking the spectra end-to-end before combination). Noise pre-scans were not available in the data, therefore per-element noise estimates were made from spectral regions distant from any peaks (> 15 ppm). The coil weights calculated by the WSVD algorithm for the non-water-suppressed spectra were then applied to combine the individual elements of the water-suppressed data. The lipid region ($0\text{ppm} < \delta < 4\text{ppm}$) of the resulting 25 water suppressed coil-combined spectra was used to calculate the relative spectral phases using the Matlab SVD function, to maximise lipid signal.

Compared to the original combination method, the WSVD method decreased the coefficient of variation (mean $\pm$ SD of the % CRLB) of the lipid/water ratio (original: $7.0 \pm 7.7\%$, WSVD: $5.1 \pm 7.9\%$). There was no significant bias ($p=0.46$) measured between the new Matlab implementation of the original method and the WSVD combined ratios. Average fitting duration was 0.52 ± 0.06 minutes.

The flexibility of the Matlab based analysis pipeline was demonstrated by the evaluation of a new coil combination methods, resulting in a modest improvement in precision and fitting time.

3.4.3 Arbitrary prior knowledge constraints

Further flexibility was demonstrated in work within the group. It was proposed that as the linewidth of spectral peaks is a function of a peak's intrinsic linewidth, based on T_2 , and additional linewidth, based on intravoxel B_0 inhomogeneity (T_2'); if the relative differences in intrinsic linewidths between peaks are known, only one model parameter needs fitting to account for every peak's observed linewidth.

This fitting approach was demonstrated in the leg at 7T and in cardiac data at 3T. It was shown that for a small reduction in PCr amplitude fitting accuracy a large increase in γ -ATP amplitude precision could be gained (equivalent in some cases to doubling of peak SNR).²¹

3.5 References

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Chapter 4: Detection of myocardial inorganic phosphate

4.1 Introduction

Chapter 4 details attempts to measure the chemical shift and amplitude of the inorganic phosphate (P_i) peak in cardiac ^{31}P spectra. Resolution of the position of the P_i peak enables in vivo (intracellular) pH measurements, whilst amplitude measurements enable saturation transfer measurements of the rate of ATP production via ATP synthase and calculation of its Gibbs free energy.¹ However, signals that arise from the left- and right-ventricular blood pools, which surround the interventricular septum, mostly obscure the spectral peak arising from intracellular inorganic phosphate.

In Section 4.2, the problem of overlapping peaks is characterized. A summary of previous measurement approaches is provided (for animal and human MRS) and possible approaches for a human cardiac 7T-MRS protocol is explored.

Sections 4.3 and 4.4 describes the attempt to suppress peaks arising from flowing blood using B_0 field gradients.

In Section 4.5 saturation transfer is used to attempt to measure the chemical shift of the cardiac P_i peak in a difference spectrum.

Finally, in Section 4.6, intracellular P_i is detected in three hypertrophic cardiomyopathy (HCM) patients, using a high resolution 3D-CSI protocol at 7T.

4.2 Aims

Inorganic phosphate is a metabolite in the oxidative phosphorylation and ATP utilisation steps of cellular energy supply. It is consumed in the phosphorylation of ADP to form

ATP in mitochondria. It is reformed at the myofibrils as ATP is broken down to ADP to supply the energy for myosin actuation.²

Measurement of the chemical shift position of inorganic phosphate gives a non-invasive pH measurement of the cellular compartment containing P_i (Chapter 1 & Figure 4.1a).³

When combined with measurements of the PCr, ATP and creatine concentrations (estimated, from 1H -MRS⁴, or 1H CEST MRI⁵) it is possible to calculate the Gibb's free energy for the hydrolysis of ATP, i.e. the maximum amount of chemical work available to drive the myofibrils.⁶

While P_i is readily resolved and quantified in skeletal muscle spectra,⁷ there are several difficulties with detection of the myocardial intracellular P_i peak, P_i^{in} . Key amongst them is the low concentration (10 x lower than PCr) of P_i in the myocardium in healthy tissue.⁸ The adjacent LV & RV blood pools contain 2,3-diphosphoglycerate (DPG) which has two spectral peaks; the upfield (2-phosphate group of DPG) peak often obscures P_i at physiological pH (approx. 7.1). The blood also contains P_i , at a higher pH (approx. 7.4), causing a second P_i peak to appear in the spectrum.⁹ This second "extracellular" resonance, P_i^{ex} , is detected from the blood pools or possibly from the cardiac microvasculature.

The unresolved DPG/ P_i spectral region at about 5.3 ppm downfield from PCr is illustrated in Figure 4.1b. The 3-phosphate group of DPG contributes an unresolved symmetric triplet at 6.35 ppm, the 2-phosphate group contributes an unresolved doublet at 5.5 ppm, and there are singlets from P_i^{ex} and P_i^{in} .¹⁰ The chemical shifts of the two multiplets (DPG) and singlet (P_i) arising from blood are also pH dependent, shifting towards PCr with decreased pH.⁹

Higher field strengths provide a small benefit to peak resolution: peak broadening due to increased B_0 inhomogeneity has been shown to increase linearly with field strength in line with increased peak frequency separation, but broadening caused by multiplet splitting is not dependent on B_0 resulting in better resolved multiplets.¹¹ The separation of the two closest peaks, P_i^{ex} and P_i^{in} , at varying intracellular pH (but fixed extra cellular pH) are compared to Sparrow's resolution limit¹² for typical singlet Lorentzian linewidths at 1.5 T, 3 T and 7 T in Figure 4.1c.

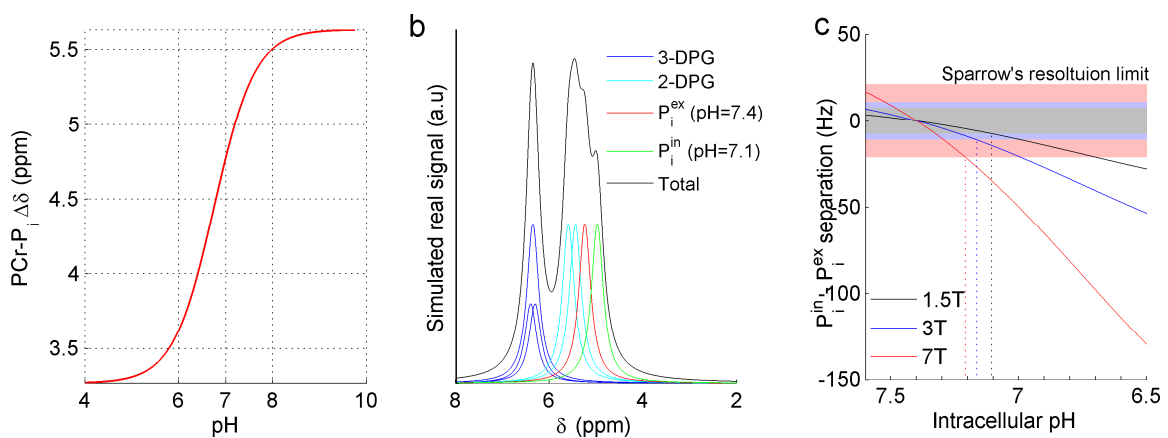


Figure 4.1 **a:** Relationship between pH and the inorganic phosphate chemical shift relative to PCr, as described by Eq. [1.55]. **b:** Simulated noiseless spectral profile of the DPG/ P_i region of a cardiac ^{31}P -MRS spectrum. The intracellular P_i peak is the right most peak, seen as a shoulder on the total broad profile. **c:** Separation of the intra and extracellular P_i peaks as a function of intracellular pH. The shaded regions represent separations too small for the two peaks to be resolvable from each other, as defined by Sparrow's resolution criterion.

Finally, it should be noted that both LV and RV blood pools are refreshed periodically during diastole, resulting in non-steady state behaviour of the magnetisation in these pools.

4.2.2 Previous measurement approaches

Attempts to measure myocardial pH by ^{31}P -MRS have been made since it was initially proposed by Moon *et al.* in 1973.¹³ Quantification of the P_i peak has been attempted in

various animal models, and occasionally in humans. Methods used to measure intracellular P_i may be categorised as follows:

- PH: Measurement in surgically excised and perfused animal hearts.
- SC: Accurate localisation using small surface coils. Performed in surgically prepared animal models, using a small (2-3cm) coil, sutured directly to the epicardium.
- D/FSW: Precise and specific localisation of a specific volume of interest (VOI). Using either B_1 - or B_0 -gradients to spatially localise to a myocardial VOI, minimising skeletal muscle and blood pool contamination.
- ST: Saturation transfer. Direct chemical exchange between γ -ATP and P_i provides a mechanism for the transfer of magnetisation. In the experiment γ -ATP is saturated, transferring saturation to the intracellular P_i ; then a difference spectrum is made with control saturation at the opposite frequency offset relative to P_i , where there is no magnetisation transfer effect. The exchanging portion of the P_i pool is visible as a peak in the difference spectrum.
- SE: Spectral editing takes advantage of the scalar couplings between spins to discriminate coupled and uncoupled spins. Spectral editing has been used to remove the obscuring 2-phosphate peak of the DPG metabolite from the spectrum leaving the P_i peak(s) to be observed unobstructed.
- FA: Prior knowledge constrained fitting of multiple peaks to a single broad peak, which effectively extracts the P_i chemical shift from the non-Lorentzian lineshape in this region of the spectrum.

- PD: ^1H decoupling, the removal of multiplet splitting caused by coupling to ^1H nuclei, results in narrower linewidths allowing the resolution of P_i from the 2,3-DPG directly. ^1H decoupling is achieved by the transmission of RF at the ^1H Larmor frequency during reception of the ^{31}P MR signal.
- Non-magnetic resonance methods, such as biochemical assays or high resolution NMR of tissue extracts.

References using one or more of these methods to measure P_i are listed in Table 4.1.

Ref	Method	Field/T	Organism	Method details	Notes on method Results	[P _i]/[PCr]	pH
14	PH	7.4	Animal-Rat	Langendorff perfused heart.	Unlocalized NMR signal.	1	7.4
15	PH	4.2	Animal-Rabbit	Perfused heart.	P _i shift as ischemia induced, P _i in perfusate obscures normoxic peak.	-	7.4
16	PH	4.3	Animal-Rat	Langendorff perfused heart.	Single P _i peak in phosphate free buffer. Acidosis observed with ischemia.	-	7.04±0.01
17	PH	4.2	Animal-Rat	Perfused heart (Blood or Krebs-Henseleit buffer).	pH difference in blood perfused (7.4) and Krebs-Henseleit perfused (7.1).	-	7.11±0.01
18	PH	4.3	Animal-Rat	Langendorff perfused heart.		-	7.01±0.04
19	SC+ST	2.3&5.6	Animal-Rat	Surgically implanted coils.	High Blood and PME contamination in non-saturation transfer experiment.	0.87	-
20	SC	1.9	Animal-Rabbit	Open chest, controlled respiration. Ischemia induced.	Reports that 2,3-DPG obscures P _i in non-ischemic tissue.	-	>6.9
21	Other		Animal-Dog	Catheter coil. Blood perfused.	2,3-DPG/P _i ratio changes under exercise stress.	-	-
22	SC	2	Animal-Cat	Open chest, controlled respiration. Ischemia induced.	300% increase in PME peak during induced ischemia. pH assigned off PME.	-	7.39
23	SC	2.1	Animal-Dog	Cardiac window, chronic close chested.	Un-localized spectra obtained with external coil.	0.6	-
24	D/FSW	1.5	Animal-Dog	Open chest. Study before/after induced ischemia.	Single peak assigned in PME region.	0.18	7.17±0.09
25	D/FSW	1.5	Human	Acute infarction patients measured.	No mention of P _i in Blood, PCr/P _i ratio lower and P _i /ATP higher in disease.	0.11	7.15
10	SE	4.3	Animal-Rabbit/Dog	¹ H coupling editing to suppress 2,3-DPG. Long T _E .	Unsure of P _i signal origin.	-	-
26	ST	4.7	Animal-multiple	Open chest, coil sutured to pericardium.	Compares saturation transfer with the infused standard.	-	7.0-7.2
27	PH	4.7	Animal-Dog	Oxyphenol perfused heart.	Reports an ATP/P _i ratio of 7 and a T1 of 2.4.	-	7.11±0.02
28	PD	1.5	Human	Proton decoupled spectrum.	Resolves three peaks: 2x 2,3-DPG and P _i . P _i assigned to myocardium.	0.138	7.14
29	D/FSW	4.7	Animal-Dog	Open chest, controlled respiration.	High resolution and small voxels. P _i is visible, but poorly resolved.	-	6.9
30	SC	2	Animal-Pig	Open chest, controlled respiration. Ischemia induced.	P _i /PME peak increase in ischemia. Reported pH from broad "PME" peak.	0.1	7.23
31	SC+ST	4.7	Animal-Dog	Open chest, controlled respiration, sutured coil.	Used suboptimal line broadening to observe distinct P _i peak.	-	7.1
32	ST	4.7	Animal-Sheep	Open chest, controlled respiration, sutured coil.	Sheep blood has low 2,3-DPG. Saturation method confirms assignments.	0.097	7.03±0.02
33	D/FSW	2	Animal-Pig	Open chest, blood perfused, sutured coil.	P _i only observed in ischemia. Data only given for ischemic case.	-	-
34	SE	2	Animal-Pig	Open chest, blood perfused, sutured coil.	"RF gradient echo" method. P _i observed in 3/5 experiments, 2,3-DPG suppressed in all.	0.24(P _i /ATP)	7.16
35	Other	2	Animal-Rat	Flow saturation of P _i . Open chest, blood perfused.	Singlet peak resolved and assigned using pH to P _i .	-	-
36	PD	1.5	Human	Proton decoupled. HCM and DCM subjects.	Resolves three peaks: 2x2,3-DPG and P _i . pH decreases in disease.	0.14	7.15±0.03
37	PD	1.5	Human	Proton decoupled + NOE enhancement, 2D CSI.	Large study size. P _i resolved in all but one subject.	0.086	7.06±0.03
38	PD	1.5	Human	Proton decoupling.	Resolves 4 peaks in the PME region. Combines with constrained fitting.	-	-
39	PD	1.5	Human	Proton decoupling. HCM subjects. 3D localised.	Subtraction of fitted 2,3-DPG peaks leaves a PME and P _i well resolved.	0.097	7.08±0.03
40	ST	2	Human	Un-localized with OVS for chest muscle.	Ignores contamination from blood but quantifies skeletal contamination.	-	7.11±0.05
41	SE	7.05	Animal-Mouse	"DEPTH" sequence. Open heart, blood perfused.	Excellent blood suppression, accurate P _i identification.	-	-
42	FA	1.5	Human	VARPRO - Fit both intracellular and extracellular P _i .	Residual difference on the two fits is very small.	0.15	7.08±0.04
43	FA	1.5	Animal- Dog	1DCSI. In vivo, blood perfused. Ischemia induced.	No pH change detected Gives an upper limit for P _i /PCr = 0.14.	-	7.13-7.17
44	PH	11.6	Animal- Rat	Excised, perfused heart. Compared with hyperpolarised ¹³ C.	Good agreement between methods. P _i conc. 4mM, PCr 20mM, ATP 10mM.	-	7.07±0.02
			Human	Inter study mean (± SD)		0.12±0.03	7.10±0.03
45			<i>Human</i>	<i>Average value in Bottomley's review.</i>			<i>7.13±0.03</i>

Table 4.1 Papers reporting attempts to identify the intracellular P_i peak, from a search of all papers containing "inorganic phosphate" in their topic since 1975 on the ISI Web of Science database, performed in December 2014. Legend for the "Method" column: PH, Perfused Heart; SC, Small coil localisation; ST, Saturation transfer; D/FSW, DRESS/Fourier shift window localisation; PD, ¹H decoupling; SE, Spectral editing; FA, Fitting approach.

4.2.3 Proposed approaches

This section examines the advantages and disadvantages of several previously-implemented and four new methods for measuring P_i in the human heart by 7T ^{31}P -MRS.

Proton decoupling

^1H decoupling has been used in 1.5 T ^{31}P -MRS for resolving the inorganic phosphate peak.³⁶⁻³⁹ The method requires dedicated RF hardware, with the ability to transmit at the ^1H frequency *during* ^{31}P signal reception, without the ^1H RF damaging the sensitive ^{31}P receive electronics. No suitable coil is available in Oxford at 3T or 7T.

The SAR limit also makes ^1H decoupling infeasible at ultra-high field strengths: the ^1H RF at 7 T causes approximately 20 times the heating it does at 1.5 T.

Saturation / Inversion transfer

Saturation (or inversion) transfer are commonly employed methods in ^{31}P -MRS (e.g. see Chapters 5, 6 and 8 for their application to study the creatine-kinase flux). The intracellular P_i may be partially saturated (or inverted) by saturating (or inverting) the γ -ATP peak. A difference spectrum with a control experiment may then be taken to leave just the saturated/inverted portion of the magnetisation. However, the low concentration of P_i combined with the slow exchange between P_i and γ -ATP, results in small perturbations of the P_i peak.⁴⁶ Combined with the $\sqrt{2}$ SNR penalty in subtraction methods, this leads to low SNR difference spectra.

Stimulated Echo Acquisition Mode (STEAM)

Single voxel spectroscopy using the stimulated echo acquisition mode (STEAM) method, which uses three sequential slice selective 90° pulses to form a stimulated echo from a

single 3D selected volume, offers inherent suppression of strongly moving tissue.³

Moving spins, such as blood in the ventricles, do not experience all three pulses required to form the stimulated echo. This is most obviously demonstrated in black-blood STEAM MRI.⁴⁷

However, STEAM acquisitions suffer a 50% SNR penalty compared to spin echo sequences. In addition, long repetition times, and therefore long scan times, are required for optimum SNR with 90° excitation, as used in STEAM. The cardiac and respiratory motion, and B_0 - and B_1 -inhomogeneity present in cardiac ^{31}P -MRS reduce the signal recorded in a single STEAM acquisition.

Black-blood via single or double inversion recovery

Single inversion recovery black blood sequences work by inverting all spins to be imaged, and then waiting an inversion time chosen to match the null point of the blood signal before acquiring an image, or spectrum.⁴⁸ In ^1H -MRI the myocardium and blood have significantly different T_1 values,⁴⁹ however at 7T the ^{31}P T_1 of DPG and PCr are very similar (3.05 ± 0.41 s vs. 3.09 ± 0.32 s),¹¹ therefore both metabolites would have the same signal null point.

Double inversion uses two inversion pulses to prepare the magnetisation.⁵⁰ The first is a non-selective pulse which inverts all spins within the transmit coil's sensitive region. The second inversion pulse immediately restores the original longitudinal magnetisation in the slice of interest. The sequence then waits for a calibrated time before the acquisition segment. In this time blood which did not experience the second inversion will flow into the slice to be imaged, simultaneously undergoing longitudinal relaxation, so that the time of imaging coincides with the blood null point.

For ^{31}P -MRS at 7T achieving global inversion is difficult with the high B_1^+ inhomogeneity and limited peak B_1^+ of small surface coils, whilst the selective inversion is also challenging because it must be achieved with minimum chemical shift artefact, which implies a requirement for a high inversion bandwidth, often SAR-intensive pulse.

SNR enhancement using SSFP

^{31}P steady state free precession (SSFP) CSI has shown a 4-5x increase in PCr SNR over “fast low angle shot” (FLASH) CSI.⁵¹ SSFP uses alternating phase excitation pulses to achieve a higher proportion of transverse magnetisation in a steady-state acquisition than FLASH. However, a signal weighting, approximately proportional to T_2/T_1 , and a strong off-resonance signal modulation must be managed.⁵²

It has been shown that the SNR enhancement for small, long T_2 species such as PCr and P_i is significant. However the short repetition times required, on the order of 5 ms, do not permit sufficient time for a high resolution readout, but only allow spectral “bins” several ppm wide. Therefore, for a simple SSFP sequence, there is very low chemical shift resolution between resonances and it is infeasible to use this method to resolve P_i , despite the SNR enhancement, from other metabolites with comparable chemical shift.

Dephasing of flowing signal using B_0 gradients

Bipolar B_0 gradients (a pair of gradient lobes with zero integral, but non zero first moment, §4.3) are used to sensitise ^1H -MRI images both to coherent flow and to random diffusion processes.⁵³ During a single gradient lobe all spins (stationary and moving) will accumulate additional phase in the transverse plane. During a second gradient lobe, with equal but negative area, stationary spins will be rephased by the same amount. Meanwhile, any spins which have moved will retain an additional phase,

dependent on the distance moved. For random movement processes the additional phase accrued by the spins in a voxel should also be random and lead to signal suppression due to signal dephasing. The turbulent flow of the blood refilling the ventricles is likely to contain a significant random flow pattern, especially when measured across large spectroscopy voxels, thus it should be possible to suppress using a single set of bipolar B_0 gradients.^{35,54}

Exogenous T_2 contrast agents

T_2 or “negative” contrast agents, typically iron based, reduce the T_2 of spins in a small surrounding region. A high molecular weight (> 20 kDa) negative contrast agent, which will not perfuse into myocardial microvasculature and extracellular space over the duration of a ^{31}P -MRS acquisition (30 minutes), may result in a very short T_2 and signal attenuation of blood-borne metabolites whilst not affecting metabolites in the myocardium.⁵⁵

Though this effect has been thoroughly demonstrated for black-blood cardiac ^1H -MRI, in which the contrast agent (e.g. Ferumoxytol [Feraheme, AMAG Pharmaceuticals, Cambridge, MA]) acts on the free water in blood, it has not been demonstrated yet in a ^{31}P acquisition in which the visible metabolites are located predominantly inside the erythrocytes.⁵⁶

4.2.3.2 Chosen methods

Of the methods discussed in the previous section, the methods chosen for implementation and further evaluation were:

- The dephasing of flowing signal using B_0 gradients, and
- Saturation transfer difference spectroscopy.

Both methods only require minor alterations to the well-validated 3D-CSI sequence, do not require B_1 -insensitive pulses, nor additional hardware. Dephasing by B_0 gradients is particularly attractive due to the high-performance gradients on the 7T Magnetom scanner, the potential to use short repetition high SNR acquisitions, and there are no requirements for prior-knowledge of metabolite relaxation time constants.

4.3 Gradient-based flow suppression: methods

Consecutive, equal time, but opposite amplitude trapezoidal gradients, known as a bipolar gradient, can be introduced between excitation and acquisition segments of a pulse sequence.

The phase of a spin isochromat at time t , under the influence of a gradient is given by

$$\phi(t) = \gamma \int_0^t \mathbf{G}(t') \cdot \mathbf{r}(t') dt', \quad [4.1]$$

where γ is the gyromagnetic ratio; $\mathbf{G}$ is the applied gradient, $\mathbf{r}$ is the position of the isochromat at time t' . In the case of equal and opposite gradient lobes, a stationary isochromat accumulates no phase, however a moving isochromat accumulates a phase dependent on its individual path along the gradient axis.⁵³ A sequence is sensitised to the n^{th} differential of isochromat motion (i.e. position, velocity, acceleration, etc.) by a non-zero “ n^{th} gradient moment”, m_n ,

$$m_n(t) = \int_0^t G(t') t'^n dt'. \quad [4.2]$$

The signal S observed in a voxel is exponentially related to the variance of a Gaussian phase distribution, $\langle \phi^2 \rangle$ ⁵³

$$S = S_0 e^{-\langle \phi^2 \rangle}. \quad [4.3]$$

A variance of π results in a >95% suppression of signal within one voxel. In phase contrast MRI the phase effect of gradients on flowing spins is characterised by the VENC (velocity encoding):

$$\text{VENC} = \frac{\pi}{\gamma |\Delta m_1|}, \quad [4.4]$$

Where Δm_1 is the change in the 'first gradient moment'.⁵³ VENC has units of cm s^{-1} ; it is the velocity of spins which results in a π phase shift. A larger first gradient moment will result in a lower VENC value.

The additional delay required to insert the bipolar gradients between the excitation pulse and the readout will cause a T_2^* -dependent signal decrease of all metabolites in the spectrum.

4.3.1 ^1H -MRI proof of principal

The bipolar gradients were implemented in a cine FLASH ^1H -MRI sequence at 3T to answer the following questions:

1. Does adding a single set of short bipolar gradients sufficiently suppress signal arising from strongly flowing spins, such as those in metabolites present in the blood?
2. Is the suppression consistent between subjects?
3. Does cardiac trigger time affect the amount of signal suppression?
4. Is the optimal trigger time dependent on heart rate?

Five healthy subjects (5 male, 31 ± 9 years, 77 ± 6.5 kg) were scanned at 3 T (3T Trio, Siemens, Germany), using a 32-channel ^1H cardiac array for signal reception and the

inbuilt body coil for RF transmission. A flow-compensated, gradient-echo, velocity encoding sequence ("BEAT_FQ_560") was used to acquire a 24-phase cardiac CINE, with 20 mm slice thickness, 64x38 in-plane resolution, and a 500x297 mm² FOV. The sequence was prospectively ECG triggered. For each subject, T_R was set so that the 24 images covered the whole cardiac cycle. The sequence acquired two images per cardiac phase, a normal image with no motion sensitising gradients and a second with bipolar motion sensitising gradients in the specified direction. Data sets were acquired with gradients set to give a VENC of 25cm s⁻¹ and 50cm s⁻¹ in both the short-axis and long-axis directions. Images were acquired in both the mid short axis view and 4-chamber long axis view.

In one subject the scans were repeated with the gradient running in the long-axis direction. The gradient amplitudes were varied to give five different VENC values from 60 to 20 cm s⁻¹.

The images were manually segmented, using custom Matlab software, to select out the left and right ventricular blood pools. The mean and standard deviation of the signal in each of the image sections (gradients on vs. off) was recorded. The amount of blood signal removed is expressed as a ratio S_{on} / S_{off} .

4.3.2 ³¹P CSI in static tissue

A single healthy subject (male, 21 years, 83 kg) underwent a ³¹P-MRS CSI examination of their quadriceps skeletal muscle at 7T. Two otherwise identical acquisitions were made, one with bipolar motion sensitising gradients, the other without the gradients and no additional time between excitation and readout, to measure the effect of the gradients on metabolite signals arising from static tissue.

The 16 channel ^{31}P receive array was placed across the anterior surface of the thighs. The standard CSI sequence (Appendix A, MDR7T pulse) was used, with a modified FOV ($150 \times 300 \times 200 \text{ mm}^3$) and a transmit voltage of 530 V. The motion sensitised sequence had bipolar gradients with an amplitude of 37.7 mT m^{-1} (limited by peripheral nerve stimulation) and a flat-top duration of 1.2 ms. The total duration for both gradient lobes was 3.152 ms and the VENC was 19.8 cm s^{-1} . These gradients were used in all subsequent experiments.

The WSVD algorithm was used to combine signals from the receive array.⁵⁷ A voxel from the anterior quadriceps muscle was selected for analysis. Spectra from both the motion sensitised and normal CSI acquisitions were analysed. An additional synthetic spectrum was generated by truncating the FID of the normal CSI to give data that would have been acquired using the T_E of the motion sensitised acquisition. Fitting was carried out using AMARES in Matlab.

4.3.3 ^{31}P CSI in the myocardium

Two healthy subjects (male, 44 & 32 years, 85 & 75 kg) underwent the same protocol, modified for a cardiac examination. The standard CSI sequence (Appendix A, MDR7T pulse, 16 channel coil) was used. For the non-sensitised CSI acquisition, a delay equal to the duration of the bipolar gradients was introduced between excitation and readout. In both acquisitions a 25mm-thick BISTRO RF saturation band was placed to suppress signal from skeletal muscle in the anterior chest wall. The acquisitions were triggered using three lead ECG, with a trigger delay set to 60% of each subject's R-R interval.

A single voxel from the mid interventricular septum was selected for fitting and analysis from both subjects.

4.3.4 Real-time “cine” sequence

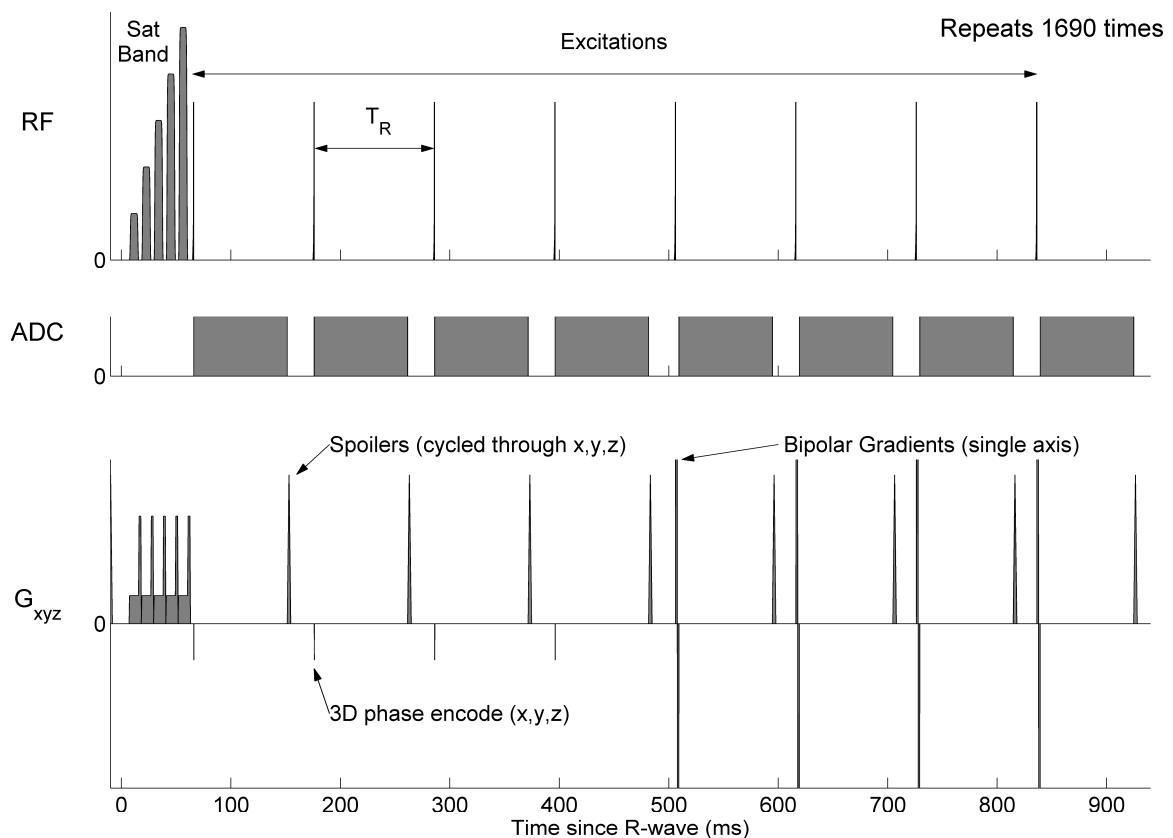


Figure 4.2 Pulse sequence diagram of a single transient (one ECG detected R-R interval) of the motion sensitised CINE CSI sequence. Within each transient the phase encode steps of each acquisition (8 shown) are equal. The bipolar gradients are present in the second half of the acquisitions in each interval.

To examine the effect of different acquisition trigger delays, the time between the ECG detected R-wave and the spectroscopy acquisition, and to mitigate the effect of varying heart rates during the scan a CINE CSI sequence was developed capable of making several independent CSI measurements in one acquisition at different phases of the cardiac cycle (Figure 4.2).

Five healthy subjects (male, 32 ± 9 years, 76 ± 7.3 kg) underwent cardiac examinations using the CINE CSI sequence. Spectral acquisitions were recorded for the maximum number of cardiac phases possible given a T_R of 110 ms. The first cardiac phase of each phase encode step was triggered from a three lead ECG. The first half of the phases

were acquired with no bipolar gradients, the second half includes bipolar gradients identical to those in the single acquisition method. The standard CSI matrix parameters were used (Appendix A, 16 channel coil, MDR7T pulse), with 20 averages at $k=0$ ($T_A = 48$ minutes). A 25mm-thick BISTRO saturation band was placed to suppress signal from skeletal muscle in the anterior chest wall, and was repeated before the first phase i.e. once per R-R interval.¹¹

Spectra from individual coil elements were combined using the WSVD method. Spectra from mid interventricular septal voxels was chosen for analysis. The cardiac spectra were fitted individually using AMARES in Matlab, with standard prior knowledge (Appendix B.1).

4.4 Gradient-based flow suppression: Results

4.4.1.1 ^1H -MRI

Visual inspection of the images collected with and without bipolar gradients showed significant suppression of the blood signal (Figure 4.3), but also an obvious modulation of blood signal suppression in different cardiac cycle phases.

Quantitative analysis of the signal in the left and right ventricles, averaged across the five individual subjects showed that maximum suppression occurred in the phase at approximately 60% of the cardiac R-R interval, with the gradients along the heart's long axis and for highest amplitude gradients i.e. lowest VENC value (Figure 4.4).

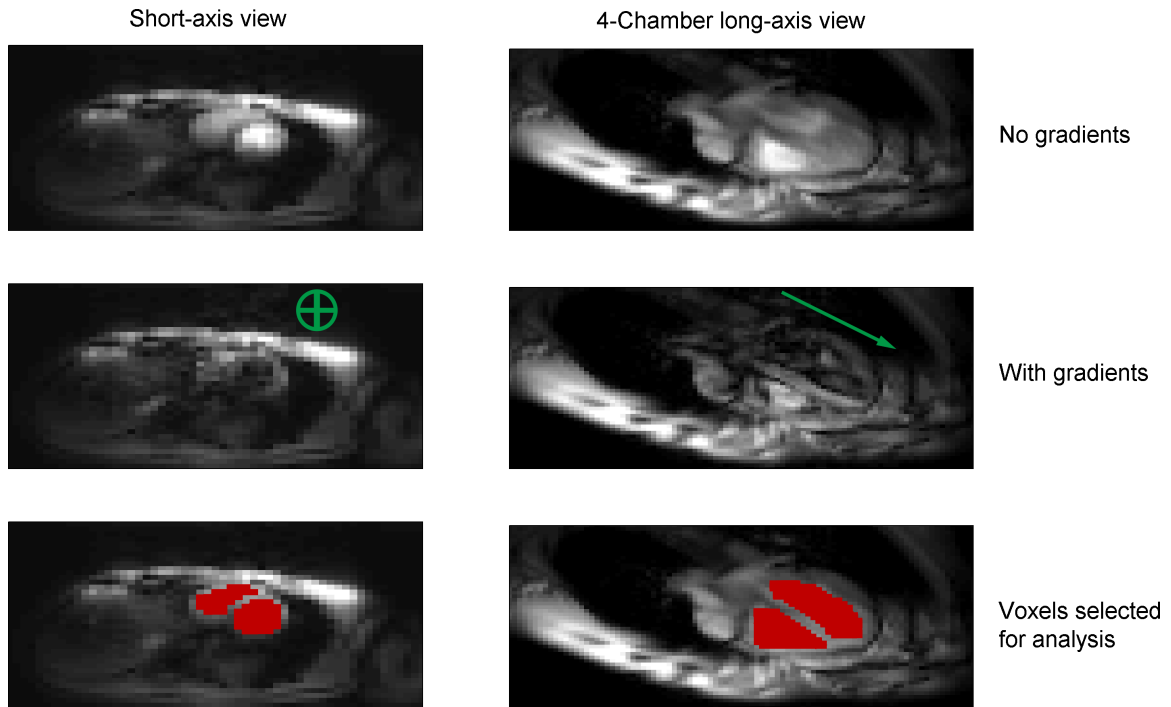


Figure 4.3 Example short and long-axis cardiac views, with and without bipolar gradients in a single subject. The bipolar gradients run along the long-axis (normal to the SA imaging plane, green markers) and have a VENC value of 25 cm s^{-1} . The voxels selected for analysis are highlighted in red.

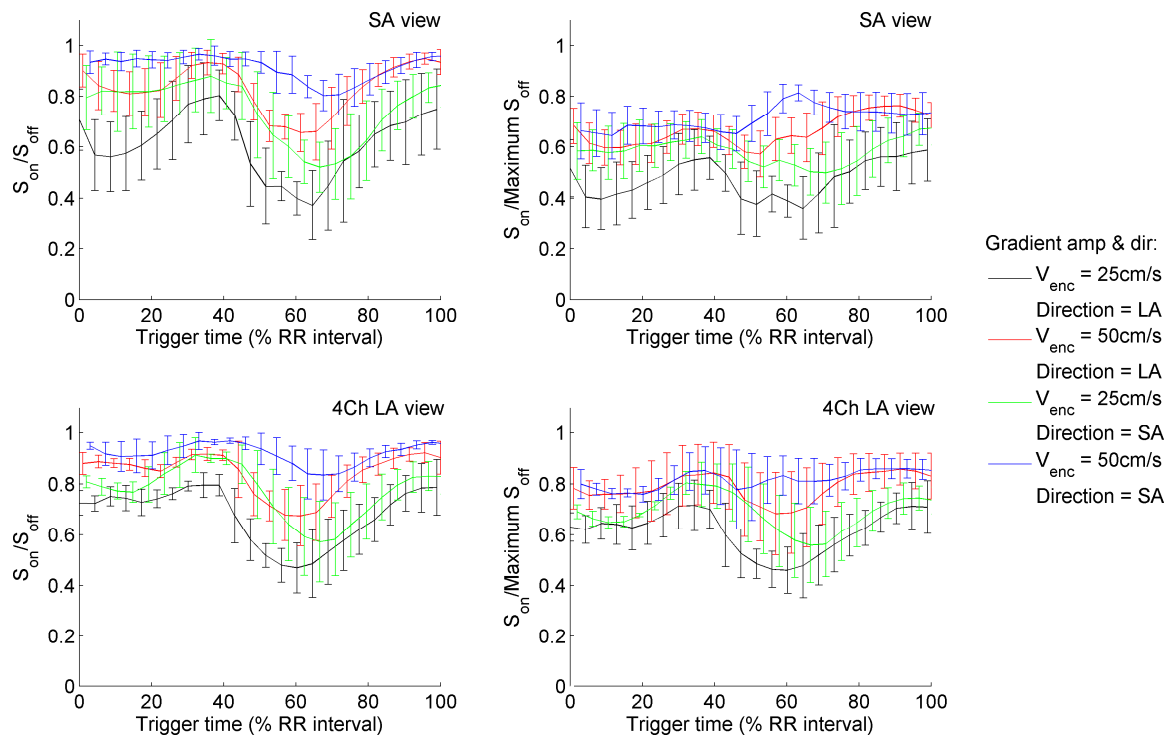


Figure 4.4 Mean results of the five subject ^1H -MRI proof of principal study. The signal of the LV and RV blood pools in the acquisitions with bipolar gradients are compared to the normal signal in the same phase ($S_{\text{on}}/S_{\text{off}}$) and the maximum normal signal ($S_{\text{on}}/\text{Maximum } S_{\text{off}}$). Results of both imaging planes and a range of gradient amplitude and directions are shown.

The extended measurement in a single subject showed a near linear relationship between gradient VENC value and the signal suppression from the gradients (Figure 4.5).

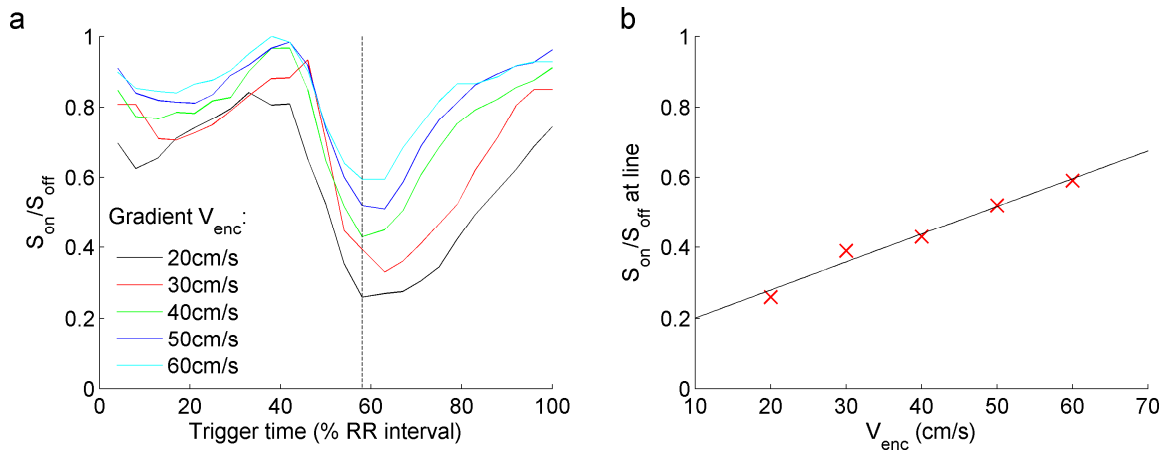


Figure 4.5 **a:** Ventricular signal suppression for five different gradient amplitudes in a single subject. **b:** The signal suppression decreases linearly with increasing VENC (decreasing gradient amplitude) and is consistent with Eq [4.2].

The results of the ^1H -MRI investigation imply that suppression of signals from blood is possible using short bipolar gradients, that the optimum acquisition time is at approximately 60% of the R-R interval (i.e. mid diastole), and that this is consistent between subjects.

4.4.1.2 Static tissue (skeletal muscle)

The addition of bipolar gradients to the CSI sequence did not cause obvious qualitative changes to the skeletal muscle spectrum. The amplitudes of the fitted metabolites from the acquisition with gradients were found to be within 13% of the amplitudes of the metabolites from the truncated FID of the normal acquisition.

The SNR of the metabolite peaks was found to decrease less than that predicted, i.e. due to T_2^* dephasing. The linewidth of the PCr peak was 25.0 Hz, giving a T_2^* of 13 ms.

The predicted signal decrease for an additional 3.15 ms gap was therefore 22%. The SNR of the sensitised and shortened acquisitions was 12% lower than the normal acquisition, corresponding to a T_2^* of 24.6 ms.

The reason for the difference in measured and predicted SNR decrease is not fully understood. The PCr SNR measurements have a precision in the range of 2% to 3%, therefore this is a notable difference. In this work the PCr lineshape is fitted as a strict Lorentzian, the relationship between T_2^* and linewidth is not the same for peaks with a Voigt or Gaussian lineshape, which could occur with high B_0 inhomogeneity or with signal contamination, and distort the T_2^* measurement.

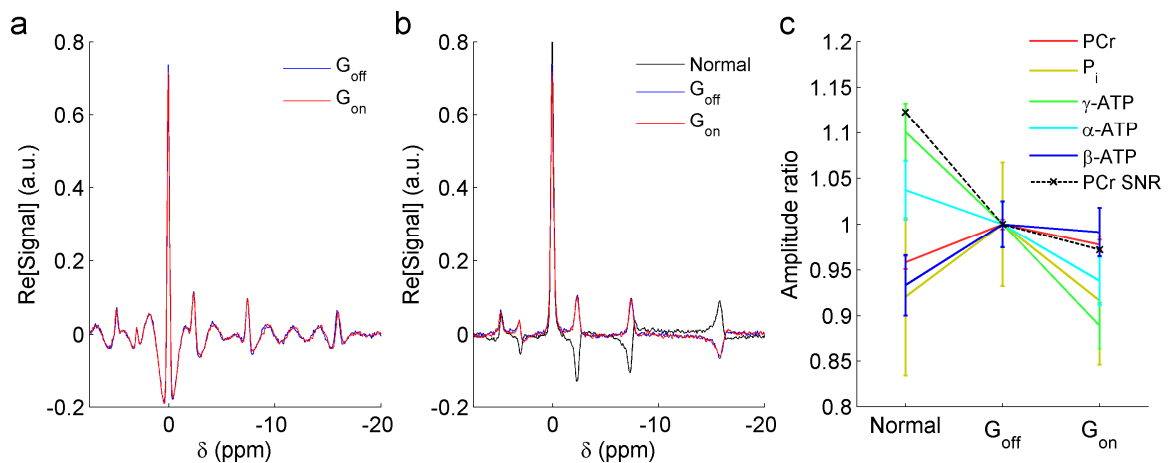


Figure 4.6 **a:** Phase corrected motion sensitised (G_{on}) and unsensitised+truncated (G_{off}) spectra from the quadriceps of a healthy volunteer. **b:** Sensitised, truncated and normal raw spectra. **c:** Fitted amplitude ratios of the metabolites normalised to the unsensitised but truncated spectra. The PCr SNR ratio is also plotted on the same axes. The error bars show the CRLB of the ratio.

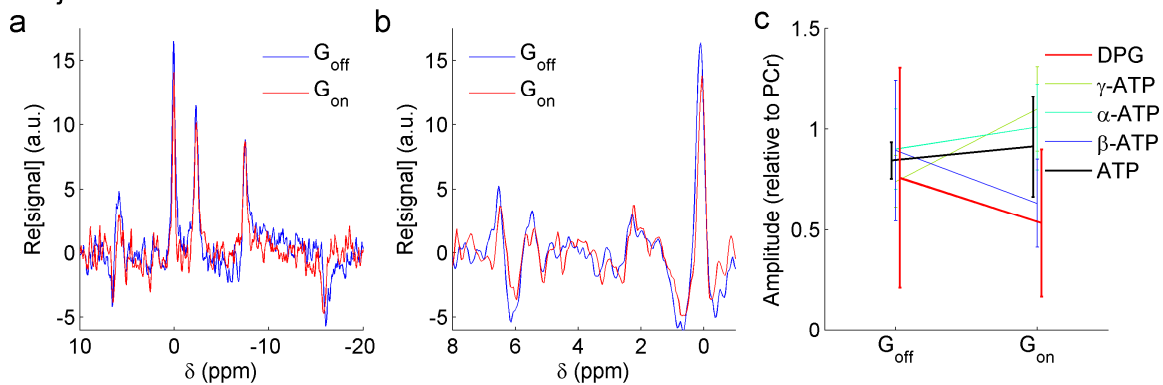
4.4.1.3 Cardiac spectra

In both subjects, septal voxels from the motion sensitised acquisition still had visible DPG peaks. However, in both subjects the DPG/PCr ratio decreased, from 0.73 to 0.54 and from 0.91 to 0.44 respectively, whilst the ATP/PCr ratio was changed less by the introduction of the gradients (Figure 4.7c&f). The low SNR (5.2 and 6.7) of the DPG

peaks results in high CRLBs on the DPG/PCr ratios. A clearly resolved P_i peak was not evident in either subject.

Average heart rates were recorded for the gated motion sensitised CSI acquisitions. The average heart rate for the first subject was 63 ± 21 bpm, the shortest R-R interval was 700 ms and the longest was 1320 ms. The average heart rate for the second subject was 45 ± 5 bpm, the shortest R-R interval was 1150 ms and the longest was 1410 ms.

Subject 1



Subject 2

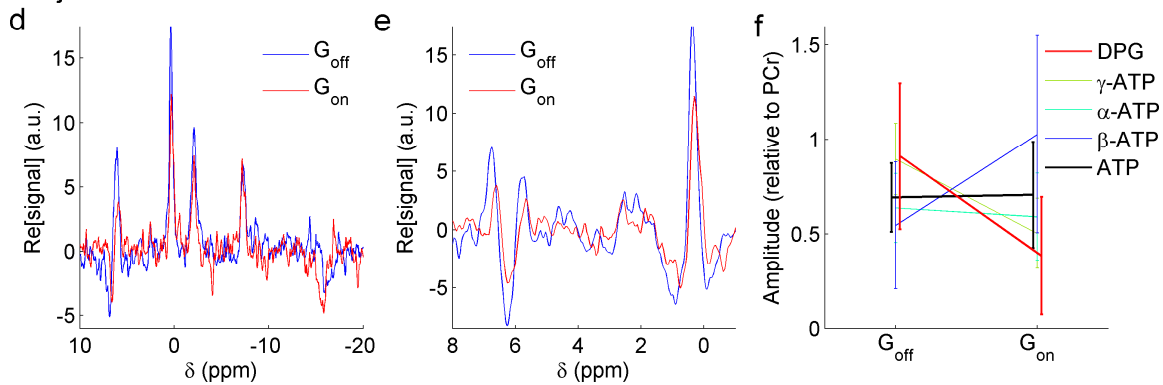


Figure 4.7 **a+d:** Raw motion sensitised (G_{on}) and unsensitised (G_{off}) spectra from the myocardium of two healthy volunteers. **b+c:** Phase corrected sensitised and unsensitised spectra, showing the DPG to PCr region. **c+f:** Fitted amplitude ratios, relative to PCr in the same spectrum, of the metabolites. In both subjects DPG shows the greatest decrease. The error bars show the CRLB of the ratio.

4.4.1.4 CINE

Across the five subjects a mean ($\pm$ SD) of 7.8 ± 1.5 phases per R-R interval were collected. The fitted metabolite amplitudes (DPG, average ATP and PCr) for each cardiac phase of each subject is displayed in Figure 4.8a-c. The maximum suppression of the

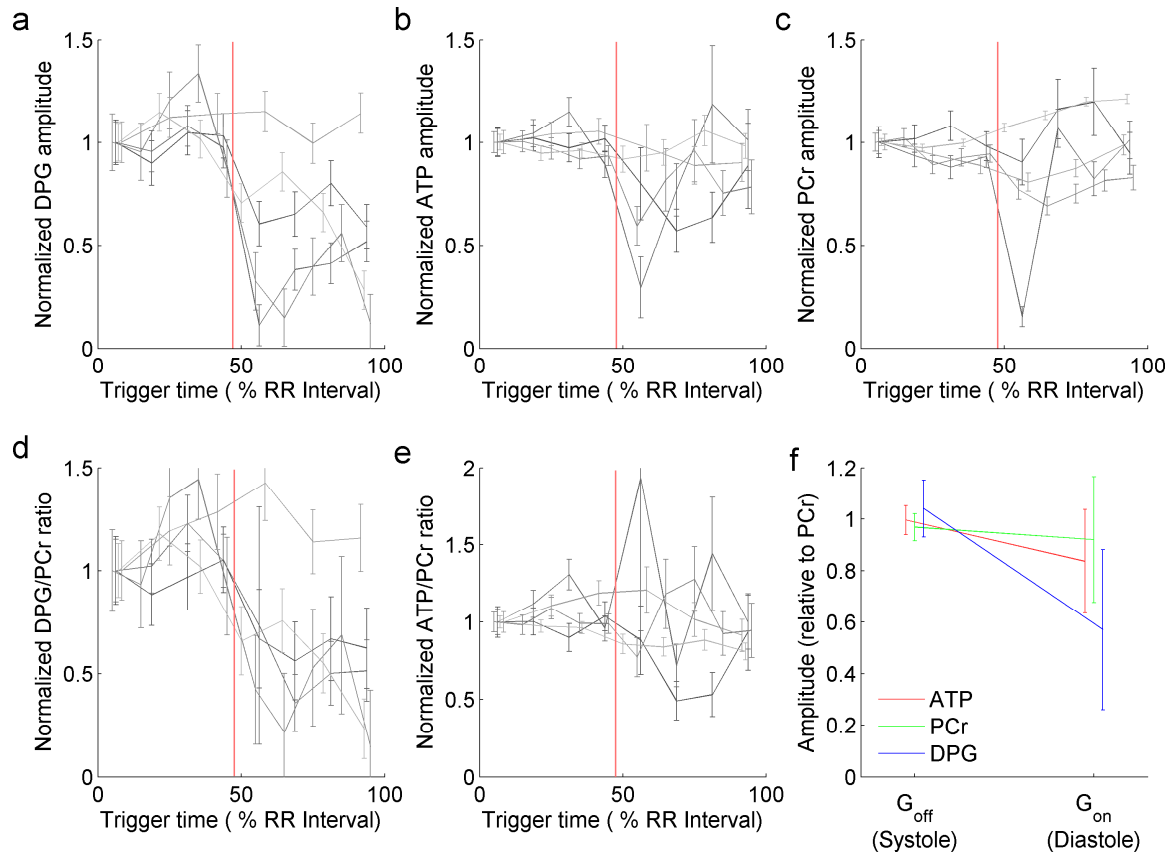


Figure 4.8 **a-e:** Metabolite amplitudes and ratios (with CRLB marked) for each phase and subject. The metabolite ratios are measured relative to the PCr peak in the same spectrum. The red bar marks the switch from unsensitised acquisitions to motion sensitised acquisitions **f:** Average metabolite ratios for the study (with inter-subject standard deviations) for the collated phases with bipolar gradients (occurring in diastole) and without bipolar gradients (occurring in systole).

