

Application of MR to Identify Metabolic and Physiological Correlates of Human Ageing and Inactivity

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UNITED KINGDOM • CHINA • MALAYSIA

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

September 2017

Abstract

Physical inactivity has been linked to poor health and disease progression, particularly in older people. This has led to an increasing interest in the effects of physical activity, physiological function and ageing. Importantly, negative health traits generally attributed to ageing, such as frailty, cognitive decline and brain atrophy, may in part result from decreased habitual physical activity levels, and be preventable with increased exercise.

Here, we use magnetic resonance imaging (MRI) techniques to quantify the cortical haemodynamic and metabolic responses to acute low/moderate intensity exercise in healthy young and older volunteers, to investigate how this response is influenced by ageing and cardiorespiratory fitness. In addition, structural MRI is used to investigate global and regional grey matter volume, and cortical thickness in young and older adults, and to assess its association with age and cardiorespiratory fitness.

In the final Chapter, methods are developed to study the impact of a 16-day limb immobilisation on brain and muscle function using ultra-high field, 7 T MRI. Functional magnetic resonance imaging (fMRI) is used to assess changes in brain motor cortex function over the course of 16 days of upper limb immobilisation, and assess whether any changes are observed associated with the loss of voluntary handgrip strength over the same time period. Muscle MRI is performed to assess changes in muscle cross sectional area, and muscle magnetic resonance spectroscopy (MRS) developed with the aim of quantifying changes in forearm muscle IMCL and EMCL content over the immobilisation period.

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Acknowledgements

I would like to take this opportunity to express my gratitude toward those who have been there for me throughout my time at Nottingham. If I were to do you all justice, this would be another thesis chapter in itself. First and foremost, a very special thanks goes to Professor Susan Francis. Sue has put up with me for 8 years now, something that deserves a medal on its own, and over that period she has continued to give me her time, effort and support. Sue truly does put the 'super' in 'supervisor'. I would also like to extend my thanks to Professor Paul Greenhaff and Professor Penny Gowland for their expertise and motivation for continually moving forward. I consider myself incredibly lucky to have had such an amazing team of supervisors.

A special mention must also go to Sally Eldeghaidy, for her expertise in statistical analysis and all things brain.

To *all* of my colleagues at the Sir Peter Mansfield Imaging Centre. You have been like a family to me from the beginning. A special thanks goes to Edward Breeds for all his support through the tough times, and all his shenanigans during the good ones, to Elena Boto for rewarding me with treats-for-words during write-up as motivation to keep going, to Richard Dury for his MATLAB wizardry, to Charlotte Buchanan for being my big sister and always looking after me, to Ben Hunt and Nicolas Geades for our moody drives through the countryside, and lastly, but definitely not least, Lesley Martin, who has been a constant joy to ~~flirt~~ work with and who will forever be my number one.

Finally, I'd like to thank my parents. To my darling mother, whose constant piss-taking has motivated me to succeed in life, but who still thinks I'm a little bit stupid. And to Pops, who I owe so much to for his limitless support and unwavering positivity, and without whom I could never have done this. I dedicate this thesis to you.

1. Introduction

1.1 OUTLINE

We are an ageing population. Forecasts suggest that within the next twenty years one quarter of UK residents will be aged 65 and over [1], and 1 in 4 children born today can expect to live to 100. However, healthy lifespan is failing to increase at the same rate as life expectancy [2]. On average, adults spend the last decade of life in poor health, often living with cognitive impairment and chronic pain. Ageing is associated with the onset of neurodegenerative diseases and a progressive decline in musculoskeletal health and functional capacity, leading to a loss of independence. In addition to prolonged suffering, the high rates of ill-health among the older population is predicted to increase the nation's overall healthcare cost by 25% [3]. A cost-effective and widely accessible preventative to improve quality of life and reduce economic burden is now urgently required.

World health statistics highlight physical inactivity as 1 of the 5 leading risk factors for chronic diseases, such as type 2 diabetes, and global mortality, accounting for one quarter of all deaths [4]. Nearly half of older adults (65+ years) are estimated to be failing to meet the recommended physical activity guidelines [5]. Sedentary ageing has been associated with a decrease in cerebral blood flow (CBF) [6, 7] which has been linked to cognitive decline, stroke [8, 9] and the onset of neurodegenerative diseases, such as dementia [10]. Evidence is also emerging that physical inactivity contributes to lipid accumulation [11-13], metabolic impairments [14, 15] and muscle mass loss [16], all of which correlate strongly with functional decline [17-20].

The substantial and growing evidence on the impact of physical activity on cognition, chronic illness and musculoskeletal health has generated an increasing interest in the effects of cardiorespiratory fitness on physiological function with age. There is now strong evidence to suggest that regular physical activity has wide-ranging health benefits which include the preservation of cognitive and musculoskeletal function with age [21-24]. Exercise has also been shown to be an effective treatment in stroke rehabilitation [25-28], improving quality of life and reducing depression.

Furthermore, it has been reported that chronic exercise promotes neuroplasticity in the human brain [29].

However, despite its clear benefits, the underlying mechanisms behind the cerebral response to exercise remain poorly understood, and more work is needed to identify optimum exercise strategies for improved brain health [30]. There is, therefore, still much reluctance among healthcare professionals and the public to embrace physical activity as a prevention and treatment strategy.

The aim of the work presented in this thesis is to provide a more comprehensive understanding of the cerebral and metabolic response to acute and chronic exercise with ageing, and investigate the impact of physical inactivity on brain and muscle function.

1.2 SCOPE OF THESIS

Chapter Two of this thesis details the theoretical principles behind nuclear magnetic resonance (NMR). **Chapter Three** outlines magnetic resonance imaging (MRI) and spectroscopy (MRS) techniques. The interaction between nuclei and an external magnetic field is described along with nuclear relaxation processes. Basic imaging and spectroscopy sequences are then introduced, followed by a description of the hardware of an MR system.

Chapter Four provides a comprehensive account of the physiological and functional imaging sequences used in the studies presented in this thesis. The cerebral vascular architecture of the human brain is introduced. Methods to quantify cortical haemodynamic and metabolic responses to exercise are then described, including arterial spin labelling (ASL), phase contrast MRI (PC-MRI), T₂-relaxation under spin tagging (TRUST) and blood oxygen level dependant (BOLD) contrast.

Chapter Five investigates how the cerebral response to an acute exercise challenge is influenced by normative ageing. Measures of cerebral blood flow, regional perfusion, brain activation and oxygen utilisation are collected at rest, during low to moderate intensity exercise and on recovery in young and older adults. This work was presented at the International Society for Magnetic

Resonance in Medicine (ISMRM), Singapore, 2016 as an oral presentation, and at the European College of Sport and Exercise Science (ECSS), Germany, 2017.

Chapter Six explores the relationship between brain architecture and normative ageing using high resolution structural MRI. In particular, the association of grey matter volume and cortical thickness with cardiorespiratory fitness is investigated.

Chapter Seven describes the development of methods to study the effect of inactivity on skeletal muscle and brain function using ultra-high field. MR spectroscopy and imaging techniques are developed for 7 Tesla to investigate muscle lipid accumulation and changes in brain motor cortex architecture and activation following a 16-day upper limb immobilisation.

Chapter Eight provides an overview of the work presented in this thesis, highlighting the results and key findings, and concludes with suggestions for future research directions.

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2. Nuclear Magnetic Resonance (NMR) Theory

This chapter presents an overview of the theory behind nuclear magnetic resonance (NMR) which is necessary to understand the work carried out in this thesis. Both the quantum mechanical and classical description of the system are described, followed by an outline of how NMR can be used to measure the intrinsic properties of molecular structure and, more recently, living tissue.

2.1 INTRODUCTION

Nuclear magnetic resonance has been a method for determining the structure of organic compounds for over 50 years. Development began in the late 1940's following Rabi's work on nuclear magnetic moments [1]. This was followed by work led independently by Edward Purcell and Felix Bloch, in which they observed that nuclei such as ^1H , can absorb radio frequency (RF) energy of a strength specific to its identity when placed in a magnetic field. Upon absorption, the nuclei begin to resonate, with different atoms within the molecule resonating at different frequencies for a given magnetic strength. This observation allowed the detailed analysis of the structure of molecules through NMR spectroscopy. In 1952, Purcell and Bloch shared the Nobel Prize in Physics for their discoveries [2].

2.1.1 Nuclear Magnetisation

Atomic nuclei possess three fundamental properties: mass (m), electronic charge (q) and spin (I). Spin is an intrinsic property of the nucleus, the evolution of which can be described by quantum mechanics. The combined spin and mass of a nucleus generates a spin angular momentum, $\mathbf{J}$. A nucleus with spin and charge generates a magnetic dipole moment, $\boldsymbol{\mu}$. Like spin, magnetic moment is also an intrinsic property of a given nuclei, with the two properties being linked by:

$$\boldsymbol{\mu} = \gamma \mathbf{J} \quad \text{Eqn. [2.1]}$$

where γ is the gyromagnetic ratio. The gyromagnetic ratio is a proportionality constant unique to each nucleus, and can take either a positive or negative value. The sign of γ specifies whether the magnetic moment is parallel to the spin angular momentum (positive) or anti-parallel (negative). For protons, ^1H , which are most widely used in magnetic resonance imaging (MRI), γ equals $267.5 \times 10^6 \text{rads}^{-1}\text{T}^{-1}$ or 42.6MHzT^{-1} .

2.2 THE QUANTUM MECHANICAL MODEL

Dirac first described the quantum mechanical model of atomic nuclei in 1930. When placed in a strong magnetic field, a nucleus acts like a dipole magnet that can align with or against the magnetic field. If the spins of the subatomic particles are paired (the number of protons and the number of neutrons are even) there is no overall net spin, this is the case for ^{12}C which is NMR insensitive. However, when the spins of the protons and neutrons are unpaired (the number of protons and/or the number of neutrons are odd), such as in ^1H and ^{13}C , the net spin of the charged nucleus can take on an integer value ($I = 1, 2, 3, \dots$), or a half-integer ($I = 1/2, 3/2, 5/2, \dots$). Only nuclei with a non-zero value of I exhibit the property of magnetic resonance. Those particles which make up the nucleus with integer spins are known as bosons, while particles with half-integer spins are known as fermions. The magnitude of the spin angular momentum is expressed by Eqn. [2.2],

$$|J| = \hbar \sqrt{I(I+1)} \quad \text{Eqn. [2.2]}$$

where $\hbar$ is Planck's constant, h , divided by 2π .

NMR can only be performed on isotopes whose natural abundance is high enough to be detected. Table 2-1 shows the spin (I) and gyromagnetic ratio (γ) for the nuclei most commonly used in NMR.

<i>Isotope</i>	Spin (I)	Gyromagnetic Ratio (γ) ($\times 10^6 \text{ rads}^{-1}\text{T}^{-1}$)
^1H	$1/2$	267.5
^{13}C	$1/2$	67.3
^{19}F	$1/2$	251.8
^{23}Na	$1/2$	70.8
^{31}P	$1/2$	108.4

Table 2-1: Spin quantum number and gyromagnetic ratio of some commonly used nuclei in NMR.

2.2.1 Spin Interaction with Static (time-independent) Magnetic Field

In the absence of an external magnetic field, the sublevels of I are degenerate. However, when a magnetic field is applied, a number of different spin state directions, m_I , are allowed, as defined by Eqn. [2.3]. There are $(2I + 1)$ spin eigenstates in total, each corresponding to a particular direction of spin state [3] given by:

$$m_I = -I, -I + 1, -I + 2, \dots + I \quad \text{Eqn. [2.3]}$$

The different spin states of I are dependent only on the type of particle.

The z-component of the spin angular momentum of a spin state m_I is defined as

$$J_z = \hbar m_I \quad \text{Eqn. [2.4]}$$

A hydrogen atom, ^1H , comprises an electron orbiting a single proton, thus has a spin of $1/2$ and can take possible spin states of $\pm 1/2$, termed “spin up” or “spin down”. Due to its high abundance in the human body and its large gyromagnetic ratio, the vast majority of clinical magnetic resonance imaging is acquired using hydrogen nuclei.

When placed in a magnetic field, B_0 , the magnetic moment, μ , has energy

$$E = -\mu \cdot B_0 \quad \text{Eqn. [2.5]}$$

showing that the energy is lowest when the magnetic moment is parallel to the magnetic field. Combining this expression with Equation 2.1 gives:

$$E = -\gamma J \cdot B_0 \quad \text{Eqn. [2.6]}$$

By definition, the magnetic field is applied along the z-axis, therefore

$$E = -\hbar \gamma B_z m_I \quad \text{Eqn. [2.7]}$$

Hence for a proton with spin states $m_I = \pm \frac{1}{2}$ in an applied magnetic field, the transition between the two spin states requires a change in energy of

$$\Delta E = \hbar \gamma B_z \quad \text{Eqn. [2.8]}$$

This energy level separation is known as Zeeman splitting, and is illustrated in the energy level diagram shown in Figure 2-1.

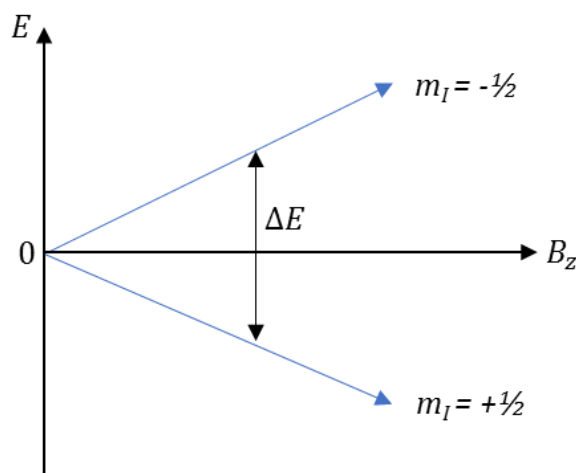


Figure 2-1: The process of Zeeman splitting, showing the energy separation between two spin states, $m_l = \pm \frac{1}{2}$, when exposed to a static magnetic field, B_z .

Transitions between the two energy states shown in Figure 2-1 can be induced by absorption or emission of a photon of frequency ν ,

$$\Delta E = \hbar\gamma B_z = h\nu \quad \text{Eqn. [2.9]}$$

$$\therefore \nu = \frac{\gamma}{2\pi} B_z \quad \text{Eqn. [2.10]}$$

where ν is the frequency expressed in Hz. Converting frequency into angular terms gives the Larmor equation,

$$\omega = \gamma B_z \quad \text{Eqn. [2.11]}$$

where ω is termed the Larmor frequency.

2.3 THE CLASSICAL MODEL

Classically, if a bar magnet is placed in an external magnetic field it will experience a torque, τ , to align with the field. Similarly, a nucleus with magnetic moment will also experience a torque in a magnetic field, causing a rotation proportional to the change in spin angular momentum,

$$\boldsymbol{\tau} = \frac{d\mathbf{J}}{dt} = \boldsymbol{\mu} \times \mathbf{B}. \quad \text{Eqn. [2.12]}$$

Substituting for $\mathbf{J}$ in Eqn. [2.12] gives,

$$\frac{d\boldsymbol{\mu}}{dt} = \gamma \boldsymbol{\mu} \times \mathbf{B}. \quad \text{Eqn. [2.13]}$$

Assuming $\mathbf{B}$ is static and parallel to the z-axis, then Eqn. [2.13] can be separated into x, y and z components as

$$\frac{d\mu_x}{dt} = \gamma \mu_y B_z \quad \frac{d\mu_y}{dt} = -\gamma \mu_x B_z \quad \frac{d\mu_z}{dt} = 0 \quad \text{Eqn. [2.14]}$$

which can be solved to give the equation of motion of the magnetic moment;

$$\begin{aligned} \mu_x(t) &= \mu_x(0) \cos \omega_0 t + \mu_y(0) \sin \omega_0 t \\ \mu_y(t) &= -\mu_x(0) \sin \omega_0 t + \mu_y(0) \cos \omega_0 t \\ \mu_z(t) &= \mu_z(0) \end{aligned} \quad \text{Eqn. [2.15]}$$

where $\omega_0 = \gamma B_z$. These equations describe the precession of the magnetic moment about the z-axis, as shown in Figure 2-2. The angular frequency of the precession is equal to the Larmor frequency derived in the quantum mechanical description, showing how the quantum and classical descriptions converge.

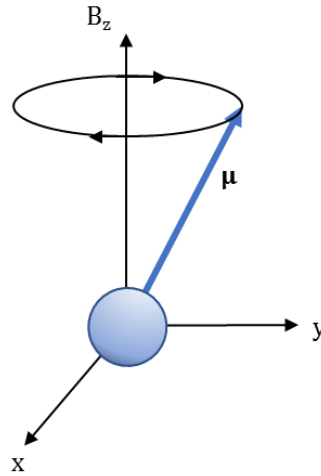


Figure 2-2: Precession of the magnetic moment, μ , around the axis of the applied magnetic field, B_z .

2.4 AN ENSEMBLE OF SPINS

So far, we have only investigated the interactions of an isolated spin in a magnetic field. However, in a real system there are many nuclei to consider. For example, in a single MR image voxel there are of the order of 10^{20} protons. Each of these spins could occupy any particular spin state allowed for that isotope. These spins cannot be analysed individually and so the bulk magnetisation is generally considered. The bulk (or net) magnetisation, $\mathbf{M}$, is described by the sum of the individual magnetic moments per unit volume, V ,

$$\mathbf{M} = \frac{1}{V} \sum_{n=1}^{N_s} \boldsymbol{\mu}_n \quad \text{Eqn. [2.16]}$$

where N_s is the total number of spins in the ensemble.

At thermal equilibrium, the presence of an external magnetic field, B_z , leads to a difference in the spin population in each state, as shown in Figure 2-3, where there is a greater number of spins in the lower energy level, $N_\uparrow$, compared to the higher energy level, $N_\downarrow$. This population difference is very small and is described by the Boltzmann Distribution;

$$\frac{N_\downarrow}{N_\uparrow} = e^{-\frac{\Delta E}{k_B T}} = e^{-\frac{\hbar \gamma B_z}{k_B T}} \quad \text{Eqn. [2.17]}$$

where ΔE is the energy difference between the spin states, k_B is the Boltzmann constant and T is the temperature of the spin system in Kelvin.

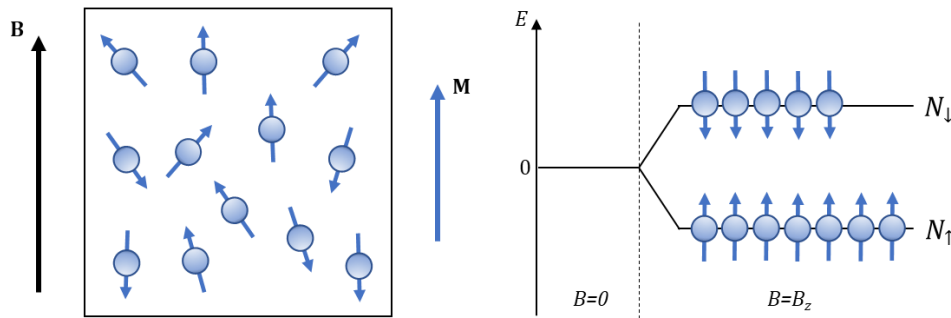


Figure 2-3: Schematic showing the concept of net magnetisation in a single voxel as a result of an applied magnetic field, B_z , leading to a spin population difference between each spin state.

In the high temperature limit, where $k_B T \gg \Delta E$, Eqn. [2.17] can be written as

$$\frac{N_{\downarrow}}{N_{\uparrow}} \approx 1 - \frac{\hbar \gamma B_z}{k_B T} \quad \text{Eqn. [2.18]}$$

Thus the overall population difference, n , can be approximated as

$$n = N_{\uparrow} - N_{\downarrow} = \frac{N_{\uparrow} \hbar \gamma B_z}{k_B T} \quad \text{Eqn. [2.19]}$$

Since there is only a small difference in number of spins between the two population states, $N_{\uparrow} \approx N_{\downarrow}$, and the total number of spins, N , is $N_{\uparrow} + N_{\downarrow} \approx 2N_{\uparrow}$. Thus the population difference, n , can be approximated as

$$n \approx \frac{N \hbar \gamma B_z}{2 k_B T}. \quad \text{Eqn. [2.20]}$$

Given that the magnetic moment of a proton can take the value of either $+\frac{1}{2} \hbar \gamma$ or $-\frac{1}{2} \hbar \gamma$, the net magnetisation of the ensemble is given by

$$\mathbf{M} = n \boldsymbol{\mu} = N \left(\frac{\hbar \gamma}{2} \right) \left(\frac{\hbar \gamma B_z}{2 k_B T} \right) = N \left(\frac{\hbar \gamma}{2} \right)^2 \frac{B_z}{k_B T}. \quad \text{Eqn. [2.21]}$$

This equation shows that the net magnetisation is directly proportional to the magnetic field strength B_z . This net magnetisation, $\mathbf{M}$, follows the same classical equation of motion previously described for a single magnetic moment, and can be written as

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B}. \quad \text{Eqn. [2.22]}$$

For the purpose of this thesis, all of the following chapters will refer to the net magnetisation, $\mathbf{M}$.

2.5 SPIN INTERACTION WITH TIME-DEPENDANT MAGNETIC FIELDS

It has been shown that, when placed in an external static magnetic field, $\mathbf{B}$ or $B_z \mathbf{k}$, spins precess about the axis of the applied magnetic field with an angular frequency of $\omega = \gamma \mathbf{B}$. Now consider applying a second, time varying

radiofrequency field, $\mathbf{B}_1(t)$ perpendicular to $\mathbf{B}_z$ which oscillates at the Larmor frequency ω_0 ,

$$\mathbf{B}_1(t) = 2B_1 \cos(\omega_0 t) \mathbf{i}. \quad \text{Eqn. [2.23]}$$

This will lead to the spins now simultaneously precessing at ω_0 about $\mathbf{B}_z$, and at ω_1 about $\mathbf{B}_1$, as shown in Figure 2-4A.

2.5.1 The Rotating Frame of Reference

To simplify the mathematics of the evolution of the magnetisation vector, $\mathbf{M}$, it is common to introduce the rotating frame of reference. The rotating frame of reference rotates about the z-axis at the Larmor frequency. This new axis system which rotates about $[x, y, z]$ is defined as $[x', y', z]$ and has unit vectors of $[\mathbf{i}', \mathbf{j}', \mathbf{k}]$. In this rotary frame of reference the time dependence of $\mathbf{B}_1$ is removed and $\mathbf{B}_1$ appears stationary as shown in Figure 2-4B.

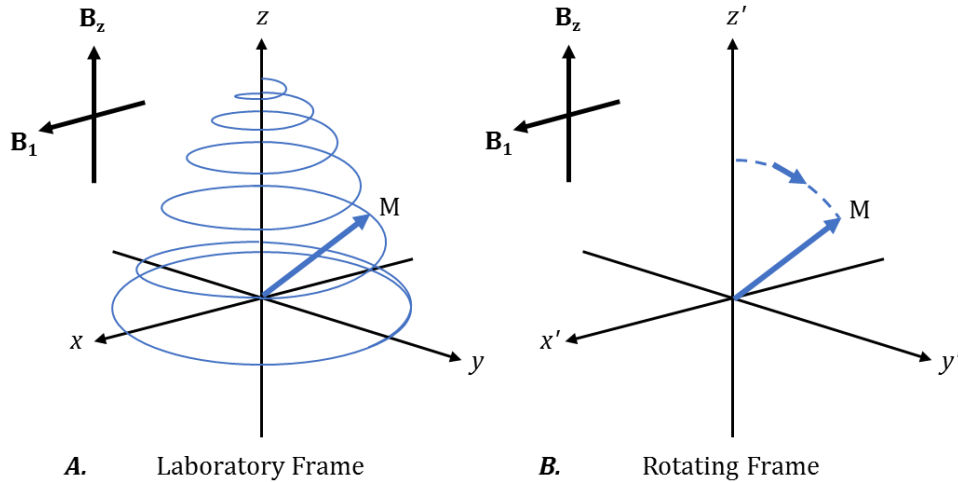


Figure 2-4: The evolution of net magnetisation after application of a time-varying magnetic field, $\mathbf{B}_1$, viewed in **A.** the laboratory frame of reference, and **B.** the rotating frame of reference.