DPG peak was detected at approximately 60% of the R-R interval, though the range was large, with >90% suppression in two subjects and a -10% suppression (i.e. increase) in one subject. Figure 4.8f shows the average metabolite ratios (relative to the PCr amplitude in the non-sensitised acquisitions) for all subjects. The DPG peak was suppressed significantly more than PCr or ATP, however the suppression had a wide coefficient of variation ($41\% \pm 26\%$). SNR did not significantly vary between the phases with bipolar gradients and the normal acquisition phases.

4.4.2 Discussion

50% suppression of the blood signal was observed in ^1H -MRI with gradients in the long axis direction with a VENC $<40 \text{ cm s}^{-1}$. The amount of suppression was strongly

dependent on the amplitude, direction and timing, relative to the R wave, of the gradient pulses. Nevertheless, suppression was consistent between all subjects scanned. The slice thickness was matched in the direction of the gradients to ^{31}P -MRS voxel sizes to ensure that the degree of intravoxel dephasing was comparable between ^1H -MRI and ^{31}P -MRS. In addition, reducing the ^1H image in-plane resolution in post processing to match spectroscopy voxel sizes in all dimensions did not affect the amount of blood signal suppression (not shown).

In static tissue the bipolar gradients produced an anticipated SNR decrease due T_2^* relaxation during the longer T_E , but all peaks were still resolvable. In the cardiac scans, suppression of the DPG peak relative to PCr and relative to the DPG amplitude in unsensitised scans was observed in both the single acquisition and CINE mode. In both cases the suppression of DPG was greater than any other peak in the spectrum. However, the combination of incomplete DPG suppression and decreased SNR meant that a P_i peak was not resolvable in any scan. The sequence should be run without any gradients for all cardiac phases to rule out phase-dependent signal modulation.

The short repetition time of the CINE sequence was not optimal for observing P_i which has a long T_1 (6.3 s).⁵⁸ A possible future development of the sequence might be to create a real-time gated sequence, which estimates the current heart rate and computes a suitable trigger delay, whilst using a longer sequence repetition time to maximise SNR.

Shortly after this work was completed, an equivalent method was implemented for hyperpolarised ^{13}C imaging in rodents.⁵⁹ The scanner system in that study was capable of 170 mT m^{-1} gradients, with a minimum VENC of 14.1 cm s^{-1} . The gradients were able

to suppress high intensity signal resulting directly from the bolus of the hyperpolarised media. As a result the myocardium was visible in the ^{13}C images.

4.5 pH using saturation transfer difference spectroscopy

A saturated spin system is one with no net longitudinal or transverse magnetisation, i.e. all spins are completely dephased in the transverse plane. Until the magnetisation recovers by T_1 relaxation, no signal can be obtained from a saturated spin system.

Chemical exchange enables the mixing of magnetisation between two otherwise independent pools. For example, in the mitochondria and at the myofibrils, chemical processes convert free inorganic phosphate into the gamma-phosphate in ATP (and vice versa), consuming (or releasing) a molecule of ADP in the process. If one saturates γ -ATP, the height of the inorganic phosphate peak may also diminish. Saturation transfer is discussed, in-depth, in Chapter 5.

Previous work in animal models and in humans at 2 T used the saturation transfer mechanism to identify the intracellular P_i peak.^{26,40} The difference is taken between two spectra: one acquired during frequency-selective saturation of γ -ATP (which transfers to P_i resulting in a smaller P_i peak) and the other during “control” saturation at a frequency equidistant from the P_i peak but on the opposite side to γ -ATP (which nominally has no effect on P_i). The difference spectra only contains signals at the resonance positions of any species to which the γ -ATP saturation has been transferred, namely PCr and P_i .

One advantage of this method is that there is no saturation transfer to peaks arising from blood. Though ATP in the blood is saturated, erythrocytes contain no mitochondria, therefore P_i is not catalytically converted to ATP in erythrocytes, nor is

the breakdown of ATP in the blood fast enough to cause saturation transfer effects on the timescale of MRS experiments.⁴⁰

The main limitation of the method is one of signal-to-noise. The P_i peak has low concentration (see Table 4.1), long T_1 (6.3 s),⁵⁸ and low SNR. Even with complete (indirect) saturation of P_i , the SNR of P_i in the difference spectrum is only $\sqrt{1/2}$ of that in a single spectrum because the noise is incoherent. In practice, P_i is unlikely to be completely saturated, giving an even lower peak amplitude (and hence SNR) in the difference spectrum. Previous measurements have established that the P_i signal is only reduced at most by $\sim 30\%$.³⁵ Therefore the SNR of the P_i difference peak is expected to be very low.

It has been suggested that the magnetisation transfer effect to P_i can be maximised by simultaneous inversion (or saturation) of both the PCr and γ -ATP peaks, which are also undergoing chemical exchange.⁶⁰ This was evaluated in skeletal muscle, alongside the conventional single saturation experiment.

4.5.1 Implementation in skeletal muscle

4.5.1.1 Methods

The method was validated by 7T 3D-CSI of skeletal muscle in the thigh. A single healthy subject (male, 23 years, 80 kg) was placed feet-first-supine in a 7T scanner. ^1H MRI localisers were acquired of one thigh to assist in the placement of spectroscopy volumes. Subsequently the 16-channel array coil was placed on top of both thighs for the ^{31}P acquisitions. An initial ^{31}P acquisition using modified CSI sequence parameters (Appendix A, MDR3T pulse, $16 \times 8 \times 8$, $\text{FOV} = 200 \times 340 \times 350 \text{ mm}^3$, $T_A = 11$ minutes) was made without any saturation.

Subsequently four sets of two ^{31}P acquisitions were made. In each set selective saturation of $\gamma\text{-ATP}$, and then at a control position on the downfield side of P_i , was achieved using delays alternating with nutation for tailored excitation (DANTE) pulse trains.⁶¹ DANTE comprises a train of short hard-pulses alternating with short delays, the amplitude of the pulses may be modulated for a tailored frequency response. The DANTE pulses replace the normally empty “ T_R fill” between the end of the previous readout event (and optional spoiler gradient) and the start of the next transient. The DANTE parameters were altered in each set of acquisitions: the inter-pulse delay was fixed at 250 μs , but the pulse duration was varied from 50 μs to 200 μs in steps of 50 μs . For each set the DANTE transmit voltage was set to the maximum allowable given the SAR limit (70, 50, 50 and 35 V).

Sixteen voxels were selected for analysis from one leg of the volunteer, four from each of the four rows of the central slice of the CSI grid. Voxels lay completely within the volume of the leg in the anterior-posterior direction. Coil combination used the WSVD algorithm and analysis of the spectra was carried out using Matlab AMARES, with standard prior knowledge (Appendix B.2). The pH was calculated from the chemical shifts of the fitted PCr and P_i peaks as described in Chapter 1.

In the same subject, but on a different day, the experiment was repeated with simultaneous saturation of PCr and $\gamma\text{-ATP}$ using amplitude modulated DANTE trains to create two saturation frequency bands.⁶² The other experimental parameters were the same as the first experiment. The DANTE modulation parameters were: $m = 2$, $\delta = 305$ Hz. Two DANTE pulse durations were used: 50 μs and 100 μs .

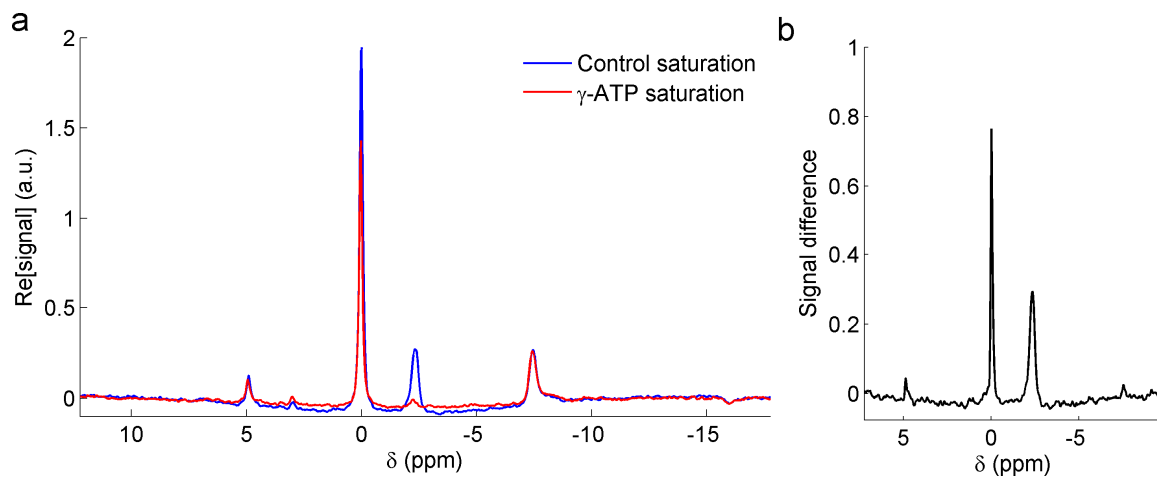


Figure 4.9 **a:** ^{31}P spectrum with control saturation (blue) and on resonance saturation (red) from a single voxel located in the quadriceps muscle of a healthy volunteer. **b:** Difference spectrum of the two spectra shown in **a**. The left most peak (~ 5 ppm) is inorganic phosphate.

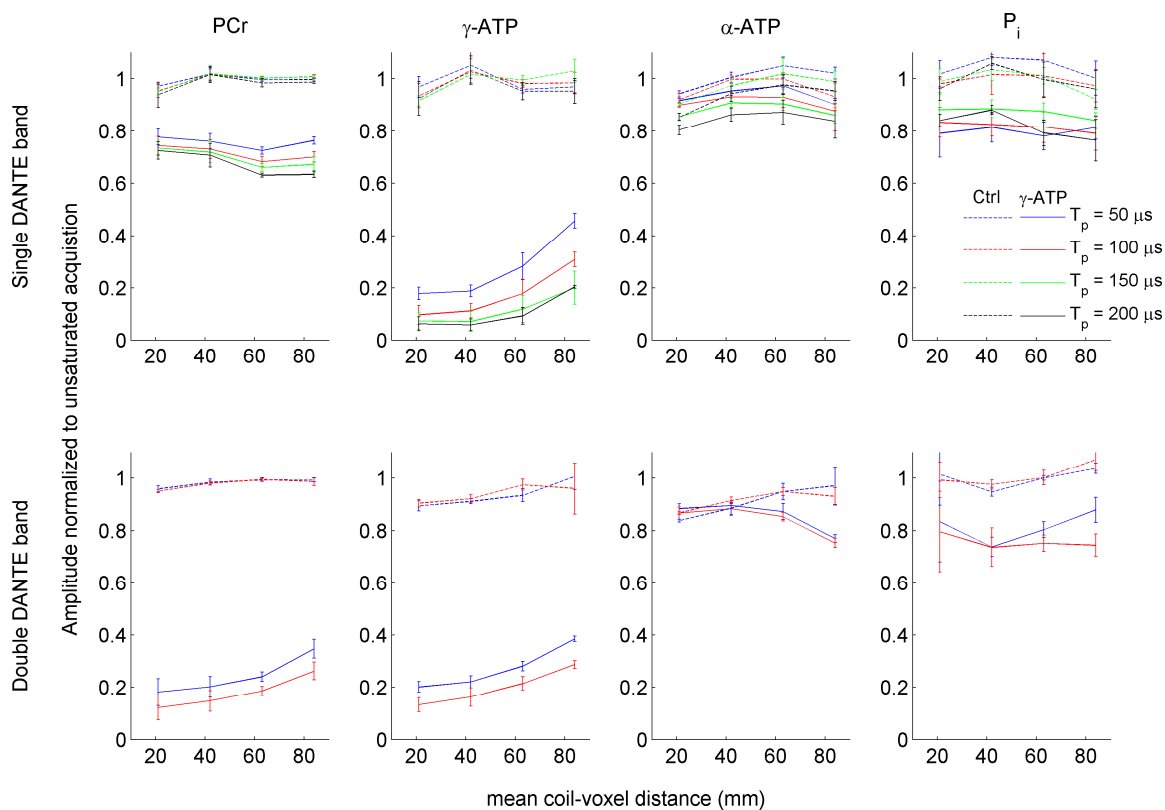


Figure 4.10 Inter-voxel mean ($\pm$ standard-deviation) saturation ratios, as a function of coil-voxel distance, for the skeletal muscle validation. The saturation ratios are shown for both the on resonance (solid line) and control saturation (dashed line) experiments. The top row shows the four fitted metabolites in the presence of a single saturation band and the bottom row of panels shows the saturation ratio for the double saturation band experiment. The mechanism for increasing saturation of α -ATP with decreasing DANTE B_1^+ is not understood. Nuclear Overhauser effects are proposed to transfer saturation between γ and α -phosphates.⁶³

4.5.1.2 Results

The difference spectra showed clear peaks at the PCr, γ -ATP and P_i resonance positions (Figure 4.9b). The average SNR of the difference spectra P_i peak in the sixteen voxels was 4.1 ± 0.4 , the P_i SNR of the control saturation spectra was 12 ± 4 . The mean PCr to P_i chemical shift was 4.90 ± 0.01 ppm, which corresponds to a pH of 7.09 ± 0.01 .

The saturation ratio, the ratio of the amplitude of the peak in the presence of saturation of γ -ATP to the amplitude with no saturation, of the PCr, γ -ATP, α -ATP and P_i peaks are shown in Figure 4.10.

At the coil-voxel distance equivalent to the coil-mid interventricular septum distance (6-8 cm) the greatest P_i saturation, and therefore greatest difference peak, is observed for the $T_p = 100 \mu s$ DANTE pulse. There was no observed difference between the saturation achieved using the single or double saturation techniques.

4.5.2 Cardiac data

4.5.2.1 Methods

A single healthy subject (male, 23 years, 82 kg) underwent cardiac saturation transfer spectroscopy to attempt to detect a myocardial P_i peak as described in the previous sections. Two ^{31}P 3D CSI acquisitions were made, the first with selective saturation at the control frequency and the second with selective saturation of γ -ATP. The standard CSI acquisition parameters were used (Appendix A, MDR3T pulse, 16 channel coil).

Frequency-selective saturation was achieved using unmodulated DANTE pulses ($T_p = 100 \mu s$, inter-pulse delay = $220 \mu s$), with a transmit voltage of 50 V.

Spectra were selected from a single skeletal muscle voxel, and 5 voxels from the mid and basal interventricular septum. After phase correction, a difference of the two

spectra were taken and inspected for a resolvable singlet peak in the 5 ppm region.

Additionally, the spectra from the 5 myocardial voxels were averaged, after frequency and phase correction, and then the difference was computed.

4.5.2.2 Results

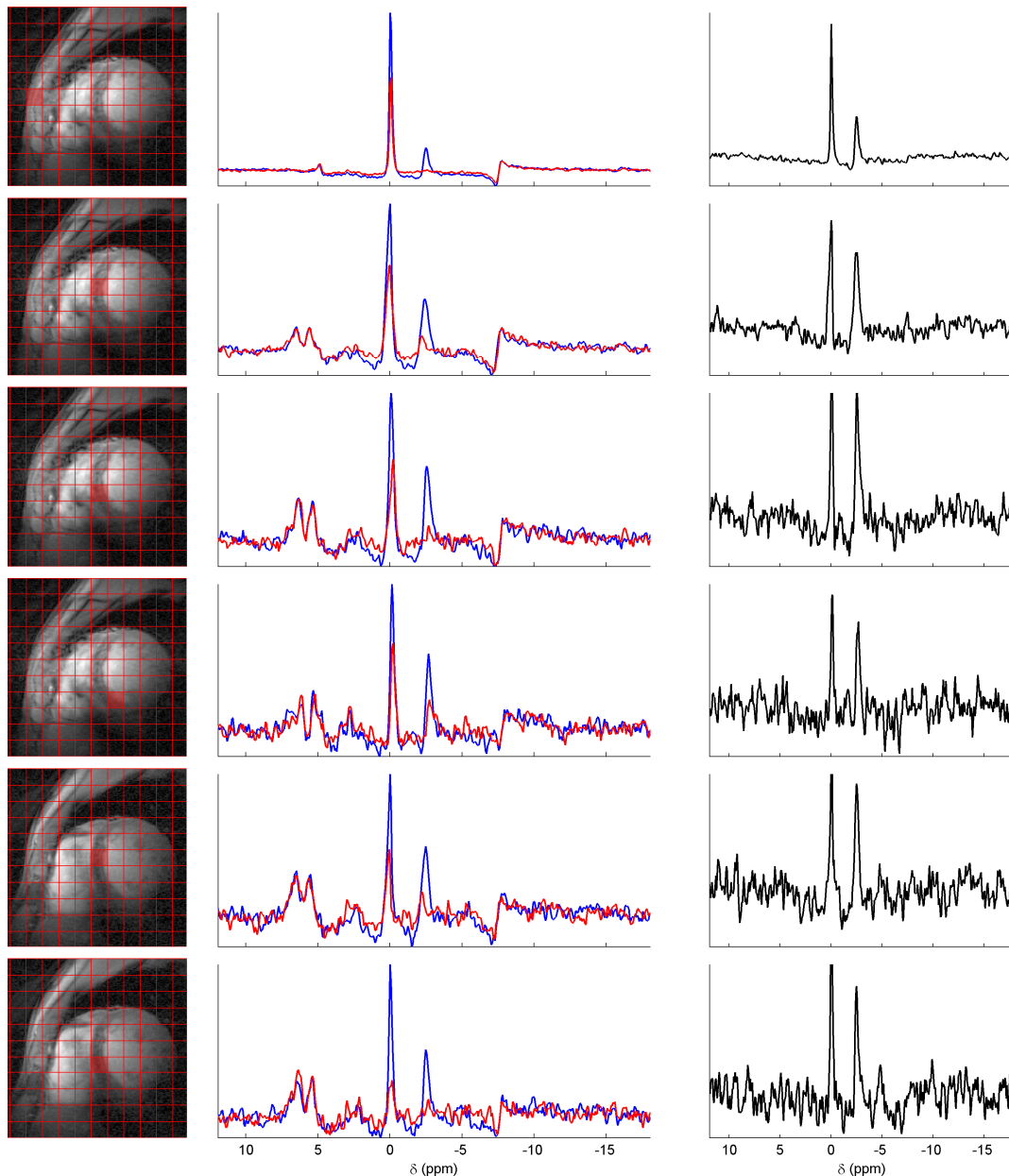


Figure 4.11 Saturation transfer spectra (blue = control, red = γ -ATP saturated)) and difference spectra (black) from 6 voxels (1 chest, 5 myocardial).

A peak corresponding to P_i was not discernible in the difference spectrum in any voxel, including the skeletal muscle voxel. The difference spectra are shown for each voxel

analysed in Figure 4.11. In the averaged spectra, which had a PCr SNR approximately 1.8x higher than the single-voxel spectra, there is a slight signal apparent in the difference spectrum at exactly 5.0 ppm (Figure 4.12). This corresponds to a pH value of 7.15.

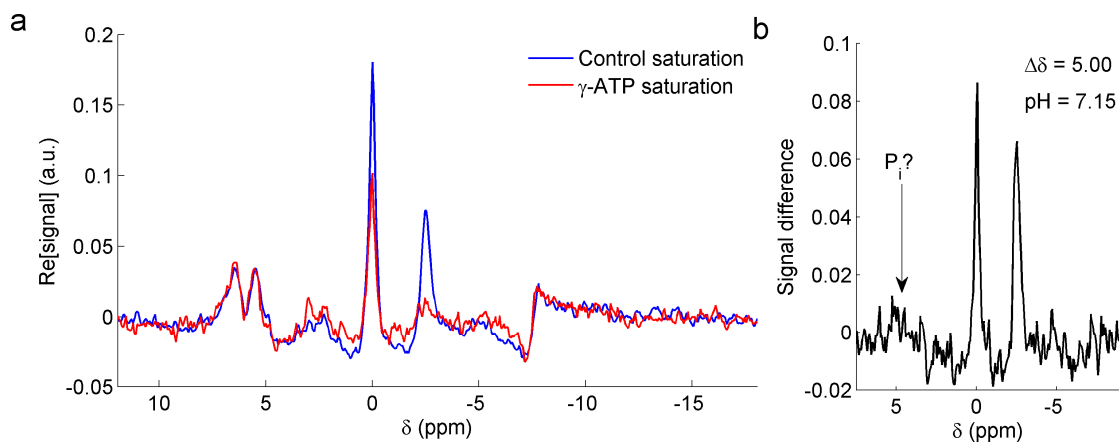


Figure 4.12 Average myocardial saturation transfer spectra (a) and difference spectrum (b) showing a possible P_i peak.

4.5.3 Discussion

The saturation transfer difference method's mechanism was successfully validated in skeletal muscle, with a difference spectrum showing a clearly resolvable peak at the location of P_i. Additionally it was demonstrated that in skeletal muscle saturation of P_i via the saturation transfer mechanism is observed throughout a large volume, spanning a range of B_1^+ values in the thigh.

However, in the single cardiac experiment attempted to-date, either insufficient SNR renders the P_i difference peak below the noise baseline or a saturation transfer effect is not observed. In the case of insufficient SNR, it seems impractical to attempt to increase the SNR by increasing the number of k-space averages: the total experiment time already exceeds 1h 15m (including localisation etc.). In addition the Biot-Savart

estimated flip-angles, ranging from 35-25° in the septum are close to the Ernst angle (32°) for the given repetition time and $P_i T_1$ estimated from skeletal muscle (6.3 s).

The only measurement of the pseudo first-order rate constant of the exchange between P_i and ATP, to-date, in the intact myocardium (rat), measures a similar value to that measured in human skeletal muscle $k_r^{ATP} = 0.1 \text{ s}^{-1}$.^{7,31} It is therefore reasonable to assume that the γ -ATP to P_i saturation transfer process is indeed taking place in this cardiac experiment.

Averaging of the spectra from several voxels raises the SNR, and more closely matches the localisation volume achieved in the published example.⁴⁰ However, even with the increased SNR, the assignment of a P_i peak in the difference spectrum is tentative, and only reasonable due to prior knowledge of the peak's location.

4.6 Resolution of P_i in patients with HCM

Severe, or late-stage, hypertrophic cardiomyopathy can cause the inhomogeneous hypertrophy of regions of the left ventricle, including the septum.⁶⁴ In patients with septal hypertrophy a ^{31}P -MRS voxel placed in the septum will contain proportionally more myocardium, and less blood, than healthy controls or HCM patients without left ventricular hypertrophy (LVH). Additionally, in heart failure it has been shown that the P_i concentration is raised from the healthy baseline level.³⁷ Combining lower blood contamination, and therefore smaller DPG and blood P_i peaks, with a larger (and therefore increased SNR) intracellular P_i peak, may result in a directly observable intracellular P_i peak.

4.6.1 Methods

Three subjects with HCM and LVH and one healthy volunteer were scanned using an extended but otherwise unmodified ^{31}P 3D-CSI sequence, using the 16-Channel array coil. Each subject's degree of hypertrophy was characterised using 3T MRI on a separate occasion. The subjects were positioned head first supine and ^1H MRI spectroscopy localisers were acquired as previously described.¹¹ The 7T 16-channel array coil was then used for the ^{31}P acquisition.

The standard CSI acquisition parameters (appendix A, MDR3T pulse, 16 channel coil) were modified to have a 18x18x12 grid with 7 averages at the centre of k-space, resulting in an acquisition time of 48 minutes. A BISTRO saturation band, with a transmit voltage of 200 V, was placed to suppress signal from the chest wall skeletal muscle.

The nominal voxel size was $13.3 \times 13.3 \times 16.7 \text{ mm}^3 = 3.0 \text{ mL}$, with a PSF broadened voxel size (defined at the PSF volume within 50% of the maximum) of 48 mL. This compares with a typical 16x16x8 CSI matrix which has nominal voxel size was $15 \times 15 \times 25 \text{ mm}^3 = 5.6 \text{ mL}$, with a PSF broadened voxel size of 110 mL. The estimated SNR of the acquisition is 1.8 times higher than the same experimental setup with the lower resolution CSI grid (16x16x8) and 28 minute acquisition time.

Spectra from mid interventricular septal voxels were selected for analysis. The signals from separate elements of the coil were combined using the WSVD algorithm. The spectra were fitted using Matlab AMARES with the standard prior knowledge (Appendix B.1) with an additional P_i peak. The P_i peak was considered resolved if the fitted peak was separated from other peaks by a frequency shift greater than Sparrow's resolution limit ($\text{FWHM linewidth} / \sqrt{3}$)¹² and the spectral matched filtered SNR was greater than 2.

The pH was calculated as described in Chapter 1 and the saturation corrected P_i/PCr ratio was calculated using Biot-Savart estimated flip-angles (Chapter 2).

4.6.2 Results

Subject maximum left ventricular thickness, the segment in which the maximum was measured, and the thickness of the mid interventricular septum, is given in Table 4.2.

#	Age	Weight (kg)	Sex	Maximum ventricle thickness (mm)	Location of maximum	Mid IV septal thickness (mm)
1	62	71	M	24.9	Basal anterior septum	18.6
2	45	70	F	14.4	Basal anterior septum	11.1
3	50	85	M	22.4	Basal anterior septum	15.0
4*	29	75	M	11.1	Apical anterior septum	10.7

Table 4.2 Subject myocardial wall thicknesses. *Healthy volunteer.

The results of the fitting and calculation of the derived parameters are given in Table 4.3. An example spectrum from the voxel with the greatest SNR P_i peak in each subject, and the location of the location of the voxel are shown in Figure 4.13.

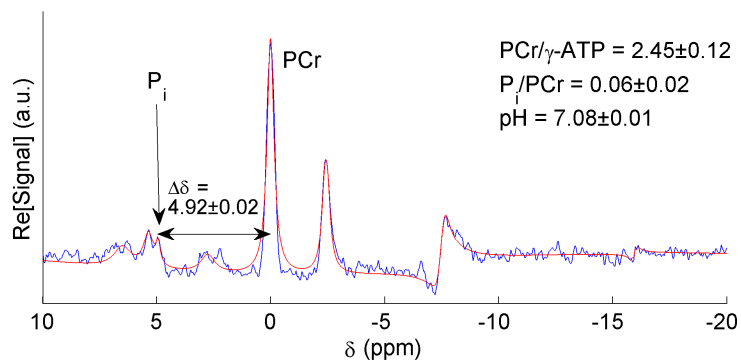
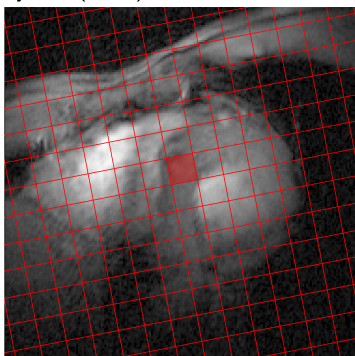
#	Voxels selected	Voxels in which P_i resolved	P_i/PCr	P_i SNR	$\Delta\delta_{P_i-PCr}$ (ppm)	pH
1	4	4	0.08 ± 0.03	3.9 ± 0.5	4.93 ± 0.03	7.10 ± 0.02
2	2	2	0.10 ± 0.07	3.0 ± 0.3	4.89 ± 0.00	7.06 ± 0.00
3	3	2	0.06 ± 0.01	3.1 ± 0.2	4.92 ± 0.01	7.09 ± 0.00
4	3	1	0.12	3.0	5.09	7.23

Table 4.3 Inter-voxel mean and standard deviation of derived parameters (P_i/PCr , SNR, $\Delta\delta_{P_i-PCr}$, and pH) from voxels which pass the inclusion criteria.

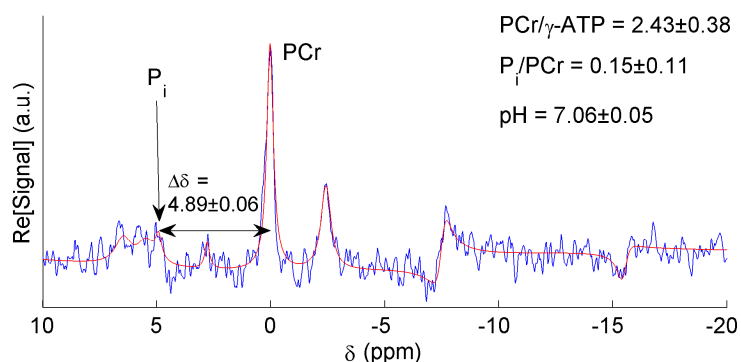
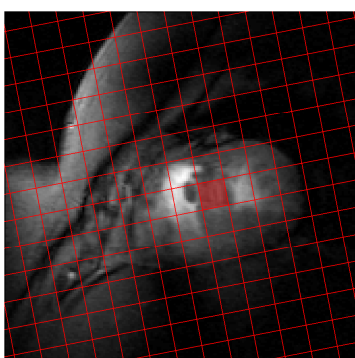
4.6.3 Discussion

P_i is resolvable in subjects with HCM and LVH using unmodified 3D-CSI acquisitions. This was achieved using higher resolution, the PSF broadened voxel size is 43% of a typical acquisition, and increasing the acquisition time. However, the SNR of the P_i peak remains low, therefore precluding the possibility of significantly reducing the acquisition time.

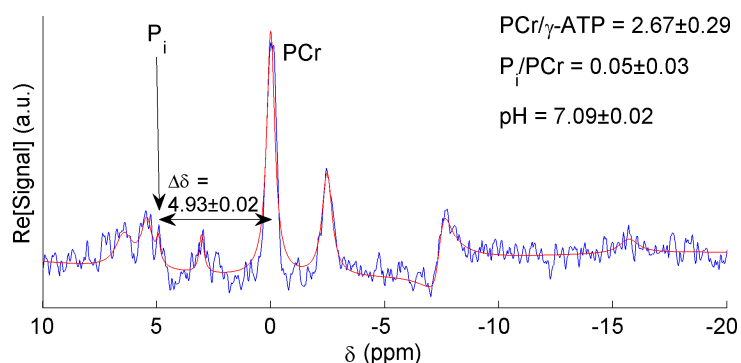
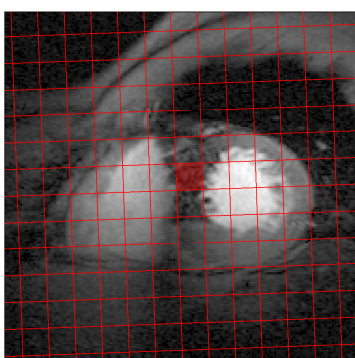
Subject 1 (HCM)



Subject 2 (HCM)



Subject 3 (HCM)



Subject 4 (Healthy)

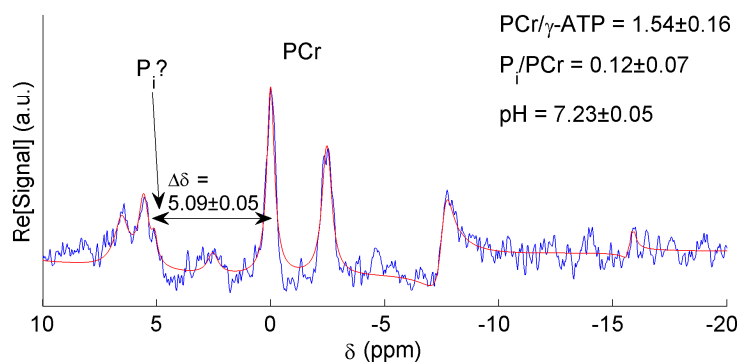
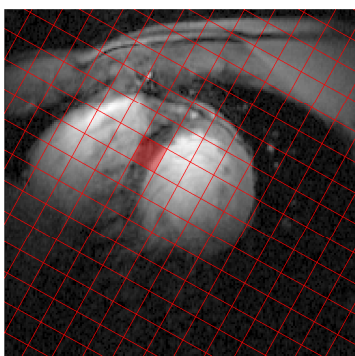


Figure 4.13 Voxel location and spectrum from the voxel with the highest P_i SNR in each of the three subjects with HCM. The P_i peak is labelled in each spectrum, and the AMARES fit (red line) is superimposed over the apodized spectrum. Derived parameters and their CRLB are given in each case.

A P_i peak was just resolved, as defined by the inclusion criteria set out above, in one voxel from the healthy volunteer. The success of the method (in both HCM and healthy subjects) might be improved by cardiac gating and B_0 shimming, which has been shown to decrease the observed linewidths in cardiac ^{31}P -MRS.⁶⁵ The current sequence does not implement cardiac gating, therefore each FID is recorded at a variable cardiac phase. If cardiac gating was enabled the acquisition of each FID could be fixed to a particular cardiac phase: either end diastole, for minimum cardiac movement, or end systole, for maximum septal thickness, might be chosen.

It remains desirable to confirm the peak assignment of P_i unequivocally. T_1 measurement or magnetisation transfer methods⁴⁶ offer direct but unfeasibly slow methods to do this as an additional scan.

4.7 Conclusion

Intracellular inorganic phosphate was detected, and the pH measured, in HCM patients with left ventricular hypertrophy by utilising the increased resolution and SNR brought by 7T ^{31}P -MRS. However, in the single tested healthy subject, the same method was only able to detect P_i in a single voxel.

The proposed flow sensitisation method using simple bipolar gradients was unable to achieve sufficient suppression of the obscuring DPG peaks to observe P_i in a 28 min scan. It is thought that the method is restricted by the limits placed on the scanner gradient system for subject safety.

We were unable to replicate the success of previous 2 T saturation transfer studies in measuring cardiac pH via P_i . (Although this method was only tested once in the heart.)

Without a method that is only sensitive to the P_i peak, the assignment of any peak remains tentative and open to dispute, especially in low SNR scans. In light of the higher P_i peak amplitude in the HCM cohort, methods which failed in healthy subjects (e.g. B_0 gradient suppression or saturation transfer) may succeed in the unhealthy cohort. Alternatively further SNR gains could be pursued using non-Fourier reconstruction (e.g. Spectroscopy with Linear Algebraic Modeling⁶⁶).

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Chapter 5: Creatine Kinase measurement by ^{31}P -MRS

5.1 Introduction

The creatine kinase energy shuttle, comprising the creatine kinase enzyme catalysed transfer and buffering of ATP in myocytes, is a major stage of the myocardial metabolic energy supply chain (§1.2.2). PCr and the gamma phosphate group of ATP, which have prominent peaks in cardiac ^{31}P spectra, undergo catalysed two-way chemical exchange in the CK energy shuttle. The exchange, which occurs on the timescale of an MR experiment, mixes components of the magnetisation of each resonance, affecting the observed signal. The observed signal of the exchanging peaks is not dependent on just the apparent T_1 (and Eq. [1.48]), but is a complex function of both MR sequence and metabolite parameters.

The first part of this chapter discusses the effect of the creatine kinase exchange process on the appearance of cardiac ^{31}P spectra and on the measured PCr/ATP ratio. The Bloch-McConnell equations, which adapt the Bloch equations to treat exchanging species, are introduced. The requirement to consider chemical exchange during saturation-correction of spectra is discussed with reference to the literature. Saturation correction of short-repetition-time cardiac spectra using established values for the exchange parameters is attempted.

The second part covers techniques used to measure the creatine kinase forward rate constant k_f^{CK} . The requirements for a successful k_f^{CK} measurement protocol at 7T are

set out, and a survey of published methods is conducted to assess their suitability for adaptation to 7T.

The final section assesses a multi-parametric fitting strategy to calculate the exchange parameters from a set of variable repetition time and saturation transfer data. From this study, it is established that the problem is underdetermined, and so it is necessary to make an independent measurement of one of the parameters. Flip-angle (or B_1^+) is selected as the most straightforward choice.

5.2 Effect of creatine-kinase-mediated exchange on the appearance of cardiac ^{31}P spectra

The chemical exchange of phosphate groups between PCr and the terminal (γ) position in ATP takes place in the slow exchange regime, i.e. $k_{\text{ex}} \ll |\Delta\nu|$. The pseudo first order exchange rate k_{ex} is approximately 0.3 s^{-1} (Table 5.3 & 5.4), whilst the peak separation $\Delta\nu$ is about 290 Hz. In this regime there is no peak coalescence, or observable peak broadening, which typifies the traditional “fast” exchange regime in NMR.¹ However, significant exchange still occurs on the time-scale of a typical MRS experiment (many seconds), and as such the effects of magnetisation exchange are observed in experiments.

When a phosphate group exchanges from PCr to γ -ATP, or vice versa, the spin state of the ^{31}P nucleus is preserved. In the classical interpretation of MR this is equivalent to a portion of the total magnetisation vector associated with the original chemical environment being transferred to the new environment. (To preserve chemical

equilibrium, a portion of the destination magnetisation vector is transferred simultaneously in the reverse exchange reaction).

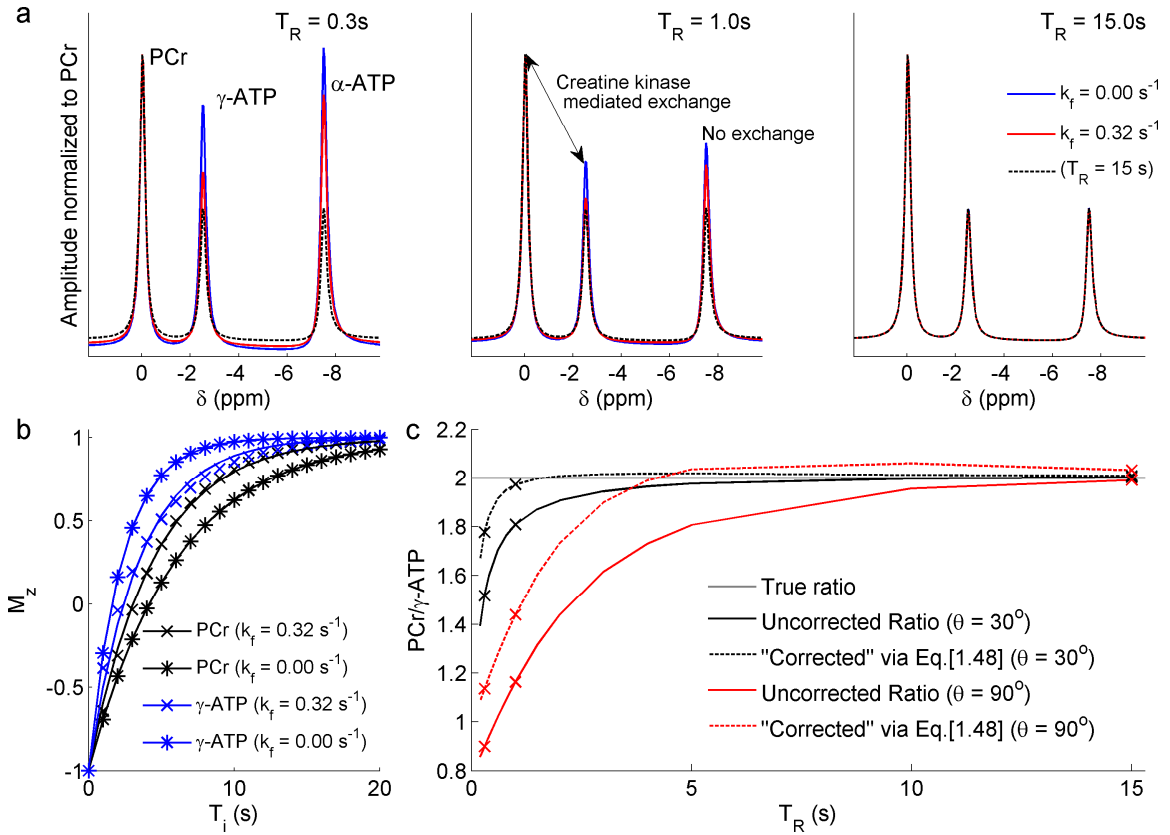


Figure 5.1 **a:** Effect of the creatine kinase on the appearance of a cardiac ^{31}P -spectrum. The spectrum is shown for three different T_R s, the final rightmost one is fully relaxed and so the three plots are all identical. **b:** Inversion recovery of the PCr and γ -ATP peaks in the presence of exchange and without exchange. The apparent (or 'observed') T_1 values are equal to the intrinsic T_1 values (6 s and 2.3 s respectively in this simulation) when $k_f = 0\text{ s}^{-1}$, but change towards each other in the presence of exchange. **c:** Uncorrected and "corrected" (via Eq. [1.48]) PCr/ γ -ATP ratio as a function of repetition time and for two flip-angles.

Due to chemical exchange a single spin might experience a range of molecular environments, and hence have several relaxation time constants, during a single MR experiment. The signal of the exchanging metabolites does not necessarily follow the partial saturation relationship described in Chapter 1; the deviation from equation [1.48] is dependent on the chemical system and pulse sequence parameters (Figure 5.1a). A T_1 , measured under these conditions, by inversion recovery, will not follow a

mono-exponential relaxation path (Figure 5.1b). Saturation correction using Equation [1.48] will fail, as it assumes a single mono-exponential relaxation constant (Figure 5.1c).

5.2.2 Bloch-McConnell equations

The evolution of a classical magnetization vector is described by a modification to the Bloch equations (Eq. [1.38]), which results in a set of coupled differential equations known as the Bloch-McConnell equations.² The equations, for a spin isochromat experiencing no RF field and neglecting T_2 relaxation processes, for a two site reaction i.e.



are as follows:

$$\frac{dM^A}{dt} = \frac{M_0^A - M^A}{T_1^{*,A}} - k_{AB}M^A + k_{BA}M^B, \quad [5.2]$$

$$\frac{dM^B}{dt} = \frac{M_0^B - M^B}{T_1^{*,B}} - k_{BA}M^B + k_{AB}M^A. \quad [5.3]$$

In equations [5.2] and [5.3] k_{AB} is the rate of exchange from site A to site B, and k_{BA} the reverse reaction. T_1^* is the so-called “intrinsic” longitudinal relaxation constant of the exchanging species, which is the relaxation constant that would be observed if there were no exchange. T_1^* (intrinsic) is not equal to T_1 (“observed” or “apparent”) which is the relaxation measured with exchange; and both are not equal to T_1' which is the relaxation measured under steady-state saturation.

From equations [5.2] and [5.3] Spencer *et al.* derived equations for the steady-state equilibrium saturation factors, SFX^A and SFX^B , for two exchanging peaks.³

$$SFX^A(T_R, \theta) = \sin \theta \left(1 + q_1 \frac{(1 - \cos \theta) \exp(D_+ T_R)}{(1 - \cos \theta \exp(D_+ T_R))} + q_2 \frac{(1 - \cos \theta) \exp(D_- T_R)}{(1 - \cos \theta \exp(D_- T_R))} \right), \quad [5.4]$$

$$SFX^B(T_R, \theta) = \sin \theta \left(1 + r_1 \frac{(1 - \cos \theta) \exp(D_+ T_R)}{(1 - \cos \theta \exp(D_+ T_R))} + r_2 \frac{(1 - \cos \theta) \exp(D_- T_R)}{(1 - \cos \theta \exp(D_- T_R))} \right), \quad [5.5]$$

$$D^+(T_1^{*A}, T_1^{*B}, k_{AB}, k_{BA}) = 0.5 \left[- \left(\frac{1}{T_1^{*A}} + \frac{1}{T_1^{*B}} + k_{AB} + k_{BA} \right) + disc \right], \quad [5.6]$$

$$D^- = D^+ - disc, \quad [5.7]$$

$$disc = \sqrt{\left(\frac{1}{T_1^{*A}} - \frac{1}{T_1^{*B}} + k_{AB} - k_{BA} \right)^2 + 4k_{AB}k_{BA}}, \quad [5.8]$$

$$q_1 = 0.5 \left[\left(\frac{1}{T_1^{*A}} - \frac{1}{T_1^{*B}} - k_{AB} - k_{BA} \right) / disc - 1 \right], \quad [5.9]$$

$$q_2 = -1 - q_1, \quad [5.10]$$

$$r_1 = 0.5 \left[\left(\frac{1}{T_1^{*B}} - \frac{1}{T_1^{*A}} - k_{AB} - k_{BA} \right) / disc - 1 \right], \quad [5.11]$$

$$r_2 = -1 - r_1. \quad [5.12]$$

In this series of equations θ is the flip-angle, $T_1^{*,x}$ is the intrinsic T_1 of species x and T_R is the repetition time. However, for more complex exchange processes, those with more than two species (n-site), including cyclic exchange regimes, the equations may be fully and succinctly expressed in matrix form, as described by Ouwerkerk and Bottomley.⁴

$$\frac{d\mathbf{M}}{dt} = \mathbf{A}\mathbf{M} + \mathbf{C}, \quad [5.13]$$

where the magnetisation $\mathbf{M} = (M_{s_1}, \dots, M_{s_n})$ and $\mathbf{A}$ is a matrix with elements:

$$A_{ij} = \left\{ \frac{1}{T_1^{*,S_i}} + \sum_{j \neq i} k_{S_i S_j} \right\}, \text{ for } i = j; \quad [5.14]$$

$$A_{ij} = k_{S_i S_j}, \text{ for } i \neq j. \quad [5.15]$$

The elements of vector $\mathbf{C}$ are

$$\mathbf{C} = \left(\frac{M_{0,S_1}}{T_1^{*,S_1}}, \dots, \frac{M_{0,S_n}}{T_1^{*,S_n}} \right). \quad [5.16]$$

5.2.2.1 Implementation of an n-site Bloch-McConnell simulator

For sequence simulation and fitting of experimental data, equation [5.13] was implemented for n-site exchange in Matlab, permitting the evolution of the magnetisation vectors of all exchanging peaks to be followed during nutation by arbitrary RF and longitudinal and transverse relaxation processes. The function takes as inputs: the complex RF in units of Hz, the time raster, the frequency offsets, and T_1 and T_2 constants, and relative concentrations of the exchanging peaks and the rate constants of exchange. The simulation is precompiled to the Matlab “MEX” format for speed using the Matlab CODER toolbox.

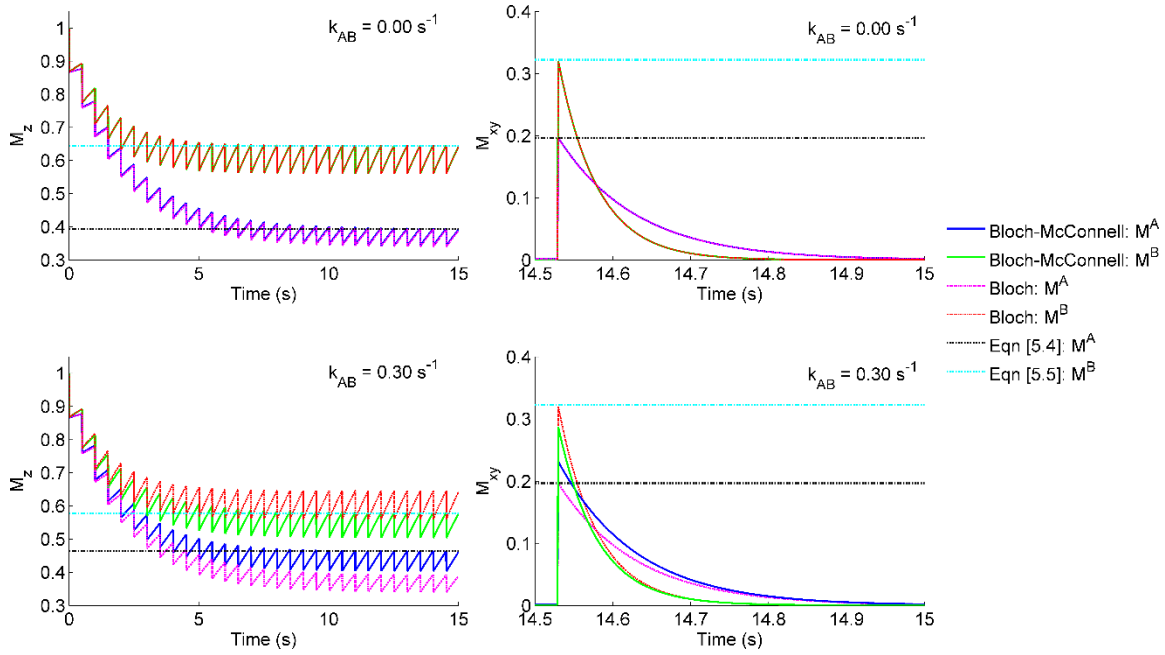


Figure 5.2 Schematic of the validation of the Bloch-McConnell simulation against equations [5.4] and [5.5] for a two pool exchange case (Eq.[5.1]). The Bloch-McConnell simulation was also compared against a previously implemented and validated Bloch simulation. The plot shows the approach to steady-state for two exchanging peaks (A: $T_1^* = 6$, $M_0 = 1.5$, $T_2 = 0.1$ s; B: $T_1^* = 2.3$, $M_0 = 1$, $T_2 = 0.05$ s; $\theta = 30^\circ$, $T_R = 0.5$ s). The simulation matches both the saturation factor equations and the Bloch simulation when there is no exchange. However, with exchange, the Bloch-McConnell simulation diverges from the Bloch-Simulation, but matches the saturation factors. The Bloch-McConnell simulation of 15 s of magnetisation evolution in this figure, for two peaks, on a 10 μ s raster, took 2.12 s on a desktop computer with an Intel i7-3770 (3.40 GHz) CPU and 32 GB RAM running Windows 7 64-bit.

The simulation was validated against equations [5.4] and [5.5] for two pool mixing and a previously implemented Bloch equation simulation when the exchange was set to $k_{AB} = 0 \text{ s}^{-1}$ (Figure 5.2).

5.2.3 Literature viewpoint

The one-pulse experiment is the most common ^{31}P -MRS sequence in the literature.

Ernst and Anderson established that in such an experiment the magnetisation rapidly approaches a cyclic steady state.⁵ They derived expressions for the calculation of the observed magnetisation in the steady state, taking into account both longitudinal and transverse magnetization effects (Eq. [1.47]).

Ernst's equation is derived from the Bloch equations, which unlike the modified Bloch-McConnell equations (Eqs. [5.2] & [5.3]), do not account for the effect of exchanging peaks. In a series of papers,^{3,6-8} bringing together theoretical calculations, simulations, in vitro and in vivo experiments Spencer and colleagues explored the effects of two and three pool exchange on the one pulse NMR experiment.

Using the matrix formulation of the Bloch-McConnell equations (Eq. [5.13]), Spencer and Fishbein analysed the effect of the exchanging networks on the saturation factors, as compared to those calculated by Eq. [1.48], the T_1 measured by a partial saturation method relative to the intrinsic T_1 , and finally the observed equilibrium magnetisations and ratios of exchanging peak concentrations.⁸

The saturation factors were sensitive to the parameters of both the exchange network and the sequence (excitation flip-angle and T_R). The exchange-affected factors followed the same functional form as the Ernst formula-derived factors (Figure 5.3a). The values converge at short and long T_R values.

T_1 measured by a partially saturated method, T_1^S , defined in 8 as

$$T_1^S = \frac{-T_R}{\ln\left(\frac{SF - \sin\theta}{SF \cos\theta - \sin\theta}\right)}, \quad [5.17]$$

in which T_R is the repetition time, θ is the flip-angle, and SF the metabolite saturation factor as calculated using Eq. [1.48], was found to diverge significantly from the intrinsic T_1 of the species, with T_1 values close to the T_1^* being measured at very short T_R (<100 ms) (Figure 5.3b).

Finally, and with most relevance to cardiac ^{31}P -MRS, it was found that using saturation factors derived from Eq. [1.48] to correct partially saturated peak amplitudes, before using them to calculate a metabolite ratio, could result in a significant over estimation of the ratio (Figure 5.3c).

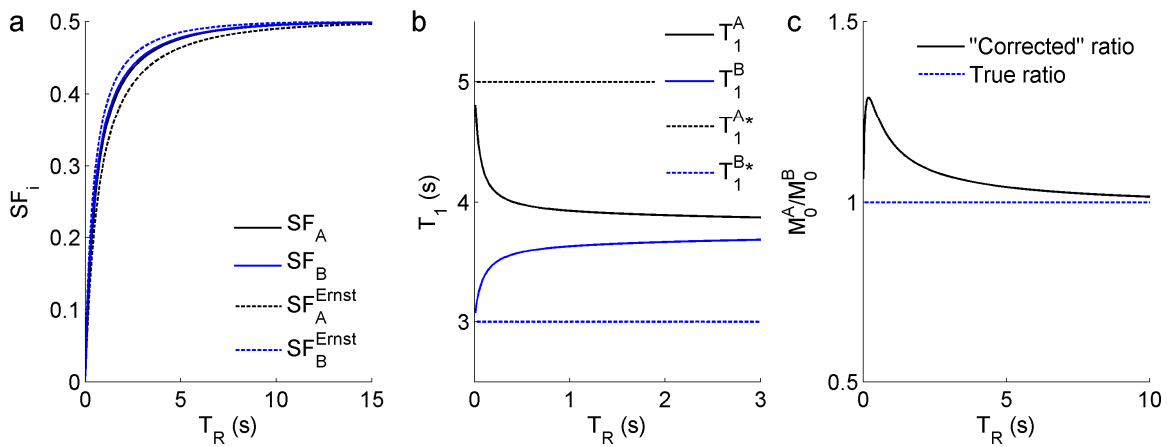


Figure 5.3 Illustration of the effects described in reference 8 (Figures 2,4 and 8). **a:** Calculated saturation factors as a function of repetition time calculated using Eq. [1.48], and Eqs. [5.4] and [5.5]. **b:** Apparent and intrinsic T_1 -relaxation constants as measured by Eq. [5.17]. **c:** PCr/ATP ratio calculated using the Ernst partial saturation formula. $M_0^A/M_0^B = 1$, $T_1^A = 5$ s, $T_1^B = 3$ s, $k_{AB} = 1$ s $^{-1}$, $\theta = 30^\circ$.

As a consequence of the exchange-affected saturation factors tending to the value of the non-exchanging saturation factor as $T_R \rightarrow 0$ or $T_R \rightarrow \infty$, the corrected metabolite ratio also tends to the true value in these limits. However, as Spencer and Fishbein note,

in this specific case, to be within 5% of the true metabolite ratio, which requires a T_R of <100 ms, there is a large – up to 20 times – SNR penalty, compared to the highest SNR at any T_R .⁸

These observations from theoretical calculations and Bloch-McConnell simulations were validated in an in vitro (phantom) creatine kinase system and in rat muscle, in vivo.^{6,7}

Spencer and Fishbein concluded that:

“the goals of achieving high SNR and of avoiding large systematic errors [in T_1 and metabolite ratio measurements] due to chemical exchange are ... mutually inconsistent”.

Ouwerkerk and Bottomley provided a contrasting viewpoint.⁴ Whilst they agreed that exchange processes would render the observed T_1 relaxation process multi-exponential, and that the multi-exponential behaviour will depend on both sequence parameters and chemical parameters of the system, they identify that there might be very little error in the measured PCr/ATP ratios in skeletal muscle and cardiac ^{31}P -MRS.

Ouwerkerk and Bottomley’s analysis of in vivo muscular ^{31}P -MRS, affected by the creatine kinase exchange process, was conducted using a numerical simulation of the same Bloch-McConnell equations that Spencer and colleagues used. But by using established chemical and physiological values specific to the specific creatine kinase exchange system (Table 5.1), they showed that, while a measured T_1 does vary significantly, longitudinal relaxation remains nearly mono-exponential and therefore saturation factors calculated from the Ernst formula (Eq. [1.48]) do not differ greatly from the truth except at short T_R values ($T_R < 300$ ms).

Furthermore, Ouwerkerk and Bottomley established that, with suitable calculation of the expected errors, it was feasible to select sequence parameters which produced an

acceptable amount of error (<10%) in the Ernst-formula-derived saturation factors whilst retaining near optimal (>95%) SNR.

This is seen in the recreated calculations for this work (Figure 5.1c), for certain conditions with high SNR ($T_R = 0.5 - 1$ s, $\theta = 30^\circ$), low error (<5%) is expected in the PCr/ATP ratio. However, it is also possible to select parameters that appear reasonable ($T_R = 1$ s, $\theta = 90^\circ$), but which give lower SNR and create higher (18%) errors in PCr/ATP ratio.

Ouwerkerk and Bottomley proposed that the magnitude of the third term of equation [5.4], $q_2 (1 - \cos \theta) \exp(D_- T_R) / (1 - \cos \theta \exp(D_- T_R))$, provides a measure of the error expected to arise from a chosen set of parameters. For example, using the parameters in Table 5.1, the term evaluates to 0.006 (0.6%) for $T_R = 1$ s, $\theta = 30^\circ$ but to 0.05 (5%) for $T_R = 1$ s, $\theta = 90^\circ$.

Simulation parameters	Value in heart muscle
[PCr]:[ATP]	1.5:1
$T_1^{*, \text{PCr}}$	6.0 s
$T_1^{*, \gamma\text{-ATP}}$	2.0 s
k_f^{CK}	0.32 s ⁻¹

Table 5.1 Creatine kinase system chemical and MR parameters from reference 4. The value for the pseudo first-order rate constant has been updated from 0.27 s⁻¹, in light of more recent measurements (Table 5.3). These values are used for simulations and figure generation throughout this chapter.

Despite continued publication from both sides a consensus could not be reached. In my opinion it is likely, as Spencer *et al.* conclude, chemical exchange can be the source of large systematic errors in the analysis of MRS data.⁹ However, for cardiac ³¹P-MRS, including MRS of healthy and ischaemic hearts, and dynamic PCr depletion experiments,

providing experimental parameters are chosen to minimise error, the one-pulse experiment remains valid for myocardial energetic measurements.¹⁰

5.2.4 Saturation correction of short repetition time spectra

The 16-channel receive array (Chapter 2), produces a low B_1^+ field in human subject's hearts. Using the "MDR7T" pulse (§2.4.2.2) that spans the whole ^{31}P cardiac spectrum results in a maximum flip angle in the interventricular septum of $\sim 10\text{-}15^\circ$, which is lower than the $\sim 30^\circ$ typically desired. To obtain high SNR with this coil and pulse, one possibility would be to collect CSI data with a much shorter T_R than the 1 s that is typically used. For an observed PCr T_1 of 3 s and a flip-angle of 10° , according to the Ernst and Anderson formalism (Eq. [1.49]), the optimum SNR occurs with a T_R of approximately 50 ms.

Hardware and sequence limitations mean that the minimum possible T_R is 330 ms, whilst maintaining 2048 spectral samples, and 130 ms, when the spectral samples are reduced to 512. With a 6000 Hz bandwidth at 7T, 512 samples provides a spectral resolution of 12 Hz or 0.1 ppm, further reduction would impact spectral analysis. However, data acquired at these two repetition times was found to have significantly different PCR/ATP ratios (after saturation correction by Eq. [1.48]) compared to data collected previously with a T_R of 1 s.

We hypothesised that these differences were a result of the creatine kinase exchange mechanism. Both Ouwerkerk and Bottomley, and Fishbein and Spencer, predict incorrect saturation-correction of metabolite ratios via Eq. [1.48] due to chemical exchange at short T_R values.

Simultaneous fitting of data from with different repetition times and from multiple subjects to Bloch-McConnell sequence simulations might enable an accurate equilibrium PCr/ATP ratio to be calculated, without *a priori* knowledge of the intrinsic relaxation time constants or the true concentration ratio.

5.2.4.1 Methods

Five healthy subjects (5 female, 58.0 ± 7.6 kg, 33.4 ± 6.0 years), underwent ^{31}P -MRS at 7T as described in Chapter 2, using a 28 minute acquisition-weighted 3D-CSI protocol. The 16 channel array coil was used. To mitigate the low flip angle produced in the interventricular myocardium ($15.7 \pm 1.3^\circ$), as estimated by the scaled Biot-Savart field map, consecutive acquisitions were made with $T_R = 130$ ms & 330 ms .

The measured uncorrected PCr/ATP ratios from all 10 acquisitions were least-squares fitted using equations [5.4] and [5.5], to calculate a single value of $T_1^{*, \text{PCr}}$ and $T_1^{*, \gamma\text{-ATP}}$ and an $M_0^{\text{PCr}} / M_0^{\text{ATP}}$ (PCr/ATP) ratio per acquisition. The T_R is known and the flip-angles were estimated using a dual- T_R partial saturation method from the α and β -ATP peaks and previously measured T_1 values.¹¹ The exchange rate was estimated from literature values (Table 5.3).

5.2.4.2 Results

The uncorrected PCr/ γ -ATP ratios of the 130 ms and 330 ms data show a significant difference (t-test, $p=0.004$). Standard saturation correction (applying Eq. [1.48] with literature T_1 values and the sequence T_R) raises the average uncorrected value of 1.37 to 1.86 (for the 130 ms acquisition), and from 1.71 to 2.00 (for 330 ms). There is still a significant difference between these values (t-test, $p=0.043$).

Using the simultaneous fitting method and including the effect of exchange gives mean ($\pm$ SD) PCr/ γ -ATP values of 2.01 ± 0.46 (at 130ms) and 2.11 ± 0.38 (at 330ms), which are not significantly different (t-test, $p=0.36$). Similarly the simultaneously fitted and exchange corrected PCr/avg-ATP ratios are 1.95 ± 0.41 (130ms) and 1.97 ± 0.38 (330ms). The per-subject exchange corrected values are plotted in Figure 5.4b, and range from 1.08 to 2.68. Fitted intrinsic T_1 values are 5.4 s (PCr) and 1.8 s (γ -ATP).

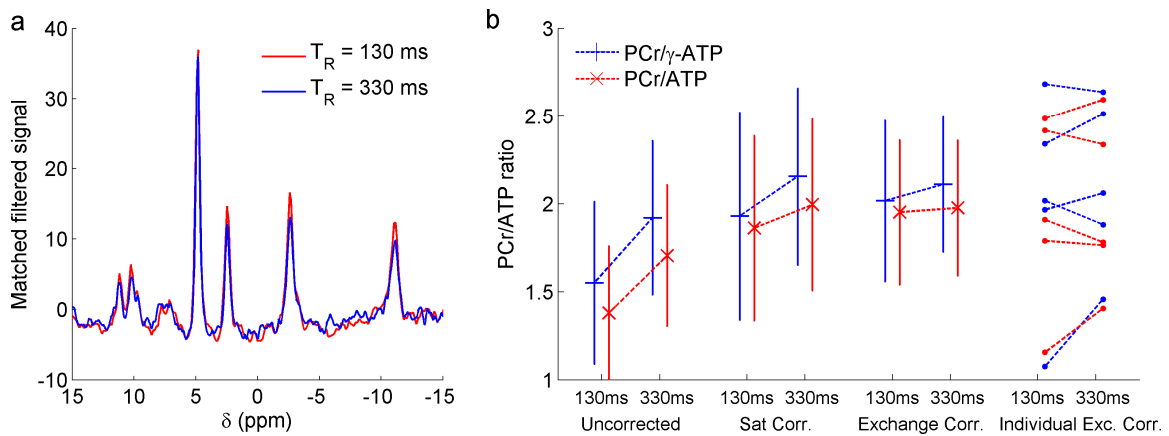


Figure 5.4 Correction of short TR spectra. **a:** Spectra from consecutive acquisitions ($T_R = 130$ ms and 330 ms) localised to the interventricular septum of a single subject. **b:** Inter-subject means and standard deviations for the five subjects, without correction, with standard independent saturation correction and with a combined correction including exchange effects. The final column gives the individual subject values at both repetition times. The blue and red lines signify the PCr/ γ -ATP ratio and the PCr/avg-ATP ratio (i.e. $3 \times \text{PCr} / [\alpha\text{-ATP} + \beta\text{-ATP} + \gamma\text{-ATP}]$).

5.2.4.3 Discussion & Conclusions

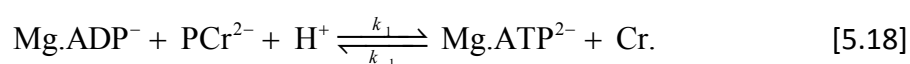
Using simultaneous fitting of multi- T_R data collected from five subjects it is possible to increase the accuracy of the corrected PCr/ATP over standard saturation correction. The fitting required flip-angle estimates derived from literature T_1 values for α -ATP and β -ATP and a literature value for k_f^{CK} , which could if inaccurate introduce significant errors. This approach is not feasible for a single repetition time, or for data from a single subject, because the least-squares problem is severely underdetermined.

5.3 Measurement of the in vivo creatine kinase rate constants by ^{31}P -MRS

Accurate measurement of the exchange kinetics of the creatine kinase system are central to understanding its role in the normal and diseased heart in vivo. The kinetics of a first-order chemical reaction are characterised by the rate constants of the forward k_f and backwards k_r reactions. Magnetic resonance offers several techniques to explore the kinetic parameters of reactions. For the rates and longitudinal relaxation times of biologically interesting molecules, magnetisation transfer (MT) is typically the most sensitive technique.¹² Enzymatic reactions are generally too fast for isotope flux experiments, which also are difficult in vivo, and too slow for MR linebroadening experiments.¹³

5.3.1 Magnetisation transfer measurements

The creatine kinase catalysed reaction, representing the reaction process at each end of the cyclic creatine kinase energy shuttle process, may be written¹⁴



Magnetisation transfer experiments, first described by Forsen and Hoffman¹⁵ and shown in a biological context in *E. coli* cells by Brown *et al.*¹⁶, enable the measurement of the pseudo-first-order rate constants for the exchange of the terminal phosphate group of ATP with PCr, and vice versa. For the CK reaction the rate constants are defined as:

$$k_f^{\text{CK}} = k_1[\text{Mg.ADP}^-][\text{H}^+], \quad [5.19]$$

$$k_r^{\text{CK}} = k_{-1}[\text{Cr}]. \quad [5.20]$$

The fluxes are:

$$CKflux_f = k_f^{CK}[PCr^{2-}], \quad [5.21]$$

$$CKflux_r = k_r^{CK}[Mg.ATP^{2-}]. \quad [5.22]$$

The rate constants measured by magnetisation transfer experiments are always effective first order rate constants, even if the underlying reaction is not first order.¹²

The flux measured is also unidirectional: it is the microscopic rate of exchange of two nuclei between reacting species; and is distinct from the overall (macroscopic) net flux, which is the difference between the forward and reverse unidirectional flux.^{12,17}

Magnetisation transfer experiments can be split into steady-state saturation transfer and temporal magnetisation transfer. In the former, selective saturation of one or more exchanging species is used to continuously perturb the system equilibrium. The observer waits for the system to establish a new equilibrium position, or steady-state, before recording the magnetisation. In the latter, a temporary perturbation is made and a time-course experiment is conducted to observe the magnetisation's reaction to the perturbation, and return to equilibrium.

5.3.1.1 Steady-state saturation transfer

Saturation transfer (ST) is a simple and commonly employed MT method. (See this section and section 5.3.2.1 for a summary of experiments described in the literature.)

The method uses a selectively saturating sequence module to completely saturate one of the exchanging species, e.g. species B in an exchanging AB system. The saturating RF field is designed to continuously saturate the magnetisation of B, i.e. remove all net magnetisation, whilst minimising the direct effect on the magnetisation of species A.

The saturation effect introduced at B is transferred to A, via chemical exchange, reducing the longitudinal magnetisation of A, by a proportion dependent on the chemical exchange properties. This results in a new equilibrium magnetisation level of A. From the view point of spin state populations, saturation of B equalises the populations of α and β states of the B nuclei, where at equilibrium there would normally be more α spins. Exchange then randomly swaps nuclei between species B and A, resulting in a net flux of β spins to A. The result is that the ratio of α to β spins is also decreased across A nuclei, therefore A also appears to become somewhat saturated. Longitudinal relaxation of A is simultaneously trying to return the magnetisation to the unsaturated equilibrium state (more α states). At some value of M_z^A , the rate of saturation by exchange equals the relaxation rate and a new steady-state is established.

The relationship between the saturated and unsaturated magnetisation and the determination of the two pseudo-first-order rate constants may be derived by imposing the boundary conditions $dM^A/dt = 0$ and $M^B = 0$ on Eq. [5.2]

$$\frac{dM^A}{dt} = \frac{M_0^A - M^A}{T_1^{*A}} - k_{AB}M^A + k_{BA}M^B :$$

$$M_{SS}^A = \frac{R_1^A}{R_1^A + k_{AB}} M_0^A , \quad [5.23]$$

where $R_1^A = 1/T_1^{*A}$ and M_{SS}^A is the saturation steady-state magnetisation of A. The ratio of the change in magnetisation due to saturation to the unsaturated equilibrium magnetisation $\Delta M^A/M_0^A = (M_0^A - M_{SS}^A)/M_0^A$ is therefore

$$\frac{\Delta M^A}{M_0^A} = \frac{k_{AB}}{R_1^A + k_{AB}} . \quad [5.24]$$

Imposing only the second condition we may solve the linear first-order differential equation produced, in which $1/k_{AB}$ has been substituted for τ , the time constant or “residence time” of exchange:

$$\frac{dM^A}{dt} = \frac{M_0^A - M^A}{T_1^{*A}} - \frac{M^A}{\tau}, \quad [5.25]$$

$$M^A(t) = \frac{M_0^A \tau}{T_1^{*A} + \tau} + ce^{-t\left(\frac{1}{T_1^{*A}} + \frac{1}{\tau}\right)}. \quad [5.26]$$

The solution (Eq. [5.26]) describes the recovery, to the steady-state saturation equilibrium, of the longitudinal magnetisation of species A. The formula is mono-exponential; T_1^{*A} and τ may be replaced with a single time constant T_1' which is the observed T_1 relaxation constant for species A while species B is undergoing saturation.

$$\frac{1}{T_1'} = \frac{1}{T_1^*} + \frac{1}{\tau} = \frac{1}{T_1^*} + k_{AB}. \quad [5.27]$$

The full solution to Eqn. [5.25] may therefore be written

$$M^A(t) = \frac{M_0^A}{T_1' k_{AB}} + \left(M^A(0) - \frac{M_0^A}{T_1' k_{AB}} \right) e^{\frac{-t}{T_1'}}. \quad [5.28]$$

By substitution of Eqn. [5.27] in to Eqn. [5.23] the relationship between T_1' and T_1^* may be written

$$T_1' = T_1^* \frac{M_{SS}^A}{M_0^A}. \quad [5.29]$$

Finally, by rearrangement of [5.23] and substitution of either [5.27] or [5.29] an equation for k_{AB} may be formed in terms of just M_{SS}^A, M_0^A and T_1' (Eq. [5.27])

$$k_{AB} = \frac{1}{T_1'} \left(1 - \frac{M_{SS}^A}{M_0^A} \right). \quad [5.30]$$

Note that saturation of species B enables the measurement of the rate constant for the flow from A to B. This may be understood by reference to Eq. [5.23] which only contains the k_{AB} term, i.e. when B is continuously saturated, the B magnetisation is always zero, so irrespective of k_{BA} there is no net magnetisation available to return to affect the A peak.

A steady-state saturation transfer measurement of k_{AB} therefore requires the determination of the saturated and unsaturated equilibrium magnetisations and the T_1' or T_1^* of species A. To minimise the effects of direct saturation of species A by the saturating RF, M_0^A may be replaced with M_{Ctrl}^A , which is the equilibrium magnetisation of A in the presence of a saturating RF field positioned on the opposite side of A, but at the same chemical shift displacement as species B.¹⁸

Summary of historical measurements of k_f^{CK} by saturation transfer

Requiring only three directly measureable parameters, steady-state saturation transfer has historically been widely implemented to measure the CK reaction kinetics for in vitro experiments, in animals and in humans.

In vertical bore NMR scanners, where signal localisation was not required due to isolation of the sample (e.g. *E. coli* cells¹⁶; phantom models of the CK reaction¹³; and perfused, surgically excised tissue¹⁹), M_{Ctrl}^A and M_{SS}^A were measured directly, using sequences with $T_R \approx 5T_1^*$ and a calibrated flip angle excitation pulse. T_1' was measured using either inversion recovery (IR), saturation recovery or progressive saturation while maintaining selective saturation of the destination species. Alternatively T_1^* was determined after the enzyme catalysing exchange was inhibited pharmacologically.¹⁶

The first in vivo CK measurements were achieved in the intact rat brain by Shoubridge, Briggs and Radda²⁰ using similar saturation strategies to previous ex vivo experiments. Koretsky *et al.* made the first measurements in an intact rat heart, achieving the necessary localisation with surgical implantation of a small RF coil.²¹ Rees *et al.* reported the first measurement of the human skeletal muscle CK flux, using the same long T_R methods - without localisation, in the forearm of healthy volunteers.²²

Validation of the CK exchange model was a significant driving force in the early experiments. Gadian *et al.* (in frog muscle), Koretsky *et al.* (in rat heart), Shoubridge (in rat brain), Brown *et al.* (in perfused tissue), Nunnally and Hollis (in perfused hearts), Matthews *et al.* (in rat heart) and Kobayashi *et al.* (in rat heart) measured a discrepancy in forward and backwards creatine kinase fluxes.^{17,19-21,23-25} The disparity between these rates and the ATP synthesis rate, also measured by ³¹P-MRS saturation transfer, supported the hypothesis that the reaction was taking place in multiple cellular compartments, now established to be within the mitochondria and at the myofibrils.^{21,25} Brindle *et al.*¹³ and Boyd *et al.*²⁶ validated the saturation transfer measured rate against NMR isotope exchange and 2D NMR respectively; both measuring similar forward rates to saturation transfer.

Measurement from less homogenous tissue, in human subjects or large animal models, requires localisation of the signal to the volume of interest (e.g. specific brain anatomy or the myocardium) to make measurements not contaminated by surrounding tissue.

However, the long T_R measurement of M_{Ctrl}^A and M_{SS}^A and determination of longitudinal relaxation times by multiple time-point methods described up to this point are incompatible with localisation. Localisation methods require multiple repeated

transients of the sequence: when combined with long repetition times the resultant total scan duration exceeds subject tolerance and imposed ethical limits.

Hsieh and Balaban applied ^{31}P -MR imaging in the human calf, measuring CK activity, but not a quantitative rate constant, relying on partial saturation correction using the apparent T_1 .²⁷ A quantitative 2D CK rate image was formed, in the intact monkey brain by Mora *et al.* several years later, though T_1' was determined by un-localised spectroscopy in a separate cohort.²⁸ Cadoux-Hudson and colleagues achieved a similar feat in the human brain, 1D localised spectra were measured in one cohort, whilst a second was used to determine T_1' from inversion recovery.²⁹ Bottomley and Hardy combined the two measurements in a single exam in human subjects for the first time: 3D localised (2D-CSI + slice selection) spectra were measured from the brain, with a short T_R (1 s), and combined with saturation correction values and T_1' determined from unlocalised spectra.³⁰ This is the first work in which M_{Ctrl}^A and M_{SS}^A were derived from partially saturated magnetisations acquired with short sequence T_R and flip-angles $< 90^\circ$, maximising SNR per unit time and enabling two dimensional phase encoded spectra to be acquired in a tolerable time (5-6 minutes). The use of saturation factors uncorrected for exchange is justified by the small errors observed at $T_R \geq 1$ s, as Ouwerkerk and Bottomley later demonstrated.⁴ In the same paper the method was used to make the first localised measurements of k_f^{CK} in the human heart.

Chen *et al.*, using the T_1' measured by Bottomley, demonstrated the sensitivity of the CK flux to physiological stimulation in vivo for the first time.³¹ A 34% increase in CK flux was detected in a 3D localised volume in the human visual cortex during visual stimulation.

More recent measurements of cardiac and brain CK flux, starting with Bottomley *et al.*'s four-angle saturation transfer (FAST) method, have focused on time efficient determination of T_1' and T_1 , and calculation of the equilibrium magnetisation from partially-saturated spectra.³² These methods are described in detail and assessed for suitability for use at 7T below and in Chapter 8. Unlocalised steady state saturation transfer continues to be used in homogenous tissue at high field strength, where Valkovic *et al.* have demonstrated a significant advantage from ultra-high field magnets.³³

5.3.1.2 Temporal saturation

Aside from steady-state saturation transfer, Forsen and Hoffman's original demonstration of rate measurements by NMR included several time course experiments which also enable the user to determine the exchange rates. These experiments were: (1) measurement of the decay of A upon saturation of B, (2) measurement of the recovery of A after saturation of B was stopped, (3) measurement of the recovery of A after complete saturation of A, (4) the recovery of A after complete saturation of both A and B.

Method (1), described as "Time Dependent Saturation Transfer" (TDST) has been employed in perfused hearts and most recently in the human heart by Bashir and Gropler.³⁴ The time course relationship is described by

$$M^A(t) = \frac{M_0^A}{\tau} \left(\frac{1}{T_1^*} + k_f e^{-t/\tau} \right), \quad [5.31]$$

where $\tau = 1/T_1^* + k_f$, and t is the duration of the saturating pulse. The k_f may then be calculated:

$$k_f = \left(\frac{M^A(0) - M^A(\infty)}{M^A(0)} \right) \tau = \left(\frac{M_0^A - M_{ss}^A}{M_0^A} \right) \tau. \quad [5.32]$$

The advantage of the TDST experiment is that the determination of the relaxation rate constant is not dependent on accurate knowledge of the excitation flip-angle. However, the long sequence repetition times required have, to date, limited this technique to at most 1D-ISIS localisation schemes. While the steady-state measurement may make use of efficient T_1 measurement strategies developed for ^1H -MRI, TDST is inherently limited by the repeated relaxation and saturation required to measure different saturation time points.

5.3.1.3 Temporal Inversion transfer

Dahlquist *et al.* suggested a time course measurement, where instead of perturbing the equilibrium state with saturation, inversion of one or more exchanging peaks was used.³⁵ Inversion transfer, requiring two acquisitions, was demonstrated and compared to saturation transfer in perfused rat hearts by Degani *et al.*³⁶

No closed-form expression for the first order rate constants exists for the inversion transfer experiment. Rather the Bloch-McConnell equations must be fitted to the recovery curve observed by the experiment, to determine the free parameters:

$$M^A = f(k_{AB}, k_{BA}, T_1^{*A}, T_1^{*B}, M_0^A, M_0^B). \quad [5.33]$$

Flip-angle and T_R must also be included in the fit or separately determined. As demonstrated recently in human liver, skeletal muscle and brain, the parameters may be determined from the analysis of a single inversion transfer experiment.^{37,38}

Inversion transfer is also thought to drastically reduce the effect of confounding physiological mechanisms which affect saturation transfer measurements.³⁹ Steady

state saturation transfer, due to the use of long low-power saturating pulses, is sensitive to small and otherwise undetectable metabolite pools. In inversion transfer, where the labelling of exchanging pools occurs during a short inversion pulse, the effects observed due to exchange are proportional to the size of the pools.

Inversion recovery, in its simplest form, is incredibly time intensive, though recent work has addressed this.⁴⁰ The example studies have all been acquired with unlocalised FIDs, or only with OVS, and long T_R values (15/30 s). Implementing a Look-Locker type experiment⁴¹, as previously done in Oxford¹¹, for the determination of apparent T_1 values in myocardium, may represent the future of localised inversion transfer experiments, but fitting of Look-Locker data necessitates accurate knowledge of the excitation flip angle and timings of the sequence.

5.3.2 Survey of implemented CK rate measurement methods

The measurement of the creatine kinase forward and reverse rate constants, and associated fluxes, from localised myocardium of healthy and patient groups at 7T is a principal goal of the work in this thesis. Despite bringing SNR benefits, MRS at 7T suffers from greater B_0 and B_1 inhomogeneity than at lower field strengths. In the absence of signal localisation, or with large voxel volumes (hundreds of mL), the low B_0 homogeneity causes line broadening, non-Lorentzian lineshapes, and makes selective saturation more difficult. Significant B_1 inhomogeneity in a large voxel leads to both a receive sensitivity weighting of the tissue and a range of flip-angles experienced in the voxel. Due to increasing dielectric effects at higher fields, the B_1^+ and B_1^- weightings are not equal even for a transmit/receive coil, due to the B_1 “twist” phenomenon.^{42,43}

Reducing the size of the voxel, either by increasing resolution in currently implemented localisation schemes (e.g. 1D-CSI) or by implementing higher-dimensional localisation, should reduce the deleterious effect of field inhomogeneity.

Of the methods described in §5.3.1, only a few have been combined successfully with localisation schemes to enable uncontaminated determination of myocardial k_f^{CK} in humans. The methods which have been implemented in humans or in large animal (porcine and canine) models, as well as some of the basic methods, are surveyed below to assess which methods are best suited for application in the human heart at 7T.

The survey is summarised in Table 5.2. The compatibility of each method with 3D-CSI localisation was judged on the following criteria:

1. Maximum T_R required by the method. At typical resolutions for cardiac ^{31}P -MRS (16x16x8 or 16x8x8) an acquisition weighed CSI grid requires between 500-1700 transients. A T_R greater than 7.2 s will cause a single step of the method to exceed 1 hour in duration. Ideally no single T_R would exceed 2 s, allowing a 16x16x8 grid to be measured, with 5 averages at the centre of k-space, in 30 minutes.
2. Total number of steps. The minimum duration of a 3D-localised scan at a sufficient resolution, without saturation, to obtain sufficient SNR for analysis is 6 minutes. Introducing saturation, or using a non-optimal T_R degrades the SNR and increases the minimum duration to more than 10 minutes. A large number of steps will increase the total duration or may result in a compromise being required between sufficient SNR for analysis and scan duration.

The compatibility with 7T ^{31}P -MRS was assessed on:

1. Any requirement for B_1 -insensitive pulses. B_1 -insensitive adiabatic and composite pulses are challenging to implement on current hardware available at 7T, due to low peak B_1^+ and SAR limits (§5.4.3.2 and Chapter 2).
2. A requirement for prior knowledge of MR or chemical properties of the system. Does a method use an optimisation, or assumption, which requires metabolite T_1^* or metabolite pool ratios to be pre-determined? These parameters are newly characterised for the human heart at 3T,⁴⁴ and have not been measured at 7T. Additionally it is known that the pool sizes change in disease, and it has not been shown conclusively that intrinsic T_1 values are unchanged in disease; though this is the opinion of the respected group at Johns Hopkins.⁴⁴

5.3.2.1 Survey results

The FAST method (F) is the most appropriate for translation to 3D-CSI. The 1 s T_R used in the original implementation is close to the T_R typically used in cardiac ^{31}P -MRS, and it may be shortened if appropriate for the available excitation flip-angle.

As a minimum, all methods require some adaption for use at 7T. The majority of methods implemented in the literature rely on either B_1 -insensitive pulses (BIR-4 or AHP pulses) or calibration of the sequence excitation flip angle to 90° . In cardiac ^{31}P -MRS neither process is easy (see §Figure 5.7): adiabatic excitation is possible only with an optimised transmit coil (currently at a cost of receive SNR), and calibration of the flip-angle requires long T_R , but localised repeated measurements, or knowledge of the metabolite T_1 values.

Method	Reference	Method description	Localisation	Total time (min)	Compatible with:	
					3D localisation?	7T?
A	34	Time dependent saturation transfer (TDST) 1. Progressive saturation: Multiple long T_R (12 s) acquisitions to determine τ . 2. Long T_R (12 s) determination of M_{ss} and M_{Ctrl} .	1D-ISIS	60	No – long T_R is incompatible with 3D-CSI. $T_A > 1$ hour per step.	Requires flip angle calibration or 90° B_1 -insensitive pulses.
B	33	Steady-state saturation transfer 1. Inversion recovery + selective saturation to measure T_1' . 2. Long T_R determination of M_{ss} and M_{Ctrl} . 3. Determine k_f from Eq. [5.30].	None	10.5	No – long T_R is incompatible with 3D-CSI. $T_A > 1$ hour per step. See F,G and H.	Requires flip angle calibration or 90° B_1 -insensitive pulses.
C	38	Inversion transfer 1. Single inversion recovery experiment monitoring magnetisation of exchanging peaks. 2. Multi-parametric fit to Bloch-McConnell equations.	None	36	Possibly - Inversion recovery incompatible. Look-Locker compatible with 3D CSI (requires FA calibration). Low number of acquisitions.	Inversion possible with hyper-secant, WURST or GOIA pulses. Look-Locker would require flip angle calibration.
D	45	Optimised approach to steady state 1. Long T_R determination of M_0 . 2. Measure M_{ss} directly with short T_R (5.5 s) via optimised approach to steady-state equilibrium, having previously determined T_1^* . 3. Short T_R scan + step 2. to measure $T_1'^6$. 4. Determine k_f from Eq. [5.30].	None	4.3	Long T_R steps are compatible with 1D-CSI (see G & H), but are incompatible with 3D-CSI for which a single step takes longer than 1 hour.	Requires knowledge of exchange parameters i.e. intrinsic T_1 and metabolite pool size, which are not known for myocardial tissue at 7T.
E	46	Iterative T_1^{nom} method 1. Determine $T_1^{nom} - T_1$ under selective and partial saturation, with SNR optimised flip angle and T_R , from pool size and T_1^* estimate. 2. Measure M' and M at same flip angle and T_R . 3. Iteratively fit to $\frac{M}{M'} = \beta + T_1^{nom} k_f$, updating estimate of T_1^{nom} .	1D-CSI	13.6	Compatible with 3D-CSI. Low number of acquisitions (2).	Requires knowledge of exchange parameters i.e. intrinsic T_1 and metabolite pool size, which are not known for myocardial tissue at 7T.

F	32	Four-angle saturation transfer (FAST) 1. Dual angle ($\alpha = 15^\circ$ & 60° , $T_R = 1$ s) measurement of T_1' (with selective saturation). ⁴⁷ 2. Dual angle measurement of apparent T_1 (with control selective saturation). 3. Calculate M_{ctrl} and M_{SS} from partially saturated magnetisation in 1. & 2. 4. Determine k_f from Eq. [5.30].	1D-CSI	39	Compatible with 3D-CSI. Low number of acquisitions: 4.	Requires flip angle calibration or $15^\circ/60^\circ$ B ₁ -insensitive pulses.
G	48	Triple repetition time saturation transfer (TRiST) 1. Dual- T_R ($T_R = 2$ s & 10 s, $\alpha = 90^\circ$) measurement of T_1' . 2. Long T_R ($T_R = 15$ s) measurement of M_{ctrl} . 3. Optional long T_R ($T_R = 15$ s) measurement of M_0 . 4. Determine k_f from Eq. [5.30].	1D-CSI	45	Incompatible with 3D-CSI – T _A > 1 hour per step. Low number of acquisitions: 4.	Requires flip angle calibration or 90° B ₁ -insensitive pulses.
H	44	Two repetition time saturation transfer (TwIST) 1. Use literature values of T_1^* . 2. Long T_R ($T_R = 15$ s) measurement of M_{ctrl} . 3. Long T_R ($T_R = 15$ s) measurement of M_{SSI} . 4. Optional long T_R ($T_R = 15$ s) measurement of M_0 . 5. Determine k_f from Eqs. [5.29] and [5.30].	1D-CSI	30	Incompatible with 3D-CSI – T _A > 1 hour per step. Low number of acquisitions: 3.	Requires flip angle calibration or 90° B ₁ -insensitive pulses. Requires knowledge of intrinsic PCr T_1 , which has not been measured at 7T.

Table 5.2 Survey of possible methods for k_f^{CK} and CK flux measurement at 7T with 3D-CSI localisation. The localisation scheme and total time for each method is taken from the referenced work. Compatibility section is colour coded to mark incompatibility (red), compatible only with modification (orange) and compatibility (green).

5.3.3 In vivo human k_f^{CK} values

Tables 5.3 and 5.4 contain literature values for k_f^{CK} arising from intact human skeletal and cardiac muscle. The range of skeletal muscle values is larger than the tight range reported for myocardial k_f^{CK} .

Myocardium

Ref	Method	Localisation	Field (T)	N	Study mean $\pm$ SD (s^{-1})
49	FAST	1D-CSI	1.5	16	0.32 $\pm$ 0.07
50	FAST	1D-CSI	1.5	14	0.32 $\pm$ 0.06
51	FAST	1D-CSI	1.5	15	0.33 $\pm$ 0.07
48	TRiST	1D-CSI	3	8	0.32 $\pm$ 0.07
44	TwIST	1D-CSI	3	12	0.33 $\pm$ 0.08
34	TDST	1D-ISIS	3	15	0.32 $\pm$ 0.05

Table 5.3 1D localised creatine kinase rate measurements of the healthy human myocardium. A single inter-subject mean and standard deviation is given for the value of k_f^{CK} reported for healthy subjects in each study.

Skeletal muscle

Ref	Method	Field (T)	N	Study mean $\pm$ SD (s^{-1})	Study range (s^{-1})
48	TRiST	3	6	0.26 $\pm$ 0.04	-
33	Saturation transfer	3	6	0.31 $\pm$ 0.04	-
33	Saturation transfer	7	6	0.35 $\pm$ 0.03	-
52	Saturation transfer + turbo spin echo	3	30	-	0.23-0.29
53	Progressive saturation + turbo spin echo	3	23	-	0.26-0.32
54	Saturation transfer + 1D-ISIS	7	23	-	0.27-0.34
37	Inversion transfer	7	10	0.46 $\pm$ 0.09	-
38	Inversion transfer	7	7	0.26 $\pm$ 0.02	-

Table 5.4 Creatine kinase rate measurements in the human leg skeletal muscle at 3T and 7T. A single inter-subject mean and standard deviation is given if only a single anatomy was measured, whilst a range is given if a range of (similar) muscular anatomies was measured.

5.4 Multi parametric fitting at 7T.

The methods described in the previous section, apart from inversion transfer, aim to measure k_f^{CK} by determining individual parameters to solve closed form expressions, typically Eq. [5.30] , derived from the bounded Bloch-McConnell equations. The reduced number of variables required to determine k_f^{CK} by these methods reduces the number of experiments required, allowing time to be spent on multi-scan averaging required for sufficient SNR at 3T and lower field strength scanners.

7T scanners enable the user to acquire several acquisitions, all with acceptable SNR and localised to the myocardial septum by 3D-CSI, within a single scan session (< 2 hours).¹¹ This raises the possibility of determining not just k_f^{CK} but also metabolite T_1^* and M_0 values simultaneously using a multi-parametric fit of the Bloch-McConnell equations to data acquired using a tailored range of sequence parameters.

This section investigates whether a multi-parametric fitting approach to k_f^{CK} measurement is viable for cardiac ^{31}P -MRS at 7T. The conclusions drawn here will inform the choice of protocol for k_f^{CK} measurement in the human heart at 7T, which is described in detail in Chapter 8.

5.4.1 Monte Carlo sensitivity analysis

From simulation of the Bloch-McConnell equations, it is apparent that the effect of the creatine kinase energy shuttle on ^{31}P -spectra is strongly dependent on both sequence flip-angle and repetition time. Steady-state saturation transfer also sensitises the measured spectrum to creatine kinase exchange.

By determining the convergence of a multi parametric fit to simulated data, in a Monte Carlo simulation, it can be determined if it is feasible to measure the k_f^{CK} in this way, at the spectral SNR typically observed in cardiac ^{31}P -MR spectra and then to determine the best combination of sequence parameters (T_R , θ and saturation) to maximise sensitivity to k_f^{CK} .

5.4.1.1 Methods

Steady state transverse magnetisation values were determined for two exchanging species, equivalent to PCr and γ -ATP. The species had MR and chemical parameters as described in Table 5.1. The magnetisation values were calculated using a numerical Bloch-McConnell simulation of an unlocalised pulse-acquire spectroscopy sequence. Magnetisation values were determined for a 14 T_R values from 0.1s to 15 s with variable step sizes. This was done for both data with (mirrored) control saturation and on-resonance saturation of the γ -ATP peak. In the simulation the saturation was achieved by a train of DANTE pulses.⁵⁵

From the magnetisation values calculated for each set of experiment parameters, a normal distribution of 10000 values was generated with a standard deviation equal to the AMARES $\text{CRLB}_{\text{Amplitude}}$ typically observed in 3D-CSI cardiac ^{31}P -MRS at 7T.¹¹ The standard deviation for each different repetition time was adjusted to keep a constant sequence duration by multiplying the SD by $\sqrt{1/T_R}$. This was then repeated for eight other values of fitting precision ranging from 0.1 times the precision (lower SNR) to 1000 times the precision (higher SNR).

Each individual set of values, at each SNR level, from each combination of parameters tested, was least-squares fitted to an appropriate function (see below) to generate 10000 values of each fitted parameter. The mean and standard deviation of each fitted parameter for a certain combination of sequence parameters was calculated. Least-squares starting values of k_f^{CK} were offset from the true value by 100%, all other starting values were randomised around the true value.

The sets of parameters tested, details of the fitting functions and fitted parameters are detailed in §§5.4.1.3-3.

5.4.1.2 Results: Variable repetition time

Fitting convergence for an experiment consisting of the full range of repetition times generated (0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.5, 2, 3, 4, 5, 10, 15 seconds), without selective saturation of γ -ATP, and an excitation flip angle of 90° was tested.

Simulated data was fitted by equations [5.4] and [5.5] as described above. Parameters available for fitting were k_f , T_1^{*A} , T_1^{*B} , M_0^A , M_0^B and θ . The degree of convergence was analysed across all SNR levels, in turn, as each parameter was freed for fitting. This generated a series of hypothetical situations where different combination of parameters have been separately determined, i.e. in the first case only k_f was free to vary, whilst in the final situation all parameters were free to vary.

The mean and standard deviation of the fitted k_f are shown in Figure 5.5a. In the initial example where only k_f could vary (line 1) the simulation produced a good fit across all SNR levels. However, all other examples, with the exception of the case where the fitted

parameters were k_f , M_0^A and M_0^B (line 3), fitted k_f with either low accuracy, low precision or both.

5.4.1.3 Results: Variable repetition time & saturation transfer

The analysis was extended to include additional data points in the presence of steady-state selective saturation. The additional data had identical repetition times and an equal flip-angle to the original data.

This data was fitted, using the same least squares methods, to series of equations: [5.4], [5.5], [5.27], [5.24] and an adapted partial saturation equation ([1.48]):

$$M' = \frac{M_{ss} \sin(\theta) \left(1 - e^{\frac{-T_R}{T_1}} \right)}{1 - \cos(\theta) e^{\frac{-T_R}{T_1}}}, \quad [5.34]$$

where M' is the partially saturated magnetisation in the presence of steady-state saturation transfer.

The convergence was tested for two different sets of variable fitting parameters:

k_f , T_1^{*A} , T_1^{*B} , M_0^A , M_0^B (θ fixed) and k_f , T_1^{*A} , T_1^{*B} , M_0^A , M_0^B , θ .

The results of fitting this extended dataset, is shown in Figure 5.5b. With and without the fixed flip-angle and at all SNR levels, the fit of the saturation transfer data converged with an overall bias of less than 10% and with greatly reduced (> 10 times lower) standard deviation compared to data without saturation.

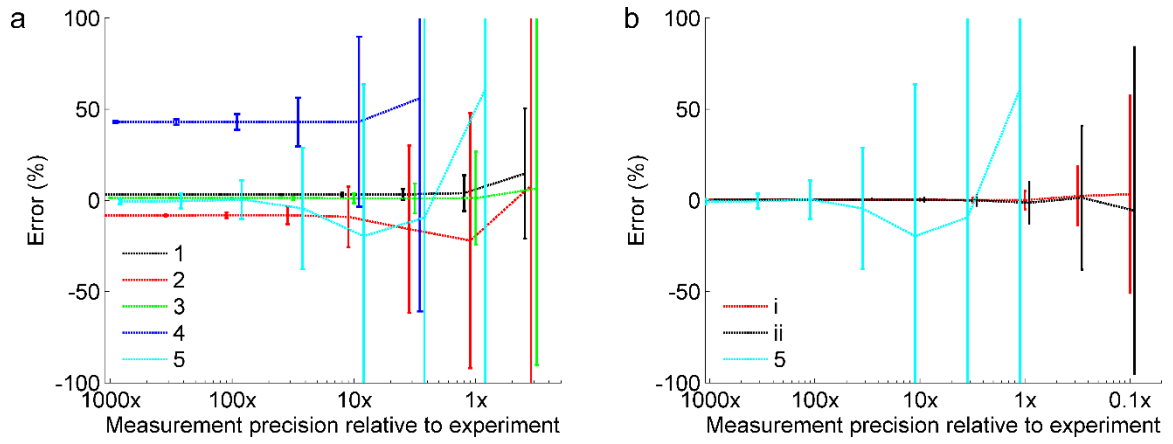


Figure 5.5 Convergence of the k_f parameter in a Monte Carlo simulation of multi parametric fitting of the exchange parameters. Two data sets, without saturation transfer (a) and with saturation transfer (b), were analysed. The fitted parameters were freed to vary in turn: 1. k_f ; 2. k_f, T_1^* ; 3. k_f, M_0 ; 4. k_f, θ ; 5. k_f, T_1^*, M_0, θ ; i. k_f, T_1^*, M_0 ; ii. k_f, T_1^*, M_0, θ . 5. Is shown in both a and b for easy comparison of the effect of saturation transfer. Points were not plotted if the magnitude of the error exceeded 100%.

5.4.1.4 Time constrained multi-parametric fitting.

It is infeasible to collect such an extensive combination of experiments from a human subject, especially combinations including acquisitions with longer T_R values ($T_R \geq 4$ s).

To assess the viability of the method for 3D localised experiments, the convergence of fitting simulated data of each combination of experiments with a total duration of under 1.5 hours was assessed. The duration of each possible component was calculated for a 16x16x8 acquisition weighted CSI grid with 5 averages at the centre of k-space.

There were a total of 58973 combinations of T_R values when including the option of including steady-state saturation transfer. The simulated excitation flip-angle was adjusted to 30°, which was closer to the Ernst angle for the shorter repetition times.

Monte Carlo simulations, as described in the previous sections were performed for each of the combinations. The standard deviation of the distribution of each individual acquisition was adjusted to account for the T_R and total duration, 1000 repeats were simulated for each combination. For combinations that took less than 1.5 hours the

standard deviation values were decreased proportionally to make all acquisitions comparable. Due to the length of calculation (32.5 hours) only a single SNR level, that equivalent to the typical in vivo cardiac experiment, was analysed.

In the initial analysis all combinations were fitted with the flip-angle variable fixed. The results were then ranked and the top 100 combinations, as selected by the lowest standard deviation with a mean bias of $< 0.5\%$, were re-analysed with the flip angle allowed to vary.

Across the top 100 combinations, the standard deviation of the fitted k_f varied from 14.7% to 22.4% with less than 0.5% bias when the fitted model included the correct excitation flip-angle. However, when the flip-angle was allowed to vary in the fitting step, the lowest standard deviation observed was 65.6% with a bias of 5.0%.

5.4.2 Multi parametric fitting of experiments

5.4.2.1 T_1 from least-square fitting of partially saturated data

The multi-parametric fitting approach was piloted by measuring the T_1 in a single voxel phosphate phantom. The phosphate T_1 was measured by both inversion recovery and a variable T_R method. Additionally the flip-angle in the single voxel was determined by fitting the $\sin \alpha$ relationship expected to FIDs excited with increasing transmit voltages. The average flip angle in the phosphate compartment of the phantom was determined to be 12.3° . The T_1 of the phosphate was found by inversion recovery to be 1.85 ± 0.04 s, which is consistent with previous measurements of the same phantom.¹¹

FIDs of the phosphate phantom were acquired with seven different repetition times (0.15 s to 2 s, variable step size). The amplitude of the phosphate signal was fitted by

the partial saturation equation with (a) the flip-angle fixed to the Biot-Savart estimated flip angle (17.1°), (b) the $\sin \alpha$ determined flip angle, and (c) with the flip-angle as a fitted parameter.

The results of the multi parameter fit were found to depend significantly on the flip-angle. Using the separately determined flip-angle the T_1 was determined as 1.91 ± 0.05 s, close to the IR determined value. With the flip-angle set to the Biot-Savart estimated value the T_1 was determined to be 2.76 ± 0.08 s. For the two parameter fit (T_1, θ) the T_1 was 0.96 ± 0.89 s ($\theta_{\text{fit}} = 10.2^\circ$).

From this simple experiment it is possible to say that (unsurprisingly) the flip-angle and measured T_1 are highly correlated, and therefore an accurate value for θ must be obtained; and the Biot-Savart estimate is not always accurate.

5.4.2.2 Fitting of in vivo data

The variable repetition time, multi-parametric fitting approach to measuring k_f^{CK} was attempted in skeletal muscle (thigh) and the myocardium in a single subject. In both cases, a series of acquisitions, with different T_R values but identical excitation flip angle, were made with mirrored control saturation, and combined with data acquired from a single T_R point with selective steady-state saturation of γ -ATP. In both tissues, the signal was localised using 3D-CSI. Skeletal muscle offered a high SNR test case for the myocardium.

To limit the number of free parameters in the least-squares fit the following attempts were made to estimate and fix or set limits on the flip-angle or intrinsic T_1 relaxation constants:

1. The flip-angle may can be estimated from the scaled Biot-Savart field map (Chapter 2).
2. The flip-angle may be calculated from the partial saturation of non-exchanging peaks (α -ATP, β -ATP and P_i [in skeletal muscle]). The T_1 of the non-exchanging peak must be known, e.g. from literature.⁵⁶
3. The intrinsic T_1 may be fixed from literature values measured in the same tissue and at the same field strength.³³ If no such data exists, as is the case for the myocardium, an upper bound may be set from literature values at a lower field strength.⁴⁴

Methods: Skeletal muscle

The quadriceps of a single volunteer (male, 84kg, 22 years) was scanned in a whole-body 7T MRI scanner. ^1H -MRI FLASH images were acquired, using a dedicated ^1H 10cm loop Tx/Rx coil, to enable localisation of the subsequently acquired spectroscopy data. ^{31}P -MRS was acquired using the 16 channel array coil, which was placed on top of the supine subject's thighs.

3D UTE-CSI data was acquired at six repetition times (0.15 s to 1.5 s, variable step size) without selective saturation and a single T_R (1 s) with selective steady-state saturation of γ -ATP. The standard 3D-CSI parameters were used (Appendix A, "MDR7T" pulse) with a FOV = 350x220x400mm³. The number of averages at the centre of k-space was adjusted to keep the duration of each acquisition approximately 10 min. The total acquisition time of ^{31}P -MRS data was 70 min.

Ten voxels were chosen for analysis, on the basis of their central position in the thigh and high quality ($\text{SNR}_{\text{PCr}} > 100$). Spectra were fitted by AMARES in Matlab, using standard prior knowledge (Appendix B.2).

The flip-angle obtained in the analysed voxels was estimated by the Biot-Savart method (Chapter 2) and independently via fitting the amplitudes of the α -ATP, β -ATP and P_i peaks to the partial saturation equation (Eq. [1.48]). T_1 values for α -ATP, β -ATP and P_i were set to 1.8 s, 1.8 s and 6.3 s respectively.⁵⁶ $T_1^{*\text{PCr}}$ was estimated to be 5.3 s as in reference 44 and, 3.0 s as calculated from the T_1' and k_f reported in reference 33.