With the time dependency of $\mathbf{B}_1$ removed, it can be rewritten as,

$$\mathbf{B}_1(t) = B_1 \mathbf{i}'. \quad \text{Eqn. [2.24]}$$

The equation of motion of the magnetisation vector $\mathbf{M}$ in the rotating frame is then given by

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \left(B_1 \mathbf{i}' + \left(B_z - \frac{\omega}{\gamma} \right) \mathbf{k} \right) \quad \text{Eqn. [2.25]}$$

which can be described by,

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B}_{eff} \quad \text{Eqn. [2.26]}$$

where $\mathbf{B}_{eff}$ is the effective magnetic field in the rotating frame of reference and is defined as

$$\mathbf{B}_{eff} = \left(B_z - \frac{\omega}{\gamma} \right) \mathbf{k} + B_1 \mathbf{i}' \quad \text{Eqn. [2.27]}$$

Eqn. [2.26] represents the precession of the magnetisation vector about $\mathbf{B}_{eff}$. When $B_1(t)$ is applied on resonance with the Larmor frequency, then $B_z = \frac{\omega}{\gamma}$ and $\mathbf{B}_{eff} = B_1 \mathbf{i}'$ resulting in the magnetisation vector precessing about the y' -axis at an angular frequency of $\omega_1 = \gamma B_1$.

If this resonant radiofrequency B_1 field is applied for a duration τ , the magnetisation vector $\mathbf{M}$ will tip through a precessional angle, θ , given by

$$\theta = \omega t = \gamma B_1 \tau. \quad \text{Eqn. [2.28]}$$

NMR experiments apply this radiofrequency field in short RF pulses. The most common pulses used in MR pulse sequences are either those which tip the magnetisation by 90 degrees, known as an excitation pulse, or 180 degrees, known as an inversion or refocussing pulse. An excitation RF pulse results in the net magnetisation being tipped into the xy' -plane with zero magnetisation in the z -plane. An inversion RF pulse causes the net magnetisation to be tipped into the negative z -plane with zero magnetisation in the xy' -plane. The effect of an excitation and inversion RF pulse is demonstrated in Figure 2-5.

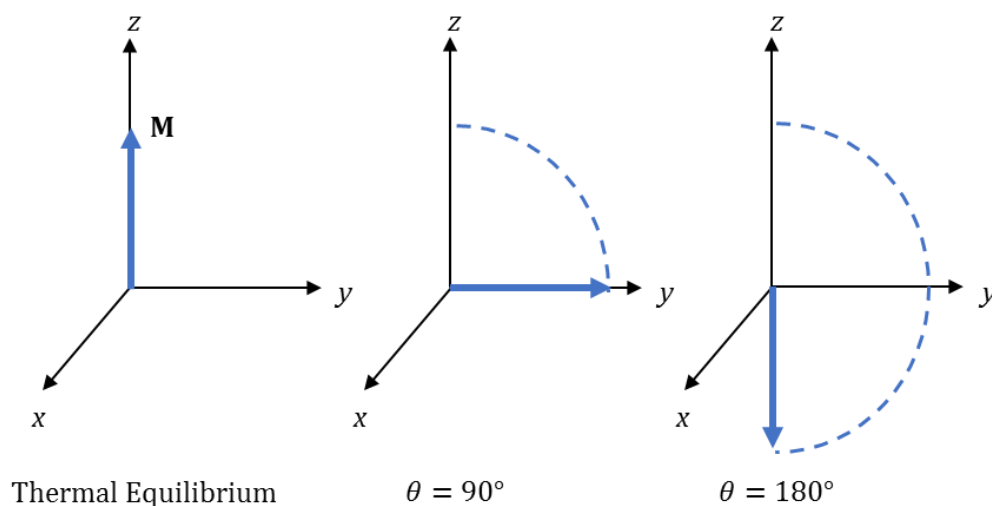


Figure 2-5: Net magnetisation in thermal equilibrium and the effect of an excitation (90°) and inversion (180°) RF pulse.

2.5.2 Free Induction Decay (FID)

The most basic form of signal detection in NMR is the Free Induction Decay (FID). When a sample is placed in a static uniform magnetic field, B_0 , at thermal equilibrium, its magnetisation aligns in the direction of the B_0 field. An RF transmitter coil surrounds the sample and generates the oscillating B_1 field. When applied at the Larmor frequency, the RF pulse excites the spins and a 90° excitation pulse tips the net magnetisation into the transverse plane. Once the RF pulse is switched off, a voltage is detected in the receive RF coil via Faraday induction. The detected signal oscillates at the resonant Larmor frequency and decays exponentially with a transverse decay constant of T_2^* , as shown in Figure 2-6.

T_2^* is dependent on the sample under examination and contains information on the environment of the spins, forming the basis of NMR experimentation.

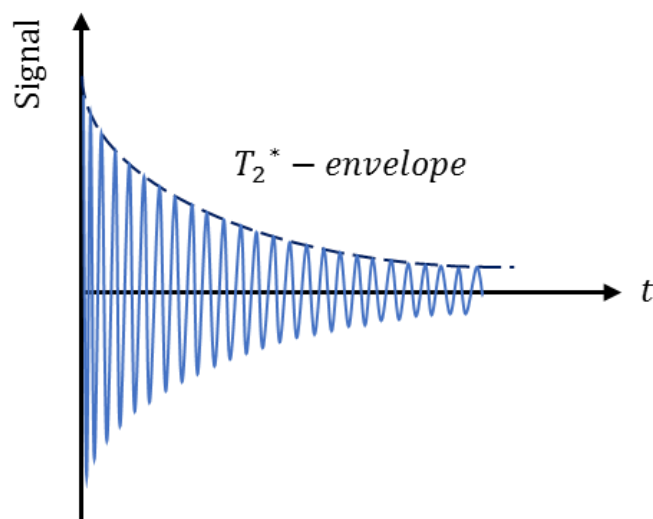


Figure 2-6: Schematic of Free Induction Decay (FID) showing the envelope decaying with the time constant T_2^* .

2.6 NMR SPECTROSCOPY

Over the past fifty years, NMR spectroscopy has been the leading technique for determining the structure of organic materials. When placed in an external magnetic field, spins within a compound do not exhibit the same resonance frequency. Rather there is a minute range of spin frequencies that is dependent on the surrounding chemical environment. The field variations that cause these frequency shifts arise from two separate sources. Firstly, the distribution of electrons surrounding an atom cause nuclei in different molecules to experience a slightly modified magnetic field. This is known as 'chemical shift' (Figure 2-7A). Secondly, neighbouring nuclei interact with one another through spin-spin coupling (Figure 2-7B). Together, these interactions allow different molecules to be identified and quantified which is the basis for many *in-vivo* NMR experiments.

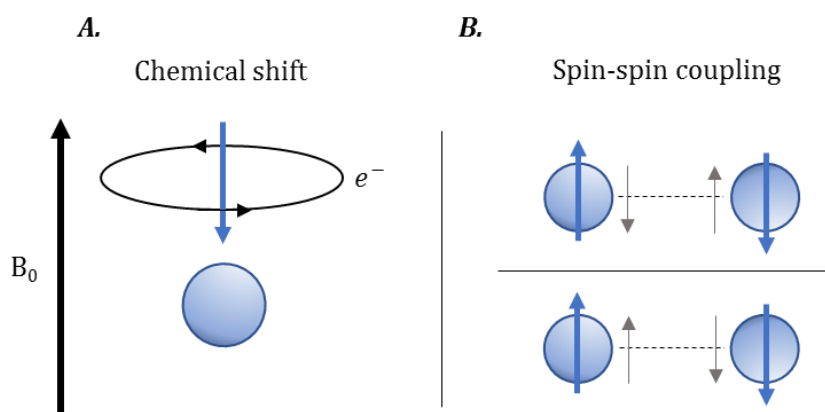


Figure 2-7: Illustration of **A.** chemical shift and **B.** spin-spin coupling.

2.6.1 Chemical shift

Based on the Larmor condition, the resonance frequency of a nucleus is determined by its gyromagnetic ratio and the external magnetic field. However, if the nucleus is within a chemical compound, the surrounding electrons partially shield it from the magnetic field to which it is exposed. The external magnetic field induces a current in the electron cloud which produces a smaller opposing magnetic field via Lenz's law. The nucleus therefore experiences an effective magnetic field offset from the external magnetic field, adjusting the frequency. This change in the frequency of the nucleus due to shielding is called chemical shift and is the cause of separation of individual peaks in MR spectra.

The effect of electronic shielding, and hence the frequency shift of the nuclei, is dependent on the strength of the magnetic field. In the order of parts per million (ppm) chemical shift can be derived by,

$$\delta = \frac{\omega - \omega_{ref}}{\omega_{ref}} \text{ppm}$$

where δ is chemical shift, ω is the frequency of the nuclei and ω_{ref} is the frequency of a reference nucleus (typically Tetramethylsilane (TMS)).

2.6.2 Spin-spin (J-) coupling

Individual peaks within the spectra can be split further into characteristic multiplet peaks through interactions with the spins of neighbouring nuclei

(Figure 2-8). The different patterns of peak depend on the number of interacting nuclei. The spin of one nucleus polarises the spins of the local electrons and the energy levels of neighbouring nuclei are in turn perturbed by the polarised electrons. This causes a constant increase or decrease in energy.

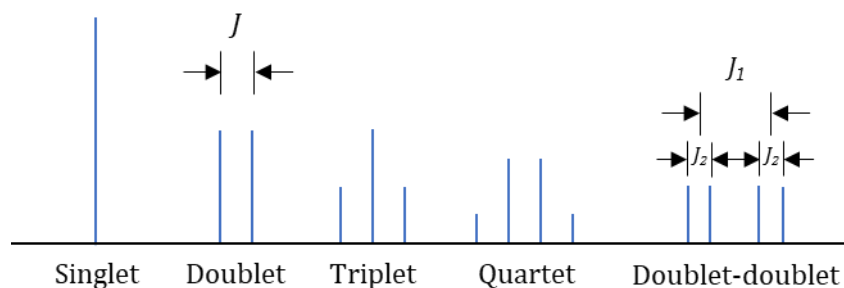


Figure 2-8: Example of peak splitting caused by coupling of simple and more complex systems.

Consider two uncoupled nuclei, A and B. Each nucleus can be in either a spin up state, α , or spin down state, β . Hence this simple spin system will contain four energy levels: $\beta\beta$, $\beta\alpha$, $\alpha\beta$, $\alpha\alpha$ (Figure 2-9A). The energy between spin state transitions determine the observed frequency (ν_1 and ν_2) which, for this example, will be two singlet peaks (Figure 2-9B).

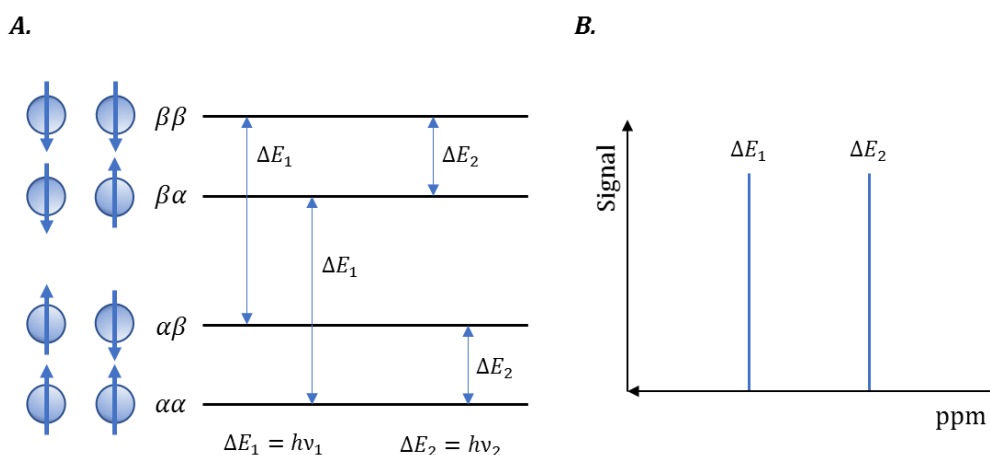


Figure 2-9: A. Energy diagram and B. corresponding spectrum for a simple uncoupled two spin system.

Now consider that nuclei A and B are coupled. The magnetic field at nucleus A will either be greater or less than the external magnetic field due to the

interaction of nucleus B. When the polarised electrons spin are aligned with B_0 there is an overall lowered energy state, and an increased energy state when the electron spins are aligned against B_0 . As a result, the energy levels of the coupled spin system will be perturbed depending on whether the electron spin alignment is in a higher or lower energy state (Figure 2-10A). The resulting spectrum reflects the splitting observed in the resonant frequencies corresponding to nuclear spin transitions which, in this case, will be two doublet peaks (Figure 2-10B). Signal intensity for each doublet peak will be split by half according to the Boltzmann distribution. The distance between two split absorption peaks is known as the J coupling constant (in units of Hertz) and is a measure of the magnetic interaction between the two nuclei.

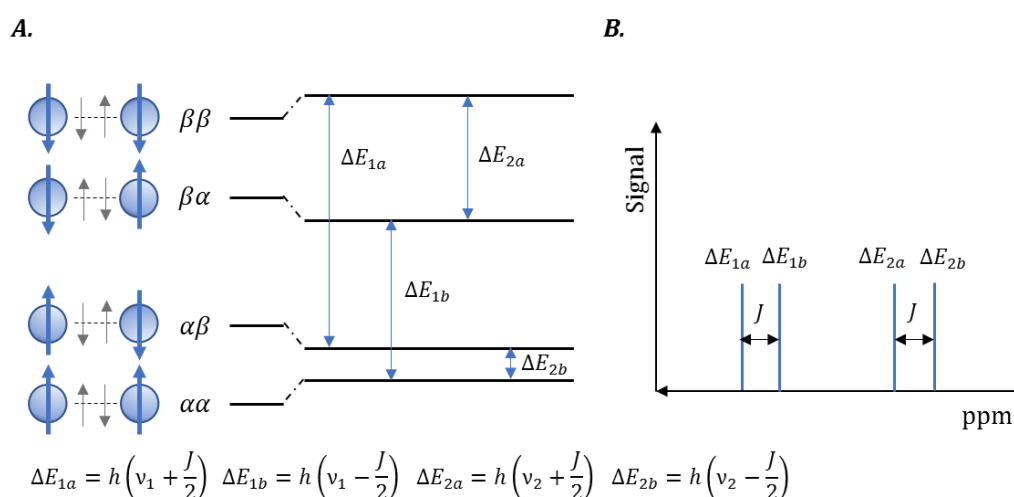


Figure 2-10: A. Energy diagram and B. corresponding spectrum for a coupled two spin system.

The complexity of the splitting pattern in a spectrum increases with the number of interacting nuclei and follows the same logic as Pascal's triangle for increasing splitting (Table 2-2).

NMR spectroscopy can be applied *in vivo* (usually referred to as magnetic resonance spectroscopy, (MRS)) to investigate and quantify metabolites in living tissue (described in more detail in Chapter 3 Section 3.8).

<i>Configuration</i>	<i>Peak ratios</i>
<i>A</i>	1
<i>AB</i>	1:1
<i>AB₂</i>	1:2:1
<i>AB₃</i>	1:3:3:1
<i>AB₄</i>	1:4:6:4:1

Table 2-2: Complexity of the splitting pattern increases with the number of coupled nuclei following Pascal's triangle.

2.7 RELAXATION AND SIGNAL ACQUISITION

At thermal equilibrium, the bulk magnetisation vector $\mathbf{M}$ lies parallel to the direction of the applied magnetic field, B_0 , and is termed the equilibrium magnetisation, M_0 . The spin system is classified such that the magnetic field is applied in the z-direction, therefore $B_0 = B_z$ and $M_0 = M_z$, referred to as the longitudinal magnetisation. There is no magnetisation perpendicular to the applied magnetic field, such that the transverse magnetisation $M_x = M_y = 0$.

As described in Section 2.5, when a time varying magnetic field $\mathbf{B}_1(t)$ is applied to the system, the spins are disturbed from their equilibrium state. Subsequently, when this RF field is switched off, spins return back to thermal equilibrium by a process known as relaxation. The time it takes for spins to relax back to thermal equilibrium forms the principle source of contrast in MR imaging, and is governed by two relaxation times – longitudinal (T_1 -) and transverse (T_2 -) relaxation.

2.7.1. Correlation time

The mechanisms behind relaxation are highly dependent on the environment that the spins reside in. As molecules move around inside a voxel, interactions between neighbouring spins cause local magnetic field fluctuations, slightly altering the frequency of precession for each spin. The

fluctuation of the frequency of the spins is described by the spectral density $J(\omega)$,

$$J(\omega) = \frac{2\tau_c}{1 + \omega^2\tau_c^2} \quad \text{Eqn. [2.34]}$$

where τ_c is the correlation time, defined as the time it takes for a molecule to rotate by one radian. Rapid fluctuations in the transverse field describe a highly mobile environment and have short τ_c with a broad spectral density. Conversely, slow fluctuations have long τ_c and a narrow spectral density (illustrated in Figure 2-12A).

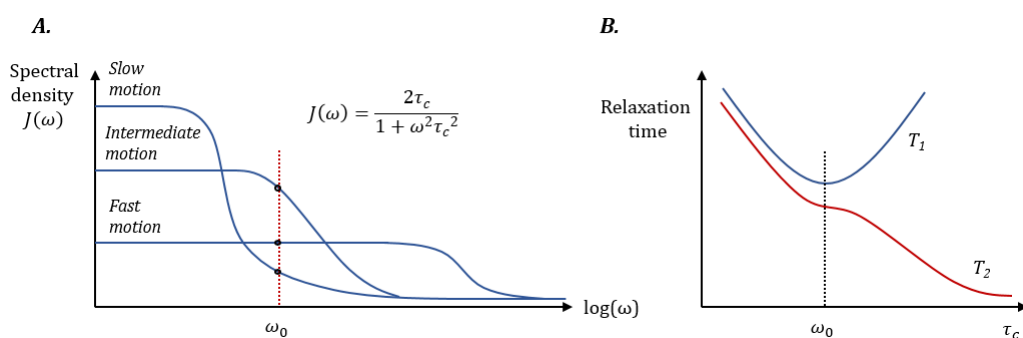


Figure 2-12: A. Spectral density function $J(\omega)$ for three molecules with varying rates of motion τ_c . **B.** Dependency of T_1 and T_2 as a function of correlation time.

The effect of correlation time on relaxation rates can be seen in Figure (2-12B). Longitudinal relaxation is caused by transient magnetic fields precessing at the Larmor frequency, as described in Section 2.7.2, therefore T_1 relaxation is optimal when the correlation time is equal to ω_0 . Transverse relaxation, as described in Section 2.7.3, is caused by transient magnetic fields precessing at any frequency and therefore T_2 relaxation is optimal at higher correlation times. Both T_1 and T_2 relaxation are inefficient at short τ_c , such as in cerebrospinal fluid (CSF).

2.7.2 Longitudinal Relaxation, T_1

The longitudinal relaxation time, T_1 , refers to the process by which M_z recovers exponentially to thermal equilibrium with its surroundings (Figure 2-13). This is also referred to as the spin-lattice relaxation as it involves the exchange of energy between the spin system and its

neighbouring molecules in the lattice. As M_z returns to M_0 , the spin system moves from a high energy state to a low energy state, and the energy it obtained from the RF pulse is dissipated as heat within the lattice. The efficiency of this energy transfer is dependent on how closely the spins are coupled to the lattice and indicates the mobility and binding of the molecules in the sample. Water has a longer T_1 relaxation time than tissue since the molecules are more mobile. This is the cause of T_1 contrast.

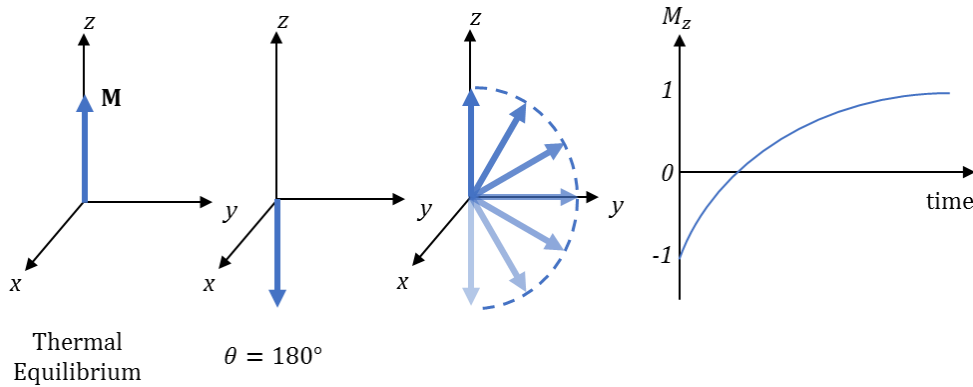


Figure 2-13: Longitudinal relaxation following a 180° pulse. The magnetisation, M , is inverted and recovers exponentially back to thermal equilibrium with a time constant of T_1 .

The rate at which longitudinal equilibrium is restored can be described by the Bloch equation:

$$\frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1} \quad \text{Eqn. [2.29]}$$

2.7.2.1 Measurement of T_1

T_1 is an important source of contrast in magnetic resonance imaging as it provides information on the concentration of macromolecules, water binding and water content [4]. In brain imaging, T_1 allows for the discrimination of different tissue types, such as grey and white matter, and CSF.

The most common method of T_1 measurement is to use an inversion recovery (IR) sequence. In this sequence an initial 180° degree inversion RF pulse is applied, which inverts the longitudinal magnetisation such

that $M_z = -M_0$. The longitudinal magnetisation will then recover towards its thermal equilibrium distribution with a time constant of T_1 .

After a time delay, TI (termed the inversion time), a 90 degree excitation pulse is applied to knock the longitudinal magnetisation at that time into the transverse plane, generating an FID of amplitude proportional to the longitudinal magnetisation existing at the time of the excitation pulse, which is detected by the receive coil. The inversion recovery sequence is shown schematically in Figure 2-14A. The null time, T_{null} , is the time at which the longitudinal magnetisation is equal to zero.

The magnetisation measured at time TI is given by the solution of the Bloch equation (Eqn. [2.29]). With the boundary conditions for an inversion recovery sequence, such that at time $t = 0$; $M_z = -M_0$, $M_x = M_y = 0$. Solving the Bloch equation gives the solution to an inversion recovery of

$$M_z(t) = M_0(1 - 2e^{-\frac{TI}{T_1}}) \quad \text{Eqn. [2.30]}$$

To accurately measure T_1 it is necessary to perform multiple repeats of the IR sequence to collect data over a range of TI times. The time between each of the repeated inversion pulses is known as the repetition time, TR. The signal measured across the range of TI values, $M_z(t = TI)$, can then be used to measure T_1 as shown in Figure 2-14B.

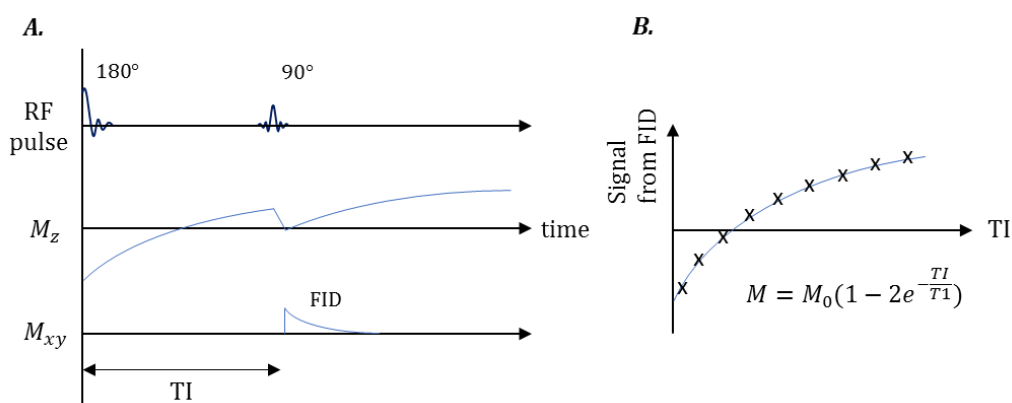


Figure 2-14: A. Pulse diagram of an inversion recovery sequence. B. Exponential fitting to measure T_1 after repeating the sequence with varying TI values.