The amplitudes of the PCr and γ -ATP peaks were fitted by the equations described in §5.4.1.3, using the Matlab Optimisation Toolbox's lsqcurvefit function. For each voxel analysed several different parametric fits were computed, all fits used the same equations and same input data:

1. The flip-angle was fixed to the Biot-Savart estimated value;
 k_f , $T_1^{*\text{PCr}}$, $T_1^{*\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$ free to vary.
2. The flip-angle was fixed to the estimated value from the non-exchanging peaks
and $T_1^{*\text{PCr}}$ was fixed to 3.0 s; k_f , $T_1^{*\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$ are free to vary.
3. The flip-angle was fixed to the estimated value from the non-exchanging peaks
and $T_1^{*\text{PCr}}$ was fixed to 5.3 s; k_f , $T_1^{*\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$ are free to vary.
4. $T_1^{*\text{PCr}}$ was fixed to 3.0 s; k_f , $T_1^{*\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$, θ are free to vary.
5. $T_1^{*\text{PCr}}$ was fixed to 5.3 s; k_f , $T_1^{*\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$, θ are free to vary.

6. The flip-angle was fixed to the estimated value from the non-exchanging peaks;

k_f , $T_1^{*,\text{PCr}}$, $T_1^{*,\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$ are free to vary.

7. No parameters are fixed; k_f , $T_1^{*,\text{PCr}}$, $T_1^{*,\gamma\text{-ATP}}$, M_0^{PCr} , $M_0^{\gamma\text{-ATP}}$, θ are free to vary.

Methods: Myocardium

The heart of a single volunteer (male, 75kg, 34 years) was scanned in a whole-body 7T MRI scanner. ^1H -MRI FLASH images were acquired, using a dedicated ^1H 10cm loop Tx/Rx coil, to enable localisation of the subsequently acquired spectroscopy data.

3D UTE-CSI data was acquired at six repetition times (0.15 s to 1.5 s, variable step size) without saturation and a single T_R (1 s) with selective steady-state saturation of $\gamma\text{-ATP}$. Modified standard 3D-CSI parameters were used (Appendix A, “MDR7T” pulse, 16 channel coil), the CSI grid = 16x8x8 and the number of averages at the centre of k-space was adjusted to keep the duration of each acquisition approximately 10 min. The total acquisition time of ^{31}P -MRS data was 70 minutes.

Four adjacent voxels in the interventricular myocardium were chosen from two slices in the mid-section of the heart for analysis. Spectra were fitted by AMARES in Matlab, using standard prior knowledge (Appendix B.1).

The flip-angle obtained in the analysed voxels was estimated by the Biot-Savart method and independently by fitting the amplitudes of the $\alpha\text{-ATP}$ peak, assuming

$T_1^{\alpha\text{-ATP}} = 1.39 \text{ s}$.¹¹ For different sets of fitted parameters, the $T_1^{*,\text{PCr}}$ was set to 5.3 s⁴⁴

and 7.36 s³⁴; otherwise an upper limit of 8 s was placed on $T_1^{*,\text{PCr}}$.⁴⁴ The amplitudes of the PCr and $\gamma\text{-ATP}$ peaks were fitted by the equations described in §5.4.1.3, using

lsqcurvefit and with the sets of fixed and non-fixed parameters as described for skeletal muscle.

Results

#	Fitted parameters	Fixed parameters	Mean k_f (s ⁻¹)	SD k_f (s ⁻¹)
1	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (Biot-Savart)	0.189	0.045
2	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$), $T_1^{*,\text{PCr}} = 3$ s	0.448	0.112
3	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$), $T_1^{*,\text{PCr}} = 5.3$ s	0.259	0.061
4	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	$T_1^{*,\text{PCr}} = 3$ s	0.472	0.110
5	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	$T_1^{*,\text{PCr}} = 5.3$ s	0.267	0.062
6	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$)	0.270	0.106
7	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	None	0.266	0.230

Table 5.5 Results of multi-parametric fitting of 10 skeletal muscle voxels. Previously measured skeletal muscle k_f^{CK} values, measured by ST, are in the range 0.26-0.35 s⁻¹ (Table 5.4).

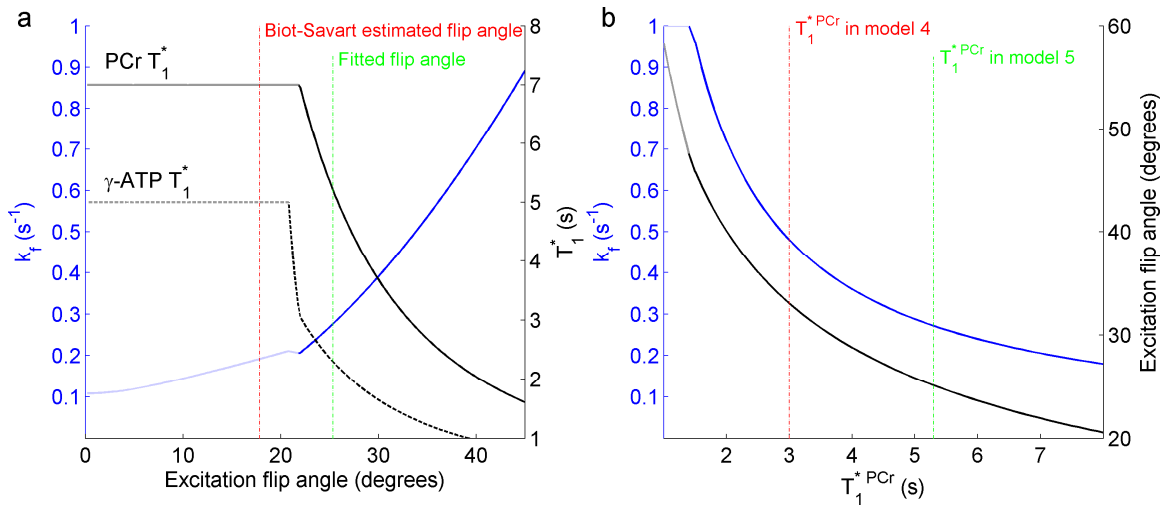


Figure 5.6 Multi parametric fitting of skeletal muscle ³¹P-MRS to determine k_f^{CK} . **a:** Effect of varying the fixed flip-angle in case 6 on three of the fitted parameters (Table 5.5). **b:** Effect of varying the fixed intrinsic T_1 of PCr ($T_1^{*,\text{PCr}}$) on the value of fitted k_f^{CK} in case 4 and 5. The lighter line colours indicate where a fitted parameter has hit a limit.

The average matched filtered SNR of the PCr peak in the analysed skeletal muscle voxels, for the acquisition with $T_R = 1$ s, was 153 ± 50 . The inter-voxel mean and standard deviation of the fitted creatine kinase forward rate constant k_f for each set of fitted parameters is given in Table 5.5. The effect of varying the two fixed parameters on the fitted value of k_f is shown in Figure 5.6.

The average matched, filtered SNR of the PCr peak in the four myocardial voxels for the acquisition with a $T_R = 1$ s was 23 ± 16 . The inter-voxel mean and standard deviation of the fitted CK forward rate constant k_f for each set of free and fixed fitted parameters is given in Table 5.6. The strong effect of varying the fixed value of the excitation flip-angle on the fitted value of k_f , in each of the four fitted voxels, is shown in Figure 5.7. Higher SNR voxels display a smaller k_f dependence on the fixed parameter values than lower SNR values.

#	Fitted parameters	Fixed parameters	Mean k_f (s ⁻¹)	SD k_f (s ⁻¹)
1	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (Biot-Savart)	0.454	0.356
2	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$), $T_1^{*,\text{PCr}} = 5.3\text{s}$	0.307	0.088
3	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$), $T_1^{*,\text{PCr}} = 7.4\text{s}$	0.232	0.065
4	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	$T_1^{*,\text{PCr}} = 5.3$ s	0.319	0.101
5	$k_f, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	$T_1^{*,\text{PCr}} = 7.4$ s	0.230	0.073
6	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}$	θ (from $\alpha, \beta\text{-ATP}$)	0.258	0.075
7	$k_f, T_1^{*,\text{PCr}}, T_1^{*,\gamma\text{-ATP}}, M_0^{\text{PCr}}, M_0^{\gamma\text{-ATP}}, \theta$	None	0.819	0.363

Table 5.6 Results of multi parametric fit of 4 adjacent voxels from the interventricular myocardium of a single subject. Literature values for myocardial k_f^{CK} are all 0.32 s^{-1} (Table 5.3).

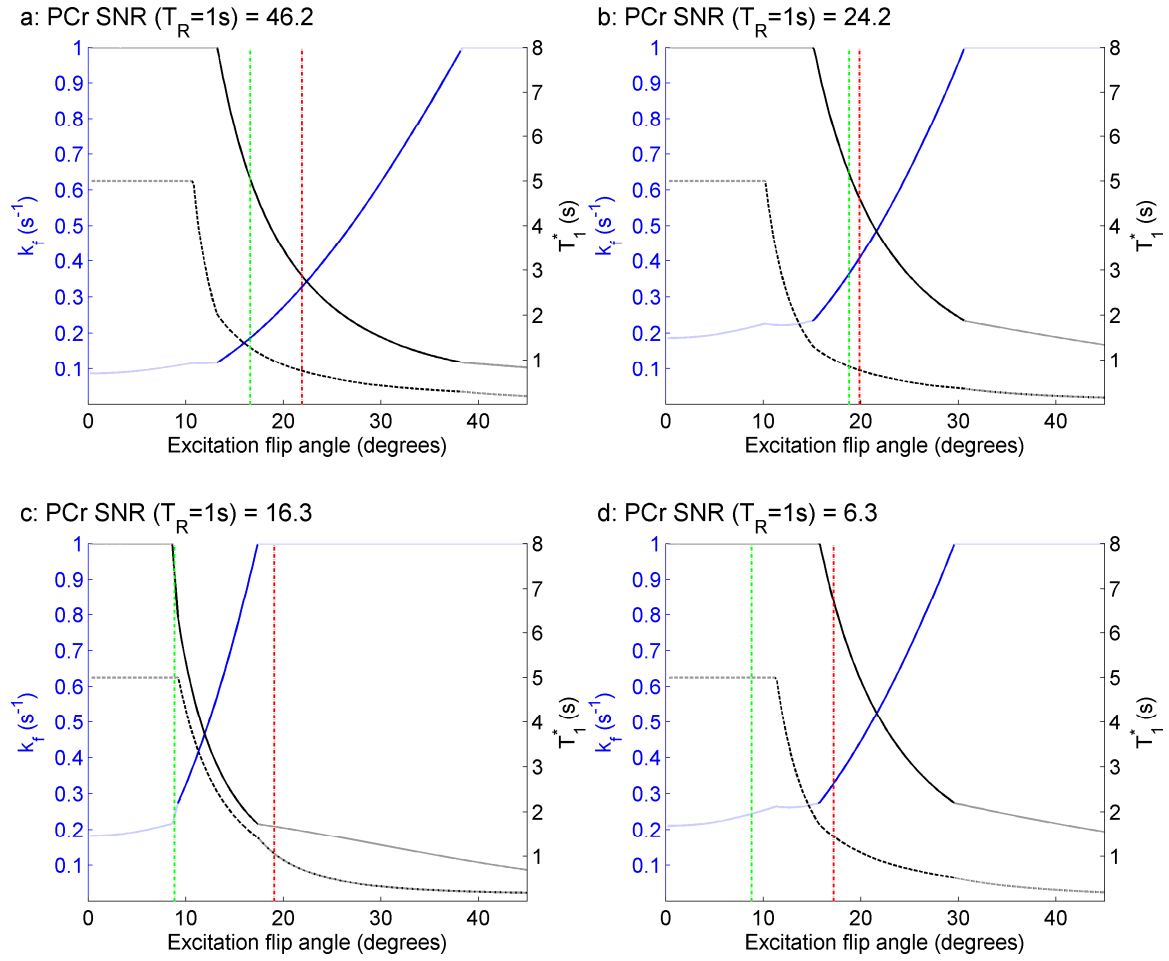


Figure 5.7 Multi parametric fitting of myocardial ^{31}P -MRS to determine k_f^{CK} . Each panel (a-d) shows the fitted k_f^{CK} for a single voxel selected from the single subject, using the fitted and fixed parameters in case 6. The PCr SNR from the 1 s T_R acquisition is noted in each case. Vertical dashed lines mark the Biot-Savart (red) and partial saturation (green) estimates of the flip-angle. The lighter line colours indicate where a fitted parameter has hit a limit. Fitted T_1^* s of PCr (solid) and γ -ATP (dashed) are shown.

5.4.3 Discussion: Direct measurement of excitation flip-angles and T_1^* in vivo

The results of both the simulations and in vivo experiments indicate that multi-parametric fitting of the Bloch-McConnell equations to variable T_R data is a viable approach for the determination of k_f^{CK} in high SNR data. But for the SNR typically observed in cardiac ^{31}P -MRS, parametric fitting of the data with all parameters unconstrained is not viable.

It is however possible to determine the forward rate constant reliably if the number of free parameters can be reduced. By determining and fixing either the value of the excitation flip-angle or $T_1^{*,\text{PCr}}$, the convergence of the fit was increased in the simulated data, and a more accurate and precise value of k_f^{CK} was measured in the in vivo data. Obtaining an unbiased k_f^{CK} by this restricted fitting method relies on accurately measuring or pre-determining one or both of these parameters.

5.4.3.1 Measurement of PCr T_1^*

The intrinsic T_1 of an exchanging species cannot be directly measured by inversion or saturation recovery. Spencer and Fishbein proposed that at very short repetition values the T_1 measured by partial saturation closely matches the intrinsic T_1 .⁸ However constraints on the sequence, the ability to accurately measure M_0^{PCr} , and the SAR limit make this impractical.

In isolated cell, perfused heart and animal models the creatine kinase enzyme may be pharmacologically poisoned. Exchange is thereby effectively stopped and the apparent T_1 equals the intrinsic T_1 , enabling direct measurement by inversion recovery. This is clearly not acceptable in humans.

5.4.3.2 Measurement of the excitation flip-angle

The excitation flip-angle, a sequence variable under the control of the sequence designer, depends on the amplitude and phase modulation of excitation pulse and the B_1^+ field. As described in Chapter 2, the surface coils used in cardiac ^{31}P -MRS produce an inhomogeneous B_1^+ . To fix the flip-angle either the B_1^+ field or flip-angle must be measured directly, or a B_1^+ -insensitive pulse used.

B_1^+ -insensitive pulses

As set out in Chapter 2, using B_1^+ -insensitive adiabatic pulses (e.g. BIR-4) to generate an arbitrary excitation flip-angle is generally infeasible for 7T ^{31}P -MRS. Recent work,⁵⁷ using quadrature excitation of a pair of 15-cm surface loops has shown that a B_1^+ -insensitive 90° adiabatic half-passage (AHP) pulses are possible across a narrow bandwidth (300Hz). However, the SAR limits the T_R to be >3 s and there would be no SAR “budget” remaining for selective saturation. The lower adiabatic limit of inversion pulses, such as hypersecant (HS^n) pulses, mean that B_1^+ -insensitive inversion is possible for inversion transfer studies.

Numerically optimised “composite pulses” offer a lower degree of B_1^+ -insensitivity compared to adiabatic pulses, though typically with a lower minimum B_1^+ and SAR requirement.⁵⁸ However, preliminary trials of composite pulses for cardiac ^{31}P -MRS showed that the minimum B_1^+ required still exceeded the $B_{1,\text{peak}}^+$ of the 10cm loop and 16 channel array coils.

Estimation of the B_1^+ field

Approaches that compute B_1^+ using the Biot-Savart law or finite-differences time-domain (FDTD) or finite integral technique (FIT) simulations, or that measure B_1 in advance in a phantom are challenging, because sample conductivity and permittivity effects make the spatial distribution of B_1 depend on each subject’s anatomy - and more so at 7T than at 1.5T or 3T.⁴³

As is evident from the significantly different flip-angles estimated by the Biot-Savart method and calculated from the partially saturated α -ATP amplitudes in the in vivo data

in the previous section, the Biot-Savart estimate is not sufficiently accurate to form the basis of a k_f^{CK} measurement technique.

Direct measurement

Measurement of either the excitation flip-angle or the B_1^+ field offers the possibility to fix the flip-angles during the multi-spectral fitting. A viable ^{31}P -MRS flip-angle or B_1^+ mapping technique, compatible with 3D-CSI would enable the use of many of the methods surveyed in §5.3.2.

Chapter 7 describes the range of possible ^{31}P -MRS flip-angle and/or B_1^+ mapping methods suitable for use with human cardiac ^{31}P -MRS at 7T. It then details a novel Bloch-Siegert-based B_1^+ -mapping technique for ^{31}P -MRS.

5.5 Conclusion

This chapter has provided a description of the effects of the creatine kinase mediated exchange of phosphate groups on the cardiac ^{31}P -MRS spectrum. The Bloch-McConnell equations have been introduced and a numerical solver tested against analytical expressions for magnetisation undergoing two-pool exchange.

A survey of the historic creatine kinase measurement methods has been completed, analysing the suitability of each method for implementation with 3D-CSI at 7T. The Four-Angle Saturation Transfer method (FAST) was selected.

Multi-parametric fitting of variable repetition time saturation transfer data was explored using Monte Carlo simulations, and phantom and in vivo experiments. Finally, it was established that due to the strong correlation between k_f^{CK} and the excitation

flip-angle, that separate flip-angle mapping is required for an accurate determination of

$$k_f^{\text{CK}}.$$

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Chapter 6: Dynamic creatine kinase measurement at 3T

6.1 Introduction

Chapter 6 describes the implementation on the Siemens platform of the triple repetition time saturation transfer (TRiST) pulse sequence.¹ This method was originally implemented on a Philips 3T system at Johns Hopkins University by Profs Bottomley and Weiss. The method is then extended to allow stress-response CK flux measurement in 80 minutes for a clinical study of the “obesity paradox” with Drs Rider and Rayner in Oxford.²

The theory and protocol for TRiST is outlined. An extension to make a second consecutive CK flux measurement in only 2 scans (instead of 3) is described. Key aspects of this sequence are validated in phantoms, in skeletal muscle and then in repeated measurements on 6 healthy volunteers. The sequence is observed to operate properly, except that the mean k_f^{CK} is 0.13 s^{-1} compared to 0.32 s^{-1} as measured by the Johns Hopkins Group.¹ Further simulations are then used to investigate this discrepancy. The results from the first 49 heart failure patients are then presented.

The chapter closes with a critical appraisal of these results in the context of the literature.

6.1.1 Repeated CK k_f measurement protocol

The TRiST method uses four spectroscopy acquisitions to measure the creatine kinase forward rate constant k_f^{CK} , M_0^{PCr} and $M_0^{\gamma\text{-ATP}}$. The total duration of the method, including acquiring ^1H localisers, is approximately 1 hour.

The creatine kinase forward flux is thought to offer the most sensitive and complete measurement of the myocardial energy metabolism status.³ Decreases in k_f^{CK} and creatine kinase flux have been observed in a range of heart failure conditions, including hypertrophic cardiomyopathy (HCM) and post myocardial infarction, compared to the healthy heart,⁴⁻⁶ even when there are no significant changes in PCr/ATP ratio.⁴

Furthermore, in healthy skeletal muscle and myocardium, it has been found that moderate exercise and stress does not change the creatine kinase flux.^{6,7} It is currently unknown whether the creatine kinase flux changes under moderate stress conditions in the failing myocardium.

Obesity causes a range of cardiac changes, including in cardiac metabolism.⁸ These metabolic effects of obesity are a possible mechanism for the increased incidence of heart failure in obese subjects. However, it has been observed that among heart failure patients, overweight and obese patients have a better prognosis compared to normal and underweight patients. This has been termed the “obesity paradox”.⁹

A study at OCMR, run by Drs Rayner and Rider, aims to characterise the metabolism of the failing heart in both normal weight and obese populations, comparing to matched controls. The study will recruit 100 subjects split evenly between the four groups. Each subject will undergo ¹H-MRI, for cardiac function, ¹H-MRS for myocardial lipid quantification, and ³¹P-MRS and TRiST for consecutive CK flux measurements at rest, which are repeated under Dobutamine-induced stress.

6.2 Theory of repeated TRiST measurements

TRiST is a saturation transfer method (Chapter 5), which comprises four excitations with variable repetition times and saturation locations. The four acquisitions are localised using 1D-CSI, with uniform k-space sampling, and 16 phase-encode steps perpendicular to the coil plane (a “loop” surface coil). The four acquisitions comprising TRiST are given in Table 6.1.

#	θ	T_R (s)	Saturation target	Scan averages	Duration (min)	Measured parameters
1	90°	>16	-	2	9	$M_{0,\text{PCr}}, M_{0,\gamma\text{-ATP}}$
2	90°	2 (T_R^{Short})	$\gamma\text{-ATP}$	18	11	$M'_{\text{PCr}}(T_R^{\text{Short}}), [M'_{\gamma\text{-ATP}}(T_R^{\text{Short}})]$
3	90°	10 (T_R^{Long})	$\gamma\text{-ATP}$	8	21	$M'_{\text{PCr}}(T_R^{\text{Long}}), [M'_{\gamma\text{-ATP}}(T_R^{\text{Long}})]$
4	90°	>16	Control	2	9	$M_{\text{PCr}}^{\text{Ctrl}}, M_{\gamma\text{-ATP}}^{\text{Ctrl}}$

Table 6.1 TRiST acquisitions. θ = excitation flip-angle, T_R = repetition time.

The repetition times are approximate as the sequence is triggered from the R-wave using ECG monitoring, after a trigger delay to the beginning of diastole. Therefore the T_R is limited to integer multiples of the R-R interval.

From scans 2&3 the PCr T_1 under steady state saturation $T_1^{\prime,\text{PCr}}$ is calculated using the two- T_R partial saturation method.¹⁰ $T_1^{\prime,\text{PCr}}$ is determined from a lookup table for the ratio of PCr magnetisation acquired at both T_R values. The lookup table is generated thus

$$R(T_R^{\text{Short}}, T_R^{\text{Long}}, T_1') = \frac{M'_{\text{PCr}}(T_R^{\text{Long}})}{M'_{\text{PCr}}(T_R^{\text{Short}})} = \frac{1 - e^{-T_R^{\text{Long}}/T_1'}}{1 - e^{-T_R^{\text{Short}}/T_1'}}. \quad [6.1]$$

Subsequently the value of $M'_{0,\text{PCr}}$ is calculated as

$$M'_{0,\text{PCr}} = \frac{M'_{\text{PCr}}(T_{\text{R}}^{\text{Short}})}{1 - e^{-T_{\text{R}}^{\text{Short}}/T_1'}}. \quad [6.2]$$

k_{f}^{CK} can then be determined by Eq. [5.30] when using control saturation

$$k_{\text{f}}^{\text{CK}} = \frac{1}{T'_{1,\text{PCr}}} \left(1 - \frac{M'_{0,\text{PCr}}}{M_{\text{PCr}}^{\text{Ctrl}}} \right). \quad [6.3]$$

The first acquisition enables the calculation of PCr concentration from an unsaturated measurement of PCr magnetisation, if suitable absolute quantification protocols are in place, thereby enabling the calculation of CK flux as well as k_{f}^{CK} .

90° excitation is achieved for just the PCr and γ -ATP peaks using frequency cycled AHP pulses.^{1,10} For each phase-encoding step, acquisitions are made in equal numbers with AHP pulses with opposite frequency sweep directions. When averaged in the reconstruction stage, the alternating frequency sweeps reduces the off resonance asymmetry of the AHP pulses. A restriction of using cycled frequency sweep pulses is that the number of averages must be a multiple of two. Combined with long T_{R} values this means that scan-time acquisition weighting (Chapter 2) cannot be used. Therefore TRiST is acquired with uniformly sampled k-space.

Selective saturation is achieved using an amplitude modulated DANTE train, played out at all times except during excitation, phase encoding and the readout event.¹ DANTE pulses provide a continuous, highly selective, saturation method, with a high Q-factor (i.e. low off-resonance saturation, also known as spill-over saturation) even close to the target frequency.

6.2.2 Consecutive measurements

A second consecutive measurement of k_f^{CK} using TriST would normally require the repetition of steps 2, 3 and 4 above, and add 40 minutes to the scan. The aim of the patient study in §6.1.1 is to make a second measurement of k_f^{CK} while the subject is undergoing dobutamine-induced stress. Due to subject tolerance and safety is not possible to administer 40 minutes of dobutamine stress, especially in heart failure patients.

It is possible to shorten the measurement if the requirement for a T_1 determination step is removed. Bottomley *et al.* proposed a similar shortening of the FAST method (used in Chapter 8) by re-using a single T_1^* measurement, which was initially determined using the preceding FAST protocol, for a subsequent series of dynamic measurements:

“FASTest”.¹¹ The reduced scan time in FASTest relies on T_1^* remaining fixed, which is reasonable because it depends on the local microscopic environment of the metabolite nuclei, which can be assumed to be identical in resting and moderately-stressed myocardium.

From the first full TriST measurement, T_1^* may be determined using a rearranged equation [5.29]:

$$T_1^* = T_1' \frac{M_0^{\text{Ctrl}}}{M_0'} \quad [6.4]$$

The subsequent repeated experiment therefore only has to determine the fully relaxed PCr magnetisation under control and on-resonance saturation of γ -ATP, to determine k_f^{CK} , as combining equations [6.4] and [5.30] gives;

$$k_f^{\text{CK}} = \frac{1}{T_1^*} \left(\frac{M_{\text{PCr}}^{\text{Ctrl}}}{M'_{0,\text{PCr}}} - 1 \right). \quad [6.5]$$

This may be achieved in two 9 minute acquisitions, equivalent to the 4th step of the original TRiST method, with the selective saturation's frequency adjusted appropriately. I.e. the full extended TRiST measurement is the four steps described in the previous section, then the stress protocol (Table 6.2).

#	θ	T_R (s)	Saturation target	Scan averages	Duration (min)	Measured parameters
5	90°	>16	Control	2	9	$M_{0,\text{PCr}}, M_{0,\gamma\text{-ATP}}$
6	90°	>16	γ -ATP	2	9	$M'_{\text{PCr}}, [M'_{\gamma\text{-ATP}}]$

Table 6.2 Additional TRiST acquisitions for dynamic measurement (under dobutamine stress).

In this way a second measurement is possible in approximately 20 minutes, less than half the original measurement duration.

During the implementation of this method at Oxford the original authors of the TRiST method published an updated shortened protocol, Two-repetition time saturation transfer (TwIST).¹² In TwIST, previously-established values of T_1^* are used to shorten TRiST to two acquisitions (not including the initial acquisition without saturation). This is equivalent to what we proposed above. Schar *et al.* further reported that T_1^* is consistent in and between healthy subjects and heart failure patient cohorts.

6.3 Methods

All scans were carried out using a 3T Trio scanner (Siemens). ¹H-MRI localisers for spectroscopy volume placement, and CINE scans to determine the EGC trigger delay for spectroscopy, were acquired using the scanner's body coil for transmit and receive. All

³¹P acquisitions were acquired using a 3T 10-cm transmit-receive loop coil (PulseTeq Ltd). The coil was matched for each subject using an RF sweep box (Morris Instruments Inc).

6.3.1 Validation of cycled AHP pulse

In private communications with the inventors of TRiST, it was established that in the original implementation on the 3T Philips platform, used in Ref. 1, excitation used a 5 ms frequency-cycled tanh/tan AHP pulse with a bandwidth of 7 kHz with a double-loop (11/17 cm) transmit coil with a separate 8-cm receive coil.¹⁰ By comparing phantom measurements of the single 10-cm loop coil at OCMR with published values for the Johns Hopkins coil¹⁰, and in private communications, it was established the our 10-cm loop coil generates in the myocardium $\sim 70\%$ of the B_1^+ of the Johns Hopkins design. Therefore, the duration of the AHP excitation pulse was extended to 10 ms, with a bandwidth of 6 kHz. Bloch simulations were performed to verify performance of this pulse over a ± 100 Hz bandwidth and 0-1000 Hz range for γB_1^+ .

The pulse simulations were validated in a phantom as follows. Fully-relaxed ($T_R = 15$ s) non-localised FIDs were acquired from a single voxel phosphate phantom (2x2x2 cm³ cube of phosphate suspended in 18 L of 73 mM saline – see Chapter 2) using the cycled 10 ms AHP pulse. The central frequency was swept from -500 Hz to 500 Hz in steps of 100 Hz for six different coil transmit voltages: 35, 70, 120, 170, 190 and 210 V. The acquired spectra were fitted using Matlab AMARES. The fitted phosphate peak amplitudes were normalised to the maximum and compared with Bloch-simulations of the sequence.

In a second experiment conducted in the same session the sequence repetition time was set to: 1, 5, 10, 15 and 20 s. The AHP transmit voltage was set to the coil maximum (210 V) and the central frequency was set to the phosphate resonance. The amplitudes of the resultant spectra were fitted using the partial saturation equation (Eq. [1.48]), using the previously established T_1 of the phosphate phantom (2.0 s at 3T), to establish the flip-angle.

Finally a Bloch-Siegert B_1 measurement (Chapter 7) was made to establish the B_1^+ experienced in the single voxel at each transmit voltage.

6.3.2 Skeletal muscle validation of TRiST

The TRiST protocol was tested in the calf muscle of two healthy volunteers (male, 33 & 30 years, 75 & 85 kg). The subjects were positioned feet first supine in the scanner with the ^{31}P loop coil positioned under one leg. The 1D-CSI grid was positioned running in the anterior-posterior direction. The protocol followed the steps in §6.2. Acquisition parameters were: FOV = 160 mm FOV, 16 matrix, 3 kHz bandwidth, 512 samples. The AHP transmit voltage was 200 V. Selective saturation of γ -ATP and the mirrored control site was achieved using an amplitude modulated DANTE train (pulse duration = 100 μs , inter-pulse delay = 810 μs , $m = 5$, $\delta = 9$ Hz).¹

The spectra were analysed using Matlab AMARES with prior knowledge for the PCr singlet and γ -ATP doublet. When fitting the saturated acquisitions, the linewidth and chemical shift of the γ -ATP peak were fixed to their values in the same voxel without saturation. k_f^{CK} was calculated using the process set out in §6.2 via Eqs. [6.1]–[6.3].

6.3.3 Validation of multiple measurements in volunteers

The validity of the second consecutive k_f^{CK} measurement was tested in six healthy volunteers (male, 31 ± 9 years, 75 ± 8 kg). After an initial unsaturated scan (the first component of TRiST), the first measurement was made with three measurements as described above. Subsequently a second measurement was made, without any change in the subject's condition or repositioning, using the additional two scan protocol.

The extended TRiST protocol was carried out as described in sections 6.2 & 6.2.2. CSI acquisition parameters were as follows: FOV = 160 mm FOV, 16 matrix, 3 kHz bandwidth, 512 samples. The CSI grid was positioned perpendicular to a transverse localiser of the heart, with the CSI delineated dimension aligned parallel to the band of skeletal muscle immediately posterior to the coil. The AHP transmit voltage was 210 V. Selective saturation of γ -ATP and the mirrored control site was achieved using an amplitude modulated DANTE train (pulse duration = 100 μ s, inter-pulse delay = 250 μ s, $m = 5$, $\delta = 9$ Hz), the inter-pulse delay was shortened from 810 μ s in the original protocol to better saturate posterior voxels with our 10-cm loop coil.

Spectra from cardiac voxels were selected and analysed on a per-voxel basis, and also after SVD-based averaging. Spectra were fitted using Matlab AMARES, with prior knowledge specifying the PCr singlet and the γ -ATP doublet. In acquisitions with selective saturation, the linewidths and chemical shift was constrained to the values fitted from the unsaturated acquisition (step 1 in Table 6.1).

The PCr/ γ -ATP and PCr matched-filtered SNR of the control acquisition (step 4 in Table 6.1) were determined. The k_f^{CK} , T_1' and T_1^* were determined as described in section 6.2.

The Creatine kinase flux was estimated from the PCr/ATP ratio and literature values for the concentration of ATP. Reproducibility of individual scans was assessed from the 4th and 5th scans, which have identical protocols here (and will be rest and dobutamine-stress in patients). The correlation of the first and second k_f^{CK} measurements was calculated for individual voxels and for averaged voxels.

6.3.4 Further sequence validation

The average cardiac k_f^{CK} measured in healthy volunteers (§6.3.3) was found to be both lower and more variable than values reported in the literature. Therefore further simulations and experiments were carried out to determine the likely cause(s).

Compared to the coil used in the original literature implementation the current coil generates lower peak B_1^+ , has greater B_1^+ standard deviation over the heart, and may have lower intrinsic SNR. To assess the effects this might have on TRiST results, a full sequence Bloch-McConnell simulation was performed for peak γB_1^+ 10 Hz to 1000 Hz in steps of 10 Hz. The effects of reducing just the excitation pulse or DANTE pulse amplitudes were calculated separately.

To assess the effect of receive sensitivity a Monte Carlo simulation was made. PCr magnetisation values arising from the full sequence simulation (with $\gamma B_1^+ = 500$ Hz) were seeded with noise corresponding to a range of spectral SNR values, for the PCr peak in the control acquisition, from 1 to 100 with a variable step size. For each SNR level 50000 individual noisy magnetisation sets were generated and used to calculate k_f^{CK} . The mean and standard deviation of each SNR level was then calculated.

Broad lineshapes and low Q-factors (high spill-over saturation) observed in the cardiac spectra prompted investigation of the effect of T_2^* on the frequency response of the DANTE pulse train because lower T_2 broadens the DANTE frequency profile.¹³ The DANTE frequency profile was measured in the single voxel phantom at 3T, for inter-pulse delay = 810 μ s (original Bottomley protocol) and 250 μ s (our protocol). The experiment was repeated twice, once with a well shimmed voxel, and then with one gradient shim deliberately misadjusted to broaden the single spectral peak observed in the spectrum. T_2^* was estimated from the linewidth of the resultant peaks. Bloch simulations of the pulse train were made to match the profiles observed from the phantom. A Bloch-Siegert B_1^+ measurement (Chapter 7) was used to determine the B_1^+ value in the single voxel, and was subsequently used in the simulations, alongside a previously measured T_1 value for the phosphate. In these phantom experiments the TRiST sequence was used with AHP excitation and a 2 s T_R , with 1.8 s of saturation and 0.2 seconds of excitation, sequence dead-time, and readout. DANTE was set to 15 V (short delay) and 23 V (long delay protocol), to match SAR.

6.3.5 Analysis of patient data

The single measurement and extended TRiST method are currently being used in two clinical studies at Oxford. Data from the completed sections of each study are presented in this section. Subjects were scanned with Research Ethics Committee approval.

6.3.5.1 Hypertrophic cardiomyopathy

Fifteen patients with a positive phenotype hypertrophic cardiomyopathy (HCM) diagnosis (11 male, 49 ± 12 years), including eleven with left ventricular hypertrophy

(LVH), and ten healthy controls underwent the single TRiST measurement (9 male, 41±18 years). The degree of hypertrophy in the interventricular septum of each subject was measured using 3T ¹H-MRI on a separate occasion. The acquisition protocol and analysis is identical to that described for the first four scans in section 6.3.3.

In addition to the normal analysis the results were also corrected for spill-over, the effect of unwanted direct saturation of PCr from the DANTE pulses, as described in reference 12. This numerical correction is calculated using Monte Carlo simulation of the full sequence. Calculation of the spill over uses Eq. 7 and the parameters in Table 2 of reference 12.

6.3.5.2 “Obesity in Heart Failure” study (rest vs stress)

Two consecutive k_f^{CK} measurements, the second under 40 µg/kg/min dobutamine stress, have been made in a total of 34 subjects so far comprising: normal weight & healthy (7), obese & healthy (17), normal weight with chronic heart failure (7), and obese with chronic heart failure (3). Recruitment is planned to continue for 25 subjects in each group. Obesity was defined as a body mass index (BMI) over 30.

The acquisition protocol and analysis was described in Section 6.3.3, except that now for the 5th and 6th spectroscopy series dobutamine was administered by a qualified physician and the rate of injection modulated to keep target heart rate of 65% maximum ($0.65[220 - \text{age}]$). CINE images were repeated during stress to adjust the spectroscopy trigger delay for the increased heart rate.

6.4 Results

6.4.1 AHP pulse

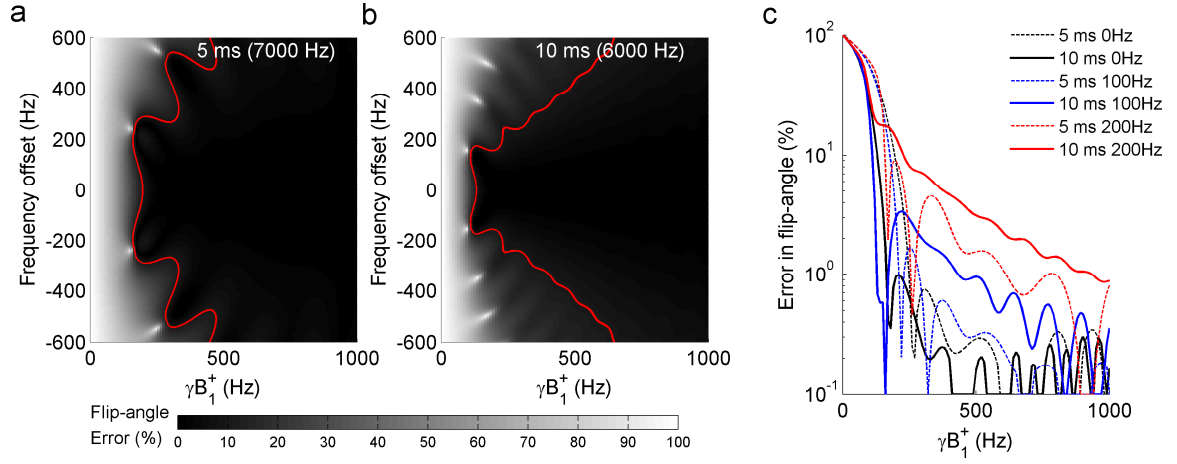


Figure 6.1 Bloch simulations comparing the 5 ms (7000 Hz) and 10 ms (6000 Hz) AHP pulses. Panel **a&b** show two dimensional (frequency offset and γB_1^+) flip-angle error plots. The darkest regions have lowest error, and the red contour is the 10% error limit. Panel **c** shows cross-sections from panel **a&b** at constant frequency offset.

Simulations of the 5 and 10 ms frequency-cycled AHP pulses determined that the adiabatic onset between 0 Hz and ± 100 Hz frequency offset was reduced by 100-150 Hz γB_1^+ (Figure 6.1). However at larger frequency offsets the 10 ms pulse produced constant flip-angle errors of 5-10%, compared to 1-2% with the 5 ms pulse.

In the experimental validation of the pulse, transmit voltages (35, 70, 120, 170, 190, 210 V) were found by Bloch-Siegert B_1 mapping (Chapter 7) to generate γB_1^+ of 125, 251, 430, 609, 681 and 753 Hz in the single voxel. The relative CRLB of the measured γB_1^+ was 0.25%.

The γB_1^+ measurements were used in pulse Bloch simulations which matched the experimentally measured frequency response of the pulse within 1% across the -200 to

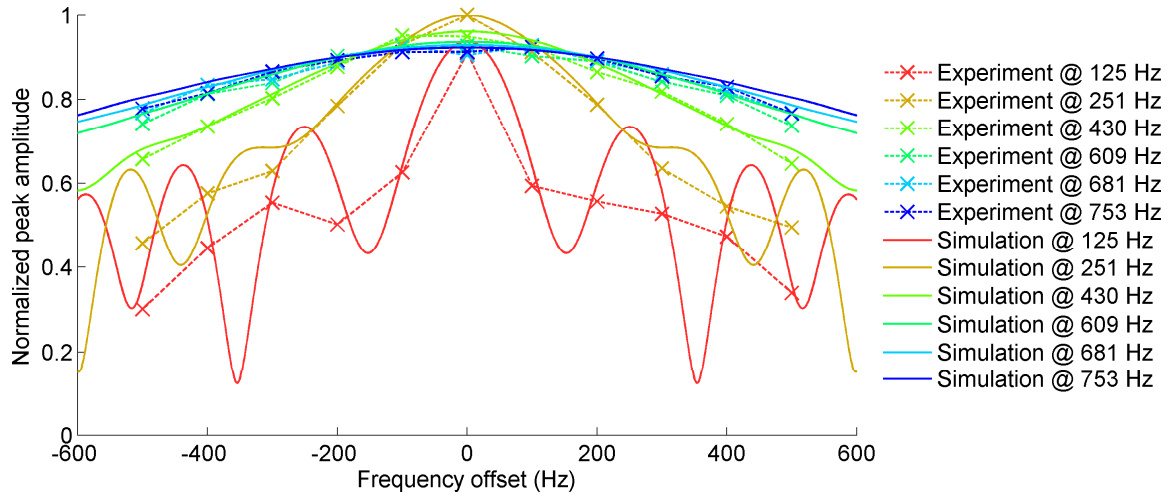


Figure 6.2 Experimentally measured and simulated frequency response of frequency cycled AHP pulses. Experiment and simulation was carried out at six different B_1^+ values, each shown in a different colour. The dashed lines linking experimental points are to draw the eye only.

200 Hz interval for all but the lowest B_1 value (125Hz). Outside that bracket most points were within 10% of the experimental values.

The flip-angle measured by the two- T_R method was 87° at 753 Hz γB_1^+ and $\Delta\omega = 0$ Hz.

The AHP pulse will provide adiabatic 90° excitation across the required 200 Hz

bandwidth providing $\gamma B_1^+ > 250$ Hz.

6.4.2 Skeletal muscle

In both subjects undergoing the TRiST protocol in their legs, 7 voxels in skeletal muscle were identified from the transverse localisers. The mean ($\pm$ SD) PCr SNR in the control saturation acquisition was 50 ± 27 (ranging from 13 to 93). Example spectra are shown in Figure 6.3a.

Consistent T_1' and k_f^{CK} values were found across the 5 voxels closest to the coil in both subjects. The average T_1' in these voxels was 2.2 ± 0.2 s, and the k_f^{CK} was 0.25 ± 0.01 s $^{-1}$. In the two voxels furthest from the coil (approx. 70-80 mm), which also contained the tibia

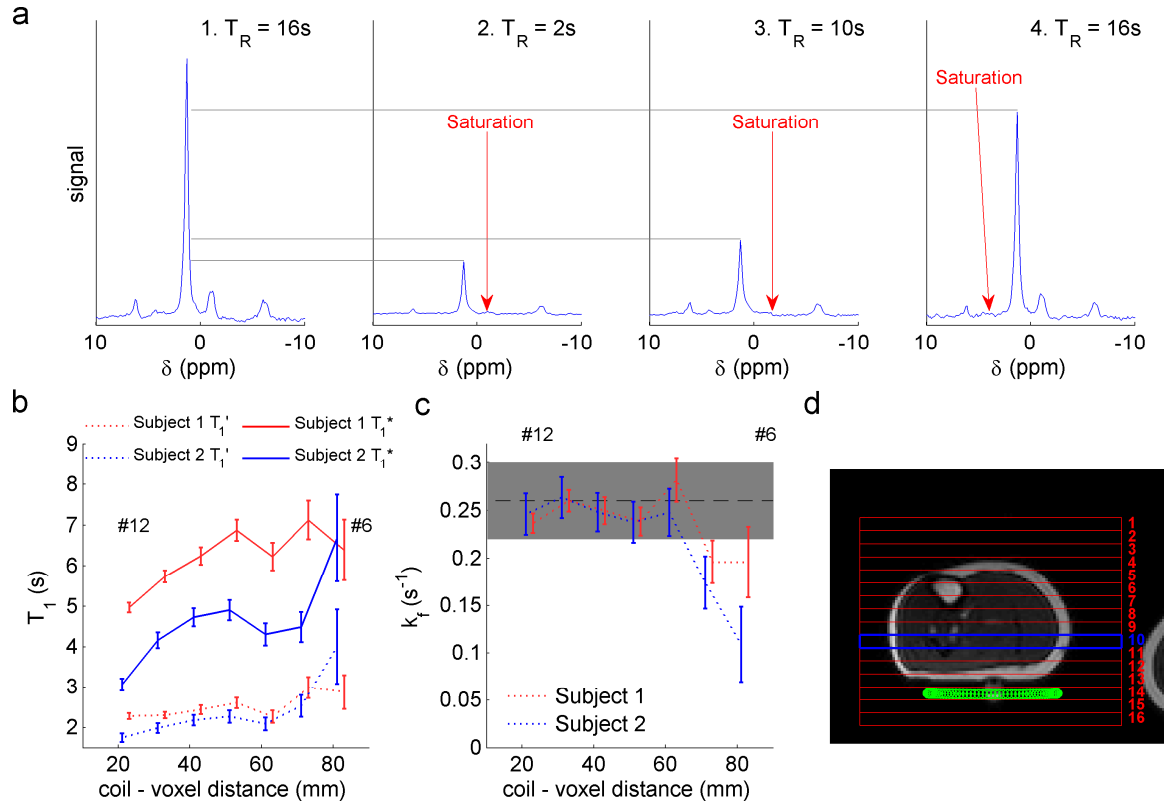


Figure 6.3 **a:** Example spectra from each component acquisition in a single subject (voxel 10 in panel **d** [blue]). The signal amplitude is scaled consistently across all axes, showing both the relative decrease in PCr amplitude and SNR during the saturated acquisitions. The frequency location of the saturating DANTE train is shown by a red arrow, if present. **b:** T_1' and T_1^* as a function of coil-voxel distance, the CRLB of each parameter are indicated by the error bars. **c:** k_f^{CK} including CRLB, as a function of coil-voxel distance. Shading denotes the range of expected values. **d:** Transverse 1H localiser image overlaid with the CSI grid (red) and the position of the loop coil (green).

and the highest amount of subcutaneous fat, T_1' was 3.10 ± 0.63 s and k_f^{CK} was

0.165 ± 0.03 s^{-1} (Figure 6.3b&c). The T_1^* was less consistent between the subjects, with

similar relative intra-subject variance to T_1' . The inter-voxel mean and standard

deviation was 6.0 ± 0.7 s in the first subject and 4.2 ± 0.7 s in the second.

Complete saturation of γ -ATP was observed in 4 out of 5 voxels in each subject (i.e. all except the furthest from the coil, where the residual γ -ATP level was 10%).

6.4.3 Multiple measurement validation

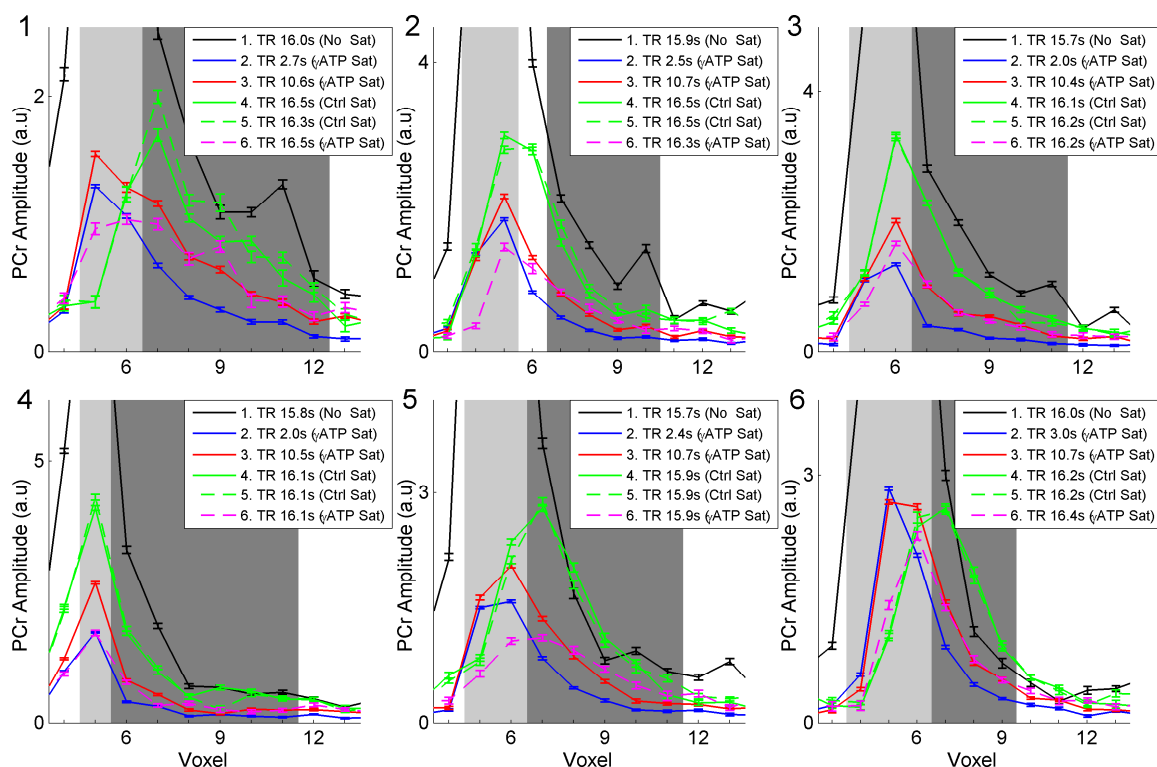


Figure 6.4 Each panel shows the fitted PCr amplitudes from a single subject. Each line in each panel is a single acquisition, the error bars show the CRLB of the fitted amplitudes. The shaded areas mark the voxels covering the skeletal muscle (light grey) and the myocardium (dark grey), as identified in transverse localiser images.

The fitted PCr amplitudes for each skeletal muscle and myocardial voxel, in each of the six subjects, are shown in Figure 6.4. The reproducibility of the per voxel amplitudes was assessed by comparing the fitted amplitudes of the myocardial voxels of the fourth and fifth acquisitions, which were consecutive and identical. A correlation and Bland-Altman plot are shown in Figure 6.5, the values were highly correlated (Pearson's R of 0.91) and the Bland-Altman plot shows a bias of 0.01 amplitude units with a coefficient of reproducibility of 0.36.

The derived parameters (PCr/ATP ratio, T_1' , T_1^* , Q-factor, SNR) calculated on a per-voxel basis, were all found to have high inter-voxel variation. For better understanding of the underlying trends in the data, each parameter is presented as an inter-subject mean

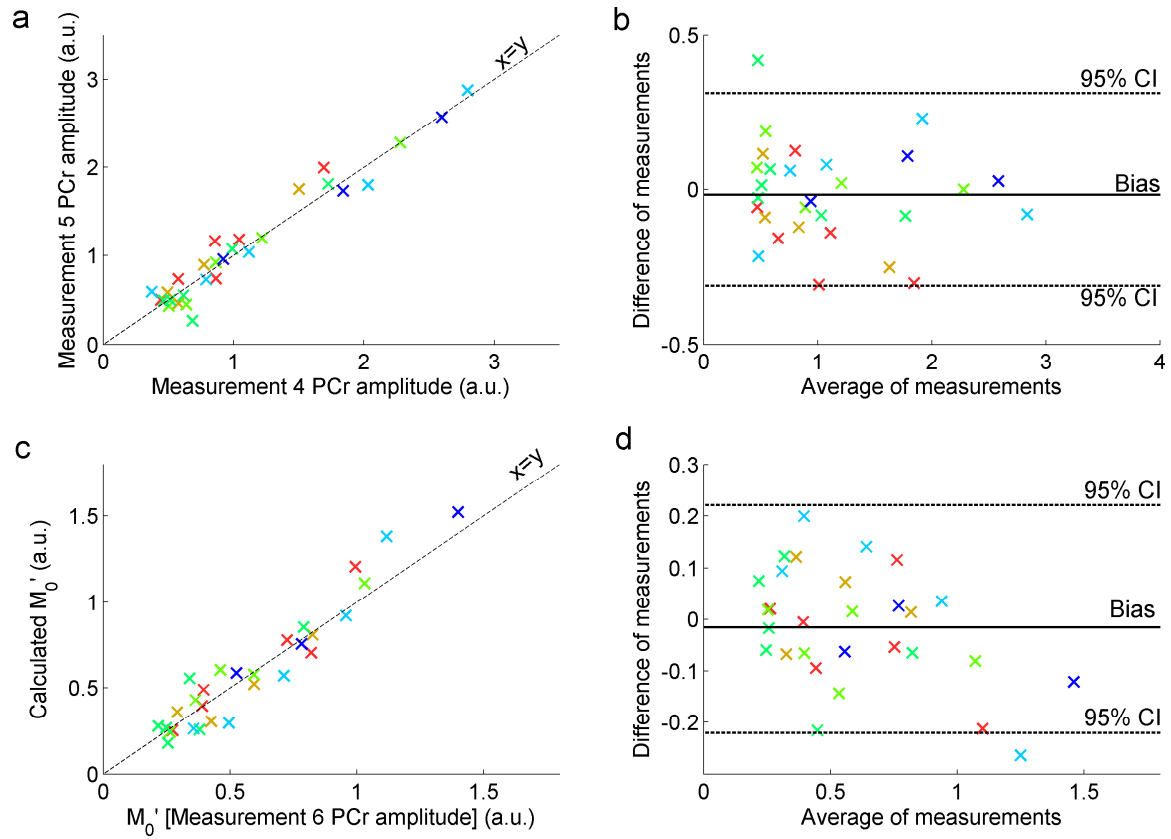


Figure 6.5 Correlation (a) and Bland-Altman (b) plots of the myocardial voxels from the (identical) 4th and 5th acquisitions. And correlation (c) and Bland-Altman (d) plots of the directly measured M_0' (PCr) (6th acquisition) and the value calculated for the first four acquisitions, using Eq. [6.2]. A different colour is used for the voxels of each subject.

and standard deviation, where voxels have been averaged together based on their relative position to the skeletal muscle-cardiac interface (Figure 6.6 & Figure 6.7). The average PCr/ γ -ATP ratio falls from 5.9 in the most anterior skeletal muscle to 2.05-2.3 in the (relatively) posterior myocardial voxels. In the same myocardial voxels T_1' and T_1^* plateau at values around 3 s and 6 s respectively. Q-factors less than 0.5 are observed in the skeletal muscle (compared to ideal values of 1.00), indicating high saturation of PCr by the DANTE train. In the myocardium, Q-factors are 0.85 ± 0.41 . The PCr SNR with control saturation (scan #4) falls from a peak average of 24 ± 10 (anterior) to 9 ± 3 (posterior).