2.7.3 Transverse Relaxation, T_2

The transverse relaxation time, T_2 , refers to the process by which the magnetisation in the xy-plane, M_{xy} , exponentially decays towards zero, the thermal equilibrium value. It is this decay which defines the envelope of the FID described in Section 2.5.2. To understand this form of relaxation, consider a 90 degree RF pulse applied to the spin system. Upon applying the pulse, the net magnetisation is tipped into the xy-plane and most spins are in phase, precessing at the same frequency. However, interactions between neighbouring spins cause microscopic local magnetic fields to be produced, slightly altering the Larmor frequency for each spin. This causes a dephasing of the spin system, and the transverse magnetisation, M_{xy} , decays to zero with a time constant given by T_2 (Figure 2-15). This process is also referred to as the spin-spin relaxation time. Natural longitudinal recovery also contributes to transverse relaxation, but $T_2 \leq T_1$.

The rate at which transverse magnetisation M_{xy} returns to equilibrium is described by the Bloch equation:

$$\frac{dM_{xy}}{dt} = -\frac{M_{xy}}{T_2} \quad \text{Eqn. [2.31]}$$

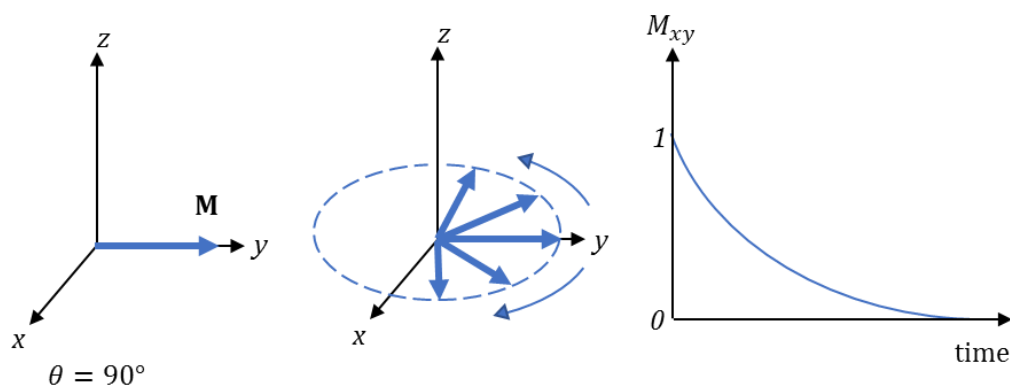


Figure 2-15: Transverse relaxation following a 90° pulse. The net magnetisation, M , is tipped into the xy-plane where the spins dephase and M_{xy} decays toward thermal equilibrium exponentially with a time constant of T_2 .

As well as the time-varying dephasing caused by spin-spin interactions, there is also a static dephasing effect caused by local field inhomogeneities

leading to the more rapid decay of the transverse magnetisation. These static processes are defined by a decay rate of T_2' . This causes the MR signal to decay faster than predicted by T_2 with the time constant of accelerated rate of dephasing known as T_2^* relaxation. These additional dephasing fields arise from inhomogeneities in the main magnetic field, spatial encoding gradients, chemical shift and differences in magnetic susceptibility between tissues. T_2^* is related to T_2 and T_2' by,

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \quad \text{Eqn. [2.32]}$$

Since T_2' processes are static in time, they can be rephased using a 180 degree refocussing RF pulse. However, natural T_2 processes vary in time and therefore cannot be rephased.

2.7.3.1 Measurement of T_2

The most common method to measure T_2 is the spin-echo sequence. This can be seen schematically in Figure 2-16A. First, a 90 degree RF pulse is applied to generate coherent transverse magnetisation such that $M_{xy} = M_0$ (Figure 2-17A). The transverse magnetisation will then decay as the spins dephase with a time constant of T_2^* due to both static T_2' and time-varying T_2 processes. Spins experiencing higher magnetic fields will precess faster than ω_0 and those experiencing lower fields precess slower. In the rotating frame, this appears as spins pointing in different directions (Figure 2-17B).

At a time, t , a 180 degree refocussing pulse is applied to the sample. This causes M_{xy} to be reversed (Figure 2-17C and D). For static T_2' processes, the fast and slow spins will rephase at time $2t$. The time-varying T_2 processes will not rephase and therefore the refocussed signal echo amplitude will be attenuated by the T_2 time constant at time $2t$. The time $2t$ at which the echo is observed is known as the echo time, TE.

The magnetisation measured at time TE is given by the solution of the Bloch equation (Eqn. [2.31]) with the boundary conditions such that at time $t=0$; $M_z = 0$, $M_x = M_y = M_0$. Solving the Bloch equations gives

$$M_{xy}(t) = M_0 e^{-\frac{TE}{T_2}} \quad \text{Eqn. [2.33]}$$

To measure T_2 accurately, it is necessary to repeat the spin echo sequence multiple times at a range of echo times, TE. The signal measured at a range of TE values, $M_{xy}(t = TE)$ is given by $S = S_0 e^{-\frac{TE}{T_2}}$ and can be seen in Figure 2-16B.

Measurement of T_2 using a spin echo scheme at increasing echo times can be inaccurate due to the effects of diffusion. Diffusion causes additional dephasing within the sample and therefore can result in the measured T_2 being shorter than expected if this effect is not taken into account. The Carr-Purcell Meilboom-Gill (CPMG) sequence [5] can be used to correct for molecular diffusion effects and minimise errors in T_2 quantification.

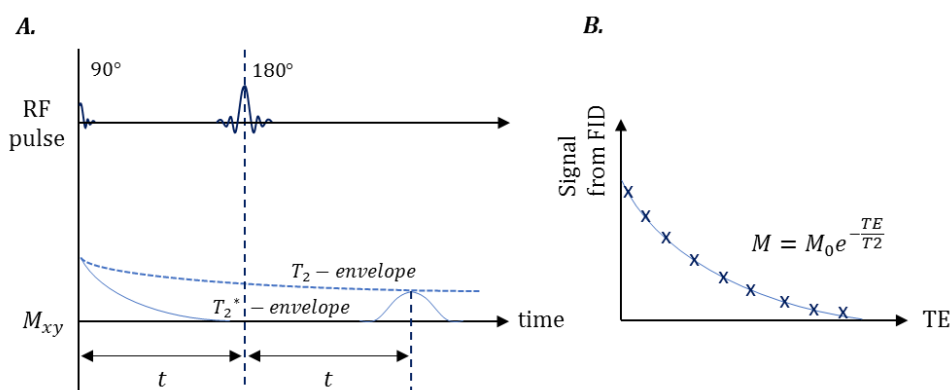


Figure 2-16: A. Pulse diagram of a spin-echo sequence. B. Exponential fitting to measure T_2 after repeating the sequence with varying TE values.

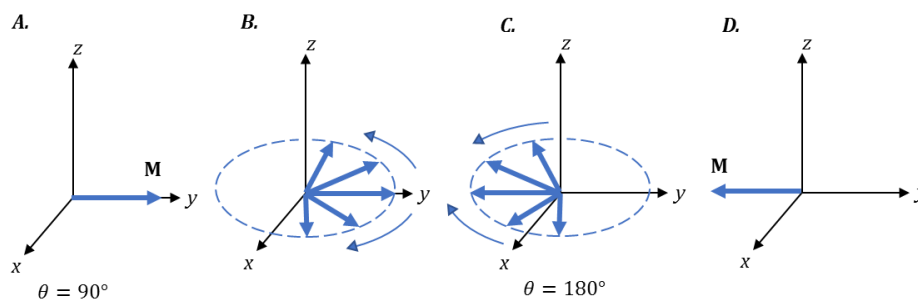


Figure 2-17: Schematic of the spin evolution during a spin-echo sequence.

A. Magnetisation is tipped into the transverse plane following an initial 90° pulse.
 B. Spins begin to dephase.
 C. Following a 180° pulse, spins are flipped and begin to rephase.
 D. Spins have rephased generating magnetisation in the transverse plane.

2.7.2.2 Measurement of T_2^*

After a 90 degree pulse an FID is generated. This signal decays with a time constant of T_2^* and the magnetisation is described by $M = M_0 e^{-\frac{t}{T_2^*}}$. By measuring the amplitude of the FID at varying times t , and applying a mono-exponential fit to the data, T_2^* can be quantified.

T_2^* weighted images are formed at increasing echo times using a gradient echo sequence, which uses magnetic field gradients to rephase the spins as opposed to a spin-echo sequence. Chapter 3 describes magnetic resonance imaging for which magnetic field gradients are used to impart deliberate variations in the B_0 field, which contribute to the dephasing of spins. If the magnetic field gradient is reversed, the spins will rephase giving rise to a gradient echo signal described by T_2^* . T_2^* contrast forms the basis for many MR applications. In particular, changes in oxygen saturation during a functional task, such as a motor movement, alters the local T_2^* in the brain, which leads to the blood oxygen level dependant (BOLD) effect used in functional MR imaging (fMRI) [6] – this will be described further in Chapter 4.

2.8 CONCLUSION

This chapter has provided a brief overview of nuclear magnetic resonance (NMR). The interactions between a nucleus and a static magnetic field has been introduced and described using both a quantum mechanical and classical model. This has been extended to an ensemble of spins and the effect of a time-varying magnetic field has been explored. Finally, the different forms of spin relaxation were outlined. How these give rise to contrast in magnetic resonance imaging will now be discussed in Chapter 3.

2.9 REFERENCES

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3. Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS)

This chapter describes the Magnetic Resonance Imaging techniques used to visualise and quantify physiological measurements during the experiments outlined in this thesis. The principle of slice selection will first be explained, followed by image encoding methods used principally within this thesis. Magnetic Resonance Spectroscopy (MRS) sequences are then outlined. Finally, the hardware used for the data acquisition and safety precautions to consider are outlined.

3.1 INTRODUCTION

Magnetic resonance imaging is a completely non-ionising form of medical imaging allowing the internal structures of the body to be visualised in a matter of minutes. The first one dimensional MR image was reported by PhD student Herman Carr in 1954 [1], however development of MRI did not evolve until twenty years later. In 1973, American chemist Paul Lauterbur expanded on Carr's findings, introducing magnetic field gradients to spatially separate signals from a sample [2]. Parallel to this, Peter Mansfield, a physicist and professor from the University of Nottingham, had begun development on slice selection [3]. In the following years, Mansfield pioneered a number of key developments including echo-planar imaging (EPI) [4], a method to produce high resolution images while reducing the acquisition time from hours to minutes. The progressive evolution of these innovations led to the adoption of MRI as a recognised medical imaging practice. In 2003, the Nobel committee acknowledged the achievements of both Lauterbur and Mansfield by awarding them the Nobel Prize in Physiology and Medicine for 'their discoveries concerning magnetic resonance imaging'.

Today, the applications of MRI have expanded far beyond structural imaging. Functional neuroimaging is a fast developing field used to quantify processes such as brain activation using blood oxygenation level dependent (BOLD) contrast [5] as well as quantification of cerebral blood flow [6], methods which are used throughout this thesis.

3.2 SPATIAL ENCODING AND MAGNETIC FIELD GRADIENTS

The previous NMR theory chapter described how to excite a signal in a sample by applying an RF pulse to generate a free induction decay. While this is useful for some NMR techniques, this alone does not provide an image. To do this, it is necessary to spatially localise the signal such that we can differentiate between regions within the sample. The Larmor equation, $\omega = \gamma B_0$, shows that the frequency of precession of the net magnetisation is linearly proportional to the magnetic field strength, B_0 , also termed B_z . Therefore, by deliberately spatially varying the magnetic field, the Larmor frequency will

also become spatially dependent in a known fashion. A magnetic field gradient, $\mathbf{G}$, can be applied along each of the three orthogonal axes, $[G_x\hat{\mathbf{x}}, G_y\hat{\mathbf{y}}, G_z\hat{\mathbf{z}}]$ to modulate the static field, B_z , with the gradient tensor describing this being

$$\mathbf{G}_r = \frac{\partial B_z}{\partial x}\hat{\mathbf{x}} + \frac{\partial B_z}{\partial y}\hat{\mathbf{y}} + \frac{\partial B_z}{\partial z}\hat{\mathbf{z}}. \quad \text{Eqn. [3.1]}$$

In the presence of a magnetic field gradient, $\mathbf{G}_r$, the spins at position $\mathbf{r}$ will experience a magnetic field strength given by

$$\mathbf{B}(\mathbf{r}) = \mathbf{B}_0 + \mathbf{G}_r \cdot \mathbf{r} \quad \text{Eqn. [3.2]}$$

where G_r has units (mT/m).

The Larmor frequency is now a function of position r within the sample and can be rewritten as,

$$\omega(r) = \gamma(B_0 + G_r r). \quad \text{Eqn. [3.3]}$$

The application of magnetic field gradients forms the basis of MR images to encode in 2D and 3D.

3.3 SLICE SELECTION

The first step to spatial encoding a sample involves slice selection. Commonly in MRI, a single plane in the sample is isolated for imaging, a process which is then repeated across slices. This exclusive excitation of spins in one slice is achieved through the simultaneous combination of a magnetic field gradient and an RF pulse with a narrow bandwidth of frequencies, known as a *slice selective* RF pulse. This slice selective RF pulse ensures that only spins with Larmor frequency within that bandwidth are excited.

Let us here describe the application of a slice selection gradient applied in the z-direction. For this situation, Eqn. [3.3] can be written as

$$\omega(z) = \gamma(B_0 + G_z z). \quad \text{Eqn. [3.4]}$$

It can be seen that the thickness, Δz , of the excited slice can therefore be controlled by varying the bandwidth of the RF pulse, $\Delta\omega$, and/or the amplitude of the slice select gradient, G_z , and is given by

$$\Delta z = \frac{\Delta\omega}{\gamma G_z}. \quad \text{Eqn. [3.5]}$$

So far, we have only considered an RF pulse with a single carrier frequency of ω_0 . To create a slice selective RF pulse with a bandwidth of frequencies, this pulse must be modulated by an audio frequency window. It is this audio frequency that defines the shape of the RF pulse. For example, if a rectangular imaging slice is required in the Fourier domain, the RF pulse is multiplied by a sinc pulse envelope. However, to produce a perfect rectangle the RF pulse would have to be of infinite length. In practice this is not possible and the pulse is truncated, leading to imperfections in the slice profile.

The application of the slice selection gradient results in the dephasing of spins. In order to rephase this effect and maximise the signal produced, following the slice select gradient a gradient of opposite polarity and half the area to the slice select gradient (by halving either the amplitude or the duration of the gradient) is applied, thus rephasing the spins to their original state. The pulse sequence used for slice selection can be seen in Figure 3-1.

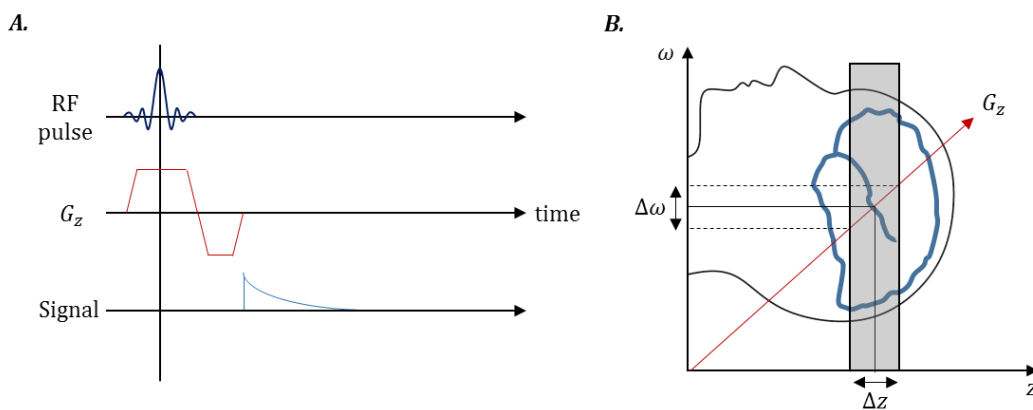


Figure 3-1: **A.** The slice selection pulse sequence showing an RF excitation pulse and a slice select gradient followed by a negative lobe of half the area to refocus the signal. **B.** A schematic of how a slice of thickness Δz is produced by combining the application of an RF pulse of bandwidth $\Delta\omega$ with a slice select gradient G_z .

3.4 PHASE & FREQUENCY ENCODING

Once a slice has been selected in the z-plane, the spins must be encoded within that slice. This in-plane encoding is achieved using phase and frequency encoding gradients.

3.4.1 Phase Encoding

Here phase encoding will be applied in the y-direction. A gradient, G_y , is applied for a short time prior to sampling. This gradient briefly modifies the spin resonance frequencies in the y-direction inducing dephasing. Once the application of this phase encoding gradient terminates, the spins then all resume precessing at the same frequency, but with different phases relative to their y-position.

The phase shift, $\Delta\phi$, introduced by a gradient G_y applied for a duration, t , is given by

$$\Delta\phi = \gamma G_y y t. \quad \text{Eqn. [3.6]}$$

3.4.2 Frequency Encoding

The final stage in spatial encoding involves encoding spins in the x-direction within the slice. This can be achieved using a frequency encoding gradient applied in the x-direction. Unlike the phase encoding gradient, the gradient, G_x , is applied during the readout and results in spatial dependency of the frequency in the x-direction.

The resulting readout signal comprises RF signals with spatial information contained within the different amplitudes, phase and frequencies. This signal is then digitalised with the raw data written into the spatial frequency domain, known as k -space.

A pulse sequence diagram showing each of these spatial encoding gradients can be seen in Figure 3-2.

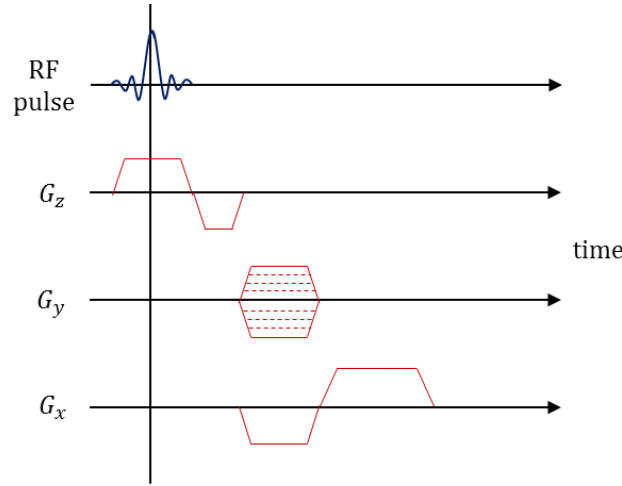


Figure 3-2: Basic pulse sequence diagram showing spatial encoding in each direction. Dashed lines of the phase encoding gradient represent separate phase encoding steps which are repeated after separate RF pulse excitations.

3.5 K-SPACE

During an MR measurement, the raw data acquired represents the transverse components of the magnetisation in the sample post excitation. The horizontal axis in the transverse plane is defined as the frequency encoding direction while the vertical axis is the phase encoding direction (as described in Section 3.4). This is known as k -space data. k -space represents the spatial frequency information of the image in both two and three dimensions. k -space and the image space data contain identical information which can be transformed in each direction using the Fourier Transform. Manipulation of k -space is the basis of fast imaging sequence optimisation.

The signal acquired from a sample, $S(t)$, during an MR pulse sequence is described by

$$S(t) = \int \rho(r) \exp \left[i\gamma \int_0^t \mathbf{G}(\tau) \cdot \mathbf{r} d\tau \right] dr \quad \text{Eqn. [3.7]}$$

where $\rho(r)$ is the spin density of the sample at position r .

The k -space vector is defined as

$$\mathbf{k}(t) = \gamma \int_0^t \mathbf{G}(\tau) d\tau \quad \text{Eqn. [3.8]}$$

Substituting this into Eqn.[3.7] gives the resultant signal

$$S(k) = \int \rho(r) \exp[i\mathbf{k} \cdot \mathbf{r}] dr \quad \text{Eqn. [3.9]}$$

The Fourier relationship between spin density and signal then becomes apparent

$$\rho(r) = \int S(k) \exp[-i\mathbf{k} \cdot \mathbf{r}] dk \quad \text{Eqn. [3.10]}$$

In 2D imaging, MRI images are spatially encoded in k -space one slice at a time. This two dimensional array, (k_x, k_y) , corresponds to the spatial Cartesian coordinates (x, y) . The connection between image space and k -space and how this relates to the field of view (FOV), spatial resolution and sampling of an image can be seen in Figure 3-3.

A. Cartesian Space

B. k -space

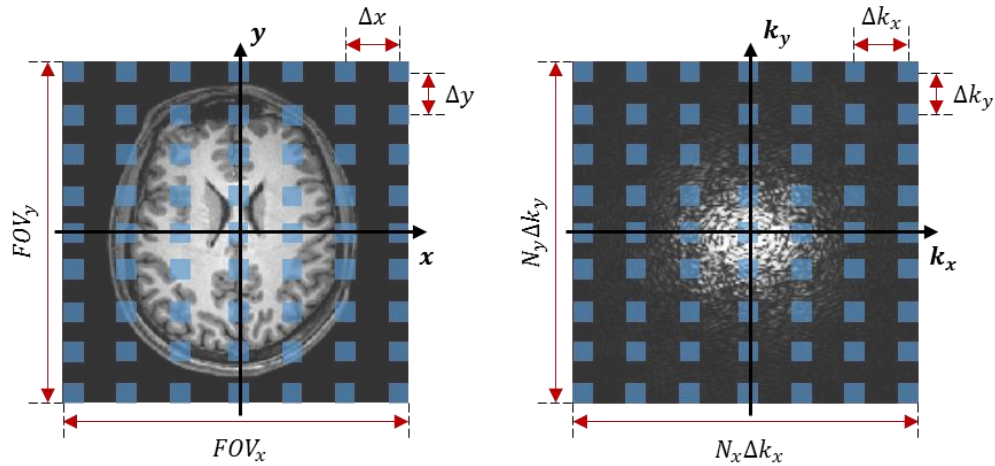


Figure 3-3: Visualisation of how **A.** image space translates into **B.** k -space. N_x and N_y are the number of sampling point in the x - and y -direction respectively.

Different areas of k -space hold different information about the resultant image. The centre of k -space provides information on contrast of the image. Conversely, the outer edges of k -space store information on the high spatial frequency characteristics of the image. Some imaging sequences utilise this

characteristic to speed up acquisition time through partial sampling of k -space, however compromises must be made with loss of signal to noise ratio (SNR) in the image and an increased susceptibility to artefacts.

3.6 IMAGING SEQUENCES

Although MRI has existed for a relatively short period of time, many advances have been made leading to improvements in both image quality and speed of image acquisition. Each image sequence uses a different combination of RF pulses and magnetic field gradients to adjust image contrast and acquisition time depending on the sample being investigated. The following section outlines the type of pulse sequences used in this thesis and how each sample k -space.

3.6.1 Gradient Echo & Spin Echo Imaging

A number of spatial localisation methods used in MR pulse sequences are based upon spin-warp imaging. This technique uses consecutive frequency and phase encoding gradients to fill k -space row-by-row. Both Gradient Echo (GE) and Spin Echo (SE) versions of this technique are used and shown schematically in Figure 3-4 and Figure 3-5 respectively.