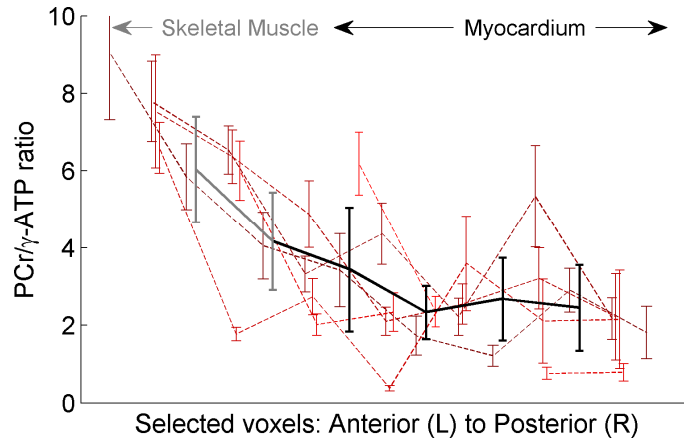


Figure 6.6 PCr/ γ -ATP ratio and CRLB of the ratio for each subject (red lines), and the inter-subject mean and standard deviation (black line). Each subject's data has been aligned so that the skeletal muscle–cardiac interface falls between the 2nd and 3rd points (read left to right) on the black line. PCr/ γ -ATP ratios greater than 10 have been excluded. All lines are only to assist the eye.

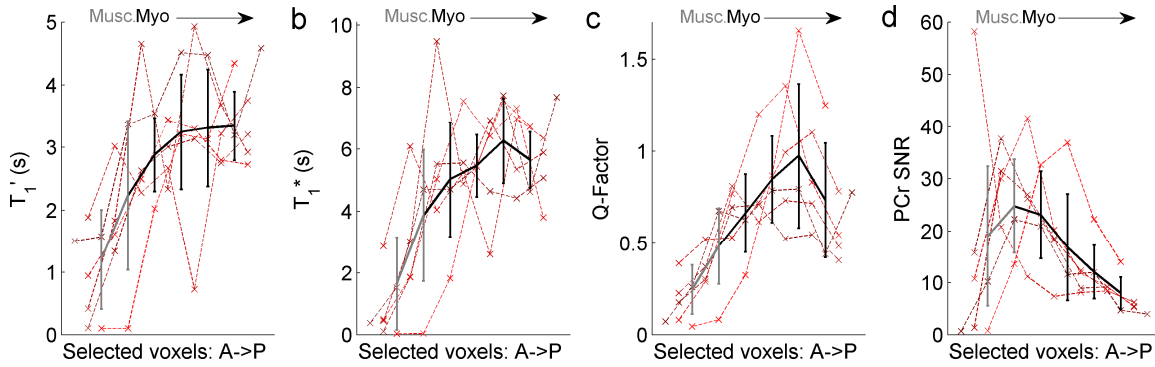


Figure 6.7 Derived parameters (**a**: T_1' , **b**: T_1^* , **c**: Q-factor and **d**: PCr SNR) for each subject (red lines), and the inter-subject mean and standard deviation (black line). Each subject's data has been aligned so that the skeletal muscle–cardiac interface falls between the 2nd and 3rd points (read left to right) on the black line. PCr/ γ -ATP ratios greater than 10 have been excluded. All lines are only to assist the eye.

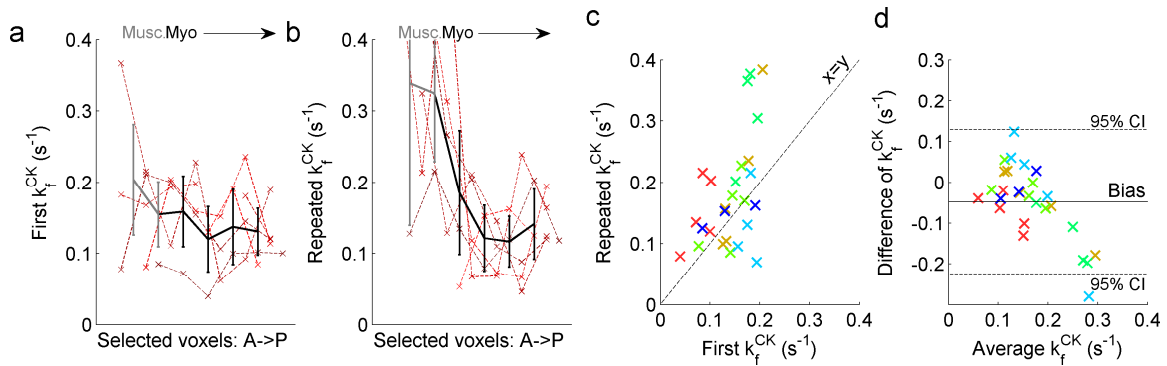


Figure 6.8 **a&b**: Per-voxel (red lines) and inter-subject average (black line) of the first and second measurements of k_f^{CK} using the extended TRIST method. **c**: Correlation plot of the two measurements for all purely myocardial voxels; each subject is shown in a different colour. **d**: Bland-Altman plot of the comparison.

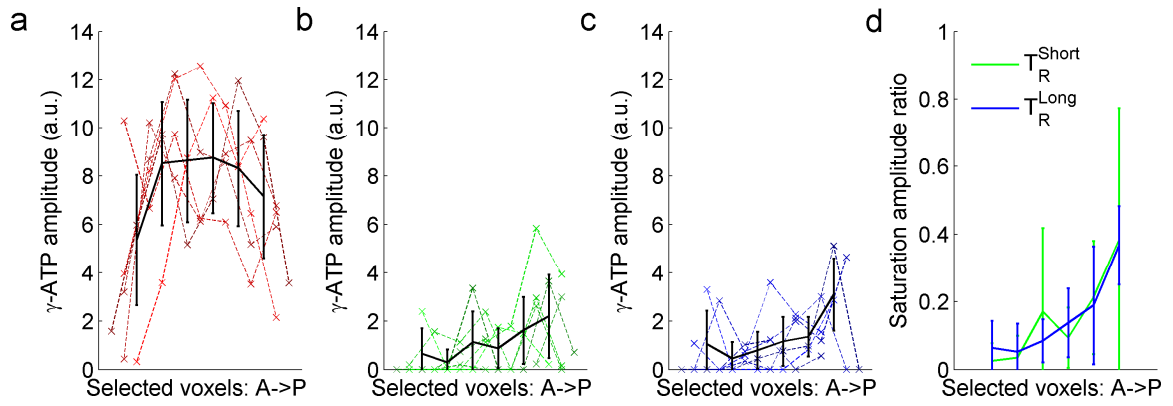


Figure 6.9 γ -ATP peak amplitude in the (a) control acquisition, (b) short repetition time acquisition and (c) long repetition time acquisition. Panel d shows the mean relative peak ratios for each of the individual saturated acquisitions.

The inter-subject average of both the first and second measured k_f^{CK} is plotted in Figure 6.8 a&b. In the myocardial region the average values for the first measurement and second measurement are both approximately 0.13, through both have high standard deviations, approximately 25% of the measured values. The per-voxel comparison of each subject's myocardial voxels has a correlation of 0.68 (Figure 6.8c), while a Bland-Altman plot of the two measurements shows a bias of -0.04, with a coefficient of reproducibility of 0.17.

The saturation ratio of γ -ATP ($M'_{\gamma\text{-ATP}} / M_{\gamma\text{-ATP}}^{\text{Ctrl}}$) observed in the two partially saturated acquisitions (#2 and #3) relative to the first control acquisition (#4) are shown in Figure 6.9d. Near complete saturation is observed in skeletal muscle voxels, but the ratio increases with increasing distance from the coil to 0.38 ± 0.07 , indicating incomplete saturation of γ -ATP in the myocardial voxels.

Averaged spectra

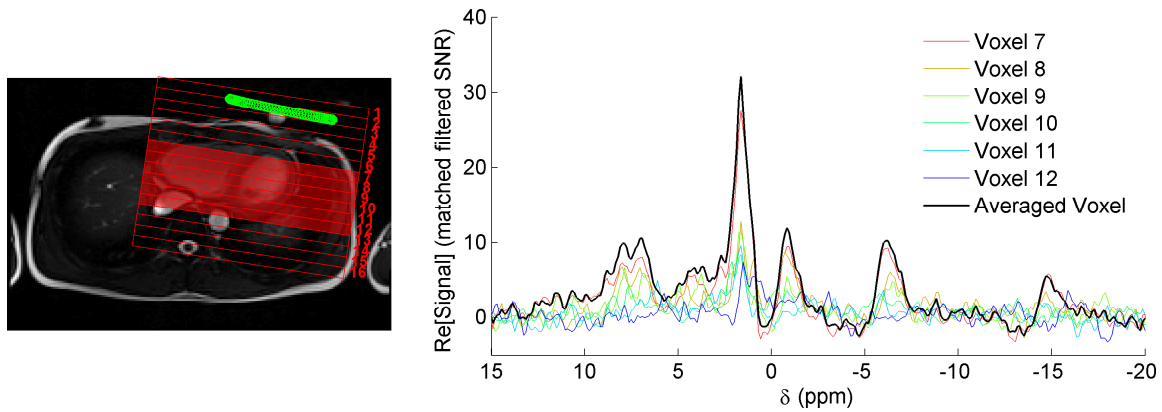


Figure 6.10 Demonstration of the effect of SVD averaging. The image shows a ^1H MRI transverse localiser of the heart. The image is overlaid with the CSI grid, with the contributing voxels highlighted, and the location of the loop coil (green). The spectrum for each contributing voxel is plotted with the SVD averaged spectrum. The spectra are normalised to the standard deviation of the baseline noise with a chemical shift less than -20 ppm.

The process of SVD voxel averaging is illustrated for a single subject in Figure 6.10.

Across the study, an average ($\pm\text{SD}$) 6 ± 1 voxels were selected in each subject as purely myocardial voxels based on localisers and having a PCr/ATP ratio < 3 . The inter-subject mean SNR in the averaged voxels, in the control acquisition, was 29 ± 7 .

The two k_f^{CK} measurements, calculated using the PCr amplitudes from the averaged spectra, are compared in Figure 6.11. The two measurements show a higher correlation (0.85 vs. 0.68), lower bias (-0.03 vs. -0.04) and a smaller coefficient of reproducibility (0.08 vs. 0.17) than the per-voxel values. Additionally the two k_f^{CK} measurements from averaged spectra show greater correlation (0.85 vs. 0.75), lower bias (-0.03 vs. -0.04) and a smaller coefficient of reproducibility (0.08 vs. 0.12) than the per-voxel values from the most apical (and highest SNR) voxel used in the averaging process. In both individual voxel and averaged analysis the method appears to overestimate the repeated k_f^{CK} at higher initial k_f^{CK} values.

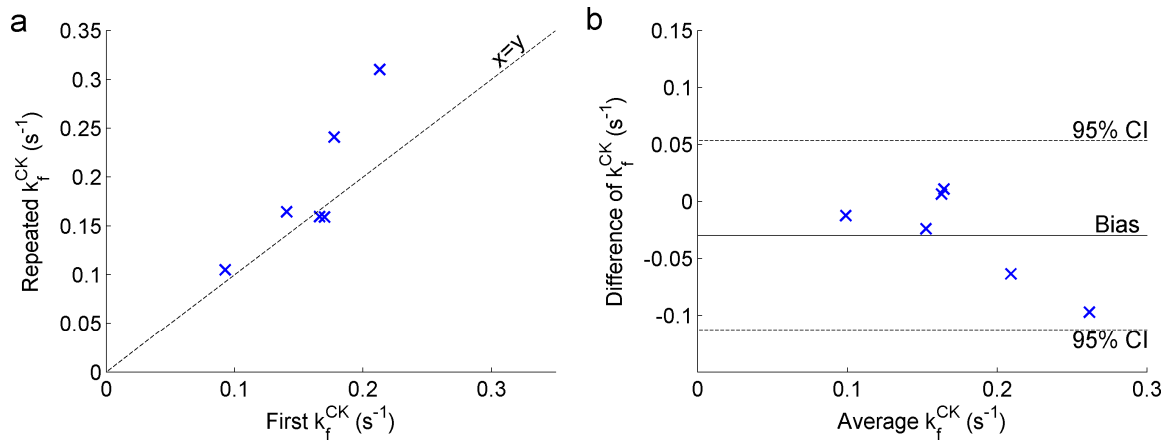


Figure 6.11 Correlation (a) and Bland-Altman plot (b) comparing the first and second k_f^{CK} measurements calculated from SVD averaged spectra. Each “x” corresponds to one subject.

6.4.4 Further sequence validation

Bloch-McConnell sequence simulation showed that the magnitude of error in k_f^{CK} measurement would be greater than 5% if the γB_1^+ was below 210 Hz. Separate simulation of the effect of each RF component (AHP and DANTE pulses) showed that, despite adiabatic excitation by the AHP pulse needing $\gamma B_1^+ > 160$ Hz, it is incomplete saturation of γ -ATP by the DANTE pulse that is the dominant source of error. The saturation ratio increases rapidly and the measured k_f^{CK} decreases when DANTE is used with γB_1^+ values below 50 Hz.

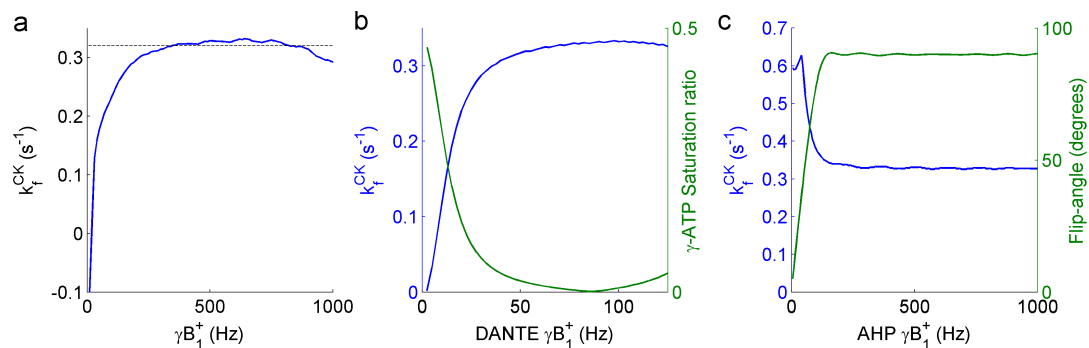


Figure 6.12 **a:** Simulated k_f^{CK} values at different γB_1^+ values. The peak γB_1^+ of the AHP and DANTE pulses are held in a ratio of 210:30, equivalent to the transmission voltages of the pulses in the experimental acquisitions. **b:** k_f^{CK} and saturation ratio as a function of DANTE peak γB_1^+ (with AHP at 500 Hz). **c:** k_f^{CK} and excitation flip-angle as the AHP γB_1^+ is varied (with DANTE at 100 Hz).

The frequency response of the DANTE pulse train in the single voxel phosphate phantom is shown in Figure 6.13. As predicted by Bloch simulations, a shorter inter-pulse delay increases the saturation bandwidth and the range of frequencies affected by spill over saturation. However the shorter inter-pulse delay achieved greater saturation (saturation ratio = 0.02 vs. 0.10), for the same SAR deposition. The T_2^* was measured as 29.7 ms and 9.7 ms in the well shimmed and deliberately poorly shimmed experiments, however no broadening of the saturation band was observed in the presence of a shorter T_2^* , rather the only effect was a 2.5% decrease in the saturation achieved. Manual adjustment of simulation parameters suggested a T_2 of ~ 200 ms in the phantom.

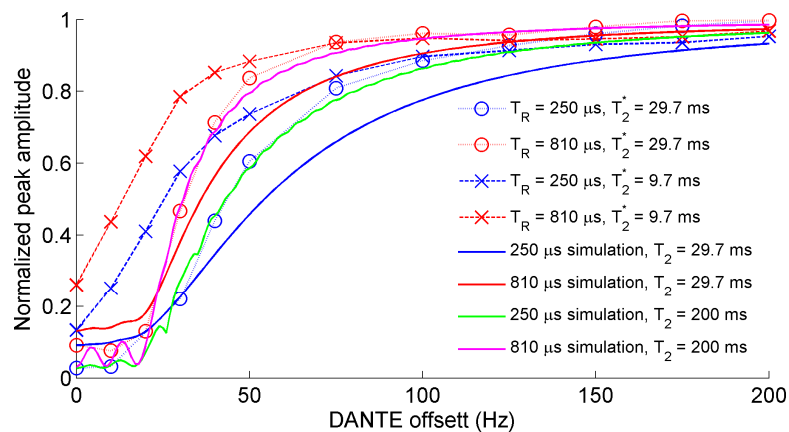


Figure 6.13 Frequency response of two DANTE pulse trains, measured in a single voxel phosphate phantom, measured with two different T_2^* values. Shorter T_2^* does not cause broadening of the DANTE frequency profile. Bloch simulations (solid lines) show that the frequency profile depends on the T_2 rather than the T_2^* .

Monte Carlo simulation of the TRiST experiment at different SNR levels (SNR of the PCr peak in the control acquisition) showed measurement bias greater than 5% at SNR values lower than 13. However the relative standard deviation (precision) of the measurement was 31% at an SNR of 20 and increased rapidly with further decreasing SNR values, surpassing 100% of the measured value at SNR values under 10.

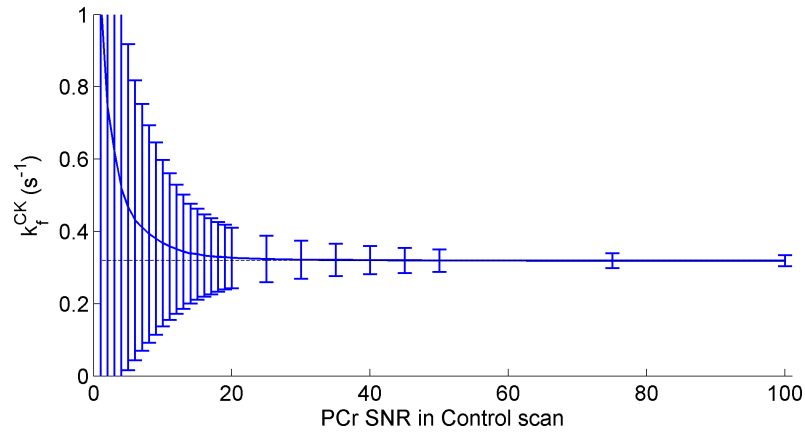


Figure 6.14 k_f^{CK} accuracy and precision as a function of PCr SNR as calculated by a Monte Carlo simulation of a full sequence Bloch simulation.

6.4.5 Patient data

6.4.5.1 Single TRiST measurement in HCM patients

The mean septal thickness of the HCM cohort was 1.65 ± 0.56 cm, 1.91 ± 0.50 cm in the sub cohort with LVH and 0.93 ± 0.22 cm in the healthy controls.

Analysis of the TRiST measurement made in HCM patients and healthy controls was done both in single voxels and using the SVD averaging method. The results (PCr/ γ -ATP ratio, PCr SNR and k_f^{CK}) are shown for the four most anterior myocardial voxels in Figure 6.15. The PCr/ATP ratio is elevated in the two most anterior voxels, in both control and HCM populations, compared to the posterior voxels and literature values for the fully relaxed PCr/ATP ratio.^{5,14} The mean ($\pm$ SD) PCr/ATP ratio in the posterior two voxels is 2.3 ± 1.3 and 2.0 ± 0.6 for the control subjects, and 1.8 ± 0.9 and 1.5 ± 0.06 for the HCM group. There is no significant difference (student's t-test, $p < 0.05$) between the two cohort's PCr/ATP ratio in any voxel. The PCr SNR decreases from anterior to posterior voxels (Figure 6.15b), the mean ($\pm$ SD) for all voxels analysed is 10 ± 7 .

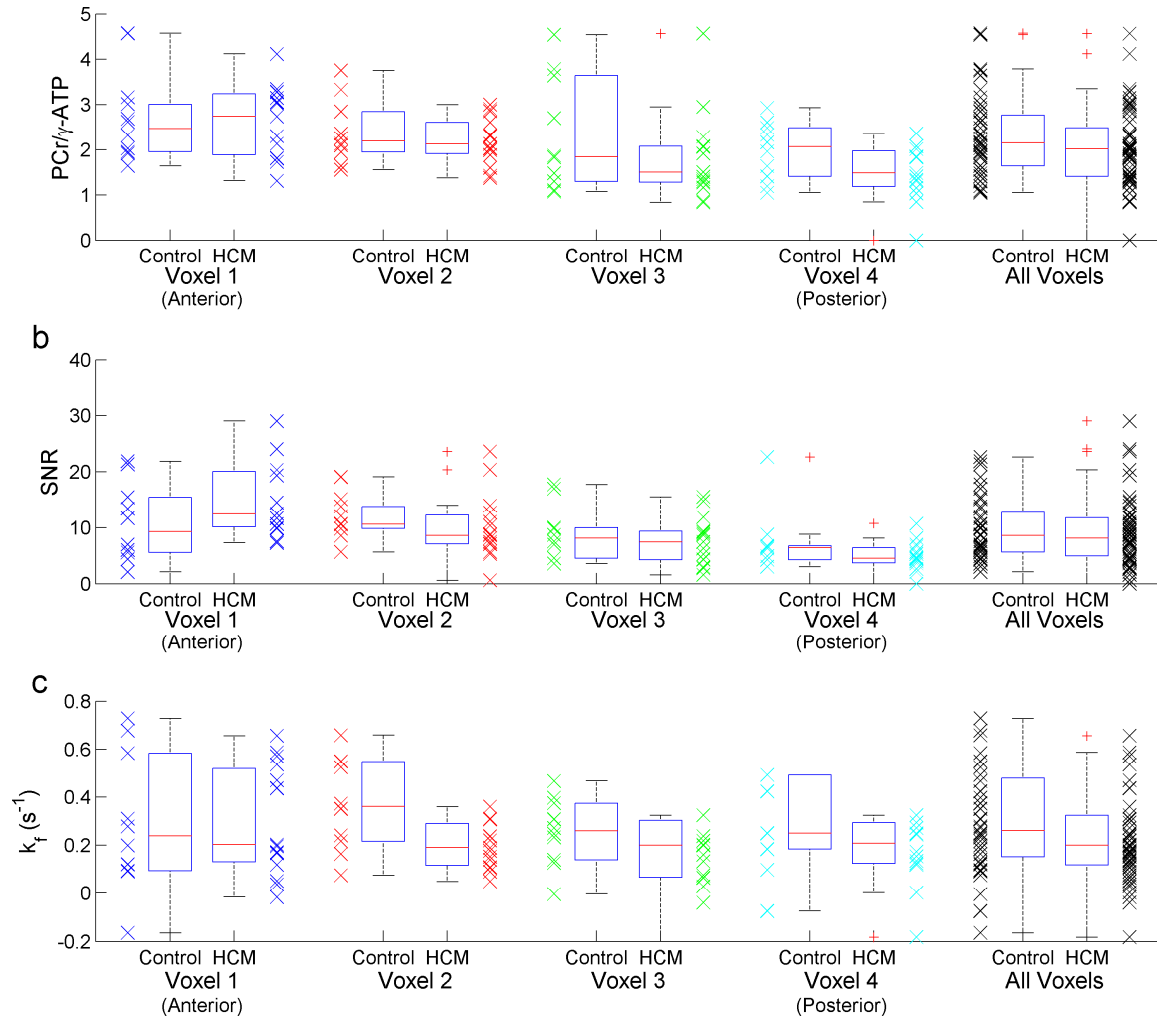


Figure 6.15 Derived parameters calculated from the 4 most anterior myocardial voxels, as identified by localisers. The fifth set shows the collected parameters from all four voxels. The box plots show the median, 25th and 75th percentiles and the range of the data, excluding any outliers (red "+"). **a:** PCr/γ-ATP ratio calculated from the unsaturated acquisition. **b:** PCr SNR in the control acquisition. **c:** Single k_f^{CK} as measured by the four first scans in the extended protocol.

The k_f^{CK} displays a broad range of values across voxels, and across subjects for each selected voxel (Figure 6.15c), with overall means ($\pm$ SD) of $0.21 \pm 0.12 \text{ s}^{-1}$ in healthy subjects and $0.15 \pm 0.11 \text{ s}^{-1}$ in the HCM cohort. There is no significant difference (Welch's t-test, $p < 0.05$) between the two cohort's k_f^{CK} ratio in any voxel collection.

The mean SNR in the averaged voxels 23 ± 13 (Figure 6.16b), was significantly higher than that of the any of the un-averaged voxel distributions. The mean PCr/ATP ratio was

2.1±1.0 for control and 2.2±0.8 for HCM subjects. No significant difference was found between the two group's metabolite ratios or SNR.

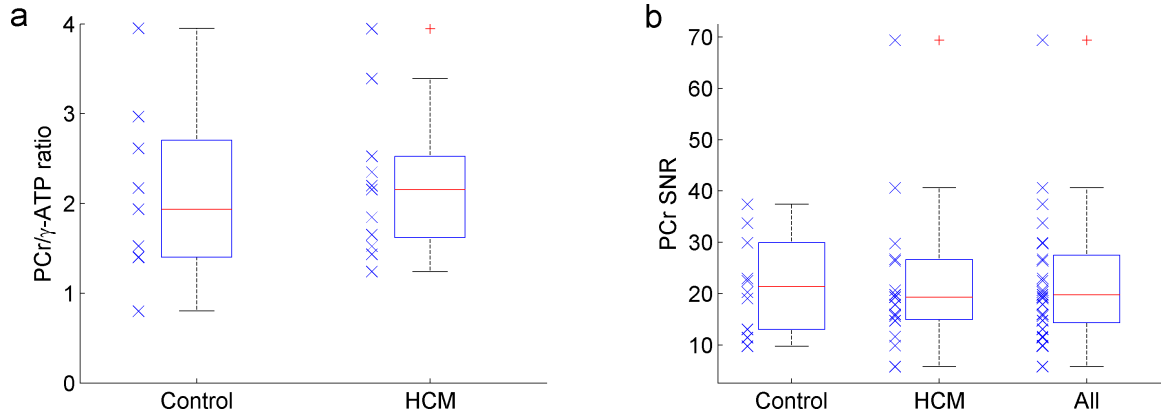


Figure 6.16 **a:** PCr/γ-ATP ratio of averaged myocardial spectra in the unsaturated acquisition. **b:** PCr peak SNR of averaged myocardial spectra in the control acquisition.

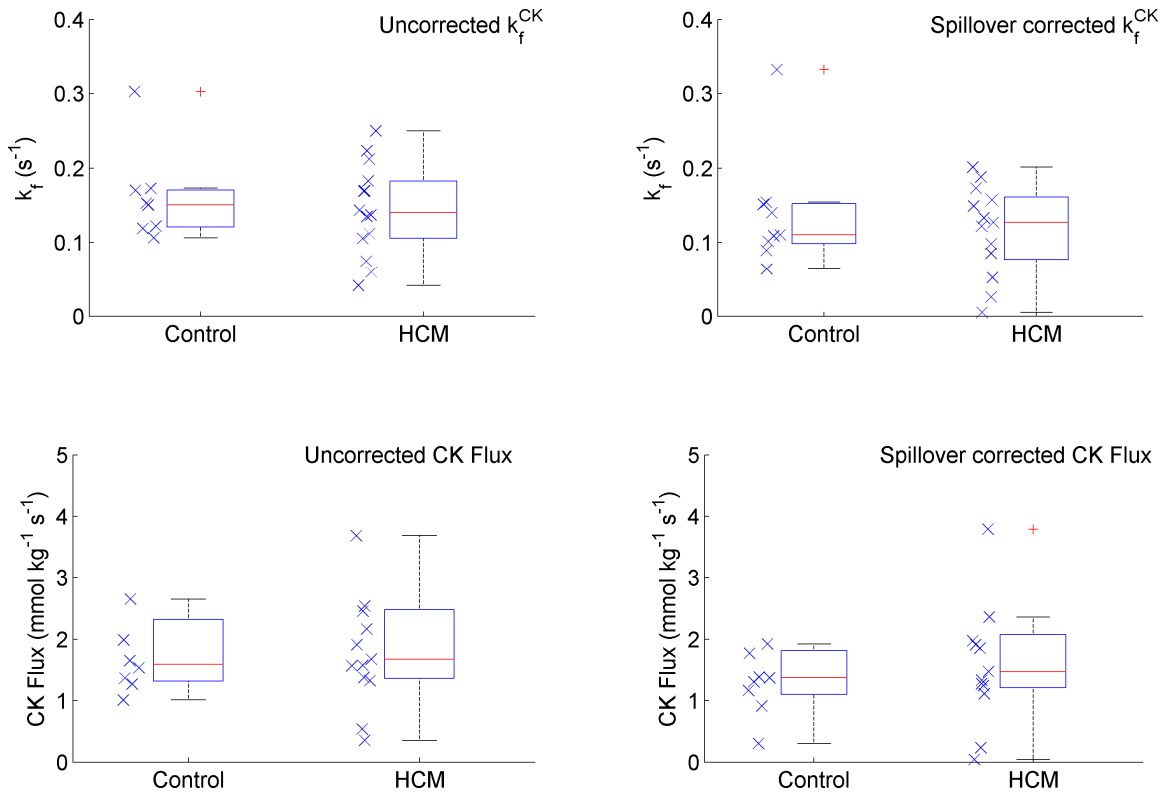


Figure 6.17 k_f^{CK} and CK flux values (with and without spill-over saturation correction [reference 12, Table 2 & Eq.7]) for the averaged myocardial spectra. The box plots show the median, 25th and 75th percentiles and the range of the data, excluding any outliers (red "+"). The blue "x"s are the individual data points.

k_f^{CK} , including spill-over saturation corrected k_f^{CK} (see §6.5.3.1 and reference 12, Table 2 & Eq.7), and CK flux values were calculated for the averaged spectra, and are plotted in Figure 6.17. Spill-over correction slightly lowered both the mean k_f^{CK} and the CK flux values, but not significantly. The mean ($\pm$ SD) uncorrected k_f^{CK} values were $0.16\pm0.02\text{ s}^{-1}$ (control) and $0.14\pm0.06\text{ s}^{-1}$ (HCM). There was no significant difference between the control and HCM k_f^{CK} values (Welch's t-test $p = 0.47$) or the CK flux values ($p = 0.95$).

6.4.5.2 Repeated measurements in heart failure and obese populations

Across all subjects the mean SNR of the averaged spectra was 21 ± 10 (Figure 6.18b). The mean PCr/ATP ratios were 2.3 ± 0.9 (NW), 2.2 ± 0.6 (OB), 2.0 ± 0.8 (OB+HF) and 2.2 ± 0.5 (NW+HF). No significant difference was found between the any group's metabolite ratios or SNR.

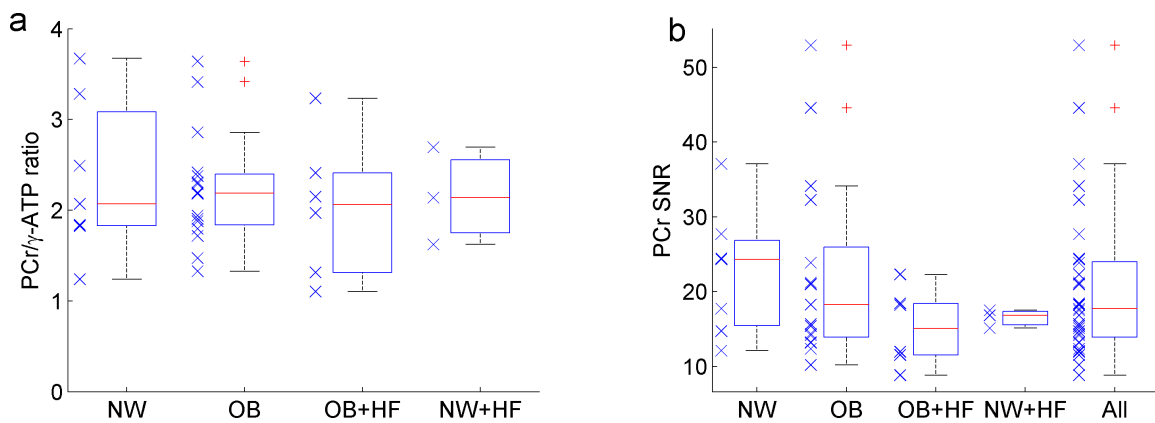


Figure 6.18 **a:** PCr/γ-ATP ratios of the average myocardial spectra of each subject cohort. **b:** PCr SNR in the control acquisition, for each cohort and all scans.

As in the HCM study, spill-over correction (reference 12, Table 2 & Eq.7) was not found to have a significant impact on the measured k_f^{CK} or CK flux values calculated. The mean uncorrected rest k_f^{CK} value (by Eq. [6.3]) for each group was $0.14\pm0.06\text{ s}^{-1}$ (NW),

$0.17 \pm 0.10 \text{ s}^{-1}$ (OB), $0.15 \pm 0.04 \text{ s}^{-1}$ (OB+HF), and $0.12 \pm 0.06 \text{ s}^{-1}$ (NW+HF). No significant difference (Welch's t-test, $p < 0.05$) was found between any cohort's k_f^{CK} value, or CK flux.

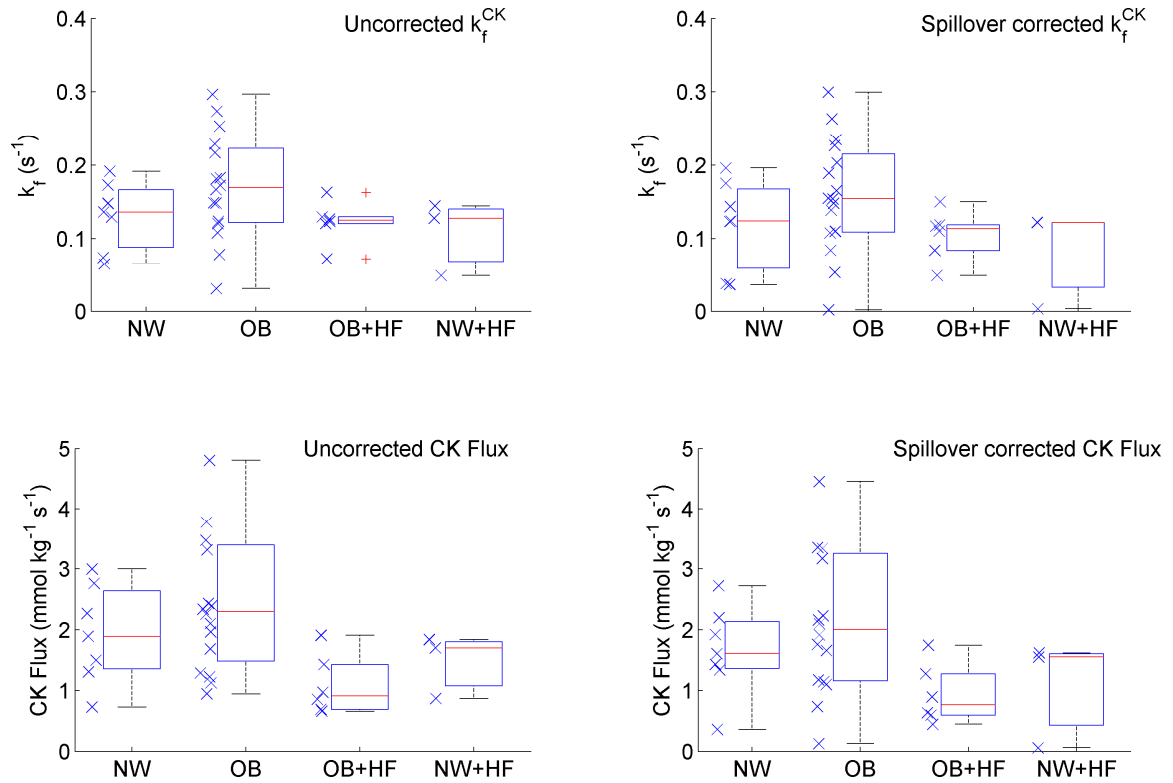


Figure 6.19 Rest k_f^{CK} and CK flux measurements (with and without spill-over correction) for each cohort, as calculated from average myocardial spectra. The box plots show the median, 25th and 75th percentiles and the range of the data, excluding any outliers (red "+"). The blue "x"s are the individual subject data points.

24 of the 34 subjects who completed the first section of the protocol completed the full protocol. The mean repeat k_f^{CK} measurement, collected during stress, for each group was $0.13 \pm 0.06 \text{ s}^{-1}$ (NW, $n = 6$), $0.13 \pm 0.09 \text{ s}^{-1}$ (OB, $n = 12$), $0.23 \pm 0.11 \text{ s}^{-1}$ (OB+HF, $n = 5$), and 0.10 s^{-1} (NW+HF, $n = 1$). No significant difference (Welch's t-test, $p < 0.05$) was found between any cohort's rest (first) and stress (second) k_f^{CK} value.

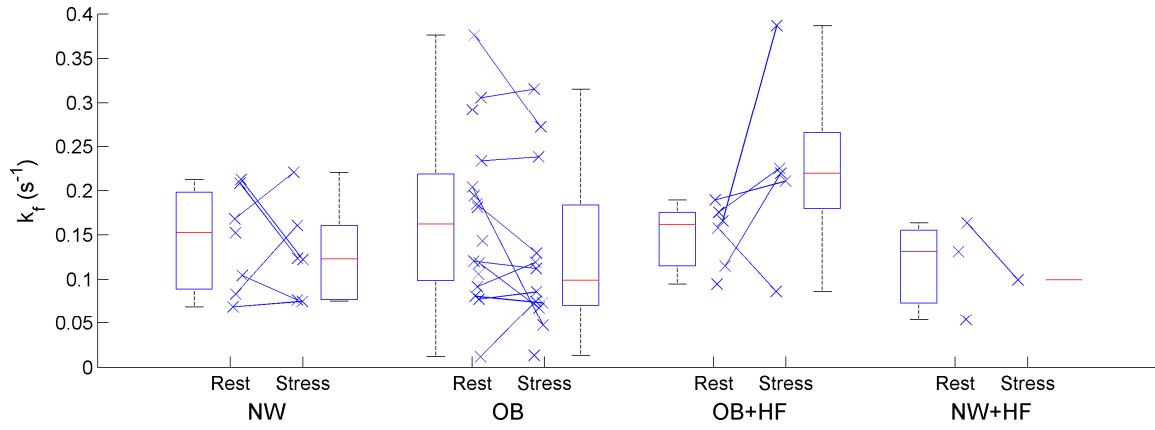


Figure 6.20 Rest and stress k_f^{CK} measurements in each cohort. The box plots show the median, 25th and 75th percentiles and the range of the data, excluding any outliers (red "+"). Blue "x"s are the individual subject data points, which are linked to show the change during stress in each subject.

6.5 Discussion

6.5.1 Underestimation of the CK rate constant in healthy subjects

The myocardial k_f^{CK} values of healthy subjects measured in this work are systematically lower than the rate constant measured in previous cardiac experiments, which have consistently measured a value of 0.32 s^{-1} .^{4,5} The rates measured in HCM and HF cohorts in this work are close to the values measured previously in such cohorts, $0.17 \pm 0.06 \text{ s}^{-1}$.^{5,12,15} The inter-subject standard deviations of k_f^{CK} are close to those previously measured with the TRiST technique^{1,12} though in this study values come from the spectra averaged from several myocardial voxels rather than single voxel analysis.

After the initial cardiac validation scans, it was presumed that because of the low spectral SNR observed, estimated by Monte Carlo simulation to cause low precision k_f^{CK} measurements, a large sample size would be required to reveal the true mean. However with 23 normal weight and healthy subjects having undergone the TRiST protocol at

Oxford, the sample size exceeds that required for equivalence testing (1-Sample Equivalence, $\delta = 0.08$, sample size > 17).¹⁶ Therefore it must be considered that a systematic bias has been introduced due to a remaining deviation from the original implementation.

6.5.1.1 Deviations from the original implementation

The Oxford TRiST implementation deviates from the published implementation in ways which could be reasonably brought in-line with the published work.

- The original coil hardware, with separate receive and transmit elements, is unavailable at Oxford: a single element transmit-receive coil is used instead. The functional receive sensitivity of the coil at Oxford extends across a larger volume than the Johns Hopkins coil, which has a smaller receive loop element. The Oxford coil will be sensitive to a greater amount of skeletal muscle close to the heart, resulting in greater spectral contamination. Additionally the use of an equal size loop for transmit and receive will result in the coil being more sensitive to magnetisation which experiences a lower B_1^+ field, predisposing it to the effects simulated in §6.4.4.
- Subjects in this study were scanned supine with the coil on top of their chest whilst in the original study the coil was set into the patient table with the subject lying prone on top of it. Whilst no difference has been found in PCr/ATP ratios measured by both methods in a small study¹⁷, it is proposed that lying prone reduces the coil to heart distance and reduces breathing motion. It was decided to scan supine to improve subject tolerance to the protocol, which lasts

approximately 70 minutes, bearing in mind the obese and heart failure cohorts and the generally unpleasant experience of dobutamine stress.

- B_0 shimming is not carried out in the Oxford protocol, whilst it is used in the original study. Experience of 1D-CSI at Oxford using the 10 cm loop transmit-receive coil, including two pilot scans of the TRiST protocol (not shown), is that B_0 shimming produces “split” PCr peaks in the spectra. Split peaks arise from the coil receive sensitive volume spanning muscle tissue experiencing sufficiently different B_0 fields to result in separately resolvable PCr peaks. Shimming, whilst decreasing the B_0 inhomogeneity across the target volume can cause greater B_0 shifts outside this volume, resulting in split peaks if both the target volume and the outer region are detected. Making the shimmed volume much larger than the coil sensitive region resulted in a shim not improved over the scanner default ‘tune up’ shim. The smaller 6 cm receive loop in the Johns Hopkins coil might as a side-effect make robust B_0 -shimming easier.
- The AHP amplitude and frequency modulation parameters have been altered from those used in the original study in order to achieve a lower adiabatic onset B_1^+ (§6.3.1). The lengthened pulse might be susceptible to T_2 effects – spin-spin relaxation causes dephasing of the spin-locked magnetisation precessing around the adiabatic pulse B_{eff} vector – resulting in a lower than expected M_{xy} .
- The DANTE modulation parameters have been modified from the original implementation. The inter-pulse delay has been decreased to maximise

saturation in regions of low B_1^+ . The shortened inter-pulse delay causes greater spill-over saturation (§6.4.4).

I believe the key to bringing the Oxford protocol fully into line with the original protocol is the coil. With a specifically designed coil, matching the original one, higher B_1^+ could be achieved – enabling matching AHP and DANTE modulation parameters to be used – and the signal localised to a smaller mostly myocardial volume – also enabling useful B_0 -shimming. Additionally the coil design could be made to be more compatible with the prone scanning position than the current coil.

The simulation contained in this chapter suggests that the only plausible mechanism for the underestimation of the k_f^{CK} rate constant arises from the effect of incomplete, or non-steady-state, saturation of γ -ATP. Incomplete saturation of the γ -ATP peak is observed in posterior voxels. Whether this is an effect of low B_1^+ , static B_0 inhomogeneity or dynamic B_0 changes due to the free breathing acquisition (estimated at ± 130 Hz)¹⁸, is currently unknown. Once again a combination of a coil which produces higher B_1^+ , but with a small sensitive volume would help.

6.5.2 Non-significant differences between HCM and healthy myocardium

This work was unable to replicate the results of Abraham *et al.* in detecting a decreased k_f^{CK} (24 % decrease) and CK flux (44% decrease) in HCM patients relative to healthy controls.¹⁹ The inter-subject mean k_f^{CK} was the same for both cohorts in this study, but the HCM population had a k_f^{CK} standard deviation three times that of the normal

population. The standard deviation of k_f^{CK} in the HCM subjects in reference 19 was over twice that of the healthy controls (0.15 vs. 0.07). In the obesity paradox study a similar increase in standard deviation was observed between the healthy normal weight and healthy obese groups (0.06 s⁻¹ vs. 0.10 s⁻¹). The increase in inter-subject variation from healthy to disease groups is also seen in PCr/ATP ratios of DCM patients in a comparison study of 3T and 7T.^{20,21}

Given the high variance, and low value of k_f^{CK} compared to skeletal muscle and previously published cardiac k_f^{CK} values, there is insufficient confidence to challenge the findings of Abraham *et al.* The precise selection criteria of the HCM cohort may also have an effect; in this study a wide range of HCM patients were admitted, some without LVH, with unmatched genotypes, and with a wide range of myocardial wall thicknesses (1.0 – 3.2 cm). In comparison, the literature study recruited from a single family, all with the same genotype (Arg403Gin mutation). Three of the literature cohort had an NYHA class of II or over. In another CK flux study⁵, the degree of heart failure was found to be important, with only those with a diagnosis of chronic heart failure showing an abnormal k_f^{CK} . The process of averaging myocardial voxels, which may span regions with variable hypertrophy and disease state, in this work might have diluted any effect further.

Whilst the scan-to-scan reproducibility is acceptable, as shown in Figure 6.5, the Monte Carlo simulations (Figure 6.14) show that the SNR achieved throughout the work contained in this chapter will result in variance on the order of the effects expected. The low SNR is highlighted by the inability of the current scans to detect a significant difference in the PCr/γ-ATP ratio between HCM and healthy populations, which has

been widely established. While the protocol is not optimised for this measurement it is a required step for the accurate determination of CK flux from k_f^{CK} . Repeated measurements to estimate reproducibility, which have so far not been attempted, will be key in determining if the variance observed in the unhealthy and obese cohorts is physiological or random.

A single dataset, collected on a healthy subject, from Johns Hopkins was shared with us, together with its intermediate analysis at Johns Hopkins. Analysis of that data with our pipeline gave an equivalent result to that from Johns Hopkins. However the SNR of this single data set is visually higher than the majority of data collected at Oxford. It is therefore proposed that a single sample (phantom or human) be scanned at both sites, to establish whether there is a significant SNR or acquisition difference.

6.6 Conclusion

An extended dynamic TRiST measurement protocol has been implemented for clinical studies at Oxford. However, after analysis of initial study results, the measured k_f^{CK} and CK flux do not match previously measured values using the same method. A series of improvement to the method have been proposed to establish if the differences are systematic bias or physiological.

6.7 References

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Chapter 7: Bloch-Siegert B_1^+ -mapping for human cardiac ^{31}P -MRS at 7T

7.1 The chapter

This chapter covers the implementation of a 31-phosphorus MRS based B_1^+ mapping technique, designed to meet the needs expressed in Chapter 5. The overall aim is to measure B_1 -maps at the same or higher resolution than typical ^{31}P 3D-CSI acquisitions, enabling a per-voxel flip-angle to be determined.

The work described in this chapter has been published as “Bloch-Siegert B_1^+ -mapping for human cardiac ^{31}P -MRS at 7 tesla” by W. T. Clarke, M. D. Robson and C. T. Rodgers (Magnetic Resonance in Medicine, 2015, doi: 10.1002/mrm.26005). In this thesis, the text in the “Introduction” and “Conclusions” sections has been expanded, and additional data has been added relating to the comparison with phantom B_1^+ measurements and Biot-Savart field estimation.

The chapter starts with a review of the difficulties of measuring B_1 -maps for ^{31}P (or equivalently for X-nuclei). The Bloch-Siegert phase based method is then introduced.

Monte Carlo simulations and propagation-of-error calculations are used to estimate the precision and accuracy of measurement strategies possible with the Bloch-Siegert method.

A Fermi pulse is chosen as the Bloch-Siegert sensitising pulse and the pulse is optimised for the specific hardware at Oxford. Validation of the Bloch-Siegert method is undertaken in point source and uniform volume phantoms, before further validation in the skeletal muscle of a single volunteer.

^{31}P Bloch-Siegert B_1 -maps are collected in 5 healthy volunteers using a 3D localised CSI sequence. Additionally the T_1 insensitivity of the method is demonstrated. Finally limitations and possible future developments are discussed.

7.2 Introduction

^{31}P -MR spectra typically have $\sim 10^{-5}$ x lower $SNR/\sqrt{t}$ than ^1H MRI, and ^{31}P -containing metabolites have T_1 relaxation times of several seconds in vivo. Therefore, it is not normally practical to obtain fully relaxed ^{31}P -MR spectra in vivo. Using a $T_R < 5T_1$ causes partial saturation (Chapter 1), so it is essential to know the flip-angle in each voxel (and the metabolite T_1) if one is to determine accurate metabolite concentrations, or metabolite concentration ratios.¹

As the phosphorus gyromagnetic ratio is only 40% of $\gamma_{^1\text{H}}$, there is less nutation of the magnetization for a given radiofrequency (RF) transmit field strength, a lower magnitude B_1^- field, and lower receive sensitivity compared to ^1H -MR. As discussed in previous chapters, to mitigate both the low SNR of cardiac ^{31}P -MRS and the detrimental effect of the low gyromagnetic ratio on the transmit performance, surface coils are often used to maximize both B_1^+ and the peak receive sensitivity. However, surface coils give large variations in B_1^+ depending on coil placement and the location of the voxel of interest in the heart.

B_1^+ -insensitive adiabatic excitation pulses have been used to obtain uniform excitation flip-angles despite this B_1^+ -inhomogeneity at 1.5T and 3T, thereby enabling absolute quantification of metabolite concentrations (Chapters 2&6).^{2,3} But in the heart at 7T, the

wide bandwidth of ^{31}P spectra, the limited peak B_1^+ and the regulatory limits on specific absorption rate make adiabatic excitation challenging.

Approaches that compute B_1^+ using the Biot-Savart law or finite-differences time-domain simulations, or that measure B_1^+ in advance in a phantom are demanding, because dielectric effects make the spatial distribution of B_1^+ depend on each subject's anatomy⁴ —and more so at 7T than at 1.5T or 3T.⁵ Therefore, at 7T, because it is challenging to use B_1^+ -insensitive adiabatic pulses and because calculated field maps are increasingly inaccurate, it is essential to be able to directly measure, for each subject, the spatial distribution of B_1^+ in the human heart, which is the focus of this work.