Gradient Echo

A gradient echo is produced by using a gradient reversal. Initially, an RF excitation pulse of angle α^0 is applied to tip the magnetisation into the transverse plane. A slice selection gradient, G_z^{slice} , is applied simultaneously with this RF pulse to spatially localise a slice. A phase encoding gradient, G_y^{phase} , and a negative pre-phasing frequency encoding gradient, $G_x^{freq.}$, then follows. This causes traversal of k -space from its origin to the desired position in the spatial domain to begin readout of the signal (A-B in Fig.3-4). During this time, the negative $G_x^{freq.}$ lobe causes dephasing of spins, resulting in the decay of the transverse magnetisation signal. A $G_x^{freq.}$ of opposite polarity and twice the amplitude is then applied during the readout period. This allows the signal to rephase and create an echo, while k -space is sampled along the k_x -direction

(B-C in Fig.3-4). The sequence is then repeated n times with G_y^{phase} of varying amplitudes, allowing for the whole of k -space to be sampled.

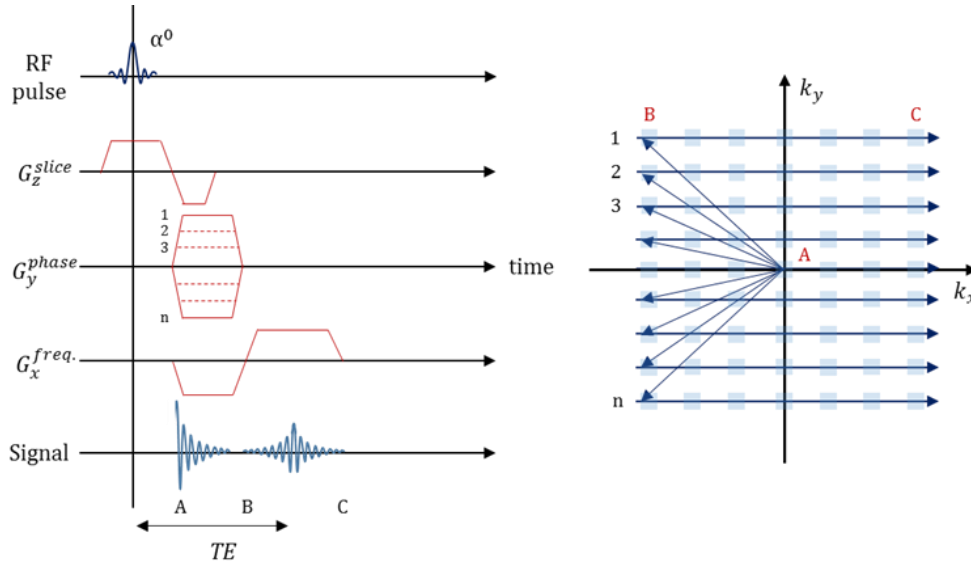


Figure 3-4: A schematic description of the Gradient Echo spin warp pulse sequence and how this translates into filling k -space.

Spin Echo

A spin echo is produced by applying a 180° RF pulse after the initial α^0 RF pulse. The spin echo sequence is similar to gradient echo, however, since a 180° refocussing pulse is applied, a positive $G_x^{freq.}$ pre-phasing lobe is initially applied. This causes k -space to be sampled from the right of the sampled area rather than the left (A-B in Fig.3-5). The 180° pulse is applied to refocus the dephased spins, inverting the k -space position (B-C in Fig.3-5) before the second $G_x^{freq.}$ is applied and readout of the signal is taken (C-D Fig.3-5). Again, this process is repeated for a number of different G_y^{phase} amplitudes allowing each row of k -space to be sampled.

There are several important differences between the GE and SE techniques. Firstly, the gradient reversal in GE imaging only refocuses those spins that have been dephased by the application of the gradient itself. Dephasing due to local field inhomogeneity, chemical shift and magnetic susceptibility differences between tissues remain out of phase and therefore image contrast

is dictated by T_2^* factors. The 180° pulse in the SE technique successfully cancels these phase shifts and therefore the SE image contrast is dictated by true T_2 relaxation. T_2^* -weighted images are more sensitive to local B_0 field inhomogeneities and therefore GE sequences can be more difficult to implement at higher field strengths. However, since only one RF pulse is necessary, the echo time is generally short for GE imaging, limiting issues at high field. Many GE sequences also use a low flip-angle excitation pulse allowing shorter values of repetition time, TR, and faster data acquisition. GE sequences form the basis for most rapid imaging techniques as described in the following sections.

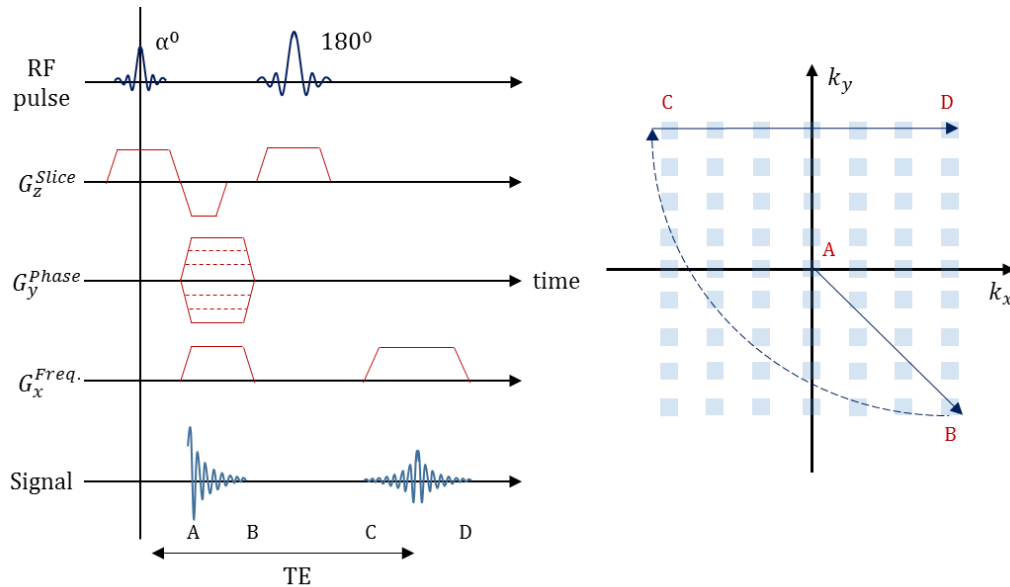


Figure 3-5: Schematic description of the Spin Echo pulse sequence and how this translates into filling k -space.

3.6.2 Turbo Field Echo Imaging (TFE)

Although faster than SE, GE acquisition times can still be long. If a 90° excitation pulse is used to achieve maximum signal, a longer TR is needed for the magnetisation to recover and the system to return to thermal equilibrium. To overcome this, smaller flip angles are used. This smaller excitation pulse leaves a large residue of magnetisation in the longitudinal axis and decreases the transverse component resulting in a quicker recovery. If the TR is reduced below the T_2 of the sample, residual transverse magnetisation is carried over

into the next repetition. RF pulses or variable gradients are used to spoil this residual magnetisation before the next excitation pulse. This causes the resulting contrast to be defined by T_1 relaxation only.

If this process of using a low flip angle with identical excitation pulses is repeated at TR a sufficient number of times, the longitudinal magnetisation, M_z , will reach a steady state. The number of pulses required to reach steady state depends on the T_1 of the tissue, TR, and the flip angle of the applied RF pulse.

Assuming that the residual transverse magnetisation is fully spoiled between repetitions, the GE steady state signal can be described by,

$$S_{SS} = M_0 \frac{\sin(\alpha) \left(1 - e^{-\frac{TR}{T_1}}\right)}{1 - \cos(\alpha) e^{-\frac{TR}{T_1}}} e^{-\frac{TE}{T_2^*}} \quad \text{Eqn. [3.11]}$$

The maximum amount of signal achieved will occur at the Ernst angle, α_E , where

$$\alpha_E = \cos^{-1} e^{-\frac{TR}{T_1}} \quad \text{Eqn. [3.12]}$$

found by differentiating Eqn.[3.11] with respect to α .

The vast majority of TFE sequences are acquired at very short TR, meaning that acquisition time is greatly reduced for 2D or 3D imaging.

3.6.3 Magnetisation Prepared Rapid Gradient Echo (MPRAGE)

The compromise in the battle for faster acquisition time through use of a small flip angle and short TR is poor T_1 -weighted image contrast. To preserve T_1 -weighting, the longitudinal magnetisation can be prepared prior to the repetitions with a 180° inversion pulse. Subsequent excitation pulses of flip angle α^0 are then applied to measure the T_1 recovery.

A schematic of the T_1 -weighted MPRAGE pulse sequence is shown in Figure 3-6.

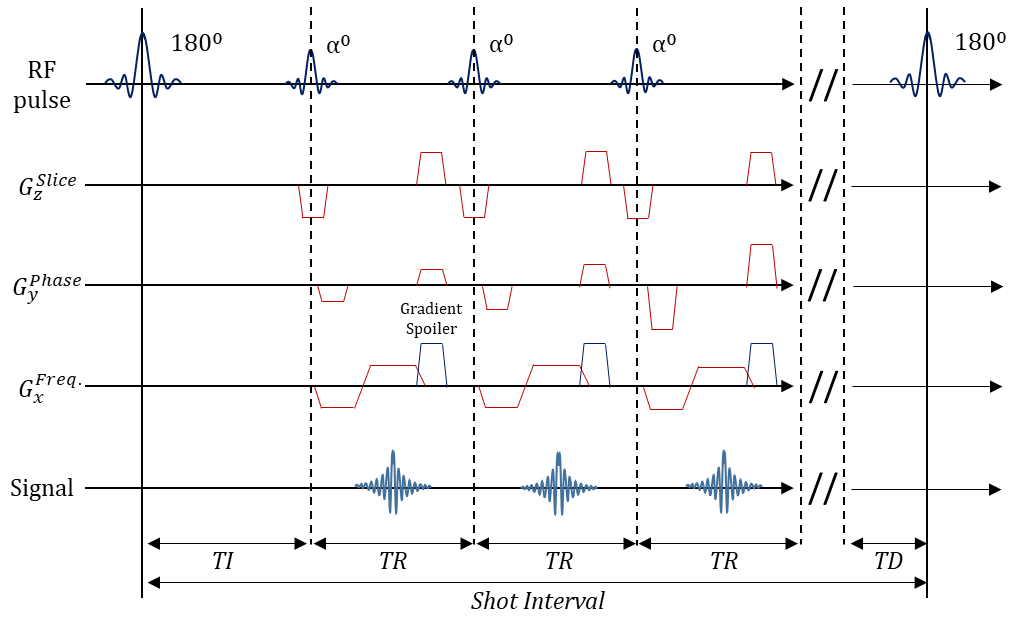


Figure 3-6: Schematic description of the Magnetisation Prepared Rapid Gradient Echo (MPRAGE) pulse sequence with an initial 180° pulse used to preserve T_1 contrast.

An initial 180° pulse applied at time $t = 0$ inverts the longitudinal magnetisation such that $M_z(t = 0) = -M_0$. The time between this inversion pulse and the first excitation pulse is defined as the inversion time, TI . The repetition time, TR , is the time between each subsequent excitation pulse of flip angle α° . The number of pulses within this train is known as the TFE factor. Gradient spoilers are applied prior to each excitation pulse to ensure all residual transverse magnetisation is destroyed. The interval between the end of the train and the next inversion pulse is known as the time delay, TD and the time between two inversion pulses is known as the shot interval.

The MPRAGE imaging technique is particularly suited to T_1 high resolution 3D imaging at 3.0 Tesla. Applications include anatomical studies and tissue quantification, such as grey matter volume and cortical thickness, as performed in Chapter 6 of this thesis.

However, at higher field (>3.0 T) inhomogeneities in B_0 can cause distortions in voxel intensity across MPRAGE data. Since tissue classification is intensity dependant, segmentation will fail. Phase sensitive inversion recovery (PSIR), also termed MP2RAGE, is a method by which the inversion recovery data is

reconstructed taking into account the polarity of the magnetisation of the sample. This correction for phase protects against B_0 inhomogeneity artefacts and allows for more robust tissue segmentation and quantification. The PSIR sequence is applied for high resolution structural imaging at 7.0 T in Chapter 7 of this thesis.

3.6.4 Echo Planar Imaging (EPI)

Echo planar imaging was first developed by Mansfield in 1977 [4] as a method to improve the temporal resolution of MRI. Unlike spin-warp imaging, EPI samples the whole of two-dimensional k -space after a single RF excitation, or shot, rather than using multiple RF pulses. This allows an entire slice to be acquired in tens of milliseconds. A GE-EPI pulse sequence and its corresponding k -space trajectory are shown in Figure 3-7.

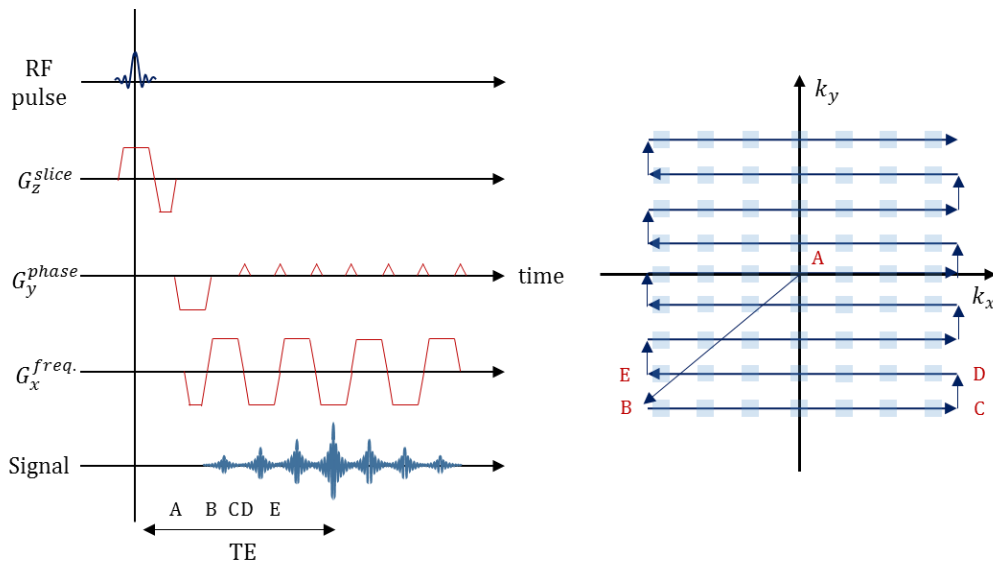


Figure 3-7: An Echo Planar Imaging pulse sequence diagram and how this translates into filling of k -space.

As with previously described techniques, an initial excitation RF pulse and slice selective gradient are applied simultaneously to spatially encode the slice. Since the sampling strategy of this particular example begins in the bottom left corner of k -space, a negative frequency and phase encoding gradient have been initially applied (A-B in Fig.3-7). Used as the readout, the frequency encoding gradient is then rapidly reversed, sweeping along k_x in both

directions, while a small phase encoding ‘blip’ gradient is applied in between to ensure k -space is sampled in ascending rows (B-C-D-E in Fig.3-7). This rapidly reversing frequency encoding gradient repeatedly rephases the transverse magnetisation to create a train of echoes as the data is sampled. Since the method traverses k -space in opposite directions, the acquired data must be reordered prior to Fourier transformation

The rapidly reversing frequency encoding gradient used in EPI can pose challenges for image acquisition. Slight imperfections in the reversing gradients make the sequences highly susceptible to the Nyquist ghost artefact, which affect images along the phase encoding direction. Also, the speed in which these gradients are applied can place heavy demands on the hardware, requiring high-performance coils with short switching times.

Echo planar imaging can be modified to acquire a 3D volume. The first method to do this was known as echo volumar imaging (EVI). This technique samples the entire 3D k -space using a single excitation pulse. However, EVI suffers from long acquisition times and is therefore now not commonly used. The more widely used method is 3D-EPI. This technique uses multiple excitation pulses, while stepping through the k_z - plane of k -space. The RF excitation pulse is applied to the entire volume, and therefore the SNR is greater for 3D-EPI compared with the 2D-EPI method.

The EPI readout provides the basis for many advanced MRI applications. Due to its high temporal resolution, EPI is widely used to measure fast changing physiological processes such as diffusion, perfusion and the haemodynamic response in functional MRI (fMRI). These applications account for the majority of the work in this thesis, and are outlined in more depth in Chapter 4.

3.7 ACCELERATED RECONSTRUCTION TECHNIQUES

3.7.1 Partial Fourier Imaging

Other methods to reduce image acquisition time include accelerated reconstruction techniques. Partial Fourier imaging is one such technique in which an entire image can be formed by sampling only selected lines of k -

space. This is possible since k -space possesses conjugate symmetry whereby the real and imaginary components of the Fourier transform are symmetric and asymmetric respectively. Figure 3-8 shows the concept of conjugate symmetry in k -space.

Theoretically, conjugate symmetry allows an entire image to be formed by sampling only half of k -space, while the other half can be estimated. However, in practice phase errors occur during image acquisition, which result in inaccurate estimations. It is therefore common for partial Fourier techniques to sample a few extra lines in the estimated half of k -space which are then used to phase correct k -space and predict more accurate values of the estimated points.

Partial Fourier imaging can be used to reduce the minimum echo time if applied in the frequency encoding direction, or reduce image acquisition time if applied in the phase encoding direction. However, since less k -space is sampled, SNR is reduced and image artefacts can be introduced.

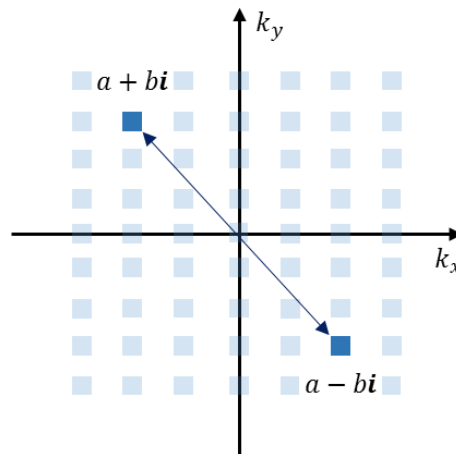


Figure 3-8: Conjugate symmetry. Two points (highlighted in blue) mirror in k -space. If the value of one is known, the other can be calculated as its complex conjugate.

3.7.2 Parallel Imaging

Parallel imaging (PI) is a method whereby undersampling and spatial localisation is assisted by the use of multiple RF receive coils. Using the known placement and sensitivity of each of these receive coils, information about the origin of the signal can be used to construct an image. This allows the number

of phase encoding steps to be reduced, thus reducing the image acquisition time. However, reducing the number of phase encoding steps while maintaining resolution means reducing the amount of k -space sampled. This leads to insufficient spatial frequency and a smaller FOV. The reconstructed image therefore includes a wrap-around artefact known as aliasing.

The most widely used technique to correct for the aliasing overlap in PI, and that which is used on the Philips scanners used to collect the data presented in this thesis, is known as sensitivity encoding (SENSE). Developed in 1999 by Pruessmann *et al.* [7], SENSE reconstructs the image data from each individual coil before unfolding and combining the partial FOV images by matrix inversion. A complete image with full FOV coverage is then produced.

To acquire an image using SENSE, an initial reference scan is performed to create a sensitivity map of each of the receive coils. This also produces aliased images for each channel. The reference scan can then be used on subsequent scans to unfold the signal superposition and produce an image with a full FOV. The SENSE factor refers to the amount by which k -space is under-sampled, which is limited by the number of receive coil elements. SENSE has been used for the majority of image acquisitions in this thesis.

3.8 MAGNETIC RESONANCE SPECTROSCOPY (MRS)

The application of NMR spectroscopy (Chapter 2 Section 2.6) *in vivo* is known as magnetic resonance spectroscopy, or MRS. MRS allows for non-invasive interrogation and quantification of a number of different metabolites from localised regions of living tissue. Performing an MRS scan is similar to that of an MRI sequence with a few additional steps preceding data acquisition.

3.8.1 Localisation

Typically, MRS is acquired from a single voxel within the tissue of interest. While MRI utilises frequency and phase encoding, localisation of a voxel in an MRS experiment is performed using a combination of band-selective RF pulses and magnetic field gradients. The most common techniques for localisation are Point RESolved Spectroscopy (PRESS) and STimulated Echo Acquisition Mode

(STEAM). Both methods use a combination of three RF pulses alongside three magnetic field gradients in each direction and rely on a spin echo (Section 3.6.1). The two techniques can be seen in Figure 3-9.

During a PRESS sequence, a spin echo is followed by an additional 180° RF pulse generating a second spin echo. Here, localisation is achieved as the initial slice-selective 90° RF pulse is applied in one plane with the following slice-selective 180° pulses applied in the remaining two planes. Signal will be received from the intersection of the three excited orthogonal planes. PRESS achieves 3D localisation in one scan and is relatively insensitive to motion, however, compared with other localisation techniques, the minimum TE achievable is relatively long.

A STEAM sequence is similar to the PRESS technique, however three consecutive 90° slice-selective RF pulses are applied. STEAM receives signal from the calculated effect of the final two 90° pulses. The second pulse returns spins to the z-axis, which still contains coherences from the initial excitation pulse. The final pulse then refocuses the spins. The echo time in a STEAM sequence can be less compared to PRESS since the signal does not need to be fully decayed after the first pulse.

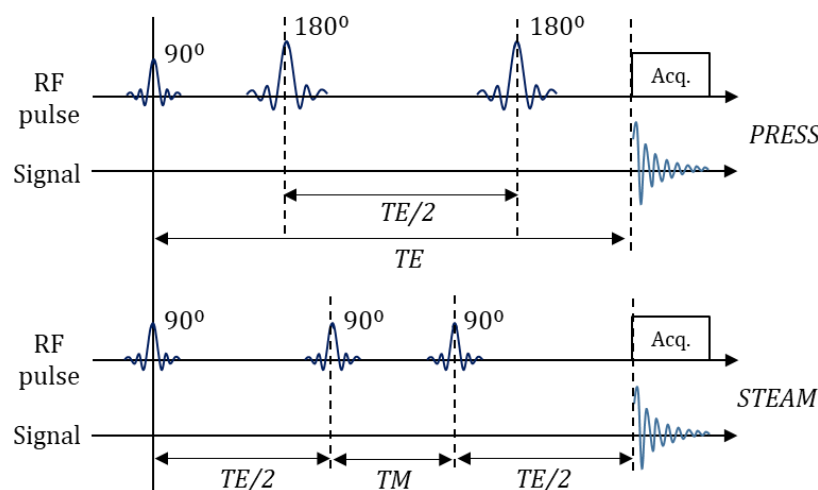


Figure 3-9: Schematic of a PRESS and STEAM localisation sequence where TE is the echo time and TM is the mixing time

3.8.2 Water suppression

Concentrations of metabolites are roughly $10^3 - 10^4$ times smaller than the water content of living tissue, and therefore water presents the dominating peak. This peak can drown the signal from the metabolites and distort the spectral baseline. While the water signal may be useful for absolute quantification, the peak is generally suppressed to improve the resolution of the metabolite peaks.

In general, a peak can be suppressed by utilising specific properties of that molecule, such as its chemical shift. Water has a different chemical shift to most metabolites and therefore common water suppression techniques rely on frequency-selective pulses. Chemical Shift-Selective (CHESS) water suppression is one such technique that performs saturation with frequency-selective pulse excitation. This method initially excites the water resonance with a 90° RF pulse before dephasing the transverse magnetisation using a spoiler gradient, achieving zero longitudinal water magnetisation prior to initiating the excitation pulse in the localisation sequence. However, B_0 and B_1 inhomogeneity within the sample may cause incomplete water suppression and therefore multiple pulses are applied to achieve broader excitation.

A second suppression method takes advantage of the different T_1 relaxation times of water and metabolites. Originally a nonselective 180° pulse is applied, followed by a delay corresponding to the T_1 of water to achieve zero longitudinal water magnetisation. However, due to the small differences in T_1 between water and metabolites, the metabolite signal is partially suppressed. This original technique was optimised to take into account the heterogeneity of water T_1 and to improve efficiency in the presence of an inhomogeneous B_1 field by finding the optimal T_1 recovery time and combining inversion pulses with the CHESS method using optimised pulse flip angles. This method is known as Water suppression Enhanced through T_1 effects, or WET.

3.9 INSTRUMENTATION

The work presented in this thesis was all performed on either a Philips Achieva 3.0 Tesla or 7.0 Tesla MR scanner. The following section briefly outlines the hardware of these systems, and the safety precautions to consider when carrying out MR studies.

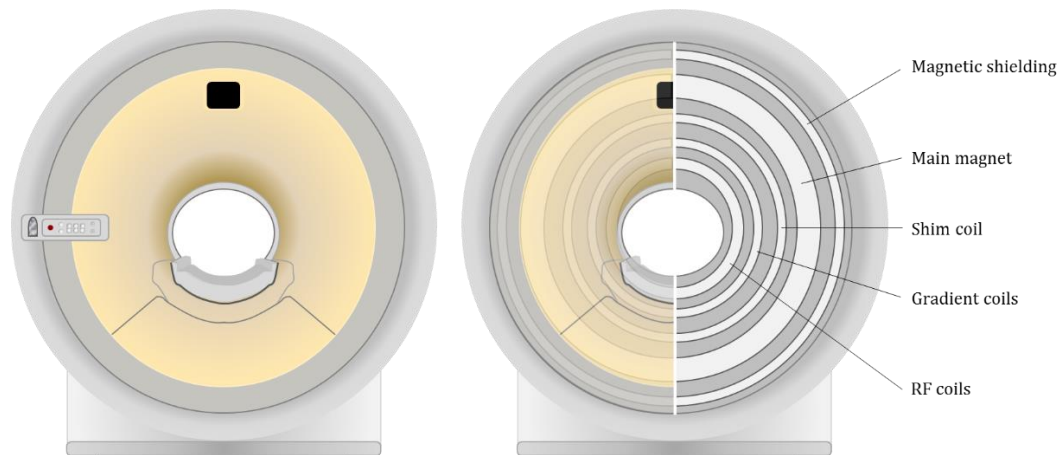


Figure 3-10: Schematic of a Philips Achieva 3T MR scanner with the positioning of the magnetic shielding, main cryogen cooled electro-magnet and the shim, gradient and RF coils.

3.9.1 Main Magnet

In order to achieve signal from spins in the human body, a high intensity magnetic field is required. To reach the magnetic field strength necessary, superconducting magnets are used. These consist of a solenoid of wire where the induced field is dependent on both the current intensity and number of loops. Superconducting wires are used to reduce the resistance and maximise the efficiency of running a high current. The coils are cooled to a superconductive state using liquid helium. Historically, the solenoid was further immersed in liquid nitrogen to reduce thermal losses, however this process has since been replaced with cryo-coolers, reducing the cost of running a superconducting magnet.

To reduce the fringe of the magnetic field, active or passive shields are installed that surround the magnet. Active shielding suppresses the fringe field within the cryostat itself. It consists of an outer superconducting coil which is

arranged such that it opposes the external field generated by the inner coil. While this can greatly reduce the fringe field outside the bore, it can also reduce the field inside the bore. Passive shielding consists of high permeability material incorporated into the walls of the scanner room. This decreases the magnetic flux lines further from the magnet. The Nottingham Philips 7.0 T scanner is passively shielded with 213 tonnes of iron.

3.9.2 Gradient Coils

The purpose of gradient coils was explained in Section 3.2. The coils are used to spatially localise the signal by producing linear variations in the magnetic field homogeneity. Three orthogonal gradients can be set up in the x, y and z direction. The amplitude of the gradient (mT/m) determines the slice thickness and FOV of the resulting image. Gradient coils are required to rapidly switch direction, and the speed at which they achieve this is known as the slew rate. For the work conducted in this thesis the gradient strengths and slew rates used were 30 mT/m and 200 T/m/s at 3.0 T and 33 mT/m and 133 T/m/s at 7.0 T. This rapid switching induces eddy currents in nearby conducting surfaces which, in turn, can distort the gradient profile. To avoid this, the gradient coils can be actively shielded, or the input gradient can be modified such that the outputted profile is the desired shape. Coil vibrations from these gradient switches are also the cause of the characteristic acoustic noise produced during an MR scan.

3.9.3 RF Coils

The radiofrequency system consists of transmit and receive coils used to excite nuclei and readout the signal produced. Transmission requires the delivery of uniform excitation throughout the sample, while reception must have the best possible sensitivity and signal to noise ratio. At 3T, a whole body RF coil is contained within the main magnet which produces a homogenous excitation across the entire scan volume. It can be used as both a transmitter and a receiver. However, since SNR decreases as $1/r^2$, smaller more flexible coils, which can be placed closer to the region of interest, are more commonly used as receivers. Although these coils are less homogeneous with a lower FOV, they

offer a much greater SNR and higher spatial resolution. The work carried out in this thesis required both brain and muscle scanning. At 3.0 T, the data acquisition for brain MRI used the inbuilt whole body transmit coil and a 32 channel receive head coil. At 7.0 T, a head volume transmit coil was used for both the brain and muscle data acquisition. Signal was then received using a 32 channel receive head coil for the brain data, while a pair of 16 channel surface coils were used for the muscle 7.0 T data. Using these multiple receive coils allowed the use of SENSE to speed up the image acquisition. The coils used in this study are shown in Figure 3-11.



Figure 3-11: *A. Philips 32 channel SENSE head receive coil used on the Philips Achieva 3T MR scanner. B. Nova 32 channel head receive coil and transmit coil used on a Philips Achieva 7T MR scanner. C. A Pair of 16-channel surface coils (MRCoils) used on the Philips Achieva 7T MR scanner.*

3.10 SAFETY CONSIDERATIONS

Although MRI poses very little risk to subjects in terms of ionising imaging techniques, such as X-ray, dangers are still present and precautions must be taken.

3.10.1 Static Magnetic Field

The strong magnetic field associated with the large magnetic field can turn any ferromagnetic object into a potentially lethal projectile. This projectile effect can cause objects to move through space at speeds of up to several metres per second. The force of attraction is dependent on a number of factors including the strength of the field and the magnetic susceptibility of the object. The static magnetic field can also cause displacement of metallic objects in the body. These may be known implants or foreign bodies picked up accidentally (e.g.

from working in a metal workshop without eye protection). The magnetic field may also interrupt the functionality of certain devices, including cardiac pacemakers and poses a potentially fatal risk. Many modern day medical devices are now developed to be MR compatible, including most heart valves.

To avoid the risks posed by the strong magnetic field, scanner sites are installed with security doors to ensure only authorised personnel have access to potentially dangerous areas, and warning signs are clearly visible. Any person to be present in or around the magnet are asked to complete a safety questionnaire designed to highlight any risk to their safety beforehand.

3.10.2 Gradient Fields

Rapid switching of the magnetic field gradients used for imaging can induce currents within the body causing peripheral nerve stimulation (PNS). This can be uncomfortable for the subject. EPI is most susceptible to PNS due to its high switching frequency of gradients. This adverse effect can be avoided by lowering the slew rate of a sequence. This was performed at 7T with the slew rate lowered from 200 T/m/s to 133 T/m/s.

3.10.3 RF Fields

The RF pulses applied during a pulse sequence transmit energy which can lead to tissue heating due to the power dissipated in the body. Heating may also occur in tattoos if the ink contains metal. Standards are set to limit the dose of energy that a subject can receive during scanning, known as the specific absorption rate (SAR). This is the RF power absorbed by biological tissue, normalised for patient body weight and generally expressed in terms of watts per kilogram (W/kg). The SAR is calculated by the scanner software before each sequence is performed, and takes into account the coil configuration and anatomic region under investigation.

Care must also be taken when positioning the subject in the scanner. Circulating currents may be induced by closed conducting loops, causing further heating. This can arise through skin contact, and so subjects are positioned without their arms and legs crossing. Another potential source is

from electrical leads, so hardware in the scanner environment is minimised and isolated where necessary (e.g. patient call buzzer, VCG).

3.10.4 Cryogenics

As mentioned in Section 3.9.1, the main electromagnetic coil is cooled to superconducting levels by submerging it in liquid helium. In the case of an emergency, the magnet is quenched leading to rapid boil-off of the liquid helium, which would displace oxygen in a closed and unventilated room, potentially asphyxiating any occupants inside. The scanning suite is fitted with oxygen depletion alarms to alert scanner operators of this event.

3.11 CONCLUSION

This chapter has outlined magnetic resonance imaging and spectroscopy techniques. The hardware of an MR imaging system was described along with the safety precautions to consider. Chapter four provides a comprehensive description of physiological and functional imaging sequences used in the studies presented in this thesis.

3.12 REFERENCES

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4. Physiological and Functional Brain Imaging Techniques

Assessment of brain haemodynamics using Magnetic Resonance Imaging (MRI) is a key theme of the work presented in this thesis and a subject which has grown rapidly over recent years. The following chapter describes the imaging techniques and analysis methods used in this thesis to assess brain physiology, function and metabolism.

4.1 BRAIN PHYSIOLOGY

Like any other organ, the brain requires oxygen to metabolise and provide energy. This oxygen, along with other nutrients, is supplied via the bloodstream. Although the average adult brain represents only approximately 2% of the body's total weight, it accounts for over 20% of the oxygen consumed and demands 12 – 15% of the cardiac output [1]. The two main pairs of arteries which feed the brain are the internal carotid arteries and vertebral arteries. The vertebral arteries merge into the basilar artery and meet the internal carotid arteries at the Circle of Willis [2]. The anatomy of the cerebrovascular system that feeds the brain is shown in Figure 4-1A.

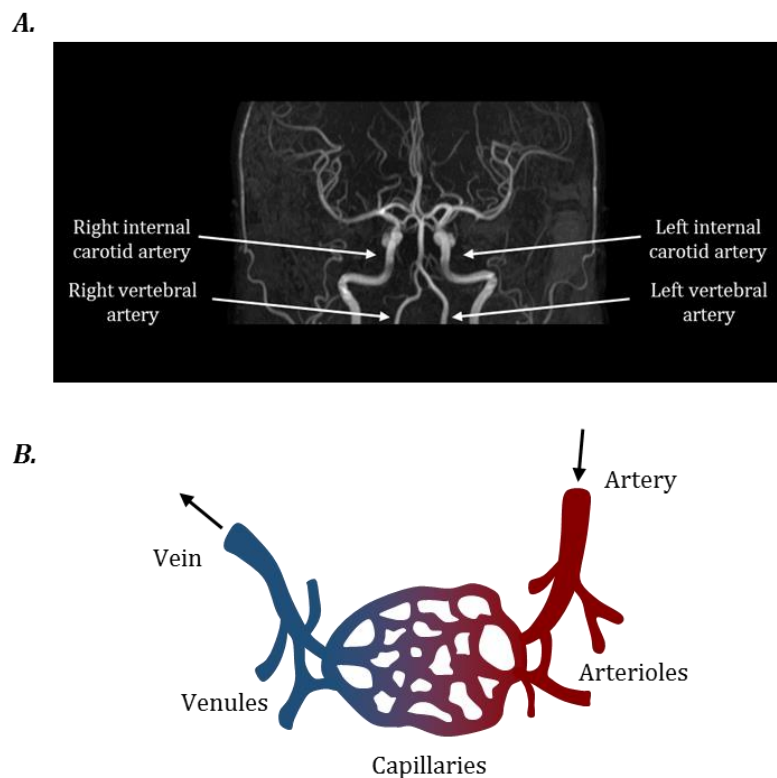


Figure 4-1: **A.** MR coronal angiogram of vessels feeding the brain. **B.** Schematic of capillary architecture, arrows indicate direction of blood flow.

These large feeding arteries divide into progressively smaller arterioles which traverse the surface until they penetrate the brain and perfuse the tissue as capillaries [3]. Within the brain, the blood–brain barrier (BBB) allows diffusion of essential molecules such as water, glucose and oxygen into the

tissue, while preventing the entry of larger, potentially harmful molecules [4]. This protects the brain from most pathogens and avoids any blood-borne infections. These capillaries continue into a network of venules and veins before joining the sagittal sinus to drain the blood from the head. The cerebrovascular architecture can be seen in Figure 4-1B.

Table 4-1 defines the primary physiological variables associated with brain function and energy metabolism that are studied in this thesis.

Variable	Abbreviation	Definition	Units
<i>Blood Flow</i>	BF	Bulk blood flow through an artery or vein	ml/min
<i>Cerebral blood flow or Perfusion</i>	CBF/f	Volume of arterial blood perfusing a given volume of brain tissue in a given period of time	ml/100g/min
<i>Oxygen Extraction Fraction</i>	OEF	Amount of oxygen extracted from the feeding blood vessels	%
<i>Cerebral Metabolic Rate of Oxygen</i>	CMRO ₂	Amount of oxygen metabolised by the brain in a given period of time	μmol/min

Table 4-1: A list of measurable quantities used to assess brain physiology, function and metabolism which can be assessed using MRI.

Blood flow (BF) is the bulk flow of blood which occurs in major arteries and veins, measured in units of millilitres per minute (ml/min). Typical blood flow through the whole brain in an adult is of 750-1000 ml/min, while blood through each carotid artery is of the order of 350 ml/min, compared to the vertebral artery which is of the order of 150-200 ml/min. It is important to distinguish between perfusion and bulk blood flow. The term perfusion describes the amount of blood delivered to the capillary bed of a volume of tissue in a certain period of time, and its units are therefore millilitres of blood per 100 g of tissue per minute (ml/100g/min). Perfusion describes blood flow

at the capillary level, and is closely related to the delivery of oxygen and other nutrients to the tissue. It is this quantity which determines whether the energy status of the tissue is likely to become compromised. Perfusion is therefore an essential parameter, and for this reason much effort has been put into methods which can quantify measurement non-invasively.

The continuous assessment of blood flow velocity can be performed using transcranial Doppler (TCD) ultrasound. However, TCD ultrasound is limited to observations of large vessel flow velocities, rather than microvascular perfusion. Regional perfusion can be measured using Computerised Tomography (CT) and Positron Emission Tomography (PET). However, these methods involve the use of ionising radiation and cannot provide continuous measures of perfusion. Blood flow can be measured with MRI using phase contrast MRI, and is described in Section 4.2.

The application of MRI measures to assess the physiological response to exercise are fully reviewed in Chapter 5. MRI is appealing to study such changes due to its non-invasive nature, and its ability to image vessels with complex geometry, and collect data at high spatial resolution. Furthermore, MRI quantifies both blood velocity and vessel diameter, the product of which provides a true measure of blood flow. Two main approaches have been developed for the assessment of perfusion using MRI: bolus tracking which requires the injection of an intravenous contrast agent (typically gadolinium) and Arterial Spin Labelling (ASL), which uses endogenous contrast based on the magnetisation of water. Since ASL does not require a contrast agent it can be repeated to study dynamic changes in the brain. This method has been used in Chapters 5 and 7 of this thesis, with the basic principles behind ASL being described in Section 4.3.

One of the important parameters defining oxygen consumption in the brain is the oxygen extraction fraction (OEF), which describes the percentage of oxygen removed from the blood by tissue during its passage through the capillary network. To date, most quantitative imaging studies mapping tissue OEF have been conducted using oxygen-15 based PET imaging techniques [5]. The advent of Blood Oxygenation Level Dependent (BOLD) imaging by Ogawa

et al. [6] opened new opportunities to non-invasively study brain haemodynamics. BOLD contrast utilises the differences in magnetic susceptibility between deoxygenated and oxygenated blood, with oxygenated blood being diamagnetic and having a magnetic susceptibility similar to the tissue, and deoxyhaemoglobin being paramagnetic. Due to this effect, the change in T_2^* can be used to identify active brain regions. However, BOLD does not directly measure OEF. Recently, T_2 -relaxation-under-spin-tagging (TRUST) MRI has been developed [7] to quantify the oxygen extraction fraction (OEF). This technique is outlined in Section 4.4.

In summary, functional magnetic resonance imaging (fMRI) techniques measure physiological and metabolic responses as a *consequence* of neural activity, i.e. not neural activity directly. They provide the advantage that they yield regional information of brain perfusion and metabolism, either at rest or in response to a task. Due to recent advances in MR imaging, it is now possible to assess these measures entirely non-invasively in a single scan session. In the following sections, the methods used to measure the physiological variables highlighted in Table 4.1 are described.

4.2 PHASE CONTRAST (PC) MRI TO ASSESS BRAIN BLOOD FLOW

Phase contrast MRI (PC-MRI) is a non-invasive method used to visualise and quantify motion in an imaging volume. It utilises the concept that the phase shift experienced by spins flowing in the direction of a magnetic field gradient is proportional to their velocity of travel. Thus for any vessel, velocity of blood flow and its cross-sectional area can be quantified from which the blood flow through the vessel is deduced in ml/min.

PC-MRI typically acquires two data sets, with flow sensitivity varied between these acquisitions, and controlled by the strength of an applied bipolar gradient pair of equal magnitude and opposite polarity. The first data set is acquired without flow sensitivity while the second is acquired with a flow sensitive sequence of a defined velocity encoding, V_{ENC} . Stationary tissue spins will therefore experience no phase shift, while moving blood spins experience a non-zero phase shift. The two data sets are then subtracted, producing a

phase contrast image. Since the phase shift is proportional to the spin's velocity, quantitative assessment of flow velocity can be achieved.

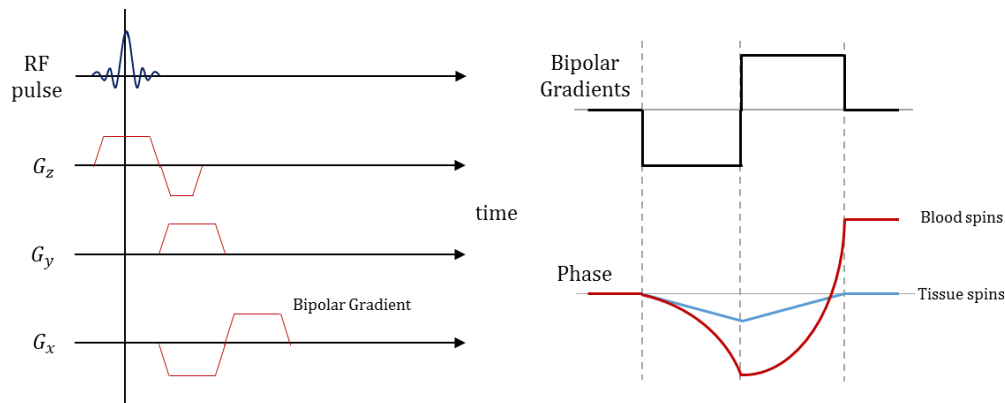


Figure 4-2: Pulse sequence diagram for PC-MRI and the effect of the bipolar gradients on the phase of blood and tissue spins.

Care must be taken when implementing a PC-MRI sequence as incorrect estimates of the V_{ENC} may result in velocity aliasing in the vessel of interest. Using this strategy flow encoding can be implemented for 2D [8] and 3D [9] PC-MRI acquisitions. More recently 4D PC-MRI [10] has been introduced, which offers a volumetric imaging technique allowing the visualization and quantification of complex flow fields.

4.3 ARTERIAL SPIN LABELLING (ASL)

Arterial spin labelling (ASL) is a non-ionising and completely non-invasive MR technique for the quantification of tissue perfusion [11]. In ASL, a volume of arterial blood water is magnetically labelled creating an endogenous contrast agent [12, 13], and so no injected contrast agent is necessary. The labelled blood can then be tracked to the tissue region and perfusion quantified.

ASL requires the acquisition of two images. A 'label image' is acquired for which the inflowing blood water is magnetically labelled, and a 'control image' for which no label is applied. As the signals from the static tissue are identical in both, any differences between the two images is attributable to the magnetised blood flow [14]. The magnetic labelling of the inflowing blood water supplying the imaging volume is typically achieved using an inversion RF pulse (for pulsed ASL – see below). After a time termed the post-label delay

(PLD), labelled blood is delivered from the labelling plane to the capillaries and perfuses into the tissue. Subtraction of the label from the control image will result in a perfusion weighted (PW) image of the tissue (Figure 4-3).

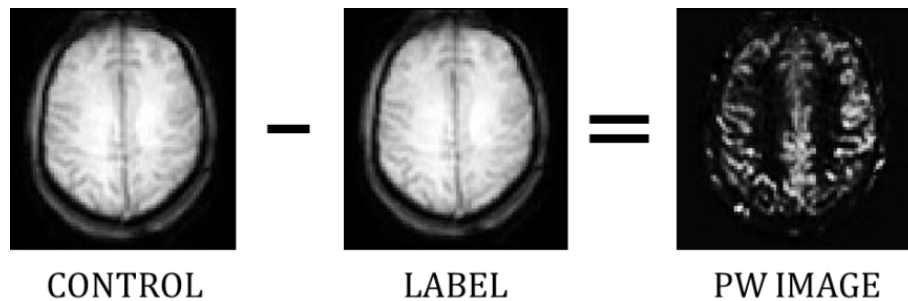


Figure 4-3: Basic method of generating a perfusion weighted (PW) image, shown for an axial slice through the brain.

However, the label signal decays with the longitudinal relaxation of blood and then tissue after it exchanges, reducing the PW signal to a minimal level within 1-2 seconds. The signal change measured by ASL when assessing perfusion is therefore inherently low, between 0.5–1 % in grey matter at 3.0 T, and increasing to 1-2 % at 7.0 T due to the lengthened tissue and blood relaxation time. Thus for sufficient signal-to-noise ratio (SNR), multiple (control-label) pairs are acquired and averaged to produce a final perfusion weighted image. The perfusion weighted image signal is affected by many parameters other than perfusion including the T_1 relaxation time of both blood and tissue, which is also dependent on the magnetic field strength, and the method used to label the blood. All these parameters must be accounted for when interpreting a PW image.

ASL techniques can be used to not only measure perfusion, but also to assess the transit time of the labelled blood, this is the time for blood to arrive at the imaging volume from the labelling slice. Care must be taken when analysing ASL data. Since ASL is a subtraction technique, it can be highly sensitive to subject motion causing artefacts. This is most commonly manifested as a peripheral ring of high signal intensity. Methods have been developed to detect and discard imaging volumes which have been corrupted by large movements. This is described in greater detail in Chapter 5.

There are three main variants of the ASL technique - Pulsed ASL (PASL), Continuous (CASL), and pseudo-Continuous ASL (pCASL). The differences between these methods arise in how the magnetic label is produced, as summarised in Figure 4-4 and described below.

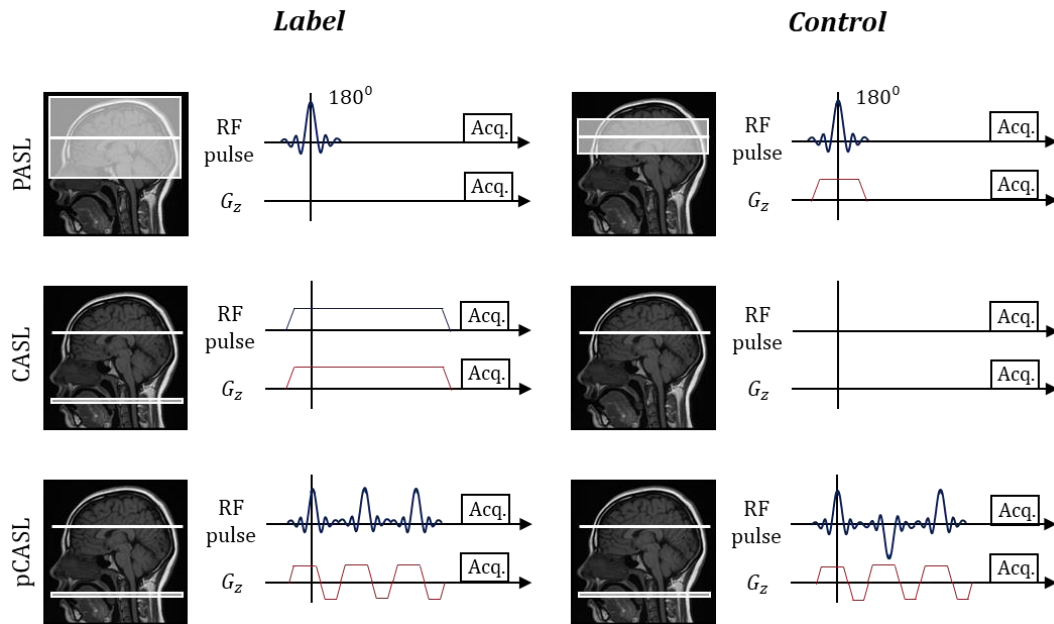


Figure 4-4: Illustration of the most common ASL schemes. Grey square indicates the labelling slab, white line indicates the imaging slice and Acq. the image acquisition.