There is a limited choice of existing B_1^+ -mapping methods that are compatible with cardiac ^{31}P -MRS at 7T. The dual-TR, flip-angle measurement method of Chmelik *et al.* is effective in skeletal muscle, but it relies on knowledge of the metabolite T_1 values.⁶ This is particularly limiting for the highest SNR peak, phosphocreatine (PCr), whose apparent T_1 depends on the creatine-kinase exchange rate⁷, which is known to change markedly in heart failure.⁸

A second potential method is actual flip-angle imaging (AFI), which has been used for musculoskeletal ^{31}P -MRS at 7T.⁹ However, that protocol used a total T_R of 4 s; a single average, $16 \times 16 \times 8$ matrix, acquisition weighted, three-dimensional (3D) chemical shift imaging (CSI) acquisition would take 40 min, leaving insufficient time for the main acquisition in a patient study.

The above methods illustrate some of the classic difficulties associated with ^{31}P -MRS and X-nuclear MRS in general, where long, and potentially unknown T_1 values, combined with low SNR, limit the ability to measure accurately a fine change in signal magnitude, hampering the translation of methods from ^1H -MRI and ^1H -MRS. I, therefore, introduce a novel method for B_1^+ -mapping in multivoxel spectroscopy based on the Bloch-Siegert shift.¹⁰ This approach encodes the B_1^+ measurement in the phase of the magnetisation, so it is independent of the T_R and of metabolite T_1 values.

^{31}P -MRS, and other X-nuclear systems, typically have complex multi-peak spectra, therefore, I explore how a Bloch-Siegert module can be applied for multi-peak spectroscopy and derive expressions for the accuracy and precision of Bloch-Siegert spectroscopy B_1^+ -mapping.

Finally, I optimize a protocol for cardiac ^{31}P Bloch-Siegert spectroscopy B_1^+ -mapping at 7T. I validate this protocol in phantoms, in human skeletal muscle and demonstrate it in the hearts of five normal volunteers at 7T.

7.3 Theory

7.3.1 Bloch-Siegert B_1^+ Mapping

Bloch-Siegert B_1^+ -mapping uses an additional off-resonance RF pulse (a “Bloch-Siegert pulse”) inserted between excitation and readout in a pulse sequence. During this Bloch-Siegert pulse, there is a transient Bloch-Siegert shift in the Larmor precession frequency.^{11,12} This causes an accumulated phase shift of the transverse magnetization and hence alters the phase of the spectral peak. The Bloch-Siegert pulse is placed at a

large frequency offset ($\omega_{\text{RF}} \gg \gamma B_1^+$) from the measured peak, to minimize additional excitation of magnetization by the Bloch-Siegert pulse (“direct excitation”), and to give a linear relationship between the Bloch-Siegert phase shift and B_1^+ .¹³ In this linear regime, the additional phase ϕ_{BS} (in radians) accumulated by a species over the duration T_p of the Bloch-Siegert pulse is

$$\phi_{\text{BS}} = 2\pi \int_0^{T_p} \frac{|\gamma B_1^+(t)|^2}{2\omega_{\text{RF}}} dt = \frac{\pi \gamma^2 B_{1,\text{normalized}}^2}{\omega_{\text{RF}}} \times (B_{1,\text{peak}}^+)^2, \quad [7.1]$$

where t is time (in s), ω_{RF} is the frequency offset (in Hz) from the Bloch-Siegert pulse to the metabolite of interest, γ is the gyromagnetic ratio (in Hz T⁻¹), $B_1^+(t)$ is the time dependent pulse amplitude of the Bloch-Siegert pulse (in T), $B_{1,\text{peak}}^+$ is the maximum amplitude of the Bloch-Siegert pulse (in T), and $B_{1,\text{normalized}}^2 = \tilde{B} = \int_0^{T_p} B_1^+(t)^2 dt / (B_{1,\text{peak}}^+)^2$ is the normalized pulse-envelope squared-integral of the Bloch-Siegert pulse.

In the original ¹H imaging Bloch-Siegert B_1^+ -mapping method¹⁰, two images are acquired with Fermi-shaped¹⁴ Bloch-Siegert pulses at equal and opposite frequency offsets $\pm\omega_{\text{RF}}$ relative to the water resonance. In each voxel, the images have phase $\phi_i = \phi_0 + \phi_{\text{BS}}$, where ϕ_0 is the normal phase due to the coil transmit and receive phases, B_0 inhomogeneity, sequence dead-time, flow, etc. B_1^+ is then obtained from the phase difference between the two images

$$\phi_2 - \phi_1 = \left(\phi_0 + \phi_{\text{BS}} \Big|_{+\omega_{\text{RF}}} \right) - \left(\phi_0 + \phi_{\text{BS}} \Big|_{-\omega_{\text{RF}}} \right) = 2\phi_{\text{BS}}. \quad [7.2]$$

Using Eq. [7.1], $\gamma B_{1,\text{peak}}^+$ is given by:

$$\gamma B_{1,\text{peak}}^+ = \sqrt{\frac{(\phi_2 - \phi_1) \omega_{\text{RF}}}{2\pi \tilde{B}}}. \quad [7.3]$$

7.3.2 Approaches When There Are Multiple Peaks

In ^{31}P -MRS, and X-nuclear MRS in general, multiple resonances are observed and there is often no dominant peak (unlike ^1H -MR where the water peak dominates). Therefore, for a single choice of Bloch-Siegert pulse centre frequency, each peak in the spectrum will experience a different frequency offset, ω_{RF} , from the Bloch-Siegert pulse to that metabolite, and therefore, according to Eq. [7.1], a different Bloch-Siegert phase shift.

As the peaks in a spectrum are at well-defined ω_{RF} , it is possible to measure B_1^+ from the difference in the Bloch-Siegert phase shift between multiple peaks in a single spectrum (with a single Bloch-Siegert pulse frequency), or from the totality of Bloch-Siegert phase shifts of multiple peaks in multiple spectra (with different Bloch-Siegert pulse frequencies for each acquisition). It is not immediately apparent whether incorporating signals from multiple peaks is beneficial when determining B_1^+ . And it is unclear whether it is more efficient to devote the whole measurement time to a single spectrum (with maximal SNR) using one Bloch-Siegert pulse frequency or whether it is better to acquire several spectra (with lower SNR) at several Bloch-Siegert pulse frequencies.

To investigate, I calculated the accuracy and precision of a representative selection of measurement strategies using Monte Carlo simulations and the propagation-of-errors method, applied to each strategy in turn as follows:

1. Deduce an expression that computes γB_1^+ from the measured metabolite phases, e.g. Eq. [7.3]. (And where relevant, generalize to an arbitrary number of metabolite peaks.)
2. Estimate experimental accuracy (bias) by Monte Carlo simulation using this expression. For each peak in a simulated spectrum, generate an array of 10^6 normally distributed phases with mean equal to the phase value from Eq. [7.1] (for the appropriate Bloch-Siegert-pulse-to-metabolite-frequency-offset ω_i and B_1^+) and with standard deviation $\Delta\phi$. Express the resulting accuracy as a percentage deviation from the true B_1^+ ,

$$\varepsilon_B = 100 \frac{1}{N} \sum_{i=1}^N (B_1^i - B_{1,\text{true}}^+) / B_{1,\text{true}}^+. \quad [7.4]$$

3. Compute experimental uncertainty $\Delta\gamma B_1^+$ by the propagation of errors:¹⁵

$$\Delta q = \sqrt{\sum_i \left(\Delta i \frac{\partial q}{\partial i} \right)^2}, \quad [7.5]$$

where $\partial q / \partial i$ is the partial derivative of q with respect to the i^{th} measured variable and Δi is the uncertainty in the measurement of i . Express the uncertainty as the coefficient of variation in percent

$$(C_v = 100 \cdot \Delta\gamma B_1^+ / \gamma B_{1,\text{true}}^+).$$

Each measurement strategy was considered for use in 7T cardiac 31P-MRS. The simulation variables were set as follows to the study means in reference¹⁶, because the hardware and acquisition method were identical to those used here. The PCr, γ -, α -, and β -ATP peaks were simulated at frequencies of 0 Hz, -300 Hz, -900 Hz, and -1950 Hz, respectively. Single peak simulations used only the PCr peak (at 0 Hz). The phases ϕ

were calculated for a 3.5 ms Fermi pulse with $B_{1,\text{peak}}^+ = 277$ Hz placed at $\omega_{\text{RF}} = \pm 2000$ Hz.

The phase standard deviation $\Delta\phi$ was set equal to the Cramér-Rao Lower Bound of the phase, calculated as described in Chapter 3 and Cavassila *et al.*¹⁷, for the study mean in Rodgers *et al.*¹⁶, divided by the square-root of the number of scans per measurement ($\text{CRLB}_\phi / \sqrt{N}$). Each measurement strategy was also evaluated for $\Delta\phi$ values corresponding to the mean SNR $\pm$ standard deviation in Rodgers *et al.*¹⁶ The derived equations and results are given in Table 7.1.

7.3.2.1 Method A: Dual-Acquisition +/–

This approach is equivalent to the original Bloch-Siegert B_1^+ -mapping MRI technique.¹⁰

Two acquisitions are made with Bloch-Siegert pulses at offsets symmetrically either side of the target metabolite. This approach computes B_1^+ using signal from a single peak.

7.3.2.2 Method B: Dual-Acquisition On/Off

Single-peak (B')

This approach uses one acquisition without a Bloch-Siegert pulse and one acquisition with the Bloch-Siegert pulse on one side of the resonances. B_1^+ is computed from a single peak.

Multi-peak (B'')

This acquisition also allows simultaneous use of data from all peaks that satisfy the linearity condition: $\gamma B_{1,\text{peak}}^+ \ll |\omega_i|$. (N.B. each peak may have a positive or negative offset.) I combine the measured B_1^+ values from each resonance peak in a maximum likelihood sense, which is equivalent to the outcome that would be achieved from least-squares fitting of the linear Eq. [7.1] to the simulation data. The equations generated

can be expressed for any number of resonances n at offsets ω_i from the Bloch-Siegert pulse.

7.3.2.3 Method C: Single-Acquisition Method

Measurement of B_1^+ from a single Bloch-Siegert sensitized acquisition may be achieved for a multi-peak spectrum, providing the first-order phase correction and excitation phase profile are known. For n peaks, a single acquisition is made with a Bloch-Siegert pulse placed so that the linearity condition is satisfied for all the peaks to be included in the measurement. To calculate B_1^+ Eq. [7.2] is applied to the phase differences between each pair of peaks and this set of B_1^+ values may be averaged in a maximum-likelihood sense.

Measurement strategy	Peaks (³¹ P equiv)	Scan 1	Scan 2	B_1^+ determination equation	Propagation of error	Bias ± SD (Hz) SNR=18 (μ-1σ)	Bias ± SD (Hz) SNR=31 (μ)	Bias ± SD (Hz) SNR=44 (μ+1σ)
A: Dual-acquisition +/-	1 (PCr)	3.5ms Fermi +2000Hz	3.5ms Fermi -2000Hz	$\sqrt{\frac{(\phi_2 - \phi_1)\omega_{RF}}{2\pi\tilde{B}}}$	$\sqrt{\frac{\omega(\Delta\phi_+^2 + \Delta\phi_-^2)}{8\pi(\phi_+ - \phi_-)\tilde{B}}}$	2.21 ±53.7	0.52 ±27.9	0.28 ±21.5
B': Dual-acquisition on/off	1 (PCr)	3.5ms Fermi +2000Hz	No Bloch-Siebert pulse	$\sqrt{\frac{(\phi_{on} - \phi_{off})\omega_{RF}}{\pi\tilde{B}}}$	$\sqrt{\frac{\omega(\Delta\phi_{on}^2 + \Delta\phi_{off}^2)}{4\pi(\phi_{on} - \phi_{off})\tilde{B}}}$	8.19 ±76.0	2.44 ±39.5	1.32 ±30.3
B'': Dual-acquisition on/off	4 (PCr, γ-ATP, α-ATP, β-ATP)	3.5ms Fermi +2000Hz	No Bloch-Siebert pulse	$\frac{\sum_{i=1}^n \pi^{-\frac{1}{2}} \omega_i^{-2} \sqrt{\frac{(\phi_i^{on} - \phi_i^{off})\omega_i}{\tilde{B}}}}{\sum_{i=1}^n \omega_i^{-2}}$	$\sqrt{\frac{\sum_{i=1}^n (\Delta\phi_i^{on})^2 + (\Delta\phi_i^{off})^2}{4\pi\tilde{B}(\sum_{i=1}^n \omega_i^{-1})^2 (\phi_i^{on} + \phi_i^{off})\omega_i}}$	9.03 ±70.1	3.97 ±36.5	2.27 ±28.0
C: Single-acquisition	4 (PCr, γ-ATP, α-ATP, β-ATP)	3.5ms Fermi +2000Hz	No scan	$\frac{\sum_{i=2, j=1}^{n, i-1} \sqrt{\frac{\phi_j - \phi_i}{\tilde{B}} (\omega_j^{-1} - \omega_i^{-1})^2}}{\sqrt{\pi} \sqrt{\omega_j^{-1} - \omega_i^{-1}}} \frac{\sum_{i=2, j=1}^{n, i-1} (\omega_j^{-1} - \omega_i^{-1})^2}{\sum_{i=2, j=1}^{n, i-1} (\omega_j^{-1} - \omega_i^{-1})^2}$	†	8.67 ±153.8	7.34 ±83.1	5.58 ±63.9

Table7.1 Comparison of possible Bloch-Siebert spectroscopy B_1^+ measurement strategies applied to ³¹P-MRS. A fuller description of the methods are given in the multi-peak approaches subsection of Theory. The values used to calculate the bias and standard deviation are taken from the study means of ref. 21, the bias is calculated using Eq. [7.4] with $\gamma B_{1,true}^+ = 277\text{Hz}$. † A generalized closed form could not be found for the uncertainty, $\Delta\gamma B_{1,peak}^+$, of Method C (the single-acquisition method), however an expression may be found by expanding the equation for determining B_1^+ for a specific number of peaks and then applying Eq. [7.5].

7.3.3 Bloch-Siegert Pulse Design

An ideal Bloch-Siegert pulse maximizes ϕ_{BS} , for some anticipated range of B_1^+ , at the same time minimizing direct excitation, and minimizing signal loss due to any additional dead-time between excitation and readout (when the transverse magnetization experiences T_2^* relaxation). Providing one remains in the linear regime

($\omega_{RF} \gg \gamma B_1^+$, maximum $\gamma B_1^+ \approx 1200$ Hz) and assuming that the SAR contribution from the excitation pulse is negligible, Eq. [6] in Duan *et al.*¹³ shows that the Bloch-Siegert phase difference is limited only by the SAR limit. Therefore, an optimized pulse must be chosen by minimizing direct excitation at the target frequency offset and by minimizing T_2^* -induced signal losses.

A commonly employed Bloch-Siegert pulse is the Fermi pulse. The pulse is defined by Eq. [4.14] of Bernstein *et al.*¹⁴:

$$B_1^+(t) = \frac{A_F \exp(i\omega_{RF}t)}{1 + \exp\left[\frac{(|t| - T_0)}{\alpha}\right]} \quad [7.6]$$

where A_F is the B_1^+ -field peak amplitude, ω_{RF} is the pulse frequency offset and T_0 and α are two adjustable parameters having the dimension of time.

7.4 Methods

All experiments used a Magnetom 7T scanner (Siemens, Erlangen, Germany). Localizers were acquired with a 10 cm 1H loop coil (Rapid Biomedical, Rimpar, Germany). This was replaced by a T/R switch and preamplifier module (Virtumed LLC, MN, USA) connected to the 10-cm ^{31}P transmit–receive loop coil for the ^{31}P acquisitions. The ^{31}P coil was tuned and matched using an RF sweeper (Morris Instruments Inc, Ottawa, Canada) for

each subject. All subjects in this study were recruited in a manner approved by the local Research Ethics Committee.

7.4.1 Fermi Pulse Optimization

The suitability of a range of Fermi pulses was examined using a Bloch simulation grid search. T_p was varied from 1 ms to 10 ms in steps of 0.5 ms, T_0 was varied from $0.1T_p$ to $0.5T_p$ in steps of $0.005T_p$ and α was varied from $0.01T_p$ to $0.3T_p$ in steps of $0.001T_p$. Spin evolution was simulated in Matlab (Mathworks, Natick, MA) at γB_1^+ values ranging from 100 Hz to 1000 Hz in steps of 100 Hz. The deviation from the ideal flat stop-band profile was quantified as

$$\Delta M_{xy} = \frac{1}{1000} \sum_{\omega=1500}^{2500} \left(M_{xy}^{\omega} \right)^2. \quad [7.7]$$

The minimum ΔM_{xy} of the shortest pulse which maintained a $\Delta M_{xy} < 0.01$ across all simulated peak B_1^+ values had $T_p = 3.5$ ms, $T_0 = 0.875$ ms, and $\alpha = 0.224$ ms. These parameters were used for all experiments presented below.

7.4.2 Point-Source Phantom Validation

The Bloch-Siegert effect was demonstrated in a saline-filled (18 L of 73 mM NaCl) phantom containing a single, small cube ($2 \times 2 \times 2$ cm³), of 1.8 M K₂HPO₄, which gives one singlet signal that originates only from the cube. The optimized Fermi pulse ($T_p = 3.5$ ms, $T_0 = 0.875$ ms, and $\alpha = 0.224$ ms) was inserted between the excitation RF pulse and the 3D phase-encoding gradients of a chemical shift imaging (CSI) sequence (Fig. 7.1a).^{16,18} The Fermi pulse offset was swept from -10 kHz to +10 kHz.

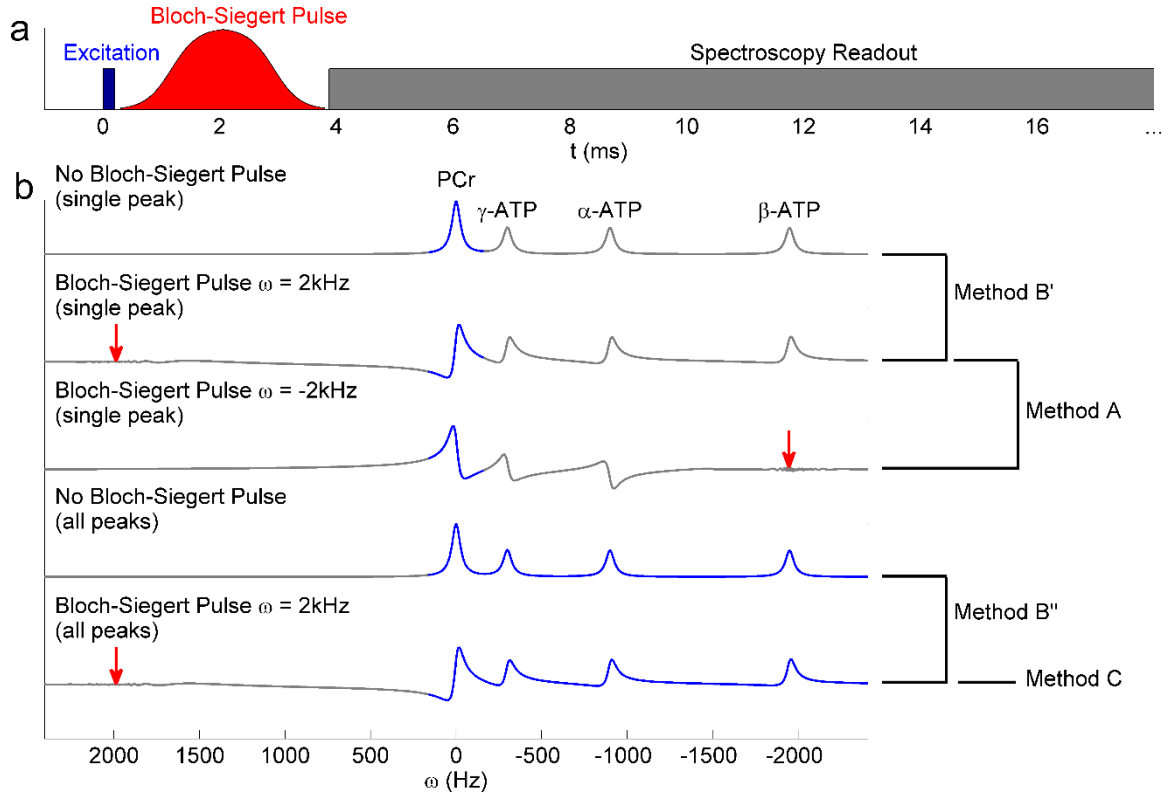


Figure 7.1 **a:** Pulse sequence timing diagram for a Bloch-Siegert spectroscopy B_1^+ -mapping sequence. The Bloch-Siegert sensitizing pulse is inserted between the excitation pulse and the readout module. In this work, the readout module consists of 3D phase encoding gradients, acquisition of a free induction decay, spoiler gradients and a final delay to produce the desired T_R . The sequence is adapted from that in Figure 1 of reference 16. **b:** Illustration of the real part of ^{31}P spectra acquired for each of the measurement strategies detailed in the Theory Section and corresponding to a row in Table 1. The peaks used for analysis in each case are shown in blue. The position of the Bloch-Siegert pulses are shown by red arrows. The phase accumulated by each peak is proportional to the inverse of its frequency offset from the Bloch-Siegert pulse (Eq. [7.1]). Note that when the Bloch-Siegert pulse is placed at -2 kHz, the β -ATP peak is almost entirely saturated.

Acquisition parameters were: $4 \times 4 \times 4$ CSI matrix, $80 \times 80 \times 80 \text{ mm}^3$ field of view, 2048 spectral points, 6 kHz bandwidth, 200 μs hard excitation pulse with a transmit voltage, i.e., the RMS voltage at the “coil plug” on the patient table, of 270 V. The voxel covering the $2 \times 2 \times 2 \text{ cm}^3$ phosphate cube was selected for analysis. Spectra were fitted using a custom Matlab implementation of the advanced method for accurate, robust and efficient spectral fitting (AMARES) algorithm¹⁹, with prior knowledge specifying a single unconstrained peak.²⁰

A $\gamma B_{1,\text{peak}}^+$ value was measured using a series of fully relaxed, nonlocalized free induction decays (FIDs) with a 4-ms hard pulse transmitted at 10–200 V. Under these conditions, where $T_R > 5T_1$, the amplitude of the observed FIDs will follow a $\sin \alpha$ relationship. The FIDs were fitted in Matlab, and then a sinusoid was fitted to the complex peak amplitudes with two adjustable parameters: the maximum signal amplitude, and the B_1^+ -per-volt scaling factor that relates the (known) applied transmit voltage to the observed signal amplitudes (i.e. flip-angle). $\gamma B_{1,\text{peak}}^+$ may be calculated as the product of the scaling factor and the Bloch-Siebert pulse transmit voltage and subsequently used to predict the phase response for the Bloch-Siebert pulse.

7.4.3 Uniform Phantom Validation

Bloch-Siebert B_1^+ -mapping was validated against a multi-TR magnitude reference method in a uniform phantom. The phantom was a $120 \times 270 \times 270 \text{ mm}^3$ box containing 0.04 M $\text{K}_2\text{PO}_4(\text{aq})$, giving rise to a singlet signal from throughout the whole volume of the phantom. The T_1 of the phantom was separately determined to be 8.57 s. The acquisition parameters were: 300 μs hard pulse excitation transmitted at 270V, 6 kHz bandwidth, 2048 spectral points, $150 \times 320 \times 320 \text{ mm}^3$ field of view, acquisition weighting, $16 \times 8 \times 8$ resolution, the first dimension perpendicular to the plane of the coil. The multi-TR method was adapted from a previously published dual- T_R method⁶, acquiring spectra at eight TRs (0.5, 1, 2, 3, 4, 6, 8, 10 s), to ensure functional sensitivity to a broad range of flip-angles simultaneously. The number of averages at $k = 0$ was adjusted for each acquisition, to achieve similar high SNR for a “best possible”

validation; acquisitions times were between 80 and 120 min each (giving a total of 12.5 h run overnight). The partial saturation equation,

$$\frac{S(\theta, T_R, T_1)}{M_0} = \sin(\theta) \frac{1 - e^{-T_R/T_1}}{1 - \cos(\theta) \cdot e^{-T_R/T_1}}, \quad [7.8]$$

was fitted to the magnitude data to determine the flip-angle, γB_1^+ was calculated from the relationship $\theta = 180^\circ \cdot T_p \cdot \gamma B_1^+ / 500$ where T_p is the duration of the hard excitation pulse.

Bloch-Siegert acquisitions used the same parameters, with the optimized Fermi pulse inserted as in Figure 7.1a. Two acquisitions were made with the pulse placed at +2 kHz and -2 kHz offsets. T_R was 500 ms, with 13 averages at $k = 0$, resulting in a total duration of 2×14 min.

To avoid artefacts due to phase-wrap present in the raw phase maps, phase differences were calculated using complex division and the Matlab function `atan2()` that returns phase between $-\pi$ and π ,

$$\Delta\phi = \phi_1 - \phi_2 = \text{atan2}[\text{Im}(e^{i\phi_1}/e^{i\phi_2}), \text{Re}(e^{i\phi_1}/e^{i\phi_2})] \quad [7.9]$$

then γB_1^+ was computed using Eq. [7.3]. Voxels with $\Delta\phi < 0^\circ$ (indicating either very low γB_1^+ or the presence of uncorrected wrap artefacts in the phase difference map, i.e., true $\Delta\phi > 180^\circ$), voxels outside the phantom, or voxels with a $\text{CRLB}_\phi > 20^\circ$ (indicating low SNR) were excluded.

7.4.4 In Vivo Validation (Thigh)

Full details of the acquisition strategy analysis are given in “Simulation results” below (§7.5.1). Method A (dual-acquisition +/-) showed highest precision and accuracy and was used for all measurements in this work.

Bloch-Siebert B_1^+ -mapping was compared with a published dual-TR flip-angle mapping method⁶ in a healthy volunteer's quadriceps (male, 28 years). Acquisition parameters were: 300 μ s hard pulse excitation transmitted at 270 V, $200 \times 200 \times 200$ mm³ field of view, $16 \times 8 \times 8$ resolution (16 perpendicular to the coil), acquisition weighting, 6 kHz bandwidth and 2048 spectral samples.

In the dual-TR comparison, excitation was centred on α -ATP (rather than PCr), to avoid complications due to the creatine-kinase mediated exchange of PCr and γ -ATP.¹ A literature value of $T_1^{\alpha\text{-ATP}} = 1.8$ s was used to calculate the flip-angle according to the published method.²¹ Three acquisitions were made with TRs of 2.2 s, 0.73 s, and 0.37 s, using 10, 20, and 43 averages at the centre of k-space for acquisition times of 25, 15, and 15 min, respectively, as recommended in Chmelik *et al.*⁶

Two Bloch-Siebert acquisitions were made with the optimized Fermi pulse ($T_p = 3.5$ ms, $T_0 = 0.875$ ms, and $\alpha = 0.224$ ms) placed at +2 kHz and -2 kHz relative to PCr; excitation was centred at PCr. T_R was 500 ms, with 25 averages at $k = 0$, resulting in a total duration of 2×12.5 min.

Fitting was performed with AMARES in Matlab using standard prior knowledge (Appendix B.2), without fixed relative phases. γB_1^+ was calculated as described above

(§7.5.4). Voxels were excluded from the comparison if the voxel was centred outside the thigh, $\Delta\phi < 0^\circ$ or $\text{CRLB}_\phi > 10^\circ$.

7.4.5 Cardiac Scans

Cardiac ^{31}P Bloch-Siegert B_1^+ -mapping was demonstrated in five healthy subjects (male, 25–46 years, 75–85 kg, 1.79–1.93 m). The scan protocol followed Rodgers *et al.*¹⁶, with subjects positioned head-first supine.

Two Bloch-Siegert acquisitions were made with the optimized Fermi pulse placed at +2 kHz and -2 kHz from PCr, and excitation by a 200 μs hard pulse centred on PCr. The excitation pulse voltage was set to achieve a flip-angle of 30° at the mid-interventricular septum [the Ernst angle for a $T_R = 500$ ms and a PCr $T_1 = 3.09$ s].¹⁶ Acquisition parameters were: $240 \times 240 \times 200$ mm³ field of view, $16 \times 8 \times 8$ resolution (16 perpendicular to the coil), acquisition weighting with 20 averages at $k = 0$, 6 kHz bandwidth and 2048 spectral samples. Total scan time was 2×10.5 min.

Fitting was performed with AMARES in Matlab using standard prior knowledge (Appendix B.1), without fixed relative phases. γB_1^+ was calculated as described in “Uniform phantom validation” above. Voxels were excluded if $\Delta\phi < 0^\circ$ or $\text{CRLB}_\phi > 15^\circ$.

Phase wrap, caused by the upper output limit of Eq. [7.9] was unwrapped manually.

In three subjects, the scans were repeated with the central frequency adjusted to $\alpha\text{-ATP}$ to allow comparison of γB_1^+ values obtained using different peaks.

7.5 Results

7.5.1 Simulation Results

The precision and accuracy of the single-peak measurement strategies (A and B') are shown in Figure 7.2. Methods A and B' show <10% error and <10% uncertainty for $1000 \text{ Hz} < \omega_{\text{RF}} < 3000 \text{ Hz}$ and $\gamma B_1^+ > 150 \text{ Hz}$. Method B' has both precision and accuracy $\sqrt{2}$ worse than Method A. These calculations do not take into account the effects of direct excitation, which is shown in the Bloch simulations. The error caused by the direct excitation is generally larger than the underlying error arising from $\Delta\phi$, emphasizing the importance of effective pulse design.

The analysis of the multi-peak methods (B'' and C) results in smoothly varying functions (Fig. 7.3). Increasing $\gamma B_{1,\text{peak}}^+$ always improves the precision and accuracy. In Figure 7.3a (Method B'') minimizing $|\omega_i|$, maximizes ϕ_{BS} and improves the precision and accuracy, while adding additional peaks ($\omega_{2,3,\dots}$) with a larger $|\omega_{\text{RF}}|$ has only a small effect. In Figure 7.3c, Method C shows lower accuracy and higher bias than the other methods when all the offsets have the same sign. However, if the Bloch-Siegert pulse is placed symmetrically between two peaks, an identical profile to Method A is calculated with a $\sqrt{2}$ improvement in both accuracy and precision for a matched scan duration, although this will only be possible in situations where the inter-peak separation is sufficient to avoid direct excitation.

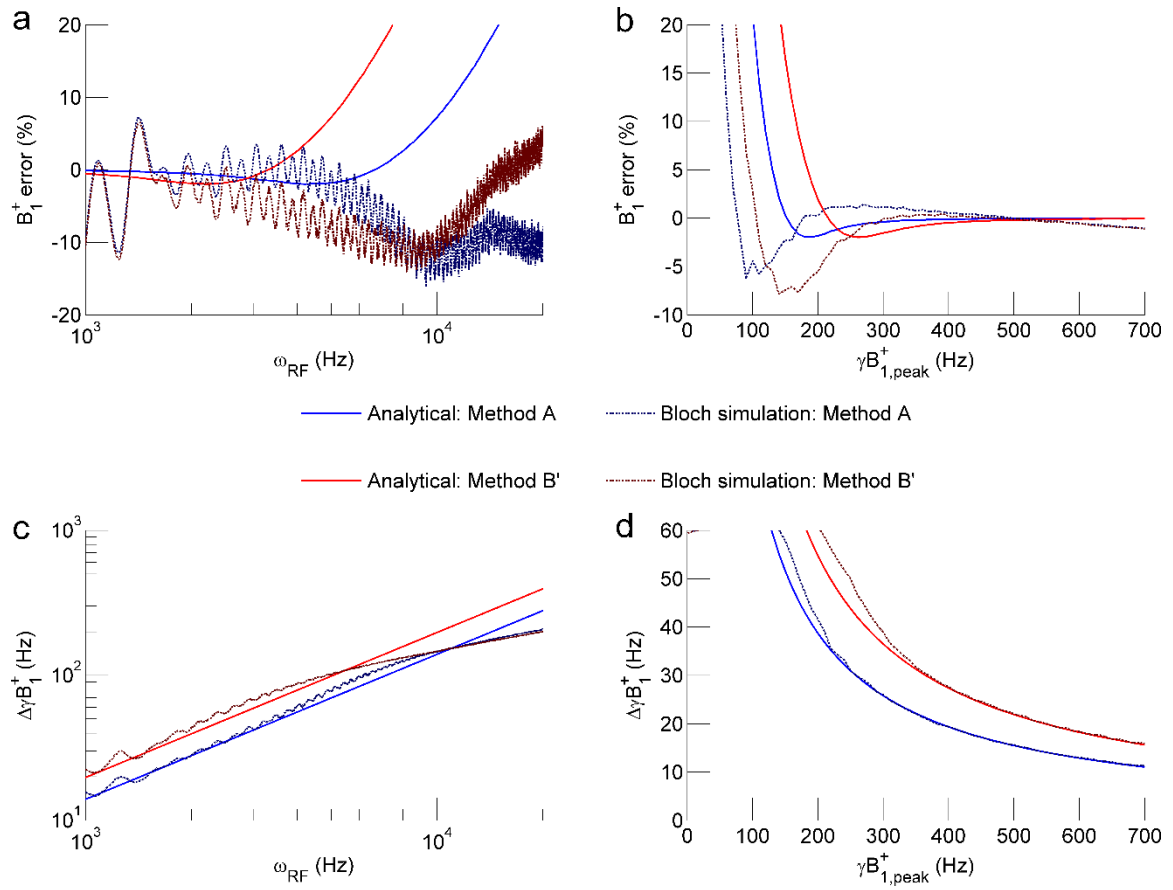


Figure 7.2 Precision and accuracy of the single-peak acquisition strategies [Method A (blue line) and Method B' (red line)] computed from the analytical expressions in Table 1. A numerical Bloch simulation of both methods, with the optimized Fermi pulse, is shown for comparison (dashed lines). Note that the differences apparent in the Bloch simulations in panel (a) and (b) arise due to direct excitation and correspond to the oscillations in B_1^+ fractional error in Figure 7.4c. The magnitude of the bias introduced scales with the simulation γB_1^+ (277Hz). The standard deviation calculated analytically is not affected by direct excitation. **a:** The fractional error, as defined by Eq. 7.4 of the simulated mean from the true γB_1^+ (277Hz) as a function of Fermi pulse offset from the central frequency ω_{RF} . **b:** The fractional error from $\gamma B_{1,true}^+$ as a function of $\gamma B_{1,peak}^+$. **c,d:** Standard deviation of γB_1^+ , $\Delta \gamma B_1^+$ as a function of pulse offset and $\gamma B_{1,peak}^+$.

Table 7.1 summarizes the methods considered for our 7T cardiac ^{31}P -MRS application.

All the measurement strategies show reasonable accuracy (<10% error) and most show an acceptable precision. Method A (dual-acquisition +/-) shows better precision and accuracy than any other method (0.2% error and 10% standard deviation). Methods B'' and C, were simulated with only positive offsets from the Bloch-Siegert pulse for our specific cardiac application. The greatest peak separation (PCr to β -ATP) in 7T ^{31}P -MRS is

1950 Hz, which is smaller than the separation required to maintain acceptable levels of direct excitation using the optimized 3.5 ms Fermi pulse. In Bloch simulation, using the optimized 3.5 ms pulse, a separation of 1950 Hz ($\omega_1 = -975$ Hz, $\omega_2 = 975$ Hz) gives a B_1^+ error of 19%.

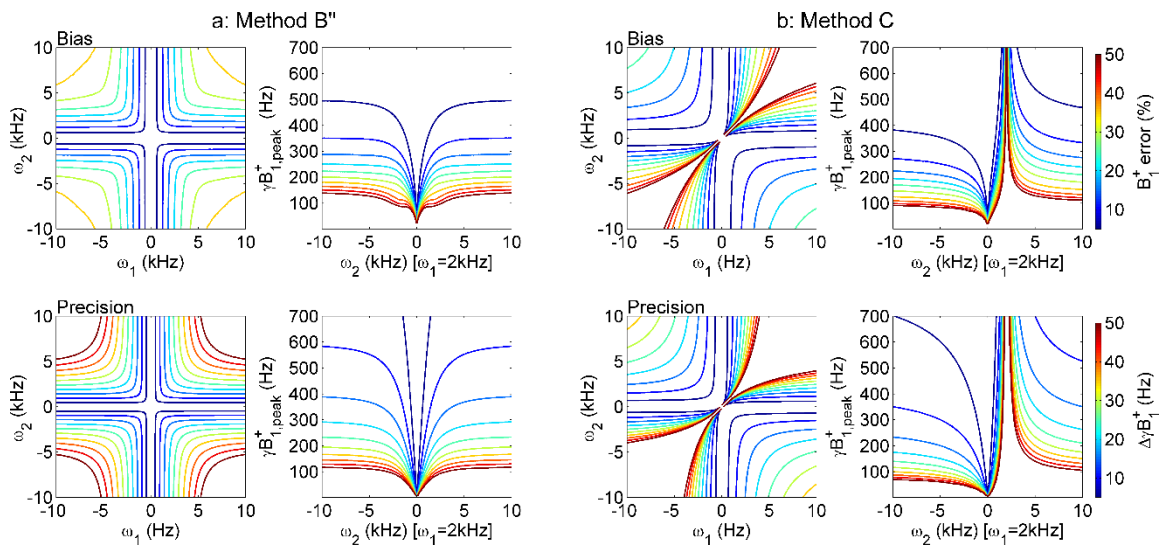


Figure 7.3 Fractional B_1^+ error (Eq. [7.4]), “Bias”, and standard deviation, “Precision”, of the multipeak acquisition strategies for a two peak spectrum. **a:** Method B^{''}. **b:** Method C. The error and standard deviation are plotted as functions of the individual peak offsets from the Bloch-Siegert sensitizing pulse, ω_1 and ω_2 ; and as the second peak position ω_2 (with ω_1 fixed at 2 kHz) and $\gamma B_{1,\text{peak}}^+$. The calculations do not take into account any direct excitation caused by the Bloch-Siegert sensitizing (Fermi) pulse, therefore, the optimum separation will be a compromise between minimizing excitation and minimizing error and standard deviation. Method C (multipeak single-acquisition) shows a $\sqrt{2}$ improvement over Method A when the Bloch-Siegert pulse can be placed symmetrically between the peaks. Method C has worse performance than Method B^{''} if the peaks are on the same side of the Bloch-Siegert pulse, while Method B^{''} is independent of the sign of ω_1 .

7.5.2 Pulse Design

The Fermi pulse selection criteria were to minimize the pulse duration whilst minimizing the direct excitation of the magnetization in the interval $1500 \text{ Hz} < \omega_{\text{RF}} < 2500 \text{ Hz}$.

The minimum of the direct excitation metric, ΔM_{xy} (Eq. [7.7]), fell below 0.01% for pulses with $T_p \geq 3.5$ ms (Fig. 7.4d). Thus the criteria, to select the shortest pulse whilst also minimizing direct excitation, were satisfied by selecting the T_0 and a parameters

corresponding to the ΔM_{xy} minimum of the parameter range with $T_p = 3.5$ ms. The resulting optimized Fermi pulse had $T_p = 3.5$ ms, $T_0 = 0.875$ ms, and $\alpha = 0.224$ ms, and was used for all experiments and simulations in this manuscript. Figure 7.4 shows a range of possible Fermi pulses, the degree of direct excitation, and the B_1^+ error from each.

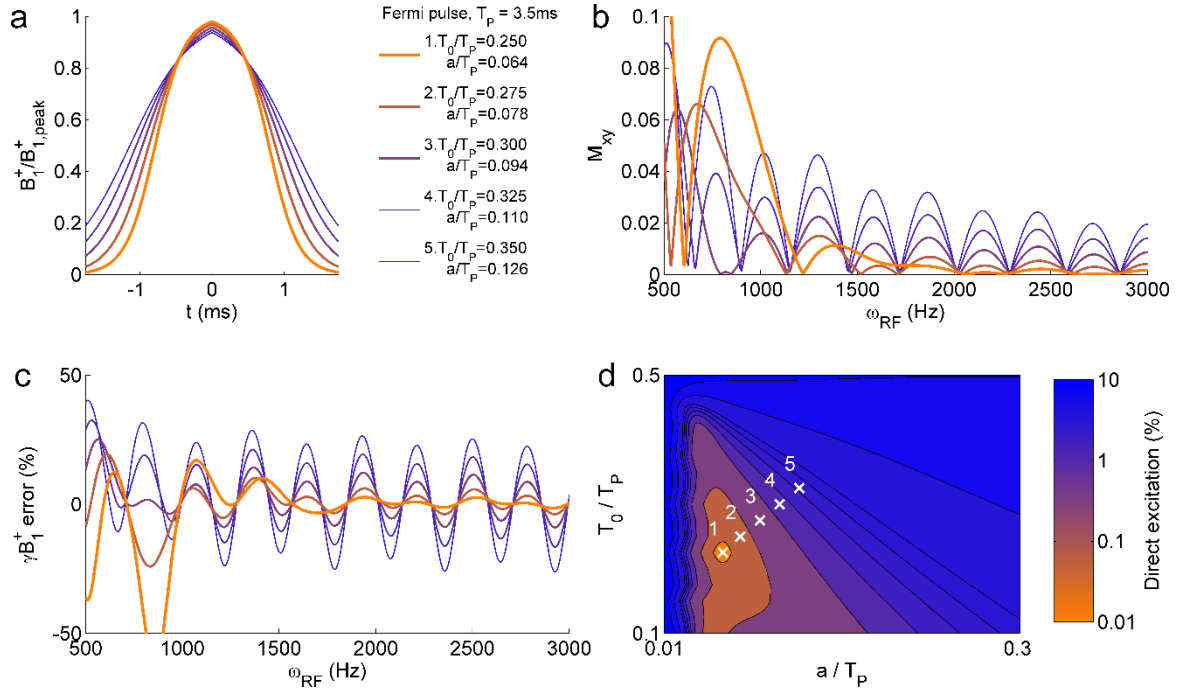


Figure 7.4 **a:** Example Fermi pulse envelopes, with fixed duration $T_p = 3.5$ ms, but varying the shaping parameters T_0 and a . **b:** Bloch simulation of five Fermi pulses showing the transverse magnetisation immediately after the pulse. $\gamma B_1^+ = 1000$ Hz. The orange line (1) is the pulse chosen for the experimental section of this work. **c:** Percentage error in calculation of γB_1^+ as a result of the direct excitation caused by the Fermi pulse. **d:** Percentage deviation of the transverse magnetisation from 0 as defined by Eq. 7.7 for a 3.5-ms Fermi pulse as a function of the shaping parameters T_0 and a . The “x” mark the locations of the pulses used to generate the lines in panels a–c.

7.5.3 Point-Source Phantom Validation

The phosphate peak phase and magnitude in the single voxel phantom are displayed in

Figure 7.5a. The predicted Bloch-Siegert phase, calculated using the B_1^+ separately

measured by nonlocalized, fully relaxed FIDs, and Eq. [7.1], and the directly measured

Bloch-Siebert phase of the single phosphate peak agree within 20% for $|\omega_{\text{RF}}| > 1000$ Hz and within 8% for $1000 \text{ Hz} \leq |\omega_{\text{RF}}| \leq 3000 \text{ Hz}$. As $|\omega_{\text{RF}}|$ increases from 1000 Hz the CRLB_ϕ is constant but the Bloch-Siebert phase ϕ_{BS} decreases, resulting in a higher relative uncertainty. When $|\omega_{\text{RF}}| < 1000$ Hz direct excitation of the phosphate peak by the Fermi pulse is observed, which causes the magnitude to drop substantially. For $|\omega_{\text{RF}}| > 1000$ Hz the magnitude varies by $< 1.5\%$, indicating that direct excitation has been minimized.

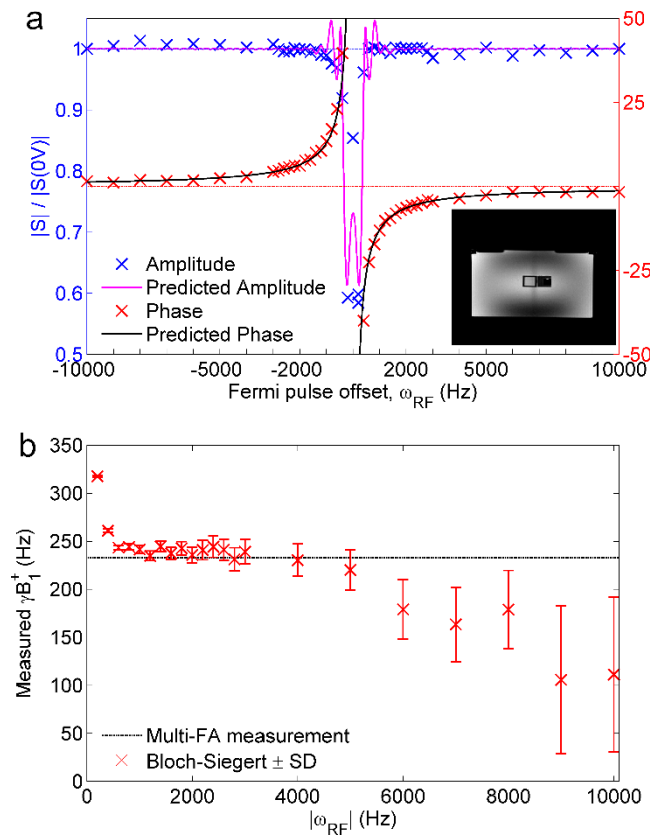


Figure 7.5 **a:** Fitted phase (red “x”) and amplitude (blue “x”) of a single peak in the presence of a Fermi pulse, as a function of Fermi-pulse offset. The amplitude shows saturation as the narrow passband of the Fermi pulse overlaps the single phosphate peak. The phase closely follows the phase predicted by Eq. [7.2] using a separate B_1^+ measurement (solid black). The measured amplitudes are closely approximated by a Bloch simulation of the pulse sequence (magenta). The inset shows a transverse ^1H image of the phantom, comprising a small cube of phosphate suspended in a tank of brine. **b:** γB_1^+ calculated using the pairs of symmetric offsets from the same experiment. The values calculated by the Bloch-Siebert method match those from a nonlocalized multi-flip-angle method in the range 500–4000 Hz.

Figure 7.5b confirms that γB_1^+ calculated from the Bloch-Siegert phases closely approaches that given by the Multi-FA reference method when $500 \text{ Hz} \leq |\omega_{\text{RF}}| \leq 4000 \text{ Hz}$.

7.5.4 Uniform Phantom Validation

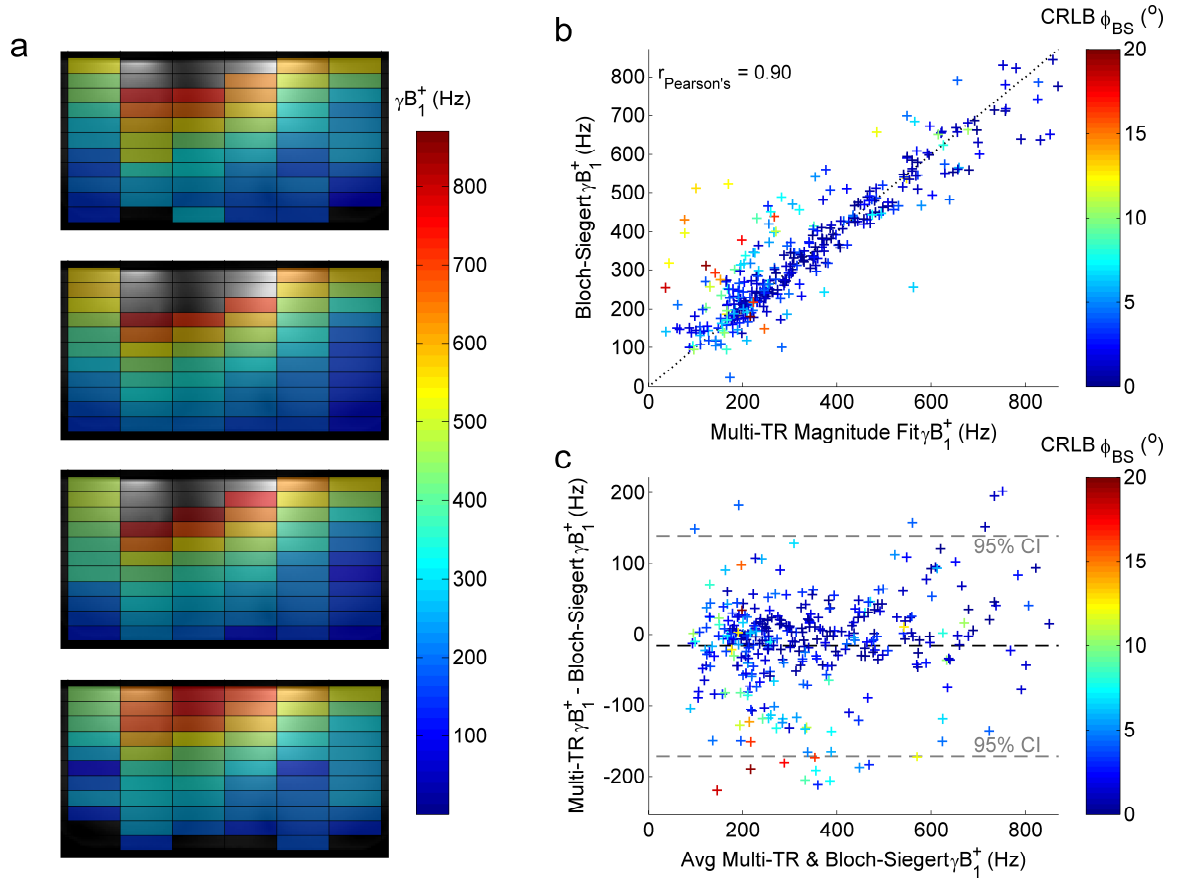


Figure 7.6 **a:** Bloch-Siegert B_1^+ maps, overlaid on ^1H localizer images, across four central slices of the $16 \times 8 \times 8$ CSI grid of the uniform phantom. Each rectangle indicates the measured value in a single ideal voxel. The maps are masked for $\text{CRLB}\phi$, phase wrap and by the limits of the phantom. **b:** Scatter plot of the per-voxel fitted multi-TR validation method against the value measured by the Bloch-Siegert method. The colour indicates the $\text{CRLB}\phi$ on the fitted phase difference and Pearson's correlation coefficient $r = 0.90$. **c:** Bland-Altman plot (average of two methods versus difference) of the validation and Bloch-Siegert methods. The colour indicates the $\text{CRLB}\phi$ on the fitted phase difference and lines show the mean difference and the $\pm 95\%$ confidence intervals.

Bloch-Siebert B_1^+ maps across the four central CSI slices covering the uniform phantom are shown in Figure 7.6a. The dynamic range of the Bloch-Siebert γB_1^+ was 0 Hz to 873 Hz (corresponding to a π phase shift). Figures 7.6b&c show the per-voxel correlation and Bland-Altman plots of the Bloch-Siebert method and the multi-TR method. A strong correlation (Pearson's $r = 0.90$) was observed. The Bland-Altman plot shows the Bloch-Siebert method measured γB_1^+ 15 Hz lower than the reference method on average, with 95% confidence intervals of ± 154 Hz. The bias and confidence intervals are equivalent to -3.1% and 31.8%, respectively, of the γB_1^+ value (484 Hz) of a centrally located voxel in the phantom.

7.5.5 In Vivo Validation (Thigh)

Figure 7.7 shows a Bloch-Siebert B_1^+ map across a central CSI slice covering a volunteer's quadriceps and example spectra from a centrally located voxel. The Bloch-Siebert γB_1^+ dynamic range was 0 Hz to 873 Hz. In the per-voxel correlation and Bland-Altman plots (not shown) of the Bloch-Siebert and Dual-TR methods⁶, good correlation is observed (Pearson's $r = 0.84$), despite the 37.5% scan time reduction from the reference to Bloch-Siebert method. The Bland-Altman plot shows a -25 Hz lower mean γB_1^+ from the Bloch-Siebert method compared with the Dual-TR method, with 95% confidence intervals of ± 211 Hz. The bias and confidence intervals are equivalent to -6.9% and 33.4%, respectively, of the γB_1^+ value (631 Hz) of a centrally located voxel in the skeletal muscle.

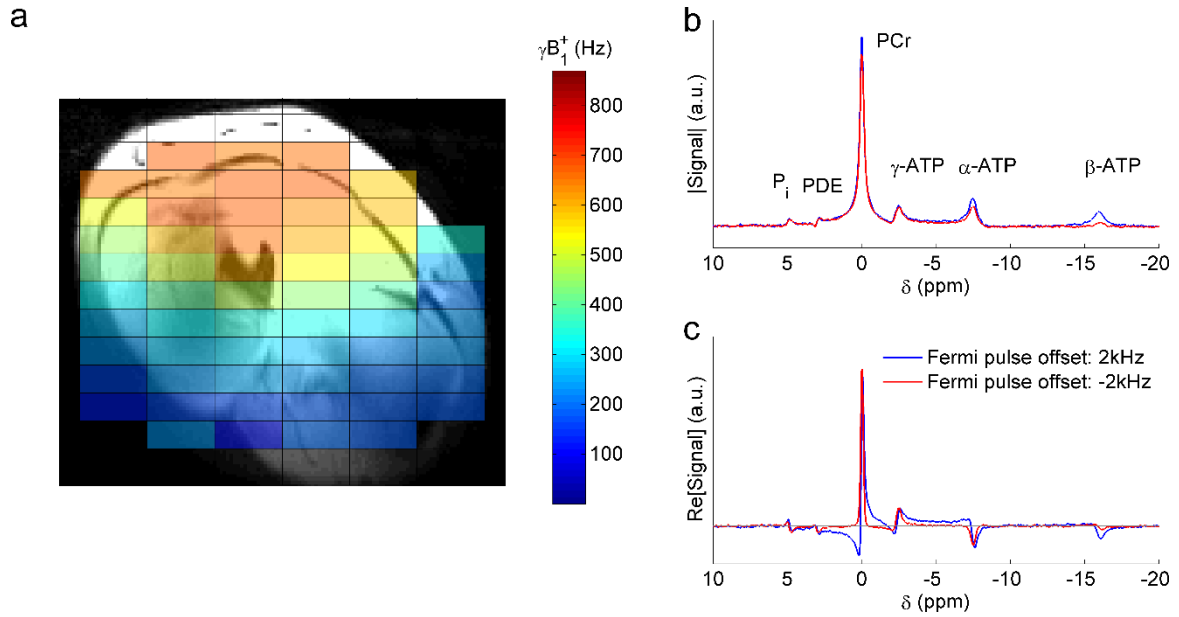


Figure 7.7 **a:** Bloch-Siegert B_1^+ map from a central slice of an $16 \times 8 \times 8$ CSI grid in a transverse plane of a healthy volunteer's quadriceps. The map is overlaid on a ^1H localizer registered to the CSI grid. **b,c:** Example magnitude and real spectra from a central voxel in **a**. The maps in **a** are calculated from the phase of the PCr peak. The phase difference between the PCr peak of the spectrum with a 2 kHz offset (blue) on the Fermi pulse and the -2 kHz offset (red) is 55.6° corresponding to a value of $\gamma B_1^+ = 484$ Hz.

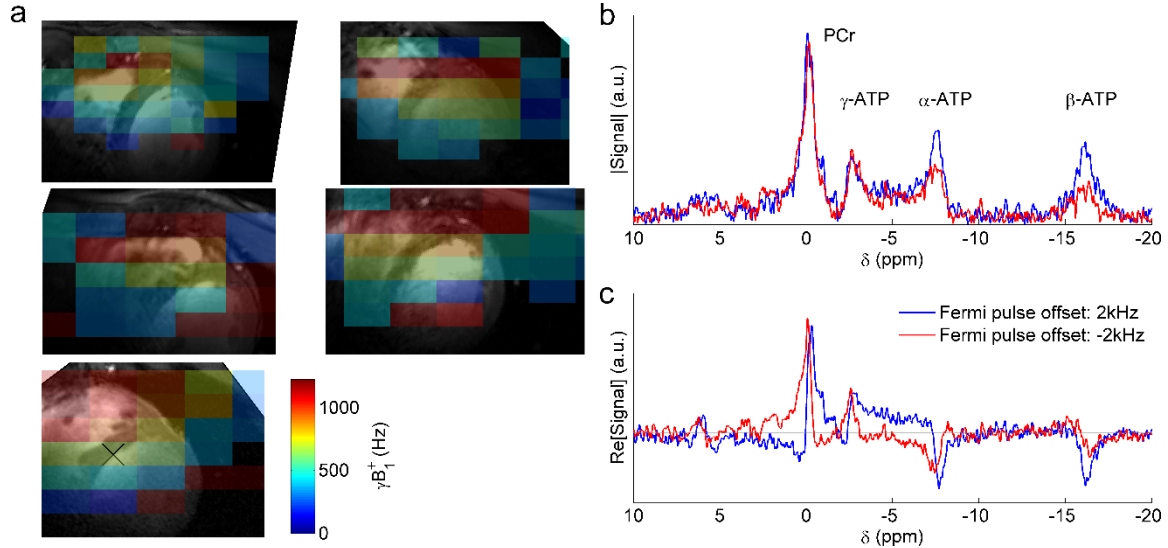


Figure 7.8 **a:** Bloch-Siegert B_1^+ maps from slices of an $16 \times 8 \times 8$ CSI grid in a mid-short axis plane of five healthy volunteer subjects. The maps are overlaid on ^1H localizers registered to the CSI grid. **b,c:** Example magnitude and real spectra from a mid-interventricular septal voxel, marked by a black "x" in the lowest B_1^+ map in **a**. The maps in **a** are calculated from the phase of the PCr peak. The phase difference between the PCr peak of the spectrum with a 2 kHz offset (blue) on the Fermi pulse and the -2 kHz offset (red) is 86.9° corresponding to a value of $\gamma B_1^+ = 605$ Hz.

7.5.6 Cardiac Scans

Five mid-septal cardiac γB_1^+ maps are shown overlaid on localizers in Figure 7.8. The Bloch-Siegert γB_1^+ dynamic range was 0 Hz to 1231 Hz. The short-axis localizers were manually segmented to extract voxels in the heart and skeletal muscle in the three CSI slices corresponding to the mid, apical and basal segments of the myocardium. In the mid-septal slices 39.6 ± 16.0 (mean $\pm$ SD) voxels were selected. After masking by CRLB_ϕ 30.6 ± 13.7 voxels remained. The B_1^+ maps are smoothly varying from 100–1200 Hz without visible boundaries between skeletal muscle, myocardium and the ventricular blood pools. B_1^+ drops-off with increasing distance from the coil. Data for the adjacent apical and basal slices, after masking by CRLB_ϕ , had 28 ± 10.1 and 21.6 ± 15.6 voxels suitable for analysis, respectively, which were used in the subsequent comparisons. Bloch-Siegert measurement using α -ATP instead of PCr is compared in Figures 7.9a,b. The mean Pearson's correlation was 0.62. The Bland-Altman plot shows a bias of 22.3 Hz with 95% confidence intervals of ± 561 Hz. This result confirms the T_1 -independence found in Sacolick *et al.*¹⁰ and is an indication that the B_1^+ values measured in this work are correct.

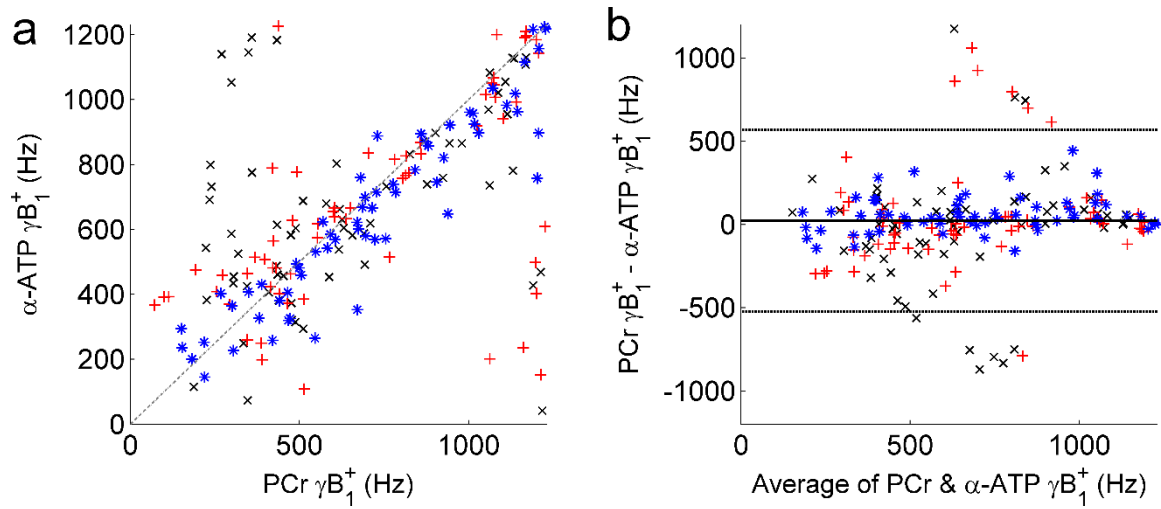


Figure 7.9 **a:** Three volunteer comparison of consecutive Bloch-Siegert B_1^+ maps, collected with the Fermi pulse placed symmetrically around the PCr peak and then subsequently the α -ATP peak in three of the subjects in the study. **b:** Bland-Altman plot of the metabolite comparison. The means and 95% confidence intervals are calculated from all points shown.

7.6 Discussion

In this study, I proposed a Bloch-Siegert spectroscopy B_1 -mapping technique suitable for ^{31}P -MRS in the human heart at 7T. It is straightforward to register the B_1^+ maps with other ^{31}P scans using the same 3D-CSI readout.

7.6.1 T_1 Insensitivity

Bloch-Siegert methods are known to be T_1 -insensitive.¹⁰ I verified this for our ^{31}P -MRS method by comparing B_1^+ maps derived from PCr ($T_1 = 3.09\text{s}$) and α -ATP ($T_1 = 1.39\text{ s}$) in three normal volunteers. This is an advantage over multi-TR and dual-angle methods, which rely on metabolite T_1 values to calculate B_1^+ .

In normal volunteers, one could use cardiac ^{31}P T_1 values from the literature in a multi-TR or dual-angle method.¹⁶ Yet, in studies of patients with cardiac disease, or in protocols involving exercise or pharmacological stress, these T_1 values may be different,

as has been reported for other nuclei.²² Recording 3D-localized myocardial T_1 values at 7T took 45 min in Rodgers *et al.*¹⁶—too long to be a calibration step in patients.

The problem of T_1 -changes in disease is particularly acute for the highest SNR peak: PCr. Chemical exchange through the creatine-kinase shuttle dominates the apparent T_1 s of PCr and γ -ATP,¹ and the forward rate constant for this process changes markedly in disease.²³ Therefore, T_1 -sensitive B_1 -mapping methods should only be applied to nonexchanging peaks such as α -ATP or β -ATP, but these peaks have lower SNR than PCr. In contrast, exchange mediated phase effects during the short (3.5 ms) Fermi pulse are predicted to be <0.1% by Bloch-McConnell simulation (not shown). Consequently, Bloch-Siegert methods can be applied to the highest SNR peak: PCr.

The Bloch-Siegert shift is independent of the excitation pulse or T_R of the host sequence.¹⁰ Therefore, a Bloch-Siegert B_1 -mapping sequence can be run with the Ernst flip-angle²⁴ and a short T_R to maximize its SNR efficiency (i.e. $SNR/\sqrt{t}$). Bloch-Siegert 1H MRI B_1 -mapping was shown to be more SNR-efficient than dual-angle B_1 -mapping.²⁵ Theory predicts a similar SNR-efficiency advantage with ^{31}P -MRS.

I believe that Bloch-Siegert B_1^+ -mapping approaches are the only known viable methods for human cardiac ^{31}P B_1^+ -mapping at 7T.

7.6.2 Bloch-Siegert Sampling Strategy

The framework to assess spectroscopic Bloch-Siegert measurements established that the dual-acquisition approach is optimal for cardiac ^{31}P -MRS, because there is insufficient frequency separation to place the Fermi pulse between PCr and β -ATP without creating artefacts due to direct excitation. In a situation where the peak

separation is much wider (4000 Hz) or where T_2^* is sufficiently long to permit longer, narrower bandwidth Bloch-Siegert sensitizing pulses, placing the pulse between two peaks will give an improvement in accuracy and precision of $\sqrt{2}$ over the analogous Method A measurement, or equivalently, it would achieve the same results but in half the total scan time. Furthermore, the single-acquisition Method C could still be attractive for time limited acquisitions, e.g., hyperpolarized ^{13}C -MRS.

7.6.3 In Vivo B_1 Maps

I believe these are the first ^{31}P -MRS B_1^+ maps made in the human heart at 7T.

Therefore, there was no gold-standard method to compare against in the heart at 7T. Instead, the methods were validated in phantoms and in the leg, where it was possible to obtain reference data with a published method. In both validation experiments, the Bloch-Siegert methods accurately matched the chosen reference methods across the whole dynamic range. The final cardiac maps present a physically reasonable range 100–1000 Hz of γB_1^+ (5–60 μT B_1^+) for the experimental setup; B_1^+ decreases smoothly with increasing distance from the surface coil, and repeated scans in three volunteers on PCr and then on α -ATP showed excellent reproducibility. Therefore, I deem that the implementation of a Bloch-Siegert B_1^+ -mapping sequence has been successful.

7.6.4 Limitations

The Fermi pulse optimization presented here is valid for the range of B_1^+ and SAR/ $(B_1^+)^2$ appropriate for our coil, and for T_2 values typical in the human heart. This optimization ought to be repeated for studies that have markedly different values for these parameters. This is because there is a trade-off between using short, high-

amplitude Fermi pulses which reduce the dead-time and minimize T_2 -induced signal losses but which give higher SAR and need to be placed at a greater offset frequency ω_{RF} for acceptable levels of direct excitation, and which, therefore, produce less Bloch-Siegert phase shift for a given B_1^+ (and hence lead to lower B_1^+ precision). Or conversely, long, low-amplitude Fermi pulses which suffer greater T_2 -induced signal losses but produce lower SAR and may be placed at a smaller offset frequency ω_{RF} for greater B_1^+ precision.

The 3D-CSI sequence localizes signals to comparatively large voxels. For a surface coil, there will be spins in each voxel that experience appreciably different B_1^+ , and, therefore, experience different Bloch-Siegert phase shifts. This causes intravoxel signal cancellation and, therefore, reduces the SNR efficiency of the Bloch-Siegert method. Furthermore, because the Bloch-Siegert phase depends on $(B_1^+)^2$, the measured voxel B_1^+ value will be a weighted average of B_1^+ within the voxel, not the mean voxel B_1^+ value. If a surface receive coil is used, then B_1^- will also vary across each voxel. B_1^- inhomogeneity will weight B_1^+ values measured by any method. To confirm that the additional B_1^+ weighting due to intravoxel difference in the Bloch-Siegert phase shift is small, 512^3 spin isochromats were simulated for a voxel 10 cm from the coil using the CSI protocol and RF coil described above. The Bloch-Siegert B_1^+ deviated from the B_1^- -weighted mean of the B_1^+ values in this voxel by <7%. The drop in SNR due to phase cancellation was <10% for $\gamma B_1^+ < 500$ Hz but increased to 50% at 1000 Hz. Note that this simulation is separate to the simulations described in the Theory section.

During the preparation of this work, a single voxel spectroscopy Bloch-Siegert B_1^+ measurement technique, based on Point RESolved Spectroscopy (PRESS) was published.²⁶ The PRESS Bloch-Siegert method allowed highly reproducible measurements of B_1^+ , using the ^1H water signal, in the hearts of six volunteers. The focus of this manuscript has been to implement these promising Bloch-Siegert methods in a multivoxel X-nuclear sequence, addressing the challenges of B_1^+ -mapping in the presence of low SNR and multiple significant peaks.

7.7 Conclusions

A Bloch-Siegert spectroscopy B_1^+ -mapping technique was implemented, which is the first method capable of measuring per-subject ^{31}P B_1^+ maps in the human heart at 7T in a clinically acceptable time. I have demonstrated the optimal measurement strategy for Bloch-Siegert spectroscopic B_1^+ -mapping and have numerically optimized a Fermi pulse for our application. Our method has been validated and shown to be accurate in phantoms and in skeletal muscle. It has been demonstrated successfully in the heart in a study on five normal volunteers.

7.8 Subsequent findings

7.8.1 Comparison with coil characterisation in phantoms

Fifteen in vivo cardiac B_1 -maps have now been collected in volunteers (2 female, 31 ± 8 years, 73 ± 8 kg) as part of studies described here and in Chapter 8, and using the 7T 10 cm loop coil for RF transmission. The average measured γB_1^+ in the mid interventricular septum was 721 ± 132 Hz (41.9 ± 7.7 μT). The septal voxels were an average 64 ± 11 mm

from the centre of the coil. Adjacent anterior and posterior voxels, located on average 58 ± 18 and 76 ± 10 mm from the coil, experienced γB_1^+ field strengths of 1023 ± 156 Hz (59.4 ± 9.1 μ T) and 513 ± 265 Hz (29.8 ± 15.4 μ T) respectively.

The in vivo field measurements are approximately twice the magnitude of the single voxel phantom measurements (Chapter 2, Figure 2.4) at the same coil-to-voxel distance (Figure 7.10a). Some of the differences may be explained by the different loading of the coil by individual subjects versus the phantom (§2.5.1.4). To assess this, the measured γB_1^+ values were divided by the reference voltage in the reference fiducial attached to the rear of the coil (Figure 7.10b). This relies on an assumption that changes in loading scale B_1^+ by the same factor at all points in space.

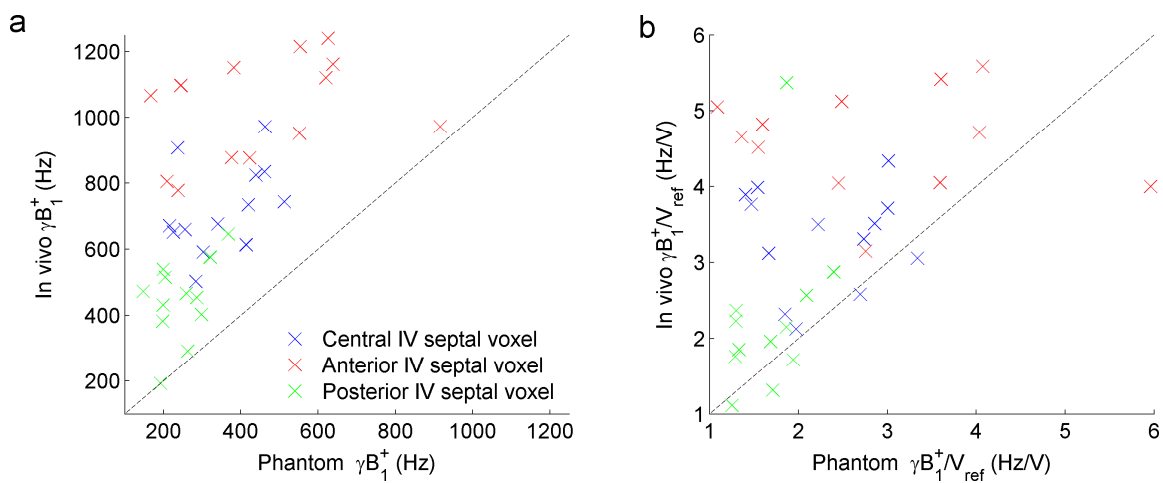


Figure 7.10 **a:** Comparison of in vivo Bloch-Siebert γB_1^+ from septal voxels in 13 volunteers acquired with the 10 cm loop coil, against γB_1^+ at the corresponding location in the phantom (see §2.3.2). **b:** The same data scaled by the reference voltage at the coil fiducial, which was measured for each subject. If coil loading acts as a single multiplicative factor, then (b) ought to be a straight line. The fact that they do not indicates that the *shape* of the B_1^+ field is different in vivo and in this phantom.

The remaining differences could possibly be explained by off-axis shifts of the in vivo voxels, variable rotation of the coil towards the B_0 axis, or the effect of varying electrical properties on the shape and magnitude of the transmit field.²⁷ Whilst the higher field

measured in vivo may be beneficial for developing future experiments, these experiments highlight the importance of direct in vivo measurement of the B_1^+ field.

7.8.2 Comparison with Biot-Savart law field simulations

The same Bloch-Siegert field maps used in §7.8.1 were compared with the scaled Biot-Savart B_1 field estimates (Chapter 2). Thirteen of the datasets were collected with data to enable comparison with Biot-Savart field maps. In these datasets, Bloch-Siegert measured γB_1^+ values from 75 voxels, in a 5x5x3 grid around a central interventricular septal voxel, were compared with the value estimated by the scaled Biot-Savart method (i.e. a computation at 0Hz that therefore neglects conductivity and permittivity effects). The Biot-Savart values were scaled to match the Bloch-Siegert pulse voltage. Data points were excluded if the Bloch-Siegert values had relative standard deviation estimates greater than 30%.

The comparison of the values made in 554 voxels had an $R^2 = 0.03$. A Bland-Altman plot (Figure 7.11) displayed a bias of -329 Hz with 95% confidence intervals of 629 Hz (300 and -958 Hz). In other words, the scaled Biot-Savart method is a very poor predictor of B_1^+ in vivo at 7T.

Whilst both the Biot-Savart and Bloch-Siegert methods estimate values for the in-vivo B_1^+ of the same order of magnitude. The low correlation indicates that the shape of the Biot-Savart predicted field diverges significantly from that measured by Bloch-Siegert mapping. A possible reason for this is the effect of the subject dielectric properties of the sample, which at high field (and high frequency) increasingly affect the spatial

distribution of B_1^+ (and B_1^-).²⁷⁻²⁹ The fiducial cannot capture changes in the spatial profile of B_1^+ and B_1^- at other locations.

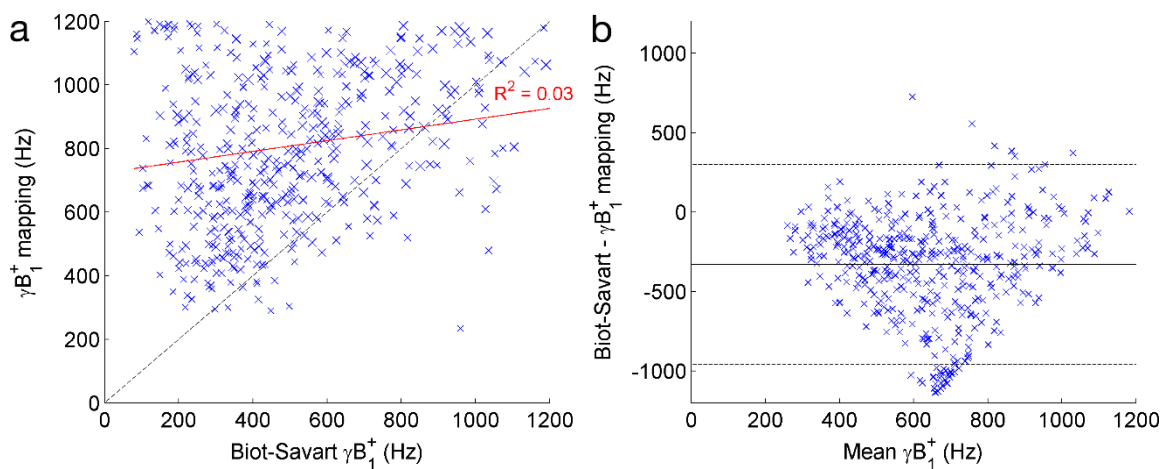


Figure 7.11 Correlation (a) and Bland-Altman (b) plots comparing the γB_1^+ values estimated by the scaled Biot-Savart method and the Bloch-Siegert B_1^+ -mapping method.

Biot-Savart estimates provide a basic method to estimate the flip-angle and saturation correction. However, in light of this data, it appears that the Biot-Savart correction method is not appropriate for the 10cm loop coil at 7T. More work will be required to enable accurate saturation correction for 31P-MRS at 7T.

7.9 Future work

Use of the Bloch-Siegert B_1 mapping sequence in a broader range of subjects, including repeated measurements on the same subject, have brought additional insights into the combination of the Bloch-Siegert technique with CSI localisation (in §§7.8.1-2). As discussed above, it is apparent that the typical B_1^+ of the 7T 10cm loop coil is significantly higher than was estimated in the simulation section of this chapter: the average of $\gamma B_1^+ / V_{\text{RMS}}$ in the mid interventricular septum of 15 subjects results in a B1-efficiency of 2.9 Hz V⁻¹ (1.19 $\mu\text{T W}^{-1/2}$) rather than the values of 1 Hz V⁻¹ (0.41 $\mu\text{T W}^{-1/2}$)

used in the simulations above. The higher B_1^+ results in greater phase cancellation due to intra-voxel dephasing and results in phase wrap occurring close to the VOI. With this updated information it would be valuable to re-optimize the Fermi pulse, shortening the length of the pulse to decrease dephasing due to T_2^* relaxation, while ensuring that a low direct excitation is maintained.

Observations of peak amplitudes in Bloch-Siegert sensitised CSI sequences have found that there is a difference, by up to 30%, in PCr amplitude between the positive and negative Fermi pulse frequency offsets. With surface coils this amplitude difference is observed in vivo and in large uniform phantoms but not in the point source phantom (as in Figure 7.5); yet it was not observed in any phantom or subjects with a volume coil at 3T. In addition the sign of the difference is dependent on the particular coil (i.e. for the 10cm loop the positive offset scan has higher amplitude, but for the 16 channel array the negative pulse offset produces a higher amplitude). It is possible that the change in amplitude is a function of intra-voxel dephasing and is effected by the interaction between the Bloch-Siegert induced phase and that of the coil's receive B_1^- field.

However, initial experiments have shown that the effect persists even for high resolution CSI matrices where intravoxel dephasing should be reduced. More investigation is required to account for the effect and mitigate the SNR penalty in the scan with decreased amplitude. The validity of the B_1^+ measurement, confirmed by the comparison between the multi-TR method and the Bloch-Siegert method in the uniform phantom which experiences this effect, remains valid.

Future development of the pulse sequence centres on creating a single interleaved acquisition, with positive and negative offset Fermi pulses alternating every transient.

This would simplify offline reconstruction, enable the possibility of online reconstruction and minimise the effect of physiological variation (movement, temperature changes etc.) on the sequence.³⁰ The requirement to measure only a single resonance, and the degradation of Bloch-Siegert B_1 maps at low spatial resolution, make the Bloch-Siegert sequence a candidate for ^{31}P imaging, which has been demonstrated in skeletal muscle at 7T.^{31, 32} For successful ^{31}P imaging in the heart, B_0 inhomogeneity must be minimised, and a reliable ^{31}P refocusing pulses must be designed.

7.10 References

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Chapter 8: Creatine kinase measurement at 7T

8.1 Introduction

This chapter deals with the implementation of a modified four-angle saturation transfer (FAST) method for the measurement of myocardial k_f^{CK} at 7T. The implementation is built according to the aims set out in Chapter 5, comprising a method built around the existing UTE 3D-CSI sequence, and achieved in a scan time acceptable for research in volunteers and patients with mild-to-moderate heart disease, i.e. 90min. This chapter builds on the observations made at the end of Chapter 5, where it was established that knowledge of the excitation flip-angle is essential for k_f^{CK} measurement at 7T. This can be achieved either measuring it or by using B_1 -insensitive pulses. Here, we use the new Bloch-Siegert B_1 -mapping protocol from Chapter 7 to measure B_1^+ in the heart.

This chapter describes our aims for a 7T CK measurement and presents a systematic literature review of the previous implementations of the FAST method.¹

The second part presents simulations and preliminary experiments, before introducing a novel “BOAST” hybrid of FAST with Bloch-Siegert B_1 -mapping.

The chapter closes with a validation study in skeletal muscle of 2 volunteers and in the heart of 10 volunteers.

8.2 The FAST method

The Four-angle Saturation Transfer (FAST) method was developed at Johns Hopkins University for skeletal muscle and cardiac creatine kinase k_f and ATP flux measurement at 1.5T.¹ It has subsequently been deployed in a number of studies at the same site in

patients with heart failure (HF), hypertrophic cardiomyopathy (HCM) and after myocardial infarction (MI).²⁻⁴ These studies established the first measurements of the creatine kinase forward rate constant in the in vivo human myocardium. The average k_f^{CK} value for a normal population across the three studies (n=45) is $0.32 \pm 0.04 \text{ s}^{-1}$. The value reported across two studies for subjects with chronic heart failure (n = 27) was $0.19 \pm 0.05 \text{ s}^{-1}$. The k_f^{CK} reported for patients with left ventricular hypertrophy (LVH), but no heart failure (n=10) was $0.36 \pm 0.04 \text{ s}^{-1}$ and for post myocardial infarction (MI) (n=15) $0.31 \pm 0.08 \text{ s}^{-1}$, neither were significantly different from the normal population.

These studies also used the FAST k_f^{CK} and the PCr concentration⁵ to compute the creatine kinase flux, that is the total amount of ATP created per second through the CK shuttle mechanism. Values for a normal and chronic heart failure population are $3.2 \pm 0.5 \text{ mmol kg wwt}^{-1} \text{ s}^{-1}$ and $1.25 \pm 0.3 \text{ mmol kg wwt}^{-1} \text{ s}^{-1}$, thereby increasing the statistical significance between the groups by using ATP flux rather than k_f^{CK} . The sensitivity increase, by combining both concentration and rate information, shows a significant difference (in CK flux) between normal subjects and patients with LVH and normal subject and patients post MI (see Table 8.1).

Ref	Group	N	PCr/ATP	[PCr] mmol kg ⁻¹	[ATP] mmol kg ⁻¹	k _f s ⁻¹	CK flux mmol kg ⁻¹ s ⁻¹
4	Normal (Rest)	16	-	10.1±1.3	5.7±1.3	0.32±0.07	3.2±0.9
4	Normal (Stress)	6	-	9.9±1.2	5.6±1.4	0.33±0.09	3.3±1.2
4	CHF	17	-	8.3±2.6	5.2±1.3	0.21±0.07	1.6±0.6
3	Normal	14	1.9±0.3	9.4±1.1	5.5±1.3	0.32±0.06	3.1±0.8
3	LVH	10	1.3±0.3	6.1±2.0	4.7±1.3	0.36±0.04	2.2±0.7
3	LVH+CHF	10	1.3±0.3	7.2±3.7	5.0±1.1	0.17±0.06	1.1±0.4
2	Normal	15	1.9±0.5	9.6±1.1	5.5±1.3	0.33±0.07	3.2±0.8
2	MI	15	1.7±0.3	5.4±1.2	3.4±1.1	0.31±0.08	1.7±0.5
All†	Normal	45	-	9.7±0.7	5.6±0.8	0.32±0.04	
All†	CHF	27	-	7.9±2.1	5.1±0.8	0.19±0.05	1.3±0.3

Table 8.1 Summary of results from the FAST method combined with absolute concentration measurements in references 2-4. †Weighted arithmetic means of the normal and chronic heart failure (CHF) groups from all three studies.

FAST protocol and data analysis

The original implementation of FAST used B_1^+ -insensitive BIR-4 (or BIRP⁶) pulses to produce constant flip-angles of 15° and 60° in spite of the inhomogeneous B_1^+ from a surface coil. Pulse-acquire experiments with each flip-angle were repeated twice: first with saturation of the γ -ATP peak, second with saturation mirrored around the PCr peak (“control saturation”), to produce two separate dual-angle T_1 measurements.⁷ In this way, the relaxation time under steady state saturation (T_1') and control saturation (written simply as “ T_1 ” in this Chapter), may be calculated thus:

$$T_1' = -T_R / \ln \left[\frac{\sin \alpha - R' \sin \beta}{\cos \beta \sin \alpha - R' \cos \alpha \sin \beta} \right], \quad [8.1]$$

where $R' = M'(\alpha)/M'(\beta)$, is the ratio of the partially-saturated PCr amplitudes with the two flip-angles α and β . T_1 may be determined analogously from R . The appropriate version of Eq. [8.1] may be substituted into the partial saturation equation (Eq. [1.48]) to calculate M_0 and M_0' values from either of the PCr amplitudes $M(\alpha)$ or $M(\beta)$ ($\alpha = 15^\circ, \beta = 60^\circ$) recorded under the same saturation conditions:

$$M_0 = \frac{M(\alpha)[\cos\beta - \cos\alpha]}{\sin\alpha[\cos\beta - 1] - R \sin\beta[\cos\alpha - 1]}, \text{ or } \frac{M(\beta)[\cos\alpha - \cos\beta]}{\sin\beta[\cos\alpha - 1] - \sin\alpha[\cos\beta - 1]R^{-1}}. \quad [8.2]$$

The equivalent expression for M_0' is formed by substituting R' for R and M' for M .

Subsequently k_f^{CK} may be calculated from Eq. [5.30]:

$$k_f^{\text{CK}} = \frac{1}{T_1'} \left(1 - \frac{M_0'}{M_0} \right).$$

In the original cardiac application of FAST, localisation was by 1D-CSI. There is, however, no fundamental limit to the localization used with FAST.

8.2.2 The “BOAST” (Bloch-Siebert + FAST) method

As discussed in Chapter 2 and Chapter 5, arbitrary flip-angle adiabatic pulses are not feasible to apply in the heart using the surface coils available in our lab at 7T.

Furthermore, Biot-Savart flip-angle estimation is inaccurate (§5.4.3.2). Thus the strategy for 7T implementation must be to measure the flip-angle or B_1^+ in the heart.

Chapter 7 introduced Bloch-Siebert B_1^+ mapping for ^{31}P -MRS. It is possible to obtain Bloch-Siebert B_1^+ maps with the same CSI parameters as desired for the four FAST acquisitions, thus avoiding issues with spatial registration.

We propose the following “BOAST” (Bloch-siebert fOur Angle Saturation Transfer) protocol to measure 3D-resolved k_f^{CK} in the heart at 7T:

1. ^{31}P B_1^+ map:
 - a. Positive-offset Bloch-Siebert acquisition;
 - b. Negative-offset Bloch-Siebert acquisition;
2. Calculate transmit voltages required for chosen excitation pulse to achieve desired α and β flip angles in target voxels;
3. Optional unsaturated acquisition at α or β flip angle for frequency calibration and direct saturation measurement;
4. Acquisition at β flip angle with γ -ATP saturated;
5. Acquisition at β flip angle with control saturation;
6. Acquisition at α flip angle with γ -ATP saturated;
7. Acquisition at α flip angle with control saturation;
8. Calculate k_f^{CK} using Eqs. [8.1], [8.2] and [5.30].

Despite the inhomogeneous transmit profile of surface coils, this protocol facilitates the measurement of k_f^{CK} values across a range of CSI localised voxels, without the use of adiabatic pulses. However, as detailed below (§8.2.4.3), the precision of the measurement within any one voxel is dependent on the actual flip-angles achieved for the α and β acquisitions, which may be set exactly for one voxel, but which will vary according to the coil transmit profile elsewhere.

8.2.3 Aims for 7T

The move from 3T to 7T field strength scanners brings an SNR increase of 2.8x to cardiac ^{31}P -MRS.⁸ In regards to creatine kinase k_f^{CK} measurements, this increase in SNR might be used to:

- Increase the precision of k_f^{CK} value compared to lower field scans;
- Decrease the total acquisition time, keeping the same precision;
- Increase the spatial resolution and/or extend the CSI localisation to more dimensions i.e. 2D or 3D-CSI;
- Or some balance of the above.

A brief qualitative description of each of these options is given below. The primary concern is the validity of the Bloch-Siegert B_1^+ mapping sequence if used with low resolution or low dimensional CSI sequences. The measurement bias introduced to the B_1^+ measurement as a result of low spatial resolution is detailed in section 8.2.4.4, and is also extended to the analysis of the errors introduced in k_f^{CK} . The consequence of these experiments and simulations is to restrict the Bloch-Siegert method to 3D-CSI with voxel dimensions less than 15 mm in each direction.

Regardless of the necessity for 3D localisation (3D-CSI or 2D-CSI + slice selection) with Bloch-Siegert B_1^+ mapping, it is also desirable to reduce spectral contamination from non-myocardial tissue e.g. blood, skeletal muscle and liver. Additionally the reduced voxel size of 3D-CSI compared to 1D-CSI will tend to reduce B_0 inhomogeneity across the voxel, resulting in an increase of T_2^* and in turn a reduction of metabolite linewidth, reducing the fitting uncertainty.

Finally the ability to shorten the sequence may be limited by: the minimum repetition time required for sequence components (§8.1.1.2), the minimum saturation pulse duration (§8.2.4.2), or the minimum number of sequence averages to form an SNR-efficient acquisition weighting profile (§2.4.1.2).

8.2.4 Protocol design

8.2.4.1 RF coil

For the implementation of BOAST, the ideal transmit element would have a high $B_{1,\text{peak}}^+$ and $B_{1,\text{peak}}^+ / \sqrt{\text{SAR}}$, for maximum Bloch-Siegert precision (Chapter 7), and a homogenous B_1^+ transmit field, to maximise the volume experiencing the optimised target flip-angle (§8.2.4.3). The ideal receive element maximises the receive sensitivity across the myocardium, for increased spectral SNR.

The transmit element of the 16 channel array has insufficient $B_{1,\text{peak}}^+$ to use it in combination with the Bloch-Siegert method, despite it having the most homogeneous B_1^+ and the highest receive SNR of the coils.

The quadrature coil has higher $B_{1,\text{peak}}^+$ and $B_{1,\text{peak}}^+ / \sqrt{\text{SAR}}$ than the 10cm loop coil, which would increase the precision of the Bloch-Siegert calculated flip-angle maps, but its lower receive SNR reduces the range of flip-angles (as described in §8.2.4.3), around the target α and β values, which result in acceptable k_f^{CK} precision. The 10 cm loop was chosen as a compromise between these extremes.

8.2.4.2 Selective saturation pulse

The original implementation of FAST, at 1.5T, used a continuous low-power (5-10 Hz γB_1^+) hard pulse for frequency selective saturation of the γ -ATP peak.¹ However, a

maximum limit on the RF amplifier un-blank time on a Siemens Magnetom 7T MRI scanner, renders continuous-wave pulses impossible.

In more recent saturation transfer methods frequency selective saturation has been achieved using amplitude-modulated DANTE pulse trains.^{9,10} Pulse trains comprise a series of identical and equally-spaced pulses. Popular choices include Hann-windowed hard pulses, sinc pulses and hyperbolic secant pulses. Amplitude modulation means that a repeating pattern of different amplitudes is applied for the pulse train. This is equivalent to multiplying the pulse train by an envelope function, which in the small-tip-angle limit is equivalent to convolving the Dirac-comb pattern of the DANTE frequency-response with the Fourier transform of the envelope function.

Bloch simulations and phantom tests established that a fixed amplitude DANTE train ($m = 1, T_{\text{on}} = 100 \mu\text{s}, T_{\text{off}} = 230 \mu\text{s}$) provided saturation across the range of B_1^+ values expected in the interventricular myocardium ($50 - 800 \text{ Hz } \gamma B_1^+$) whilst maintaining a Q-factor (the effect of undesired off-resonance saturation) of >0.95 at a 289 Hz (2.4 ppm) frequency offset, i.e. the PCr to γ -ATP separation at 7T. The complete saturation of γ -ATP ($S_{\text{sat}}/S < 0.1$) and Q-factor of 0.97 - 0.95 was confirmed in skeletal muscle and myocardium at 7T (§8.4).

8.2.4.3 Optimal flip-angles and repetition times.

The two sets of paired scans in the FAST protocol are used to calculate two effective T_1 values using the dual angle method (DAM). The DAM is popular because it does not require either an inversion of the target metabolites (required for the “gold standard” method of inversion recovery [IR]); or their saturation (required for saturation recovery [SR]).¹¹⁻¹³ IR, SR and the DAM all require a minimum of two acquisitions, though they are

often run with more to increase their sensitivity to a range of T_1 values.¹⁴ However, all except the DAM usually wait for (nearly) full relaxation of the longitudinal magnetisation between each transient, therefore the DAM, which allows $T_R \ll 5T_1$, is one of the most efficient methods known.⁷

In theory the DAM (also known as multiple flip-angle spoiled gradient echo (SPGR)), allows calculation of T_1 s with any pair of flip angles (α , β) as long as the conditions $\alpha \neq \beta$ and $\alpha, \beta \neq 0^\circ, 180^\circ$ are satisfied. However, with noisy data the precision and accuracy of the DAM will vary as a function of both flip-angles, the T_R and T_1 .

In the original FAST implementation the α and β flip-angles and T_R were selected using a combination of quantitative calculations of SNR and qualitative arguments. For this work the flip-angles and T_R were re-optimised to find the sequence parameters which give the maximum k_f^{CK} measurement precision and minimum bias. Due to the use of conventional amplitude-modulated (i.e. not adiabatic) excitation pulses this optimisation must take into account the range across which the measurement will work and how the ratio of the chosen flip angles $m = \beta/\alpha$ ($m > 1$ without loss of generality) affects this.

The optimisation of the sequence parameters was achieved using Monte-Carlo Bloch simulations of the DAM section of the FAST protocol. The sequence was Bloch simulated without localisation, for two exchanging peaks at a range of repetition times (0.3 – 2 s) and flip angles (1 – 180°), with control- and on-resonance saturation. Other parameters were matched to the best known values for PCr and γ -ATP (Chapter 5, Table 5.1). The resultant magnetisation was then used to generate a distribution of values (50000 points) at each combination of input variables. The standard deviation of the

distribution was set equivalent to CRLB measured with the 10-cm loop coil previously, and scaled appropriately for the variable T_R .⁸ Finally the values of T_1^{PCr} , M_0^{PCr} and k_f^{CK} were calculated for each repetition, in each distribution, separately, and means and standard deviations calculated.

The Monte-Carlo simulations were validated against analytical expressions (ref. 15 Eq. [9]) for the precision of DAM to measure T_1 relaxation constants. The maximum T_1 measurement precision estimated by the Monte-Carlo sequence simulation closely matched that predicted by the analytical method (Figure 8.1a). The distance between the optimum flip-angles for a set repetition time and T_1 value as calculated by the analytical method and the Monte-Carlo method was $3 \pm 1^\circ$. Examples of the Monte-Carlo optimisation plots, both with and without exchange ($k_f^{\text{CK}} = 0 \text{ s}^{-1}$ and $k_f^{\text{CK}} = 0.32 \text{ s}^{-1}$) are shown in Figure 8.1b.

An example of the simulated data is shown, for a range of T_R values, in Figure 8.2. L-curve analysis (Figure 8.3a), analysis of the minimum bias (Figure 8.4a) and standard deviation (Figure 8.4b), and the width of regions with acceptable bias and standard deviation (Figure 8.4c) showed that lower T_R values gave higher precision and accuracy. β/α ratios of 4 and 5 give similar results (Figure 8.3b), but a ratio of 4 was chosen as it gives a (slightly) larger acceptable range of B_1^+ values, relative to the target. The optimal pair of flip-angles was chosen as the point with minimum standard deviation with a bias less than 5% greater than the minimum bias. Setting a minimum T_R of 400 ms (see §8.1.1.2), the optimal protocol parameters were $T_R = 0.4 \text{ s}$, $\alpha = 16^\circ$, $\beta = 64^\circ$. This choice of flip-angles is close to that of Bottomley and Ouwerkerk⁷, although our T_R is much shorter.

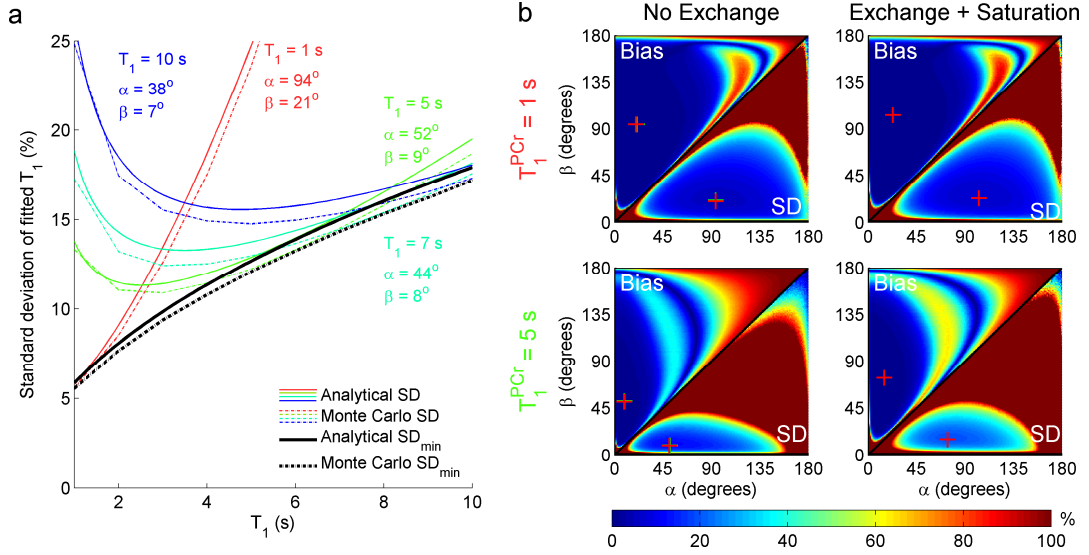


Figure 8.1 **a:** Validation of the Monte-Carlo simulations of the dual-angle method scans in the FAST protocol against analytical expressions (ref. 15 Eqs. [9]&[13]) for the precision of the DAM. Each coloured set of lines correspond to the optimal pair of flip angles for a particular T_1 value. The thick black lines are the minimum standard deviation possible at a certain T_1 . **b:** Colour plots of the accuracy (Bias) and precision (SD) as calculated by the Monte-Carlo method. The minima for the precision (red +) match closely the values calculated by the analytical expressions and also lie in regions of low bias. Note that since $m > 1$ by definition, we have saved space by combining the plots for each Bias and SD pair in a single square.

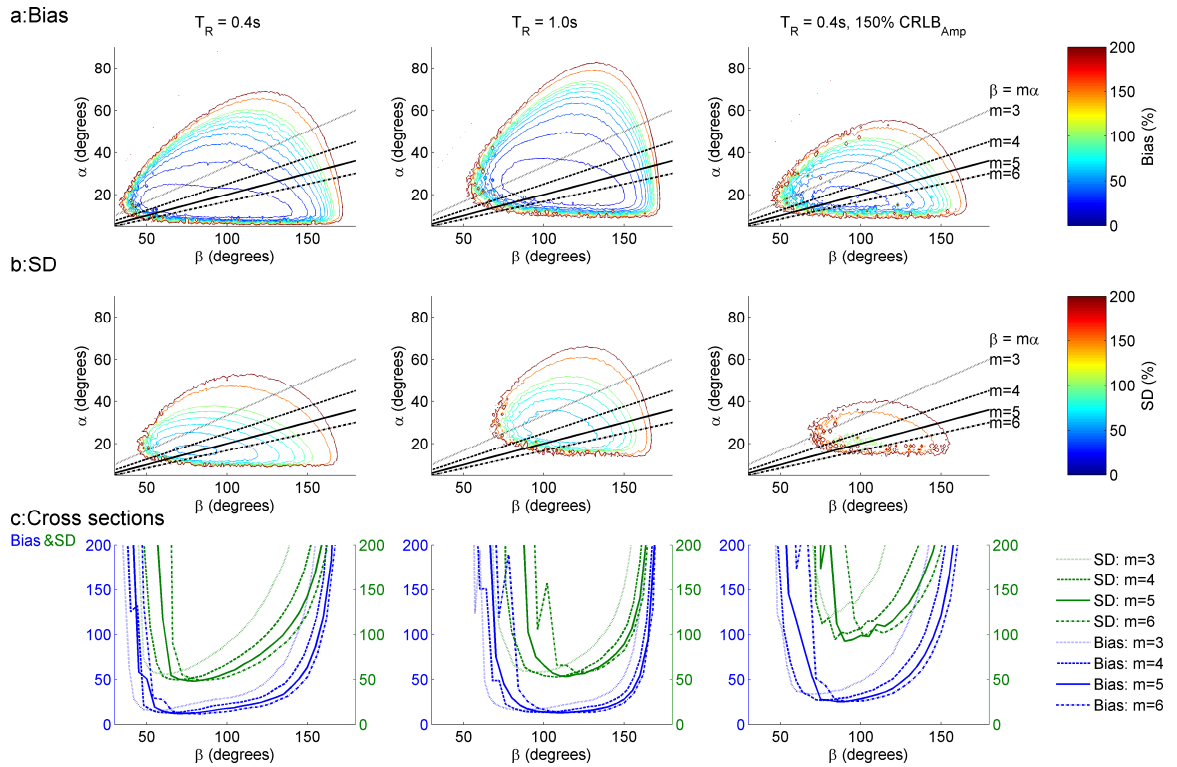


Figure 8.2 Results of the Monte-Carlo simulation of the FAST measurement protocol. **a:** The predicted measurement error (Bias) as a function of the two flip-angles, at two repetition times and at a lower SNR (50% increase in CRLB_{Amp}). The black lines mark the possible flip angles at different values of m . **b:** The predicted measurement precision, in percent. **c:** The cross sections of both bias and precision, for the values of m marked by the straight black lines in **a** & **b**.

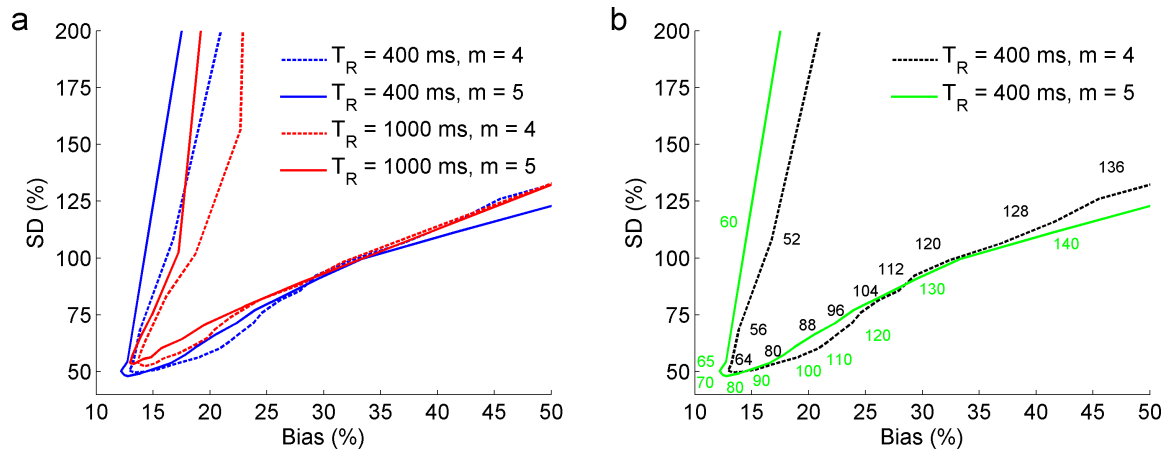


Figure 8.3 L-Curves of the cross-sections shown in Figure 8.2c. The axes are percentage bias and precision (standard deviation), the optimum measurement point corresponds to the inflection in each line. Panel **a** compares the effect of the T_R , while panel **b** shows the (minimal) effect of changing the ratio of the α and β flip-angles. The β flip-angles are marked in **b**.