4.3.1 Pulsed ASL (PASL)

Pulsed ASL (PASL) uses a discrete, short duration adiabatic RF pulse to invert a wide slab of arterial blood. There are a number of variations of the PASL scheme, the most common schemes being echo planar imaging and signal targeting radio frequency (EPSTAR) [15] and flow alternating inversion recovery (FAIR) [16], the latter being used throughout this thesis. These schemes are shown in Figure 4-5.

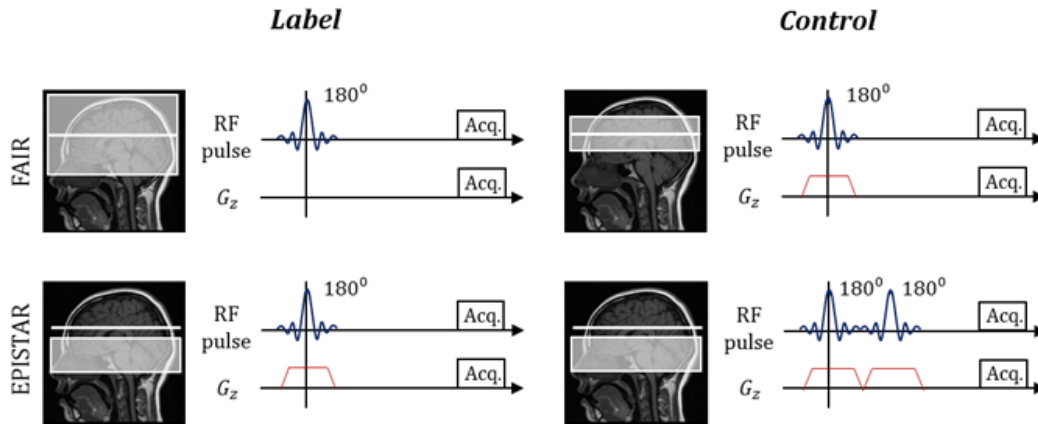


Figure 4-5: Diagram of the two most common variants of PASL schemes.

The FAIR scheme uses a non-selective inversion pulse to label a wide slab of inflowing arterial blood, this inversion is centred on the imaging volume. The control image is created using a slice selective inversion pulse centred on the imaging volume but of narrower width. The EPISTAR scheme uses a 180° inversion pulse (at double the required RF power for a 180° pulse) and a magnetic field gradient to label a slab of inflowing blood below the imaging volume. The control image is created using two successive 180° pulses, equivalent to a 360° pulse, and a magnetic field gradient. For both techniques, the RF power between label and control conditions is matched to compensate for magnetisation transfer effects. PASL schemes typically have a 95% tagging efficiency [17]. Throughout the studies in this thesis, the FAIR ASL scheme is used at both 3 and 7 Tesla.

4.3.2 Continuous ASL (CASL)

Continuous ASL (CASL) was the original ASL technique which was implemented using a separate neck labelling coil. CASL uses a flow-driven adiabatic inversion pulse to invert the arterial blood magnetisation as it flows through the labelling plane, usually placed below the imaging volume [18]. This technique combines a long, continuous low power RF pulse with a slice selective gradient applied in the direction of blood flow to invert the targeted arterial blood water spins entering the image volume. The advantage of CASL is it provides higher SNR compared to the other ASL variants, however the long

continuous RF delivery results in the deposition of a large amount of RF energy within the tissue (high SAR) and strong magnetisation transfer effects compared to PASL, limiting the practical use of the CASL scheme.

4.3.3 Pseudo-continuous ASL (pCASL)

Pseudo-continuous ASL (pCASL) is an intermediate technique introduced to exploit CASL's high SNR but overcome its high RF energy and magnetisation transfer effects. This method mimics CASL's flow-driven adiabatic inversion tagging but rather than use a long continuous RF pulse, it applies a train of small, short duration, discrete RF pulses. The control state is achieved by alternating the sign of the RF pulses to nullify the average B_1 whilst matching label and control conditions to compensate for magnetisation transfer effects. However, the TR of the pCASL is relatively long, longer than that of PASL, limiting its use for functional studies, and it has high SAR compared to PASL, which becomes limiting at 7 T. The pCASL sequence was therefore not used for work shown in this thesis.

4.3.4 Modelling the ASL Signal

To quantify the perfusion weighted images a basic physiological model is required. A model can be seen in Figure 4-6. This compartmental model illustrates the vascular architecture within the brain, beginning in a large artery and moving through the arterioles to the capillary bed.

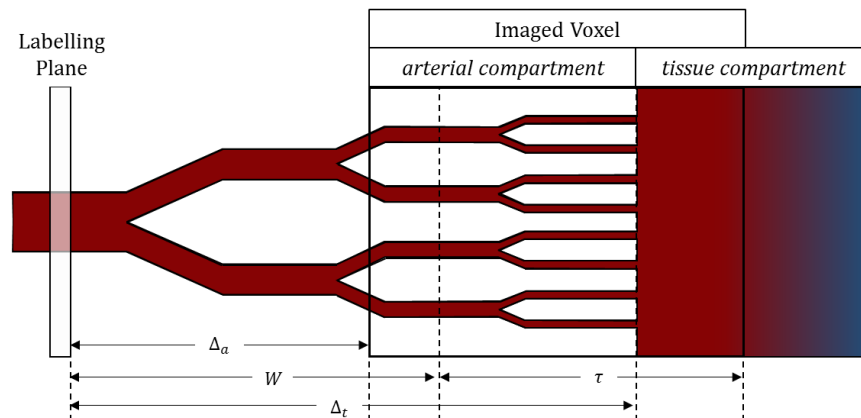


Figure 4-6: The ASL compartmental model described in Francis et al. [19]. Δ_a and Δ_t are the time it takes for the labelled spin to reach the arterial and tissue compartments respectively. W is the post label delay (PLD) and τ is the labelling duration.

Pulsed ASL data can be quantified using a combination of the single compartment model [16, 20] and a modified Bloch equation which incorporates a flow component f [21]. In the perfusion weighted image, the total magnetisation in a tissue voxel, $M(t)$, is described by;

$$\frac{dM(t)}{dt} = \frac{-M(t)}{T_1} + f \left(M_b(t) - \frac{M(t)}{\lambda} \right) \quad \text{Eqn. [4.1]}$$

where $M_b(t)$ is the magnetisation of the inflowing arterial blood water, λ is the blood:brain partition coefficient, $fM_b(t)$ is the magnetisation flowing into the imaging compartment and $f \frac{M(t)}{\lambda}$ is the magnetisation flowing out of the imaging compartment. It is assumed that no perfusing blood leaves the imaging compartment during the image acquisition, therefore $f \frac{M(t)}{\lambda} = 0$ and Eqn. [4.1] can be simplified to

$$\frac{dM(t)}{dt} = -\frac{M(t)}{T_1} + fM_b(t) \quad \text{Eqn. [4.2]}$$

Using the integrating factor method, this equation can then be solved to give

$$M(t) = \frac{1}{e^{t/T_1}} \int e^{t/T_1} fM_b(t) dt \quad \text{Eqn. [4.3]}$$

At a time $t > 0$, $M_b = 2M_0e^{-t/T_1}$ where M_0 is the equilibrium magnetisation of the arterial blood, therefore

$$M(t) = \frac{1}{e^{t/T_1}} \int_0^t 2f M_0 e^{-t/T_1} e^{t/T_1} dt \quad \text{Eqn. [4.4]}$$

which when simplified gives

$$M(t) = \int_0^t 2f M_0(t) e^{-t/T_1} dt \quad \text{Eqn. [4.5]}$$

This describes the simplest model of the signal difference for a FAIR scheme between selective and non-selective acquisitions. However, this model does not take into account the time it takes for the labelled blood to reach the tissue compartment, this is known as the tissue transit time (the time period Δ_t in

Figure 4-5), which is important when considering the changes in vasculature that occurs with ageing.

The transit time of the blood to the tissue can be accounted in the modified Bloch equation [22]. Assuming uniform labelling efficiency and in-plane saturation of the image volume ($\Delta M_{tissue}(0) = 0$), then the difference in tissue magnetisation ($\Delta M_{tissue}(t)$) can be described as:

$$\Delta M_{tissue}(t) = 2\alpha M_0 \frac{f}{\gamma \left(\frac{1}{T_{1app}} - \frac{1}{T_{1b}} \right)} e^{\frac{-\Delta_t}{T_{1b}}} \left(e^{\frac{-(t-\Delta_t)}{T_{1b}}} - e^{\frac{-(t-\Delta_t)}{T_{1app}}} \right) \quad \text{Eqn. [4.6]}$$

$$\text{for } \Delta_t \leq TI \leq W_t + \Delta_t$$

and

$$\Delta M_{tissue}(t) = 2\alpha M_0 \frac{f}{\gamma \left(\frac{1}{T_{1app}} - \frac{1}{T_{1b}} \right)} e^{\frac{-\Delta_t}{T_{1b}}} \left(e^{\frac{-W_t}{T_{1b}}} - e^{\frac{-W_t}{T_{1app}}} \right) \quad \text{Eqn. [4.7]}$$

$$\text{for } TI > W_t + \Delta_t$$

where Δ_t is the tissue transit time, W_t is the time during which labelled spins arrive at the capillary bed, α is the label efficiency ($\alpha = 1$ for ideal inversion) and $\frac{1}{T_{1app}} = \frac{1}{T_{1tissue}} + \frac{f}{\lambda}$. Figure 4-7 shows an example ASL signal curve with a given transit time at 3.0 and 7.0 T. Acquiring data with a range of PLD values allows fitting for f , W_t , and Δ_t using Eqn. [4.6] and Eqn. [4.7].

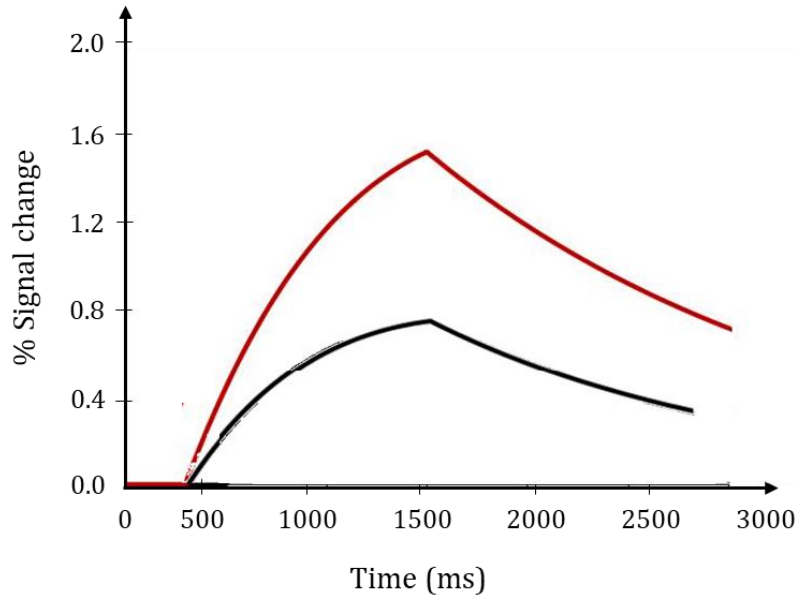


Figure 4-7: ASL signal curve for perfusion of blood at 3.0 T (black) and 7.0 T (red).

4.3.4.1 Single PLD ASL

The majority of ASL measurements are conducted using a single PLD between the label/control module and image acquisition. The advantage of this method is its simplicity in implementation and modelling of the perfusion signal, however it does not take into account transit time. As discussed above, transit time is difficult to evaluate and can cause errors when calculating perfusion values. This issue is especially important to address when investigating ageing [23]. Buxton *et al.* [24] suggested several strategies to minimise the influence of transit time and the arterial blood signal which arises at short PLDs, including waiting a sufficient PLD of ~ 1200 ms for the entire arterial labelled bolus to reach the tissue. However, since the ASL signal decays with the T_1 of the tissue post labelling, using too long a PLD can be costly to the resultant SNR.

Since perfusion is a dynamic process, it is preferable to repeat the ASL acquisition at a number of different PLD's to measure and account for transit time effects. However, while this allows for mapping of both perfusion and transit time effects, the method is inherently slow. This method is used at 3 T in Chapter 5 of this thesis.

4.3.4.2 Multiphase ASL

Alternatively, ASL data can be collected at multiple PLDs acquired in a single TR period to quantify both the relaxation time and transit time simultaneously in a single acquisition. This can be achieved using a multiphase or Look-Locker (LL) readout scheme [25] in which several low flip angle readouts are acquired following the labelling pulse (Figure 4-8). The advantage of this approach over single PLD methods is that this scheme provides temporal information without increasing the scanning duration (i.e. in a single TR period). However, since the application of multiple low flip angle readout pulses attenuates the recovery of magnetisation, this results in inherent lower signal per shot for a given PLD, and requires a more complex model for perfusion quantification.

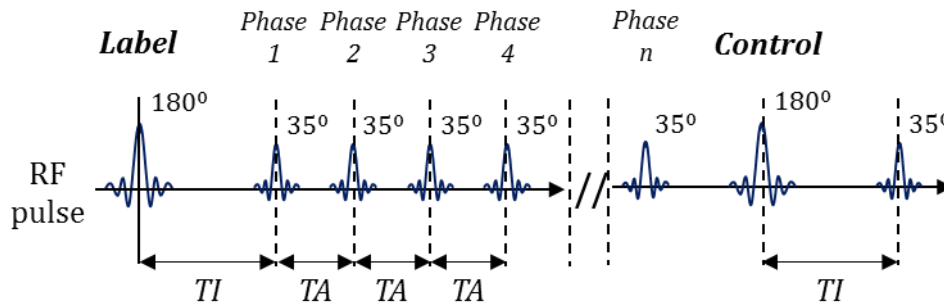


Figure 4-8: Schematic of the multiple TI Look-Locker ASL sequence.

Analysis of the signal from a Look-Locker readout scheme requires iterative fitting to a compartmental model (Figure 4-6) [19]. The model is divided into 3 compartments; Compartment 1 - arterial blood outside the imaging voxel, Compartment 2 - arterial blood inside the imaging voxel and Compartment 3 - capillary bed and blood exchange with the tissue inside the imaging voxel. The effect of the RF pulses on the blood spins is monitored in small time increments. The absolute signals are modelled for each readout pulse and the perfusion difference signals (the difference between the selective and non-selective recovery curves) are calculated. During image acquisition, arterial blood in Compartment 1 experiences an inversion recovery and is unaffected by the image readout RF pulses, while compartments 2 and 3 are modulated by the image readout RF pulses. For the FAIR scheme, the magnetisation of the blood following a selective RF pulse can be described by

$$\begin{aligned}
 M_{in}(t) &= M_{b,0} \left(1 - 2e^{-\frac{t}{T_{1b}}} \right) & \text{for } t < \Delta_a \\
 M_{in}(t) &= M_{b,0} & \text{for } t > \Delta_a
 \end{aligned}
 \tag{Eqn. [4.8]}$$

and following a non-selective RF pulses

$$\begin{aligned}
 M_{in}(t) &= M_{b,0} \left(1 - 2e^{-\frac{t}{T_{1b}}} \right) & \text{for } t < \Delta_a + W \\
 M_{in}(t) &= M_{b,0} & \text{for } t > \Delta_a + W
 \end{aligned}
 \tag{Eqn. [4.9]}$$

where $M_{in}(t)$ is the magnetisation of the blood as it enters the imaging voxel at time t after the inversion pulse, $M_{b,0}$ is the equilibrium magnetisation of blood and T_{1b} is longitudinal relaxation time of blood.

The magnetisation of arterial blood $M_b(t)$ in Compartment 2 can be expressed in terms of $M_{in}(t)$ and the magnetisation of the blood as it exits the arterial compartment and enters Compartment 3 at time t , $M_{out}(t)$, described by

$$\frac{d}{dt} M_b(t) = \frac{M_{b,0} - M_b(t)}{T_{1b}} + FM_{in}(t) - FM_{out}(t)
 \tag{Eqn. [4.10]}$$

where F is the arterial blood flow.

The magnetisation of the capillary blood in exchange with tissue M_t at time t can be described by a modified version of Eqn. [4.1];

$$\frac{d}{dt} M_t(t) = \frac{M_{t,0} - M_t(t)}{T_{1t}} + f \left(\frac{M_{out}(t)}{\lambda} - M_t(t) \right)
 \tag{Eqn. [4.11]}$$

where $M_{t,0}$ is the equilibrium magnetisation of the tissue, T_{1t} is the longitudinal recovery time of the tissue, f is the rate of tissue perfusion and λ is the blood:brain partition coefficient. The inversion and readout RF pulses perturb the magnetisation in Compartment 3 which must be accounted for.

The experiments described in Chapter 7 of this thesis use a LL-FAIR or multiphase sequence to acquire CBF data during a handgrip task performed at 7T.

4.3.4.3 Pre-saturation and post-saturation pulses

Saturation pulses are commonly used in ASL sequences, and are applied to the imaging volume immediately prior to (pre-saturation) and after (post-saturation) the labelling pulse. The benefit of saturation pulses are that they ensure that the tissue magnetisation is the same at the start of each label-control repeat, and thus this allows a reduction in repetition time (TR) of an ASL scan as there is no need to wait for the tissue magnetisation to recover. Since the tissue magnetisation is the same at the start of each label-control repeat, this significantly simplifies the modelling process as iterative effects across the TR period do not need to be considered. In all data collected in this thesis a WET (water suppression enhanced through T_1 effects) presaturation scheme and sinc post-saturation pulse was used.

4.3.4.4 Background suppression (BS)

The major limitation of the ASL technique is its low SNR. The perfusion signal in the brain is only around 1-2% of the signal of the equilibrium tissue signal. In addition, temporal fluctuations in the signal of the label and control images due to physiological noise leads to variance in the perfusion weighted difference signal. Originally developed for MR angiography, background suppression inversion pulses were suggested to be integrated with an ASL scheme to suppress the physiological fluctuations arising in label and control [26, 27]. Ye *et al.* [28] applied BS to ASL techniques and reported a substantial reduction ($\sim 70\%$) in the variance in the perfusion weighted signal.

The timings of the BS pulses need to be carefully optimised for the post label delay time being used and the T_1 relaxation time of the tissue of interest, which is field strength dependent.

An example of a tissue magnetisation recovery curve following the application of pre- and post-saturation pulses, and two BS pulses in a single PLD ASL acquisition is shown in Figure 4-9. For a scheme comprising two BS pulses, the optimum values of TI_1 and TI_2 can be found using the longitudinal Bloch equation. At a PLD $t = TI$, the magnetisation can be written as a fraction, ϵ , of M_0 and it can be shown that,

$$\frac{(\varepsilon - 1)e^{\frac{TI}{T_1}} + 1}{2} = e^{\frac{TI_1}{T_1}} - e^{\frac{TI_2}{T_1}} \quad \text{Eqn. [4.12]}$$

This equation can then be used to calculate the optimum values of TI_1/TI_2 . Typically the tissue signal is suppressed to +5-10% of its equilibrium magnetisation to avoid the need to sign correct the ASL data prior to subtraction. Although background suppression increases the SNR and reduces the variance of ASL acquisitions, in practice BS pulses are typically 95 % efficient and so can also attenuate the ASL signal, with the signal of the blood label reduced by a factor of α^n where α is the inversion efficiency of the BS pulse and n is the number of background suppression pulses.

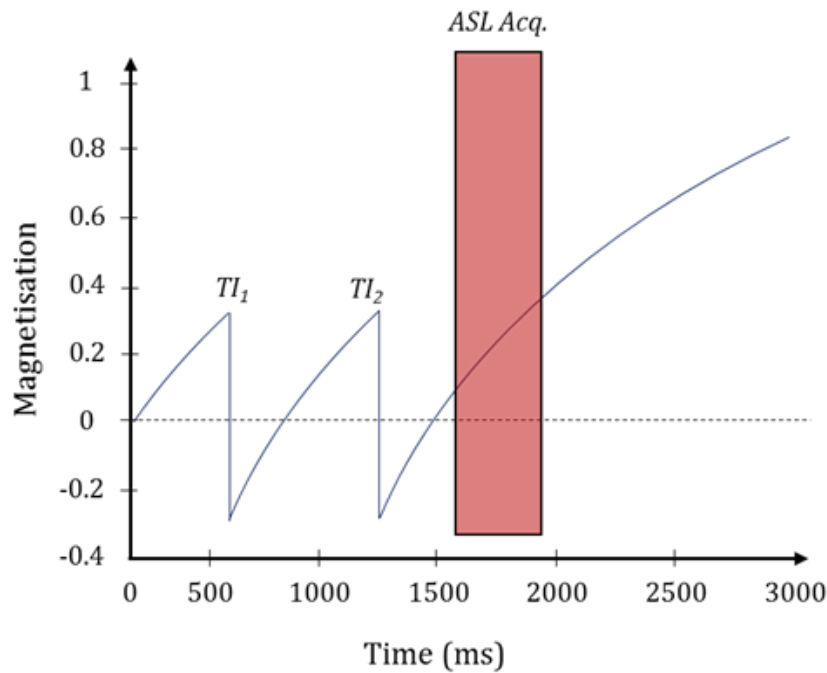


Figure 4-9: Example tissue magnetisation recovery curve shown for two background suppression pulses at time TI_1 and TI_2 . Simulation shown for BS pulses applied at TI_1 of 550 ms and TI_2 of 1250 ms, for the acquisition of multi-slice ASL data at a PLD of 1550 ms, parameters used in Chapter 5 for the ASL data acquisition collected at 3 T.

It should be noted that a multiphase or LL-ASL sequence is inherently background suppressed due to the repeated application of low flip angle readout pulses.

4.3.4.5 Vascular crushing

Blood in the large arteries can cause errors in CBF quantification, particularly at short PLDs. ASL typically focuses on tissue perfusion, however at the time of image acquisition, labelled spins may be present in both the arterial vessels and in the tissue. This signal may give rise to artificially high tissue perfusion values. One method which can be used to suppress the contamination of the perfusion signal from the blood signal is to apply a bipolar ‘crusher’ gradient pair after the RF excitation pulse and prior to the image readout. The bipolar gradient pair has equal magnitude but opposite polarity which sequentially dephases and rephases the spins, stationary spins will thus experience zero phase shift between the two gradients. However, moving spins will experience a second gradient with different magnitude to that of the first, resulting in a non-zero phase shift. Elimination of the arterial signal is based on the velocity of the spins. The phase shift acquired between the gradient pair is proportional to the spins velocity:

$$V_{ENC} = \frac{\pi}{\gamma G \delta \Delta} \quad \text{Eqn. [4.13]}$$

where γ is the gyromagnetic ration, G is the gradient strength δ is the duration of the gradient lobe, Δ is the distance between the gradient lobes and V_{ENC} is the encoding velocity. Typically defined in units of ml/min, V_{ENC} is the maximum flow velocity expected within the vessel of interest, and adjusts the strength of the bipolar gradients. Velocities above this value will be dephased, while velocities below this value will still be visible in the final image.