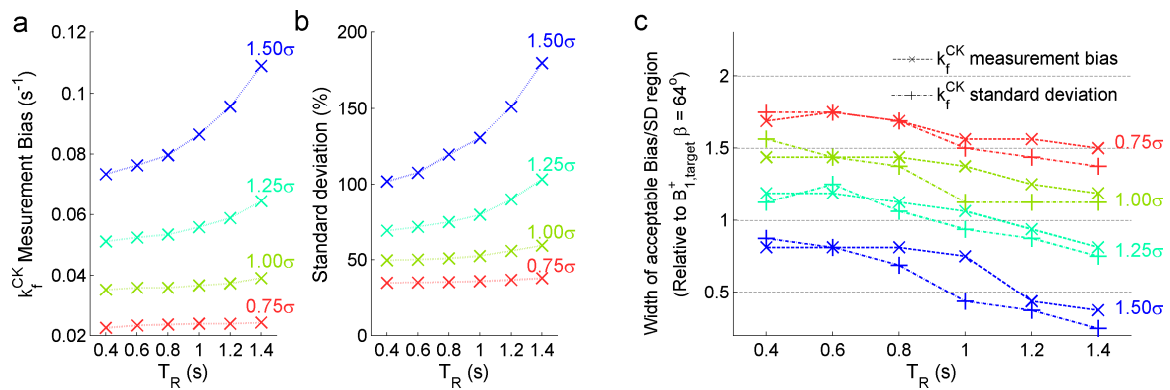


Figure 8.4 **a**: Minimum measurement bias as a function of T_R at several different noise levels: 0.75, 1.00, 1.25 and 1.50 $\times$ the experimental noise standard deviation (σ). **b**: Minimum percentage standard deviation as a function of T_R at various noise levels. **c**: Range of $B_{1,target}^+$ values, expressed relative to the optimum measurement point (chosen above), which give “acceptable” bias ($<0.1 s^{-1}$), or “acceptable” standard deviation ($<0.5 s^{-1}$), as a function of T_R .

It was found in additional simulations that lowering the SNR of the system has a significant effect on the position of the optimal flip-angle values, but not the repetition time. Changing the values of metabolite concentration ratios, or the exchange rate, only results in a small change in optimal parameters.

8.1.1.2 Maintaining the steady state saturation condition at short repetition times.

The multi-variate function describing the behaviour of the magnetisation of exchanging species may only be reduced to the relatively simple form of Eq. [5.30] under constant saturation of one exchanging species, i.e. $\mathbf{M}^B = 0$.

The results above suggest that a short repetition time maximises k_f^{CK} precision. The readout event, typically 341 ms (6000 Hz bandwidth, 2048 samples), must be shortened to maintain sufficient time for saturation. For $T_R = 0.4$ s the full sequence Bloch simulations show that the saturation time must exceed 76% of the T_R , i.e. there must be more than 300 ms of continuous saturation before each transient.

Therefore the readout time was set to 85 ms (6000 Hz bandwidth, 512 samples) for steps 4 to 8 of the protocol in §8.2.2. This maintains a reasonable spectral resolution, while maximising measurement precision.

The effect of insufficient saturation time results in a similar underestimation of k_f^{CK} as incomplete saturation (Chapter 6). It should be noted that the apparent, full saturation of γ -ATP in the resultant spectra of a single step of the protocol is not sufficient to guarantee that the constant saturation condition is fulfilled. It is possible that γ -ATP is finally saturated just before the start of the next pulse-acquire block. This would result in a saturated γ -ATP peak in the spectrum, but it would not allow time for chemical exchange and so the longitudinal relaxation of PCr would barely change. This would mean that the PCr apparent T_1 rather than T_1' would be measured, leading to an underestimate of k_f^{CK} .

8.2.4.4 CSI Resolution

The use of the discrete Fourier transform to reconstruct the k-space data results in spectra localised to voxels with signal contribution arising from a much larger volume than suggested by the nominal voxel size ($FOV / resolution$). The tissue contributing to each voxel is characterised by the point spread function (PSF) and by the field profile of the RF coil. Whilst the point spread function is symmetrical around the voxel centre, the RF coil transmit and receive fields are likely to be significantly stronger on the side of the voxel closer to the coil. B_0 inhomogeneity and tissue heterogeneity within the voxel may cause similar effects. Due to this inhomogeneity the signal in a voxel will not correspond exactly to conditions at the centre of the voxel.

The receive profile, whose effect may be seen as a shift and broadening of the voxel, is constant, between acquisitions, for the same prescribed CSI grid, coil and subject. The transmit profile, which results in inhomogeneous partial saturation of the signal, may be compounded – such as in the phase measurements of the Bloch-Siegert acquisitions, which have a square relationship to the B_1^+ field (Chapter 7). The deleterious effects of the point spread function are somewhat simplified by the use of an acquisition weighted CSI matrix, which for the cost of a wider central lobe, damps the positive and negative stopband oscillations of the unweighted PSF.

Surface coils, such as the 10 cm loop, have rapidly changing B_1 profiles. It is therefore important to assess the effect of voxel size on the Bloch-Siegert measured B_1^+ and eventual k_f^{CK} measurement.

Simulations to determine the effect of CSI grid resolution, in the case of a 1D PSF broadened, acquisition weighted CSI grid, on the Bloch-Siegert-measured B_1^+ , are shown

in Figure 8.5. A coil B_1 profile was calculated using the Biot-Savart law for a 10 cm circular loop, the 1D voxel was situated 10 cm from the centre of the coil and the B_1 profile normalized to the value at the centre of the voxel. The values of the measured B_1^+ were calculated for three different resolutions. For the calculation of the Bloch-Siebert-measured B_1^+ , the γB_1^+ was set to 300 Hz at the centre of the voxel.

The results of the simulations show that for the PSF and receive weighted Bloch-Siebert determined B_1^+ the value would be overestimated by 82%, 11% and 3% for resolutions of 8, 16 and 32 respectively. The overestimation of B_1^+ by the Bloch-Siebert method is less than the receive and PSF weighted average of the true B_1^+ because phase-wrapping in regions of space with high B_1^+ causes a partially-compensating underestimate of B_1^+ there.

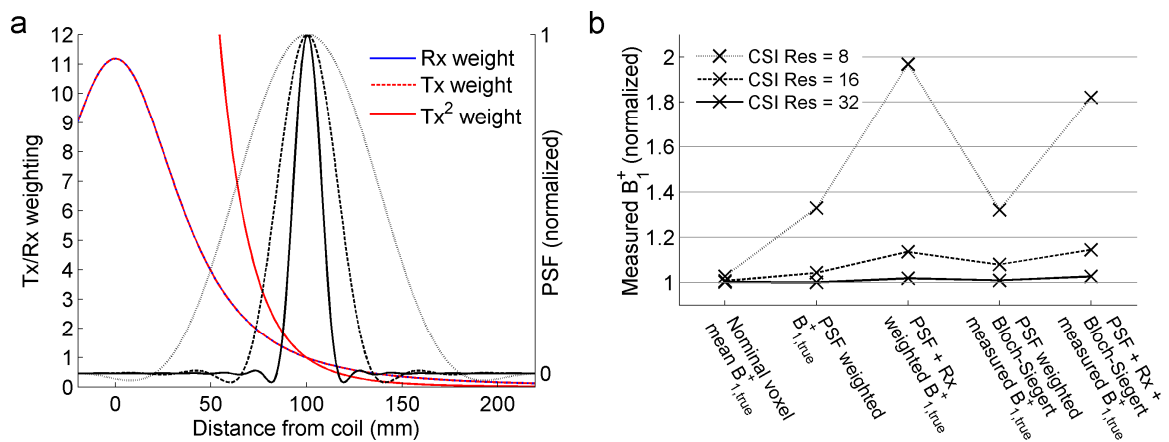


Figure 8.5 **a:** Schematic of the B_1 transmit and receive fields (neglecting dielectric effects) of a 10 cm Tx/Rx loop. Acquisition-weighted voxels at three different resolutions are overlaid. The nominal voxel widths are 30 mm (CSI Resolution = 8), 15 mm (CSI Resolution = 16) and 7.5 mm (CSI Resolution = 32). **b:** Effect of CSI voxel size (in one dimension) and “method” on the measured B_1^+ . The measured B_1^+ is normalized to the B_1^+ value at the centre of a PSF broadened voxel 100 mm from a transmit-receive coil (10 cm loop). The B_1^+ measured by the Bloch-Siebert method is a function of the square of the coil transmit profile (Tx² weight).

In addition to the simulations Bloch-Siegert B_1^+ maps were acquired, at a range of resolutions (A-P: 8, 12, 16, 20, 26, 32, 64) in the axis perpendicular to the coil (H-F: 16; all L-R: 16), from a uniform phantom previously described (§7.4.3).

The maps collected with high resolution (CSI matrix > 20, nominal voxel size < 10mm) were zero-padded in k-space to 256x256 voxels and averaged to form a high resolution “true” B_1^+ profile. The interpolated maps were used to simulate the signal arising from a FAST experiment (selectively saturated and unsaturated steady state CSI spectra of PCr and γ -ATP in exchange) at α and β flip angles scaled to the “true” B_1^+ at each of the high resolution points. The PSF and receive weighted (approximated as the normalized B_1^+ profile) simulated FAST signal was then summed for each voxel for each of the original resolution grids. A k_f^{CK} value was then calculated from the summed signal using the original B_1^+ measured at each resolution. The error in the final k_f^{CK} was expressed as a percentage error on colour-maps overlaying ^1H localisers of the phantom (Figure 8.6b).

Less than 10 % error in B_1^+ , relative to the averaged profile, was observed for resolutions ≥ 16 at all distances from the coil (Figure 8.6a). Less than 1% error was observed for the higher resolutions at distances less than 60 mm from the coil elements. At greater distances errors from low SNR dominated.

78% of voxels had an absolute k_f^{CK} error greater than 20% with the 12x16x16 resolution grid. This dropped to 55% of voxels for the 16x16x16, with high error voxels around the edges of the phantom. The resolution with minimum high error voxels (40%) was 26x16x16.

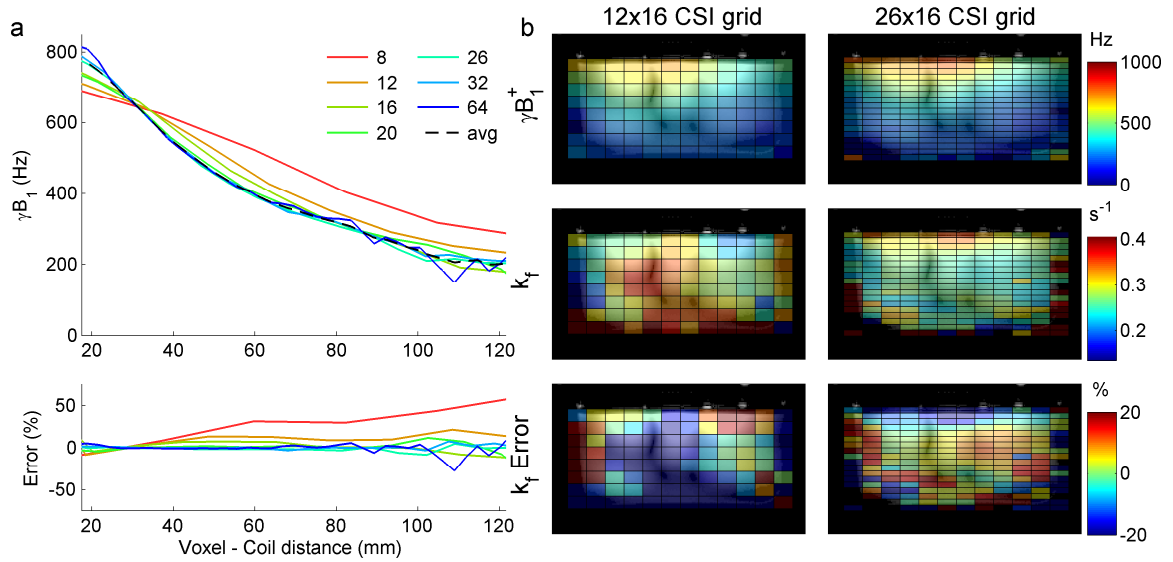


Figure 8.6 **a:** γB_1^+ profile for the central column of the CSI matrix for each resolution measured. The average B_1^+ , calculated from the resolutions $\geq 20 \times 16 \times 16$, is shown as a black dashed line. The error relative to the average is shown in the bottom plot. **b:** Colour-maps of the B_1^+ maps, simulated k_f^{CK} maps and the error in the simulated k_f^{CK} .

In these two simulation experiments significant errors are observed for CSI grid resolutions below 16 (nominal voxel size 15 – 12.5 mm). However, the variable resolution B_1^+ maps showed SNR becomes the limiting factor at high resolutions. It was therefore decided that the in vivo resolution of $16 \times 16 \times 8$ (FOV = $240 \times 240 \times 200 \text{ mm}^3$) which is the resolution typically used in vivo for ^{31}P -MRS at Oxford, was suitable for BOAST.

8.3 In vivo measurement of k_f^{CK} at 7T: Methods

Unless stated otherwise, the experiments below used a Magnetom 7T scanner (Siemens, Erlangen, Germany); localizer images were acquired with a 10 cm ^1H loop coil (Rapid Biomedical, Rimpar, Germany); this was replaced by a T/R switch and preamplifier module (Virtumed LLC, MN, USA) connected to a 10-cm ^{31}P transmit–receive loop coil for the ^{31}P acquisitions; and the ^{31}P coil was tuned and matched using

an RF sweeper (Morris Instruments Inc., Ottawa, Canada) for each subject. All subjects were recruited in a manner approved by the local Research Ethics Committee.

8.3.1 Skeletal muscle validation

Skeletal muscle in the human calf offers homogenous and isolated muscle tissue with similar physiology to the myocardium, in which the BOAST method may be tested and validated against previously determined literature values.

8.3.1.1 3T volume transmit/receive coil

A single volunteer's (male, 23 years, 84 kg) calf was scanned using a $^1\text{H}/^{31}\text{P}$ dual-tuned birdcage head coil (Rapid Biomedical, Rimpar, Germany) in a 3T Trio scanner (Siemens, Erlangen, Germany). The volume coil has a comparatively uniform excitation field, which enables comparison of the BOAST method with previous implementations of FAST using BIR-4 pulses. Using 3T (not 7T) and a volume receive coil (not a surface receive coil), will result in lower SNR and a less precise k_f^{CK} measurement than we ultimately anticipate at 7T.

The protocol followed §8.2.2. Acquisition parameters for the Bloch-Siegert scans were:

12x12x8 CSI matrix, $150 \times 180 \times 300 \text{ mm}^3$ field of view, 1024 spectral points, 4 kHz bandwidth centred at PCr, 70 V "MDR3T" excitation pulse, $T_R = 0.5 \text{ s}$, and 15 averages at $k=0$, giving a total duration for each scan of 10min. The Bloch-Siegert Fermi pulse parameters were: $T_p = 3.5 \text{ ms}$, $T_0 = 0.875 \text{ ms}$, $a = 0.224 \text{ ms}$ at 250 V. The Fermi pulse was positioned $\pm 2000 \text{ Hz}$ from PCr. The B_1^+ map was calculated as previously described in Chapter 7 and ref.16.

The acquisition parameters for the subsequent saturation transfer steps were as above, except with 512 spectral points. The $T_R = 0.4 \text{ s}$ with 21 averages at the centre of k -

space, the duration of each scan was 10 min. Selective saturation was achieved by hard DANTE pulses ($m = 1, T_{\text{on}} = 100 \mu\text{s}, T_{\text{off}} = 230 \mu\text{s}$, at 50 V). The excitation pulse was scaled using the acquired B_1^+ maps to give flip-angles of $\alpha = 16^\circ$, $\beta = 64^\circ$.

Spectra were fitted using AMARES in Matlab using standard prior knowledge (Appendix B.2). The voxels inside the leg in the four central slices of the CSI grid (head-foot direction) were analysed.

k_f^{CK}, T_1^* and M_0^{PCr} were calculated using equations [5.30], [8.1] and [8.2]. In addition the Q-factor was determined as the ratio of the PCr amplitude measured with control saturation to the PCr amplitude measured in an additional acquisition without saturation.

8.3.1.2 Surface coil (7T)

The experiment was repeated in the same volunteer's calf at 7T. The ^{31}P acquisitions were obtained using the 7T 10cm loop Tx/Rx coil. The experimental protocol again followed §8.2.2. The acquisition parameters for the Bloch-Siegert scans were: 12x12x14 CSI matrix, $180 \times 200 \times 300 \text{ mm}^3$ field of view, 2048 spectral points, 6 kHz bandwidth centred at PCr, MDR3T excitation pulse at 250 V, $T_R = 0.5 \text{ s}$, and 7 averages at $k=0$, giving a scan duration of 10 min. The Bloch-Siegert Fermi pulse parameters were: $T_P = 3.5 \text{ ms}$, $T_0 = 0.875 \text{ ms}$, $a = 0.224 \text{ ms}$, at 300 V. The Fermi pulse was centred $\pm 2000 \text{ Hz}$ from PCr.

The acquisition parameters for the subsequent saturation transfer steps were as above, except with 512 spectral points. The $T_R = 0.4 \text{ s}$ with 5 averages at the centre of k-space, the duration of each scan was 7 min. Selective saturation was achieved by hard DANTE pulses ($m = 1, T_{\text{on}} = 100 \mu\text{s}, T_{\text{off}} = 230 \mu\text{s}$, at 33 V). The excitation pulse was scaled

using the acquired B_1^+ maps to give flip-angles of $\alpha = 16^\circ$, $\beta = 64^\circ$ at the centre of the calf. Analysis of the spectra was completed identically to the 3T measurement. The central 5 slices of the CSI grid were analysed, masked as above. An additional mask was created to select voxels within 20% of the target flip-angles, these voxels were also analysed separately.

8.3.2 Cardiac validation

Ten healthy subjects (2 female, 31 ± 8 years and 71 ± 8 Kg) underwent the cardiac protocol. Subjects were positioned and localizers were acquired as previously described.⁸ The experimental protocol followed the steps set out in §8.2.2.

The Bloch-Siegert scans used modified standard CSI acquisition parameters (Appendix A, MDR7T pulse, 10cm loop coil) with a $T_R = 0.5$ s and 11 averages at $k=0$ and centred at PCr. The duration of each scan was 15:05 min. The Bloch-Siegert Fermi pulse parameters were: $T_p = 3.5$ ms, $T_0 = 0.875$ ms, $a = 0.224$ ms, at 240 V. The Fermi pulse was positioned at ± 2000 Hz from PCr.

The acquisition parameters for the subsequent saturation transfer steps were identical to the Bloch-Siegert acquisitions, except the number of spectral points was set to 512, the $T_R = 0.4$ s and the number of averages was set to 11 averages at the centre of k -space: the duration of each scan was 10:22 minutes. Selective saturation was achieved by DANTE pulses ($m = 1$, $T_{on} = 100$ μ s, $T_{off} = 230$ μ s), with a transmit voltage of 33 V. The total ^{31}P acquisition time was 81:50 minutes for the complete scan, comparable to TRiST at 3T.¹⁷

The target voxel was selected at scan time and the α and β flip-angles in that voxel scaled to the optimal values. Spectra from the target voxel were fitted using the AMARES in Matlab, using standard prior knowledge (Appendix B.2).

k_f^{CK} , T_1^* and M_0^{PCr} were calculated from the PCr amplitudes using equations [5.30], [8.1] and [8.2]. The CRLB of each derived parameter and the Q-factor were also calculated.

8.3.2.1 Multi-parametric fit

In addition to the analysis above, the amplitudes of the PCr, and unsaturated γ -ATP and α -ATP peaks were also fitted simultaneously using the analytical expression for two pool exchange (Eqs. [5.4]-[5.12]), equations [5.23] and [5.27] and the partial saturation equation (Eq. [1.48]). Variance-weighted, non-linear least squares fitting of M_0^{PCr} , M_0^{ATP} , $T_1^{*,PCr}$, $T_1^{*,\gamma-ATP}$, $T_1^{\alpha-ATP}$ and k_f^{CK} was done using 'lsqcurvefit' in Matlab.

An average literature value for the concentration of ATP in the myocardium was calculated (Table 8.2), and used to calculate [PCr]:

$$[PCr] = [ATP] \frac{M_0^{PCr}}{M_0^{ATP}}. \quad [8.3]$$

Reference	[ATP] (mmol kg ⁻¹)	[PCr] (mmol kg ⁻¹)
18	6.90±1.60	11.00±2.70
19	7.72±2.97	12.14±4.25
5	5.80±1.60	10.00±2.00
20	6.40±1.80	9.70±2.50
21	5.30±1.20	9.00±1.20
22	5.69±1.02	8.80±1.30
23	6.00±1.10	10.40±1.50
Average	5.95±0.52	9.56±0.65

Table 8.2 Reported values of [ATP] and [PCr] measured by ³¹P-MRS in human myocardium. The averages are variance weighted means of the study means.

From the per-subject PCr concentration, the creatine kinase flux was calculated as the product of k_f^{CK} and [PCr].

8.4 In vivo measurement of k_f at 7T: Results

8.4.1 Skeletal muscle

8.4.1.1 Volume coil (3T)

The total number of voxels analysed across the four central slices was 207. The average deviation from the target flip angle was $5.9 \pm 4.2\%$.

The CRLB²-weighted mean of k_f^{CK} was $0.31 \pm 0.02 \text{ s}^{-1}$. The mean percentage CRLB of k_f^{CK} was $84 \pm 150\%$, though at the centre of the volume observed values of CRLB were consistently in the 25-40% range. The average matched filtered SNR of PCr in the unsaturated scan (using the β flip-angle) was 22 ± 12 .

The average Q-factor was 0.66 ± 0.20 .

8.4.1.2 Surface coil (7T)

The total number of voxels analysed across the five central slices was 225, with 81 voxels, within 20% of the target flip, selected for a sub-group analysis. The average deviation from the target flip-angle in all voxels was $29.1 \pm 17.7\%$, and $10.1 \pm 5.8\%$ in the sub-group.

The variance weighted mean of k_f^{CK} was $0.27 \pm 0.004 \text{ s}^{-1}$ and $0.28 \pm 0.002 \text{ s}^{-1}$ in the selected voxels. The mean percentage CRLB of k_f^{CK} was $51 \pm 138\%$ and $29 \pm 81\%$, matched filtered SNR of PCr was 39 ± 20 and 41 ± 21 and the Q factor was 0.97 ± 0.21 and 0.98 ± 0.21 , for the full and masked voxel selection respectively.

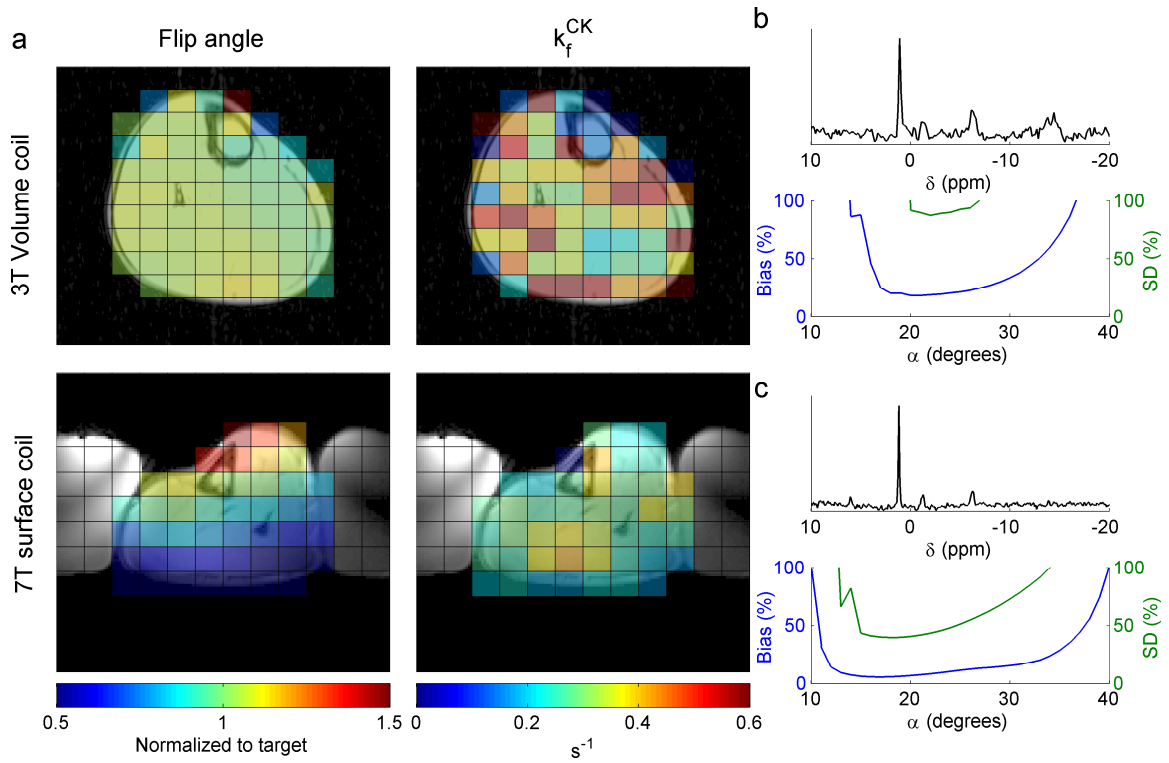


Figure 8.7 **a:** Flip-angle and k_f^{CK} maps from the central slice of a 3D-CSI matrix acquired at 3T, using a volume coil, and at 7T, using a surface coil, in a single volunteers calf. The maps are masked by the outer anatomical border. **b&c:** Example spectra and predicted bias and standard deviation of the k_f^{CK} measurement for the 3T (**b**) and 7T (**c**) acquisition. The predicted errors have been calculated using the average PCr SNR observed in each experiment.

8.4.2 Cardiac

Each of the ten subjects produced analysable spectra in the target voxel in each component acquisition (Figure 8.8). The mean PCr SNR in the acquisition using the β -flip angle, with control saturation, was 13 ± 3.4 . The median Q-factor in the target voxels was 0.95 with an inter-quartile range of 0.13.

Calculation of k_f^{CK} using the saturation transfer equations yielded an inter-subject, variance weighted, mean $k_f^{CK} = 0.35 \pm 0.05 \text{ s}^{-1}$ and least squares fitting gave $k_f^{CK} = 0.37 \pm 0.04 \text{ s}^{-1}$. The individual subject values ($\pm$ CRLB) are plotted for both methods in Figure 8.9b.

Using the results of the least squares fitting, the average literature value of [ATP], and values of PCr/ATP ratio, PCr concentration and creatine kinase flux were calculated. The per-subject values are given in Table 8.3 and Figure 8.9d. The study average PCr concentration was 11.64 ± 2.65 mmol kg⁻¹ of wet weight (wwt) and the creatine kinase flux was 4.55 ± 1.52 mmol kg⁻¹ s⁻¹.

Subject	PCr/ATP	[PCr] (mmol kg ⁻¹ wwt)	k _f (s ⁻¹)	CK Flux (mmol kg ⁻¹ s ⁻¹)
1	1.78±0.33	10.61±1.98	0.40±0.20	4.22±2.23
2	1.50±0.25	8.91±1.51	0.34±0.11	3.00±1.09
3	1.49±0.18	8.89±1.06	0.45±0.16	4.00±1.53
4	2.25±0.18	13.38±1.05	0.39±0.08	5.22±1.09
5	1.91±0.35	11.34±2.08	0.46±0.27	5.24±3.23
6	1.61±0.29	9.58±1.72	0.33±0.14	3.12±1.42
7	1.55±0.24	9.23±1.45	0.26±0.14	2.43±1.34
8	2.29±1.27	13.62±7.58	0.34±0.24	4.65±4.20
9	2.45±1.05	14.54±6.27	0.44±0.99	6.38±14.60
10	2.74±0.36	16.27±2.12	0.44±0.14	7.22±2.45
Inter-subject mean ± SD	1.96±0.44	11.64±2.65	0.38±0.07	4.55±1.52

Table 8.3 Results of the least-squares fitting of the cardiac data.

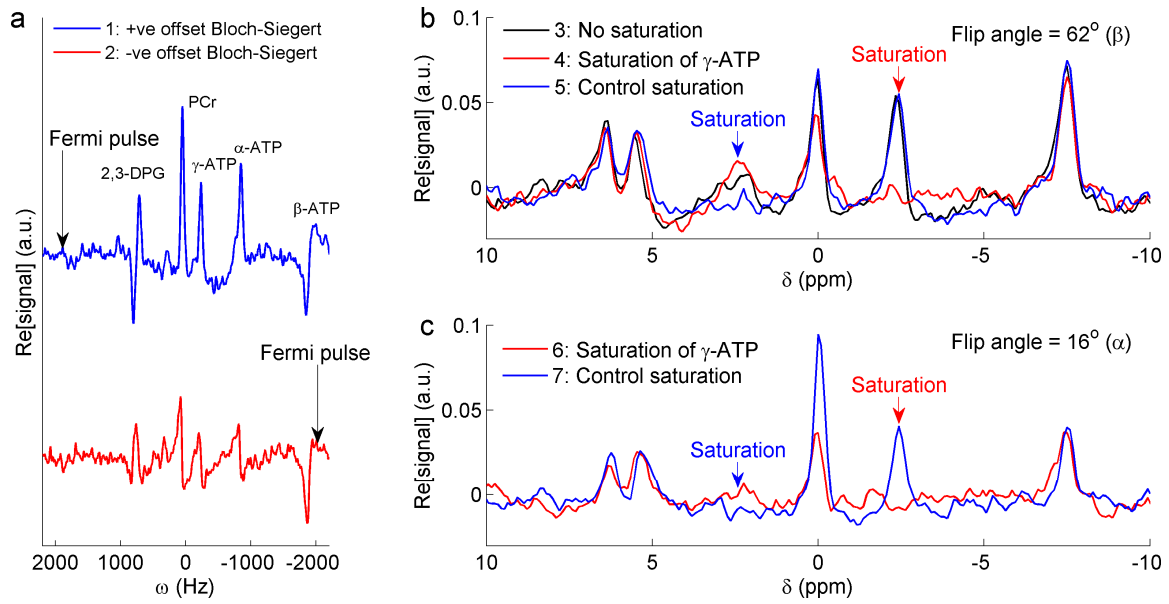


Figure 8.8 Example cardiac spectra from the septal target voxel in a single volunteer. Each group of acquisitions is shown separately: **a)** the Bloch-Siegert B_1^+ determination, **b)** unsaturated, control saturation and saturated scans at the β flip angle, **c)** control and saturated scans at the α flip-angle. PCr SNR of the β control saturation scan (blue line **b**) is 17.5.

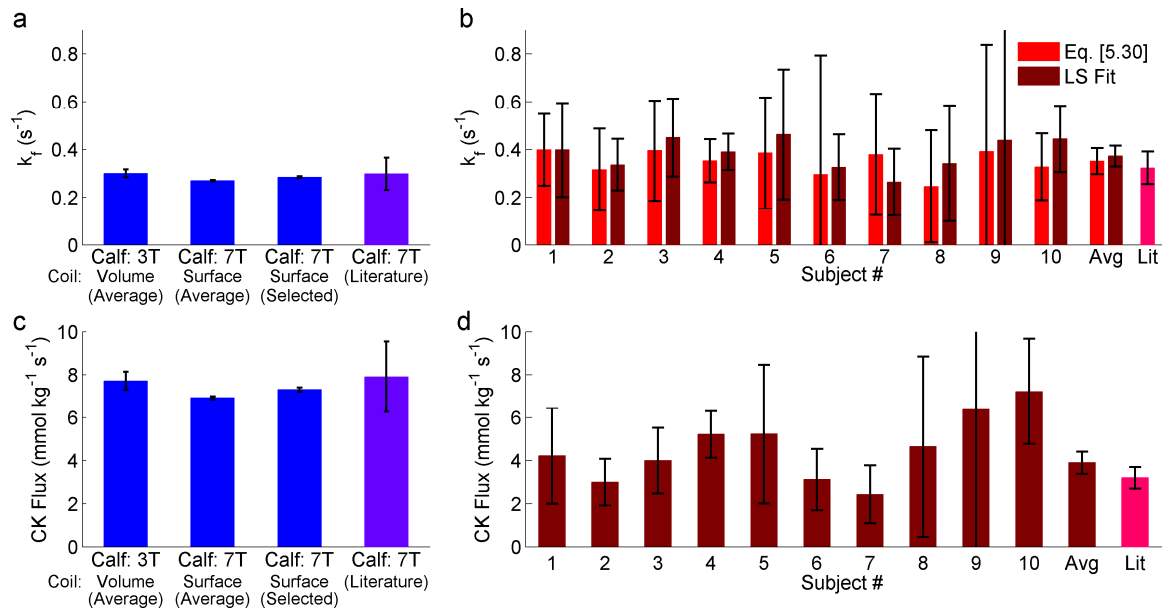


Figure 8.9 Measured k_f^{CK} values. **a:** Results of the skeletal muscle validation scans, an average literature value is provided for comparison. The 7T results for both all voxels (average) and the voxels within 20% of the target flip-angle (selected) is shown. **b:** Cardiac experiments. Results of calculation using both the saturation transfer equations and the least squares fitting are shown. **c&d:** CK Flux values of skeletal and cardiac muscle as calculated by the least-squares fitting.

8.5 Discussion

The BOAST method enables measurement of the cardiac creatine kinase rate constant and flux at 7T for the first time. The method overcomes the current inability of surface coils to reliably produce a B_1 -insensitive adiabatic pulse in the myocardium at 7T.

Instead, by combining Bloch-Siegert B_1 mapping with hard excitation pulses a known (but spatially varying) flip-angle is used.

Monte Carlo Bloch simulations of the sequence provide a small optimisation of the sequence repetition time and target flip-angles. The importance is exacerbated in low SNR conditions with the predicted bias and uncertainty rising and the range of acceptable flip angle narrowing greatly. These simulations are borne out by the results of the skeletal muscle validation. In both 3T and 7T experiments the average k_f^{CK} agrees with the literature measurements which span 0.23 s^{-1} to 0.35 s^{-1} ^{24,25} (or 0.459 s^{-1} if inversion transfer is included²⁶). However, at 3T, despite the low variation in flip-angle, the SNR is relatively low compared to 7T and a corresponding high CRLB for k_f^{CK} is observed. While at 7T the high SNR results in a relatively stable k_f^{CK} measurement despite the large spatial variation in flip-angles (Figure 8.7c). These results validate the BOAST method as viable for in vivo measurement, whilst also achieving high spectroscopy resolutions.

The k_f^{CK} measured in the cardiac experiments also closely matched literature values, despite being localised to volume significantly smaller than has previously been possible. The values in this study have been obtained from voxels centred on the myocardium, with voxel sizes, calculated as the FWHM of the point-spread-function and at the 50% sensitivity limit of a 10cm loop coil, less than half those obtained in any

previous study (110 mL vs. 298 mL). Unlike studies at lower field strengths, and the validation at 3T in this study, the amount of direct saturation of PCr by the DANTE train was negligible ($Q = 0.97$).

Least squares fitting of the data reduced the uncertainty in the measurement of k_f^{CK} and also enabled the calculation of PCr/ATP, and subsequent derived parameters, without introducing a systematic bias, which would have been the case if one attempted saturation correction of the exchanging spectra.²⁷

8.5.1 Limitations

8.5.1.1 B_1^+ error

Propagation of error analysis shows that the uncertainty in the k_f^{CK} measurement is balanced equally between the uncertainty in the measured B_1^+ ($4.3 \pm 2.5\%$ [mean $\pm$ SD]) and the uncertainty of the PCr amplitudes in steps 4-7 (Figure 8.10a). The analysis indicated that we have (nearly) optimally apportioned the scan time between recording the Bloch-Siegert B_1 -map and the later FAST acquisitions.

The simulations of the experiment at different resolutions demonstrate that the method is sensitive to the effects of the point spread function. A small bias in the measured B_1^+ results in much larger errors in k_f^{CK} (Figure 8.10b). The result of these simulations is to impose a limit on the size of a nominal voxel and the resolution of the CSI grid. Small voxels sizes result in a higher number of averages required for acceptable SNR and thus longer scan times.

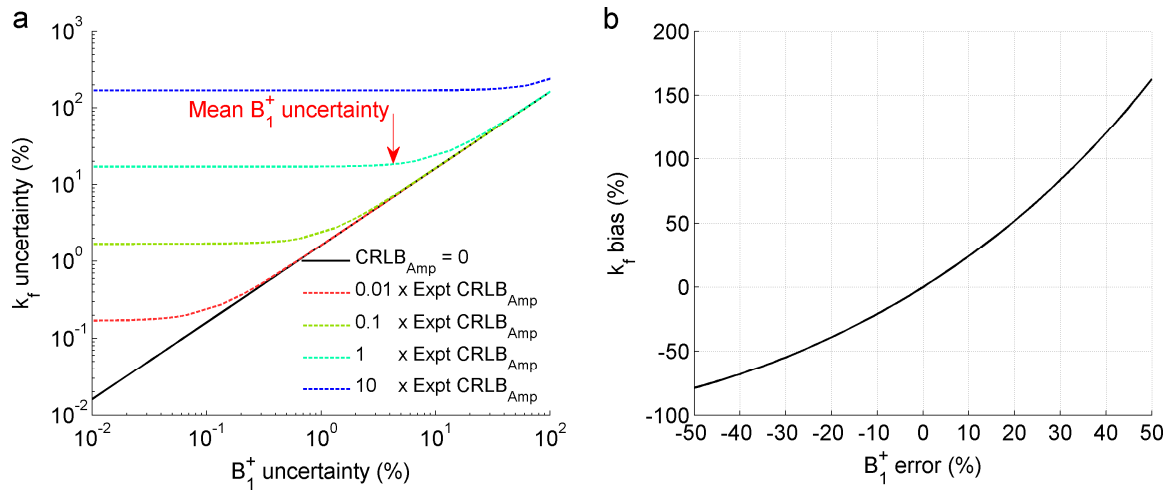


Figure 8.10 **a:** k_f^{CK} precision as a function of uncertainty in the Bloch-Siebert B_1^+ measurement precision, in the presence of a range of different FAST acquisition SNR levels. **b:** Error introduced to the k_f^{CK} measurement due to under or overestimation of the B_1^+ .

8.5.1.2 Surface coil B_1^+ inhomogeneity

As shown in Figure 8.7a, the range of B_1^+ values created by the 10 cm loop surface coil in the leg spans a large range, from approximately 50 to 150% of the target value.

When combined with the low SNR in the heart, which also has a smaller and less homogenous target anatomy, the low homogeneity of the excitation profile limits the number of voxels feasible for analysis to one or two at most. Whilst achieving the desired 3D localisation, this method therefore still falls well short of whole-heart coverage.

The situation could be improved by a change in hardware. A dedicated receive coil, such as the 16 channel flexible array has been shown to increase the receive SNR by 1.8x,²⁸ resulting in both higher measurement precision but also greater tolerated range of flip-angles. Alternatively, the increased SNR could be used to reduce the scan time by up to 3.2 times. If the optional scan without saturation was excluded the scan time would be

just 27 minutes (the minimum scan time for a single-average, acquisition weighted 16x16x8 CSI grid is 4.5 minutes), with the current measurement uncertainty.

More uniform excitation would also reduce the range of flip-angles experienced across the heart. Larger surface coils, which have more uniform excitation, driven with the current RF transmit hardware do not have high enough $B_{1,\text{peak}}^+$ for the Bloch-Siegert protocol component. Volume excitation would reduce the inhomogeneity; typical 3T body coils achieve flip-angle variation between 20-50% across the myocardium, and allow use of a dedicated receive coil.²⁹ However, either solution would require more than the 8 kW RF power available on the Magnetom 7T scanner as supplied.

8.5.1.3 Blood correction

In this work the effect of ATP signal in signal originating in the blood has been ignored.

Whilst the calculation of k_f^{CK} using the saturation transfer equations would not be affected by ATP in blood, the least-squares method relies upon the ATP amplitudes which include a contribution from the blood of approximately 11% of the saturation corrected 2,3-DPG signal. Whilst correction is theoretically possible, the flowing nature of blood means it would not follow the standard partial saturation relationship. Thus the saturation correction of partially saturated DPG peaks is difficult, and has not been attempted.

8.5.1.4 PCr concentration

Currently the PCr concentration and the creatine kinase flux are calculated using the PCr/ATP ratio and a literature value for ATP concentration. The average PCr concentration measured by this method $11.64 \pm 2.65 \text{ mmol kg}^{-1}$ is higher than the literature value of $9.7 \pm 0.7 \text{ mmol kg}^{-1}$. Using this average literature PCr concentration

would give a CK Flux of $3.73 \pm 0.64 \text{ mmol kg}^{-1} \text{ s}^{-1}$. This is closer, but still significantly different to the literature flux of $3.2 \pm 0.5 \text{ mmol kg}^{-1} \text{ s}^{-1}$.

Efforts are ongoing to establish a protocol for 7T ^{31}P -MRS absolute concentration determination in Oxford.

8.6 Conclusion

The FAST method has been successfully adapted to allow the first three dimensionally localised creatine kinase forward rate constant measurements in the human heart. The values of k_F measured in 10 volunteers ($0.35 \pm 0.05 \text{ s}^{-1}$) agreed closely with previously measured values at lower field strengths (literature mean: 0.32 ± 0.04).

Whilst 7T ^{31}P -MRS brings significant SNR benefits, tighter constraints on B_1^+ limit the range of possible excitation pulses. This study has shown that the strategy of replacing B_1 -insensitive pulses with the Bloch-Siegert B_1 -mapping technique and constant phase pulses is viable.

8.7 References

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Chapter 9: Conclusion & future work

9.1 Creatine kinase measurements

In this thesis the creatine kinase pseudo first-order rate constant has been measured at 7 tesla from tissue localised to the interventricular septum. The increased anatomical specificity will enable measurement of CK kinetic parameters in the specific myocardial tissue affected by hypertrophy in HCM. Whilst this method brings a step change from the previously obtained 1D-CSI localisation in humans, the protocol is currently only sensitive to a few voxels simultaneously.

Further development of the 7 tesla BOAST method would primarily be concerned with obtaining a more spatially homogeneous excitation flip-angle, so as to increase the range of myocardium the measurement is sensitive to in a single measurement. This could be achieved by either using hardware to achieve a more homogeneous B_1^+ field, or using pulse design to reduce the flip-angle sensitivity to the B_1^+ field. Funding is currently being sought for a whole body ^{31}P birdcage coil for 7T, which offers the most comprehensive solution to the former case. In light of the recently measured in vivo B_1^+ field both adiabatic and composite pulses may then become feasible at 7 tesla.

In addition whilst CK flux measurements have been estimated from literature values of [ATP] and the PCr/ATP ratio, for complete characterisation of the CK system absolute quantification of PCr is required. This is an ongoing aim, and is being actively addressed in the ^{31}P group at Oxford.

The studies using the TRiST based CK measurements at 3T are currently ongoing, and as such a full analysis of the patient data is not currently possible. However understanding

the measurement of low k_f^{CK} , as described in the discussion section of Chapter 6, is a priority. A replica of the Johns Hopkins coil is being sourced from PulseTeq Ltd to understand whether our choice of coil corrupted the measurement of k_f^{CK} .

9.2 B_1^+ mapping

The Bloch-Siegert based B_1^+ mapping sequence introduced in this thesis is the first known ^{31}P B_1^+ mapping method capable of being used in the human heart in vivo at 7T. Permitting fast, SNR efficient acquisition and its independence from metabolite T_1 values brings advantages over previously implemented flip-angle mapping methods.

Extensive simulation of possible Bloch-Siegert acquisition strategies found that for the specific cardiac ^{31}P -MRS case, it is best to fit only the maximum SNR peak, with the preparation pulse placed equidistant either side of the maximum SNR peak. In light of this and the desire for high resolution B_1 maps, it might be desirable to combine the Bloch-Siegert sensitising pulse with a ^{31}P imaging technique, such as Parasoglou *et al.*'s turbo spin echo method¹, which has already been demonstrated with selective saturation pre-pulses. Gradient spin echo might offer an alternative if the SAR load of TSE is too high. Sinc, Gaussian or binomial pulses can provide the necessary spectrally selective excitation for these methods.

Understanding and accounting for the differences between the measured, phantom and in vivo, and Biot-Savart estimated B_1^+ field remains a key goal. It is proposed that a series of simulations, carried out in CST Studio Suite (CST AG, Darmstadt, Germany), of the 10 cm loop coil with varying sample loading, and with varying sample electrical properties (conductivity and permittivity), should be used to understand the

relationship between the B_1^+ in phantom and in vivo, and simultaneously in the fixed coil fiducial. The Biot-Savart estimates are used throughout all ^{31}P -MRS studies at Oxford for saturation correction.² It is therefore important, in light of the results at the end of Chapter 8, to establish whether Biot-Savart estimation is suitable for this purpose, or whether improvements to the field estimations can be made, and to reconcile the challenges we have faced at 7T (and to a lesser extent at 3T) with the previously published absolute quantification results.³

9.3 Measurement of cardiac inorganic phosphate

Using high resolution CSI, saturation transfer subtraction and B_0 gradients it was not possible to detect an intracellular inorganic phosphate peak in healthy subjects at 7 T. However, using high resolution CSI a singlet peak at approximately 5 ppm from PCr, assigned as P_i was detected consistently in the interventricular septal voxels of hypertrophic cardiomyopathy patients. The high resolution sequence has room for improvement: cardiac gating would ensure a more constant voxel position relative to the myocardium, though breathing motion induced blurring would remain, without significantly affecting the repetition time. Furthermore non Fourier reconstruction, combined with optimised k-space methods could be used to increase the resolution further without increasing scan time, and with minimal SNR penalty.⁴

Confirmation of the peak assignment in HCM patients will be an important step. The most obvious method would be to exploit the magnetisation transfer mechanism between P_i and γ -ATP. Either transfer of selective saturation or inversion from the γ -ATP peak to P_i could be detected in a second repeated experiment.⁵ Though a saturation

transfer effect was not observed clearly in healthy subjects, the higher P_i peak amplitude in HCM patients could magnify the effect. This would double the length of the protocol and is therefore probably only feasible in a few subjects.

Detection of P_i remains a key goal; measuring pH in the healthy human heart using ^{31}P -MRS would be an important validation method for the proposed hyperpolarised ^{13}C bicarbonate pH measurement. Of the untested methods summarised in Chapter 4, a contrast agent (e.g. Ferumoxytol) based approach is the most promising.⁶ The use of a contrast agent to remove the blood signal would allow an arbitrary (and optimal for P_i SNR) selection of scan parameters. As it is unclear if the agent will affect the relaxation process of metabolites within erythrocytes, it would be essential to test the efficacy of such methods *ex vivo*, and possibly in animal models, before progressing to contrast-enhanced ^{31}P spectroscopy in humans.

9.4 Development of tools for ^{31}P -MRS

With its continued use, in a range of local and external clinical studies, the distributed version of the spectroscopy analysis and fitting package (including AMARES in Matlab) has improved and unified the analysis of hundreds of ^{31}P -MRS datasets. The tool brings improvement in the ease and reliability of data analysis for non-expert users, as well as allowing the latest best practice in post-processing to be easily deployed in ongoing studies. The standard reporting format facilitates pooled meta-analysis or methodological studies using the growing database of scans at OCMR.

9.5 References

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Appendix A: Standard UTE 3D-CSI acquisition parameters.

This appendix contains the listed scan parameters for the “standard” ^{31}P UTE 3D-chemical shift imaging (CSI) sequence at 7 tesla. In the main text of this thesis reference to these parameters is made in a number of method sections using the phrase “standard CSI parameters”. Brackets immediately afterwards contain a reference to this section, the excitation pulse (“MDR7T” or “MDR3T”), and the coil used (10 cm loop and 16 channel array). Any modifications to this standard are listed explicitly.

Parameter	Value	Unit
CSI grid orientation	1 st two dimensions coplanar to the short axis image.	
CSI matrix size	16 x 16 x 8 (8 in the long-axis direction)	
Field of View (FOV)	240 x 240 x 200	mm ³
Nominal voxel size	5.6	ml
Bandwidth	6000	Hz
Spectral samples	2048	
Excitation pulse	MDR3T or MDR7T (see Chapter 2)	
Repetition time (T_R)	1	s
Averages at centre of k-space	10	
“Dummy” preparation scans	4	
Total duration	28:14	mm:ss

Table A.2 Standard cardiac ^{31}P 3D-CSI sequence parameters.

The standard transmit voltages for each coil-pulse combination are listed in Table A.2. When using the MDR7T pulse the central frequency (and centre of excitation) is centred at -586 Hz (i.e. -4.9 ppm) relative to PCr to give uniform excitation of all metabolites. For

the MDR3T pulse the central frequency is set 266 Hz (2.2 ppm) from PCr, which gives uniform excitation of 2,3-DPG, PDE, P_i, PCr and γ-ATP.

Pulse	10 cm loop	16 Channel coil
MDR3T	-	400 V
MDR7T	270 V	530 V

Table A.3 Standard transmit voltages (the RMS voltage calibrated across the coil plug on the patient table) for various coil-excitation pulse combinations.

Appendix B: Standard prior knowledge

B.1 “7T Cardiac” prior knowledge

Peak	Initial values		Upper and lower bounds				Multiplet relations			Fixed relative phases
	Chemical shift (ppm)	Linewidth (Hz)	Chemical shift (ppm)	Linewidth (Hz)	Amplitude	Phase (degrees)	Relative shift	Fixed linewidth	Fixed amplitude	
β -ATP 1	-16.33	56	$-\infty$; ∞	0:0	0:0	0:360	-0.124	1	1	✓
β -ATP 2							0	1	2	✓
β -ATP 3							0.124	1	1	✓
α -ATP 1	-7.65	50	$-\infty$; ∞	0:0	0:0	0:360	-0.133	1	1	✓
α -ATP 2							+0.133	1	1	✓
γ -ATP 1	-2.47	34	$-\infty$; ∞	0:0	0:0	0:360	-0.124	1	1	✓
γ -ATP 2							+0.124	1	1	✓
PCr	0.00	33	$-\infty$; ∞	0:0	0:0	0:360				✓
PDE	2.69	60	1.5:3.0	20:100	0:0	0:360				✓
2-DPG	5.20	60	3.5:7.5	20:100	0:0	0:360			1	✓
3-DPG	6.35	60	4.0:8.0	20:100	0:0	0:360			1	✓

Table B.1 Cardiac ^{31}P -MRS prior knowledge at 7 tesla. Shading of the cells is used to indicate the linking of individual multiplet components. Bounds are denoted as “lower bound : upper bound”. Infinity indicates the absence of a constraint. All fitted signals (including individual multiplet components) were fitted using Lorentzian lineshapes.

B.2 “7T Skeletal muscle” prior knowledge

Peak	Initial values		Upper and lower bounds				Multiplet relations			Fixed relative phases
	Chemical shift (ppm)	Linewidth (Hz)	Chemical shift (ppm)	Linewidth (Hz)	Amplitude	Phase (degrees)	Relative shift	Fixed linewidth	Fixed amplitude	
β -ATP 1							-0.124	1	1	✓
β -ATP 2	-16.33	56	$-\infty$: ∞	0: ∞	0: ∞	0:360	0	1	2	✓
β -ATP 3							0.124	1	1	✓
α -ATP 1							-0.133	1	1	✓
α -ATP 2	-7.65	50	$-\infty$: ∞	0: ∞	0: ∞	0:360	+0.133	1	1	✓
γ -ATP 1							-0.124	1	1	✓
γ -ATP 2	-2.47	34	$-\infty$: ∞	0: ∞	0: ∞	0:360	+0.124	1	1	✓
PCr	0.00	33	$-\infty$: ∞	0: ∞	0: ∞	0:360				✓
PDE	2.69	60	1.5:3.0	20:100	0: ∞	0:360				✓
P _i	5.20	60	3.5:7.5	20:100	0: ∞	0:360				✓

Table B.2 Skeletal ³¹P-MRS prior knowledge at 7 tesla. Shading of the cells is used to indicate the linking of individual multiplet components. Bounds are denoted as “lower bound : upper bound”. Infinity indicates the absence of a constraint. All fitted signals (including individual multiplet components) were fitted using Lorentzian lineshapes.