4.4 T₂ RELAXATION UNDER SPIN TAGGING (TRUST)

First developed by Hanzhang Lu *et al.* [7], T₂ Relaxation Under Spin Tagging (TRUST) is a relatively recent technique for the non-invasive measurement of venous oxygenation, Y_v , and thus oxygen extraction fraction (OEF). The TRUST method uses the principle of spin labelling, similar to the ASL. However, rather than label the arterial blood, in TRUST the labelling is applied to isolate pure blood signals in a venous vessel of interest, for the brain this is in the sagittal sinus. Using flow-insensitive T_2 -preparation pulses, the T_2 relaxation time of

the blood can be found and converted into blood oxygenation using a calibration plot [29], see Chapter 5.

The TRUST pulse sequence and geometric planning to calculate Y_v in the superior sagittal sinus are shown in a schematic in Figure 4-10.

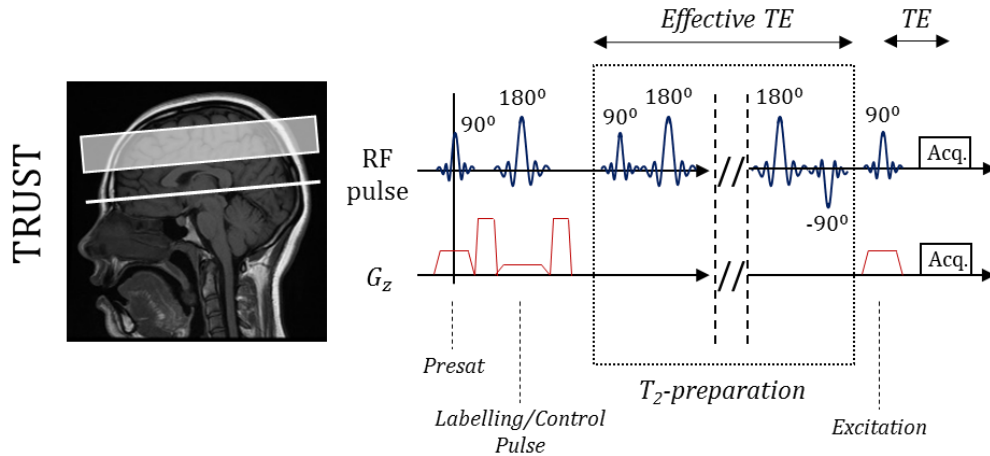


Figure 4-10: T_2 Relaxation Under Spin Tagging (TRUST) geometry planning and pulse sequence. A complete sequence varies the effective TE to modulate the T_2 -weighting.

Unlike for ASL, in TRUST the labelling slab is placed above the imaging slice to magnetically tag blood spins flowing out of the brain. A series of non-slice-selective T_2 -preparation pulses are applied prior to the excitation pulse to modulate T_2 -weighting [7]. To calculate T_2 relaxation of the venous blood, label and control scans are acquired at different T_2 -weightings by varying the duration of the T_2 -preparation sequence, defined as the effective TE (eTE). Subtraction of label and control images creates an image of the sagittal sinus across a range of eTEs.

From TRUST MRI evaluation of venous oxygenation, Y_v , the oxygen extraction fraction (OEF) can be computed. Further, by combining PC-MRI measures of blood flow with Y_v , global $CMRO_2$ can be measured quickly and noninvasively. Further details of TRUST and the quantification of Y_v , OEF and $CMRO_2$ are described in detail in Chapter 5.

4.5 BLOOD OXYGENATION LEVEL DEPENDENT (BOLD) CONTRAST

Blood oxygenation level dependent (BOLD) fMRI is a well-established technique providing high spatial resolution ($\sim$ mm) information of brain activity. It is sensitive to local changes in blood oxygenation that arise as a consequence of neuronal activation. The mechanism behind the BOLD contrast is dependent on the levels of oxygenated and deoxygenated blood leading to a change in MR signal [6].

Cerebral neuronal activation requires oxygen and glucose for energy. These nutrients are delivered to the brain via the blood and the increase in demand for energy results in an increased blood flow to the active brain region. However, the increased blood flow to the brain in response to a task is greater than the oxygen demand, leading to a net increase in oxygen at the site of neuronal activity (Figure 4-11). The origin of the BOLD signal lies in the differing magnetic properties of oxygenated (oxyHb) and deoxygenated haemoglobin (deoxyHb). Relative to brain tissue, oxyHb is diamagnetic, whilst deoxyHb is paramagnetic causing a susceptibility difference between the vessels and the surrounding tissue, distorting the magnetic field and shortening the local T_2^* . As oxygen content increases in active brain regions as a result of the enhanced local blood flow compared to oxygen consumption, T_2^* relaxation lengthens and the MR signal increases relative to baseline.

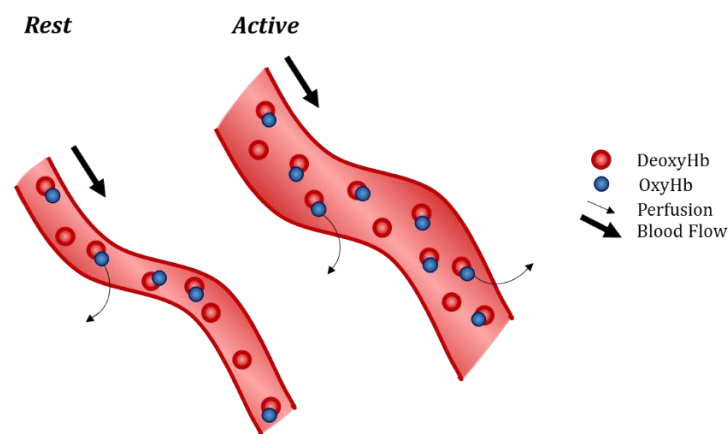


Figure 4-11: Haemodynamic changes between the rest and active state indicating the origins of the BOLD response.

When designing a BOLD fMRI study, it is important to take into account the haemodynamic response function (HRF), the lag in the oxygenation change in response to the task. Upon delivery of a stimulus, it takes approximately 6 seconds for the cerebral vascular system to respond, with the BOLD HRF shown in Figure 4-12.

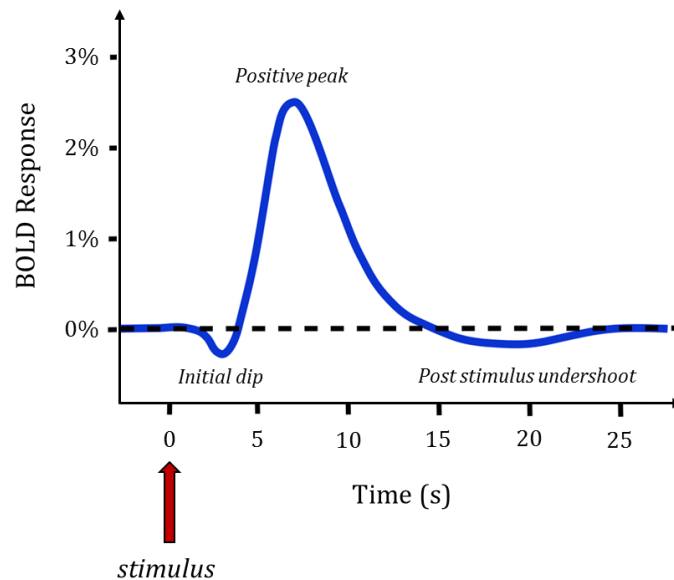


Figure 4-12: Depiction of a typical BOLD haemodynamic response function (HRF).

The BOLD HRF has been suggested to demonstrate three characteristics. First, an initial dip, however the literature is inconsistent and variable on this feature. This is believed to originate from an early increase in oxygen consumption [30] and/or local cerebral blood volume before an increase in cerebral blood flow. The duration of the initial dip is short, just 1-2 s, however it has been suggested that it may provide higher spatial resolution for localisation of neuronal activation [31, 32]. The second feature is the positive peak which dominates the curve and is used for standard fMRI measures. This delayed response originates from the disproportionate increase in CBF compared to the brain's metabolic needs. This results in a higher oxyHb to deoxyHb ratio, increasing the MR signal and forming the positive BOLD response. The duration of this peak is a few seconds. The final feature of the BOLD HRF is the post stimulus undershoot, which can last as long as 40 -50 s to a simple event-related task. Reports of the origin of this feature are

inconsistent, and it is likely to arise from multiple mechanisms, including the dilation of the venous vessels together with active inhibition [33, 34].

The magnitude of the BOLD response is dependent on the stimulus, but also the magnetic field strength and echo time used. The magnitude of the BOLD response increases from ~4% at 3.0 T to ~6% at 7.0 T. Since T_2^* weighted images are sensitive to changes in oxygenation of the blood a gradient echo (GE) EPI sequence is commonly used for fMRI. It is important to optimise the echo time (TE) to maximise the signal change between active and resting states. If the TE is too short, there will be little difference in the active and rest T_2^* curves. Similarly, if the TE is too long, there will be no signal from either state. The maximum BOLD signal occurs when the TE is equal to the resting T_2^* value of the tissue of interest (Figure 4-13). Thus since T_2^* is field strength dependent, the optimal TE is also dependent on field strength, with an optimal TE of 40 ms at 3.0 T and 25 ms at 7.0 T.

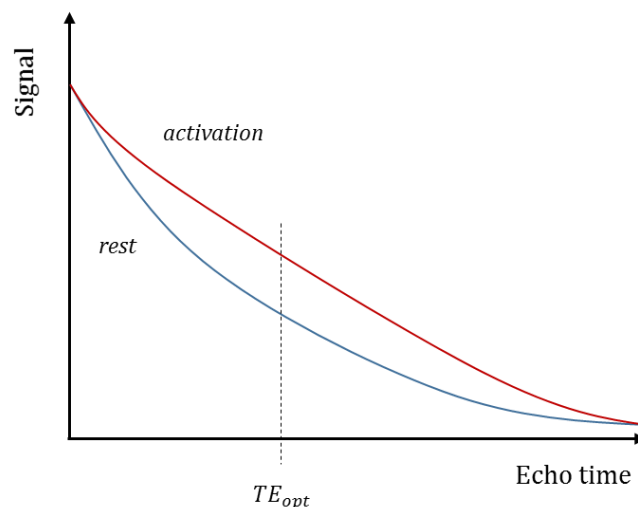


Figure 4-13: Schematic illustrating the optimal value of TE to yield the maximum BOLD contrast between resting and active states.

4.5.1 BOLD fMRI Data Analysis

There are two main designs of an fMRI experiment – a ‘block’ or ‘event-related’ design, as shown in Figure 4-14. A block design uses a relatively long, repeated stimulus (e.g. 8 – 20 s) as the ON period, during which a discrete cognitive state is maintained, this is followed by an OFF period of the order of 20 – 60s. In

contrast, an 'event-related' design uses brief, repeated discrete stimuli which are then averaged to give a measured response.

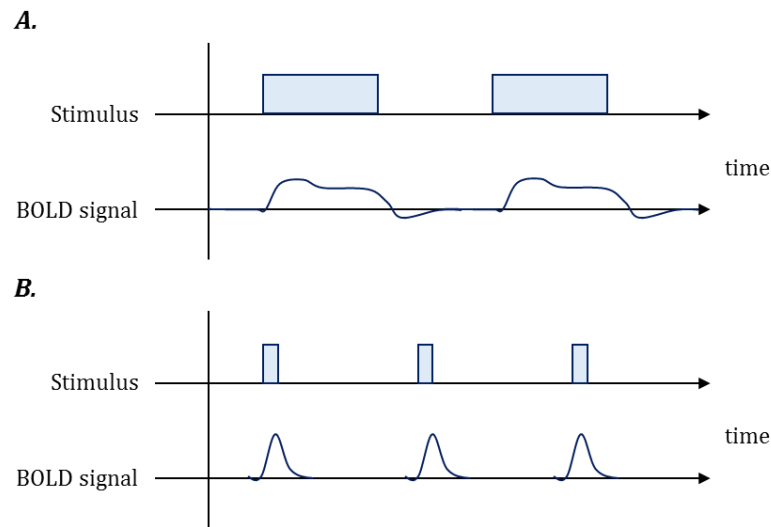


Figure 4-14: Schematic representation of **A.** a 'block' design **B.** an 'event-related' fMRI design with corresponding BOLD signal.

The basic objective of BOLD image analysis is to identify those voxels that show changes in MR signal intensity corresponding to the stimulus task design. To improve detection of active voxels a number of pre-processing steps are first performed, including image registration (realignment) and spatial and temporal smoothing of the data.

Image registration is a technique used to correct for subject head movement. Motion during an fMRI experiment causes artefacts which manifests as hyper-intense voxels at the edge of the brain. This signal can be far greater than the BOLD response thus corrupting the data. To correct for motion the image is realigned across the time series into a given data space. This is typically performed using a 6 parameter, rigid body transformation of three rotation and three translation movements.

To increase the signal-to-noise ratio (SNR), fMRI data can be spatially smoothed. Spatial smoothing reduces random noise and improves the detection of true activations. It is typically achieved using a Gaussian kernel with full-width-at-half-maximum (FWHM) of 1.5 – 2 times the voxel size. Care

must be taken when applying spatial smoothing as it reduces the resolution of the image, so a balance must be made.

Temporal smoothing is applied to the fMRI data to remove high frequency fluctuations from the time series and account for scanner drift which would compromise the detection of activations. It can be achieved using linear or polynomial detrending to remove low frequency drift. Care must be taken when applying temporal filtering so as not to remove any effects of interest, with a typical filtering limit being set to twice the task cycle duration.

Once pre-processing is complete, the data must be statistically analysed to identify areas of significant activation. The most common method is a basic multiple regression referred to as the general linear model (GLM) [35]. This approach is used in this thesis, and aims to correlate the time course of signal change in the data $\mathbf{Y}$, in terms of a linear combination of the time course of the model $\mathbf{X}$, and an error term representing random noise, ϵ :

$$\mathbf{Y} = \mathbf{X}\beta + \epsilon \quad \text{Eqn. [4.14]}$$

where β is a scaling parameter. β can be assessed to show the strength of the BOLD response and is interrogated in Chapter 7.

By convolving the input stimulus with the expected BOLD HRF, an expression for $\mathbf{X}$ can be found. This is then fitted on a voxel-by-voxel basis to determine β (Figure 4-15) and a t-statistic map is generated. Due to an inherently small signal change along with the vast number of voxels interrogated within the imaging volume, there may be a large number of voxels that are classified as active by chance. For example, if a conventional threshold of $p < 0.05$ is used to interrogate each voxel of a 100,000 voxel data set, there would be around 5000 voxels falsely declared as active (Type I error). The map is therefore corrected to account for multiple comparisons and reduce these potential false positive results.

There are two methods for controlling false positives. The first is family wise error correction (FWE). FWE controls the *chance* of false positives and corrects the p-threshold to account for the number of voxels in the data (i.e. p

$= 0.05/100,000 = 0.0000005$). However, this approach is inherently conservative and increases the chance of false negative results (Type II error) where voxels that should show activation get corrected below the threshold. The second method, false discovery rate (FDR), accounts for this and controls the *fraction* of false positives. FDR controls the number of false positives only in voxels labelled as significant. Using a threshold of 0.05, this approach ensures that 95% of voxels highlighted as activated are true positives. The FDR method is less conservative with respect to Type I errors and reduces the chance of Type II errors.

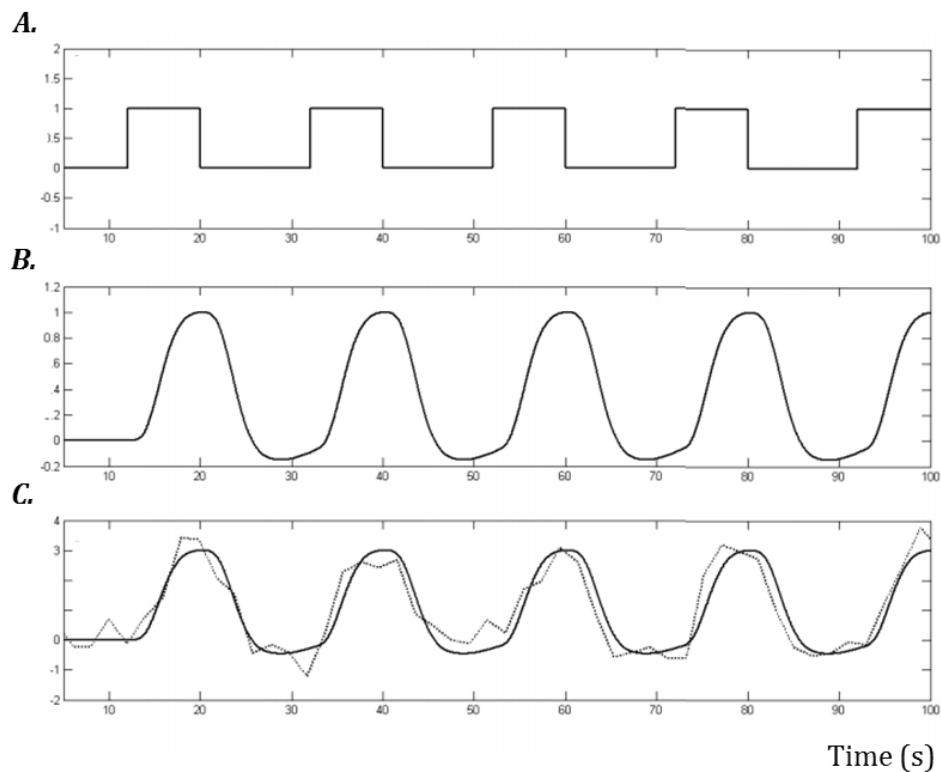


Figure 4-15: Schematic of GLM analysis for a simple ‘ON’ and ‘OFF’ stimulus. **A.** A binary representation of the stimulus. **B.** The stimulus convolved with the expected BOLD HRF curve to give **X**. **C.** The simulated BOLD response (broken line) with $\beta = 3$ overlaid on the convolved curve to show goodness of fit.

4.6 DOUBLE ACQUISITION BACKGROUND SUPPRESSION (DABS)

Often it can be informative to combine the measurement of cerebral blood flow and BOLD signal changes in a single scan. However, background suppressed ASL measures have little sensitivity to BOLD signal since the control and label

signal is inherently suppressed, and secondly the echo time, TE, of an ASL acquisition is chosen to be as short as possible to maximize the SNR of the ASL signal. To combine the benefits of background suppressed ASL with the collection of BOLD data a Double Acquisition Background Suppression, or DABS [36] scheme can be implemented. This method provides the acquisition of ASL and BOLD data in the same TR period. The DABS sequence is shown in Figure 4-16 together with the magnetisation of the images acquired. The two background suppression pulses applied at TI_1 and TI_2 improve the temporal signal-to-noise ratio (SNR) of perfusion data whilst the second acquisition is sensitive to BOLD signal changes. The first ASL acquisition is acquired with a short TE, whilst the BOLD acquisition is collected with a longer TE $\sim T_2^*$ of tissue for optimal BOLD contrast. Figure 4-15 illustrates the DABS technique using a single PLD ASL method.

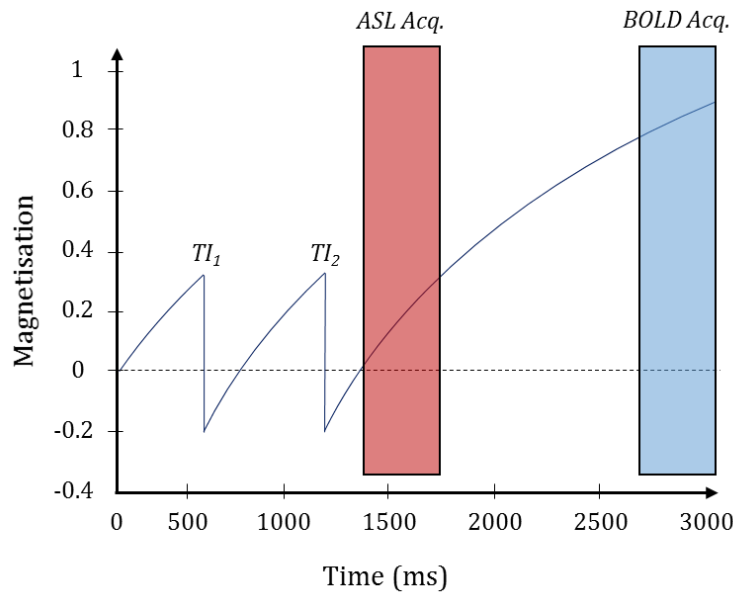


Figure 4-16: Sequence timing of DABS technique using two background suppression pulses. 1st acquisition block (red) is used for CBF analysis and 2nd (blue) for BOLD.

The advantage of using multiple PLDs in a single ASL TR period, multiphase or LL-ASL, has been described in Section 4.3.4.2. The Look-Locker readout scheme can also be combined with the DABS technique to collect multiple PLD's in addition to a BOLD readout, this scheme is termed LL-DABS. The pulse

sequence and magnetisation curve of a LL-DABS scheme is shown in Figure 4-17.

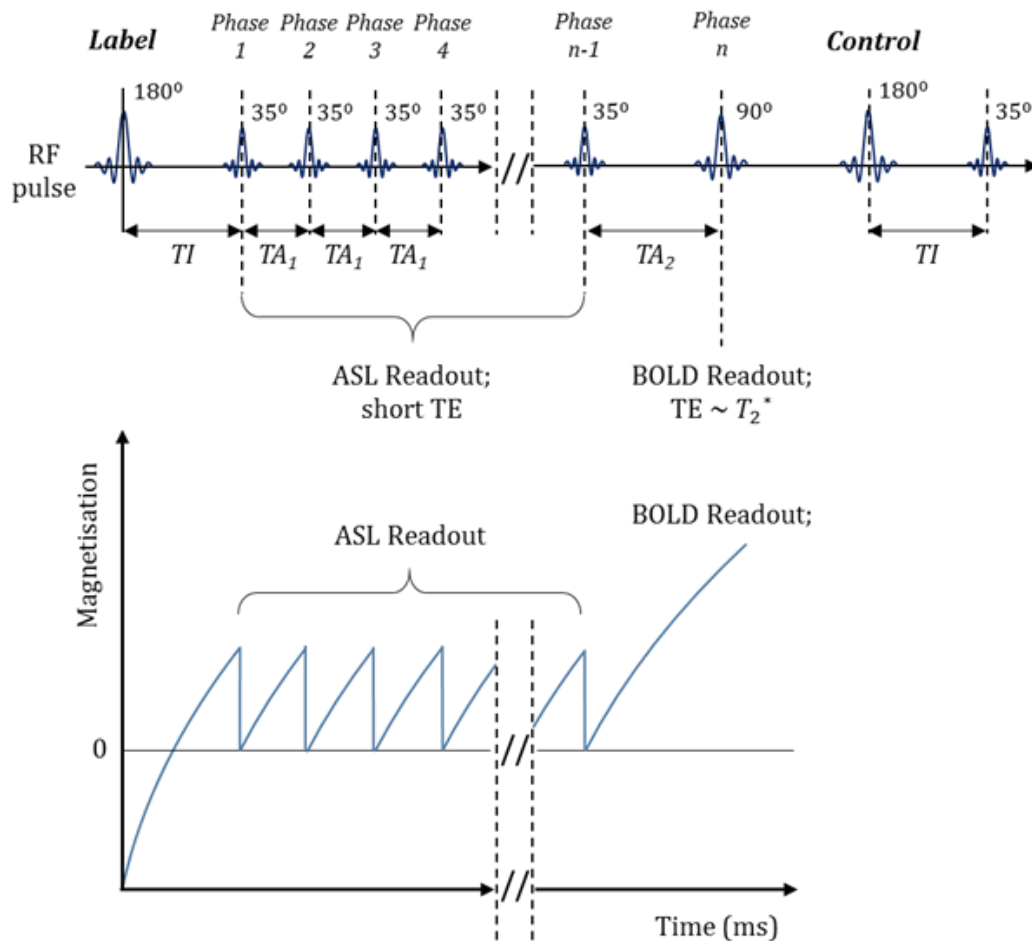


Figure 4-17: Pulse sequence and resulting magnetisation of a Look-Locker DABS scheme.

In Chapter 7 a LL-DABS scheme is used at 7.0 T to acquire simultaneous BOLD and ASL data.

4.7 CONCLUSION

This chapter has provided a comprehensive description of the physiological variables used to assess brain physiology, function and metabolism, and the MR methods by which they can be measured. The following chapter will utilise the methods to investigate the cerebral response to acute exercise in healthy ageing.

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5. Assessment of cerebral haemodynamic and metabolic response to exercise and the effects of normal ageing

The primary aim of the work described in this chapter is to quantify the cortical haemodynamic and metabolic responses to acute low/moderate intensity exercise in healthy volunteers using magnetic resonance imaging (MRI) techniques, and to investigate how this response is influenced by ageing.

First, the methods which can be used to measure changes in cerebral vascular physiology and metabolism during acute whole body exercise are discussed, and previous studies of the effects of exercise on brain physiology and function are outlined. Prior literature describing how the cerebral response to exercise changes with ageing are then reviewed and the challenges posed when performing acute exercise studies in an MR environment are highlighted. A novel MRI study to evaluate the effects of exercise and ageing on the cerebral response is then described, using MRI techniques to directly quantify changes in cerebral blood flow (CBF), brain oxygen extraction fraction (OEF), regional perfusion and BOLD contrast during low to moderate intensity exercise at 30% and 50% of maximal oxygen uptake. Responses to exercise are compared between healthy young adults and older adults to provide mechanistic insight of the physiological control and correlates of age-related changes in brain haemodynamics and metabolism.

This work was presented as an oral presentation at the 24th International Society for Magnetic Resonance in Medicine meeting, Singapore 2016, and at the 22nd European College of Sport Science meeting, Germany 2017.

5.1 INTRODUCTION

It is well established that the brain requires a disproportionate amount of resources for normal function, with strict regulation of blood flow and oxygen metabolism being critical for survival [1]. Chronic reductions in cerebral blood flow (CBF) is a major risk factor for cardiovascular disease and neurologically degenerative conditions such as Alzheimer's Disease and other forms of dementia [2]. Whilst CBF is tightly regulated by multiple control systems including cerebral autoregulation, cerebrovascular CO₂ reactivity and neurovascular coupling, a decline in CBF with healthy ageing is well documented [3, 4].

CBF, both at rest and under stress during exercise, has been an area of interest for over half a century. Its regulation in exercising humans is complex, and is likely to be affected by a number of factors as illustrated in Figure 5-1.

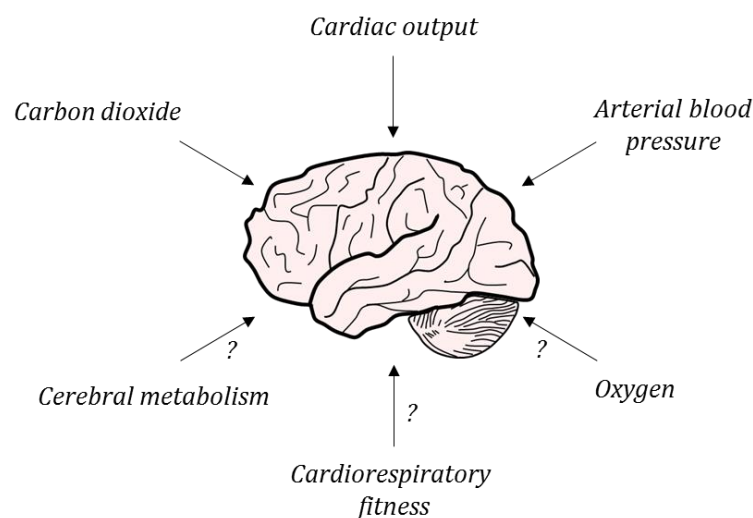


Figure 5-1: Illustration of the multiple control systems of cerebrovascular regulation during exercise in the human brain

Arterial blood pressure: Mean arterial pressure (MAP) has been seen to increase during exercise, therefore its influence on CBF should be considered. However, it is generally accepted that, as long as MAP is within the physiological range (~60–150 mmHg), CBF will remain constant.

Carbon dioxide: Arterial carbon dioxide tension (P_{aCO_2}) has been found to be a major regulator of CBF. At rest, the CO_2 -reactivity of the brain is well established [5], and during exercise, changes in P_{aCO_2} are relatively small with a slight increase during low to moderate intensity exercise contributing to a slight elevation in cerebral perfusion. At higher intensity exercise, a hyperventilation-mediated fall in P_{aCO_2} has been thought to induce vasoconstriction and thus restrict an increase in CBF. Some studies have observed that elderly individuals have a reduced P_{aCO_2} [6-8].

Cardiac output: The separate influence of cardiac output (CO) on CBF has gained prominence over recent years [9, 10]. The importance of CO in the maintenance of CBF in the context of ageing has not been adequately addressed, but in keeping with the blunted increase in CO in response to exercise with age, middle cerebral artery (MCA) blood velocity is known to be reduced in older people during exercise.

Higher aerobic fitness has been suggested to offset the age-related decrease in CBF [11], protect against cognitive decline [12] and reduce the risk of cerebrovascular and neurodegenerative diseases (e.g. stroke, dementia) [13]. Investigating cerebral responses to acute exercise in the elderly may help to pinpoint the mechanisms by which exercise training can reduce the risk of brain diseases, inform the optimisation of exercise training programmes, and assist with the identification of age-related alterations in CBF. However, there is a wide variation in the literature in the reported CBF change during exercise, likely due to the range of methodologies used. The current literature will first be described, followed by the assessment of the cortical responses to an acute exercise challenge using MRI techniques.

5.1.1 Measuring Cerebral Blood Flow and Oxygen Consumption with Radioisotope Clearance

The earliest methods to measure global CBF (gCBF) were pioneered by Kety and Schmidt in the 1940's [14] who used nitrous oxide (N_2O). This method is based on the Fick principle which states that the rate at which N_2O in the venous circulation approaches the content in the arterial circulation is

dependent upon the volume of blood flowing through the brain. It also assumes that the rate at which N₂O perfuses into brain tissue is equivalent to the difference between the amount of blood entering the brain in the arteries and the amount of blood leaving the brain in the veins [15]. Early studies in the 1950's and 1960's that used this method to assess the effects of exercise reported no change in gCBF during dynamic exercise [16, 17] for an MAP within the cerebral autoregulatory range. However, it was shown that P_{aCO_2} is a potent regulator of gCBF. These papers concluded that, in contrast to other organs in the human body, the blood flow supplying to the brain remains constant under a number of conditions, including exercise [18].

Studies using the N₂O method were reliant on a number of assumptions. Firstly, they assumed that venous drainage between the two internal jugular veins is equal. However, it has been shown that the venous drainage from the brain is asymmetric, with the larger right internal jugular vein having an estimated flow of ~400 ml/min, and the left vein draining subcortical structures with an estimated flow of only ~160 ml/min [19, 20]. Additionally it has been demonstrated that during exercise the internal jugular vein, upon which the Kety-Schmidt method relies, collapses when subjects are upright [21, 22] as is typically the case for exercise studies which have used this method.

Further methods for quantification of CBF have used the administration of an inert radioisotope (¹³³Xe) and the Fick principle. This isotope can be traced through the cerebral vascular system using extracranial detectors. However, this technique failed to provide consistent results when studying the response of CBF during exercise. For example, Thomas et al. [23] reported a median increase in CBF of 58 % in humans between rest (76 (range 55-98) ml/100g/min) and 15 minute periods of moderate dynamic exercise on a cycle ergometer (118 (range 75-164) ml/100g/min). In contrast, using a similar supine exercise protocol, Globus et al. [24] found no significant change in CBF during physical exercise, although a marked increase in cardiac output, blood pressure and right atrial pressure were found along with a mild decrease in P_{aCO_2} .

The Kety-Schmidt and radioisotope clearance methods also provide information on oxygen utilisation though, again, findings using these techniques have been inconsistent. In contrast to muscles that increase their oxygen consumption ~50 fold during intense exercise, the cerebral metabolic rate for oxygen (CMRO₂) has been reported to remain constant during cerebral activation when evaluated by the Kety-Schmidt technique, since CMRO₂ is calculated based on a constant gCBF and remains unchanged during exercise for as long as the intensity is moderate. However, using ¹³³Xe as the tracer, an increase in CMRO₂ by ~7 % is reported during prolonged exercise in hyperthermia [25, 26] and one study reports an increase ~25 % during intense uphill walking [16]. Conversely, CMRO₂ has been found to decrease ~13 % when exercise is prolonged to an extent that lowers the blood glucose level [27].

Radioisotope clearance techniques suffer similar limitations to the Kety-Schmidt method and a large proportion of the isotope can be taken up by other tissues, affecting the clearance rate and influencing CBF measures [28]. These methods also have poor temporal resolution, taking ~10 minutes to acquire a single CBF measurement, therefore transient changes are not detectable. It is also unlikely that steady state conditions remain for both the cerebral metabolic rate of oxygen (CMRO₂) and P_{aCO_2} during this length of exercising. It is expected that P_{aCO_2} increases slightly during low intensity exercise over a 10 minute duration, but during intense exercise progressive hyperventilation is likely to lower P_{aCO_2} . Further, a number of early studies involved a change in subject position from rest (supine) to exercise (upright). This results in an associated change in the anatomy of cerebral drainage, confounding alterations in P_{aCO_2} and reduced activation during exercise may in part be explained by these observations [29]. Radioisotope clearance is therefore arguably unsuitable for dynamic assessment of CBF during exercise.

5.1.2 Measuring Cerebral Blood Flow with Transcranial Doppler Ultrasound

More recently, the most common technique used to investigate cerebral haemodynamics in response to whole body exercise is Transcranial Doppler ultrasonography (TCD). This method is a type of Doppler ultrasonography that allows quantification of blood flow velocity (BFV) in any major cerebral blood vessel close to the skull by measuring the echoes of ultrasound waves moving transcranially. TCD has the advantage of being a non-invasive technique for evaluation of CBF allowing continuous, real-time recording. This method is widely used as an index of CBF, based on the principle that changes in BFV in the major cerebral arteries can be indicative of cerebral autoregulation.

Although the radioisotope literature has been inconsistent with its findings on the cerebral response to physical activity, the majority of TCD studies reveal a trend towards an increase in average blood velocity in the middle cerebral artery ($MCA V_{mean}$) during submaximal exercise [30-41]. Some studies have probed this further to show that after the initial increase in blood velocity, $MCA V_{mean}$ begins to plateau and even decrease at higher exercise intensities [40, 42]. Imray et al. [40] reported an increase in $MCA V_{mean}$ during submaximal testing but a decrease below baseline values at maximum exercise intensity. The increase in BFV from baseline during low to moderate exercise intensities was suggested to be coupled to enhanced cerebral neuronal activity [29, 30], whilst the plateau and decrease at high intensity dynamic exercise was attributed to exercise induced hyperventilation [30, 39] during which the rate of removal of carbon dioxide from the blood is increased. As P_{aCO_2} decreases, alkalosis of the blood causes vasoconstriction and therefore restricts the velocity and flow of the blood to the brain.

The cerebral and metabolic response to exercise in young adults is now becoming more widely documented, though the influence of normative ageing on these parameters has not yet been established. TCD baseline measures have suggested that global CBF and BFV decline gradually with age ($\sim 0.7\%$ / year) [11, 43-46]. The few studies that have investigated the influence of ageing on

dynamic exercise have used TCD, and they report that the age-related basal deficit in CBF remains on physical activity [7, 47-49]. However, although there is an observed decrease in BFV in the older adults during physical activity, the overall response during the exercise task has been shown to remain the same between young and older age groups, with an initial increase from baseline, followed by a plateau and a decrease at higher intensity exercise [7, 47-49]. In 2013, Fisher et al. [7] published a study showing the differences in the cerebral response to exercise between young and older adults. The authors measured the $MCA V_{mean}$ and P_{aCO_2} across a graded exercise task. Their results showed a lower $MCA V_{mean}$ and P_{aCO_2} in the older adults at baseline and across the exercise task, as can be seen in Figure 5-2. The underlying mechanisms of these age-related changes are not well known, although global brain atrophy and reduced neuronal activity have been proposed as important contributory factors [8].

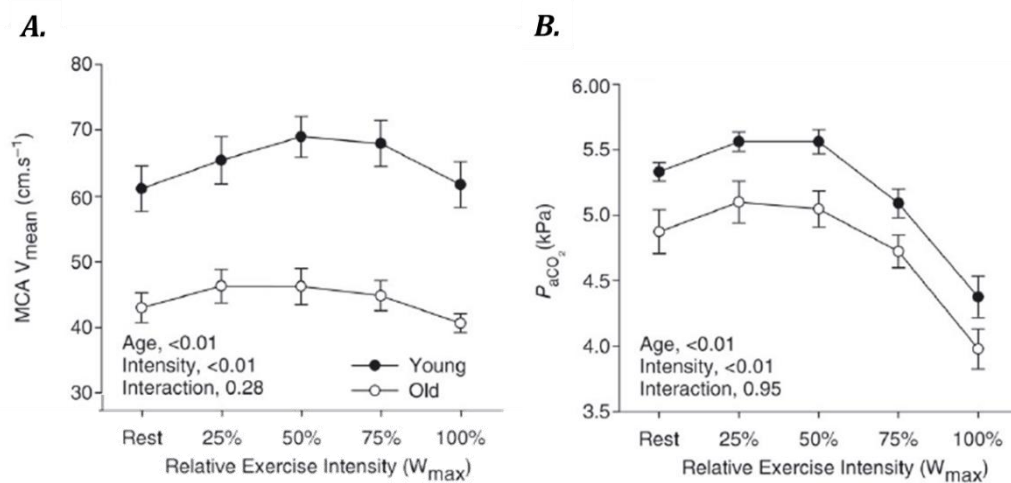


Figure 5-2: Results reported by Fisher et al. [7]. A deficit in both **A.** $MCA V_{mean}$ and **B.** P_{aCO_2} can be seen in older adults compared to young. The trends across the exercise task remain the same for each age group.

TCD is well-established for investigating cerebral haemodynamics as it has the advantage of being inexpensive and easy to use. The TCD probe can be secured directly to the subjects head (Figure 5-3A) allowing measurements to be made during an exercise task and allowing the collection of data which is not greatly susceptible to corruption by motion. However TCD does suffer limitations.

Firstly, the majority of studies have focussed measures solely on the assessment of blood velocity within the MCA. Although the MCA supplies approximately 80% of blood flow to the brain, it is one of three major feeding arteries (Figure 5-3B). Without taking into account the anterior and posterior cerebral arteries, TCD does not represent true global brain blood flow. The second limitation is the extent to which blood flow velocity measured using TCD actually represents cerebral blood flow. TCD measures flow velocity in the centre of a vessel which is assumed to have a constant cross sectional area. Any changes in the cross-sectional area of the vessel will lead to a disproportional relationship between BFV and cerebral blood flow. However, the effect of exercise on the degree of dilation of the blood vessels feeding the brain is inconsistent, and therefore controversy exists on the validity of BFV derived from TCD as a measure of CBF. Serrador et al. [50] found a high correlation between relative changes in cerebral BFV and changes in CBF over a wide range of flow velocities, suggesting that TCD determination of BFV can provide a useful indication of the real changes in the underlying CBF. However, it should be noted that since the outer diameter of the MCA is only ~3 mm [51], even small changes in the inner diameter will affect velocity. Accordingly, changes in MCA diameter may be a concern, especially during intense exercise with marked hyperventilation resulting in a lowering of P_{aCO_2} .

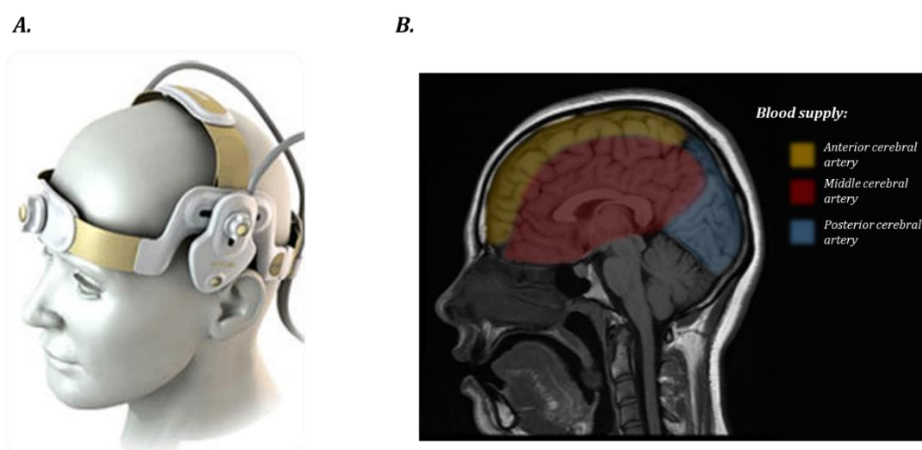


Figure 5-3: **A.** Graphic of a transcranial Doppler ultrasound setup measuring cerebral blood flow velocity. **B.** Schematic showing the arteries delivering the blood supply to different regions of the brain. The middle cerebral artery (MCA), shown in red, supplies approximately 80% of all blood delivered to the brain.

5.1.3 Regional CBF

Support for an increase in gCBF during exercise also comes from evaluations of regional CBF (rCBF) corresponding to the many regions of the brain that are involved in exercise. During a motor task it is expected that rCBF will increase in the motor cortex [52-54], supplementary motor area [55, 56], premotor cortex [57] and the insula and anterior cingulate cortex [58, 59];

Imaging techniques have now been used to evaluate CBF and cerebral energy consumption following, rather than during, intense whole-body exercise. Extensive evaluation of rCBF is possible by invasive positron emission tomography (PET) [60] and single-photon emission computer tomography (SPECT) [61] and non-invasive functional magnetic resonance imaging (fMRI) [62], (as described in Chapter 4). Such determinations of rCBF reveal that in principle, all brain activation is associated with increased flow corresponding to the relevant areas, e.g. the visual cortex during visual stimulation [63] and the motor area during handgrip exercise [64]. Thus, rCBF increases 20% for the brain as a whole and 50% for focus areas of the brain during exercise and it remains an enigma as to why the Kety-Schmidt methods determined gCBF remains stable during most types of exercise [65].

5.1.4 Regional Cerebral Oxygenation

Regional cerebral oxygenation can be measured continuously using Near Infrared Spectroscopy (NIRS). NIRS is a non-invasive optical technology that relies on the relative transparency of biological tissues to near infrared light (700-900 nm) to determine tissue oxygenation. Oxy- and deoxyhaemoglobin possess distinct absorption characteristics in the near infrared spectrum. By monitoring absorption at wavelengths where oxy- and deoxyhaemoglobin differ, it is possible to determine the concentrations of oxygenated and deoxygenated blood and total haemoglobin content. By determining the concentrations of oxyhaemoglobin, deoxyhaemoglobin and total haemoglobin, then haemoglobin-O₂ saturation (S_{cO_2}) can be calculated. During mild to moderate exercise, the NIRS derived estimate of S_{cO_2} has been shown to be maintained or increase [41, 66-70] as reviewed by Rooks et al. [71]. In

contrast, during maximal whole-body exercise such as ergometer rowing, there has been shown to be a concomitant $\sim 15\%$ lowering of S_cO_2 [72]. It has been argued that the hyperventilation-induced reduction in P_{aCO_2} is inadequate to explain this reduction in S_cO_2 during maximal exercise [66, 71], reflecting that both the lowering of P_{aCO_2} and thereby CBF and an increase in $CMRO_2$ needs to be integrated to explain the decrease in S_cO_2 .

$CMRO_2$ has been shown to be dependent not only on work intensity but also on the involved muscle mass. During handgrip exercise not associated with any changes in P_{aCO_2} , $CMRO_2$ has been shown to remain stable [73] and also during light cycling exercise [26, 67]. Yet, when exercise becomes strenuous, and especially during maximal exercise, an intensity dependent $\sim 30\%$ increase in the calculated $CMRO_2$ has been shown [26, 67, 74, 75].

5.1.5 Summary of Cerebral Responses during a cycling Exercise

The following table summarises the current findings in the literature from TCD and ^{133}Xe studies. (Adapted from Sympathetic influence on cerebral blood flow and metabolism during exercise in humans, *Thomas Seifert et al., 2011 [73]*).

	Rest	Exercise		Change		No. of studies
		Submax	Max	Submax	Max	
$gCBF$ (ml/100g/min)	57 ± 8	55 ± 6	–	NS.	–	7
$MCA V_{mean}$ (cm/s)	55 ± 6	66 ± 7	56 ± 7	+20%	NS.	20
Internal carotid flow (ICA; ml/min)	294 ± 1	347 ± 8	–	+20%	–	2
Vertebral artery flow (VA; ml/min)	95	123	–	+30%	–	1
Total CBF (ICA + VA)*2 (ml/min)	779	928	–	+20%	–	1
S_cO_2 (%)	80 ± 4	84 ± 3	65 ± 11	+5%	–18%	8
$CMRO_2$ (μ mol/100g/min)	175 ± 20	171 ± 27	227 ± 26	NS	+29%	11

Table 5-1: Human cerebral blood flow and oxygenation values at rest and during exercise, adapted from Seifert et al. [73]. Values are mean $\pm$ SD. $gCBF$, global cerebral blood flow; $MCA V_{mean}$, middle cerebral artery blood velocity; total CBF is the sum of internal carotid flow (ICA) and vertebral artery flow (VA) multiplied by 2; S_cO_2 , NIRS determined frontal lobe oxygenation; $CMRO_2$, cerebral metabolic rate for oxygen.

5.1.6 Measuring Cerebral Haemodynamics with MRI

MRI is becoming a well-established method for the quantitative assessment of physiological changes, as was outlined in Chapter 4. Techniques for assessment include phase contrast MRI (Chapter 4, Section 4.2) for simultaneous measurement of vessel blood flow velocity (BFV) and cross-sectional area, overcoming the potential inaccuracies of TCD. Whilst Arterial Spin Labelling (ASL) measures provide a technique by which to non-invasively determine regional cerebral blood flow or perfusion (Chapter 4, Section 4.3).

Recently, MRI has been shown to provide a method for measurement of oxygen utilisation. Lu et al. [76] developed the novel T₂ Relaxation Under Spin Tagging (TRUST) technique (Chapter 4, Section 4.4) to investigate oxygenation in venous vessels and hence quantify oxygen extraction fraction (OEF) and CMRO₂. Since its conception, many studies have used TRUST to measure basal metabolic rate in the human brain. Peng et al. [77] investigated the change in resting CBF and metabolic rate of the brain with normative ageing. After correcting for grey matter volume, the authors found an age-related decline in CBF at a rate of 0.12 ml/100g/min/year ($R^2 = 0.21$, $p = 0.003$). Conversely, grey matter corrected CMRO₂ increased with age ($p = 0.03$) suggesting that the brain of older adults expends more energy when compared to younger adults.

Alternatively fMRI using blood oxygen level-dependent (BOLD) contrast is a widely used technique for the assessment of those brain areas which are active in response to a task. Brain activation is associated with a larger increase in flow than in the oxygen consumption and the consequent increase in cerebral oxygenation derives the BOLD signal by fMRI, taking the different magnetic properties of oxy- and deoxyhaemoglobin into account. However, it should be emphasised that the BOLD signal is more qualitative in nature due to its origin arising from cumulative changes in CBF, CBV and oxygen consumption, as described in Chapter 4, Section 4.5.

Studies have also investigated the cerebral response to exercise using MRI, though these experiments have been constrained to small muscle groups due to the challenges faced when performing dynamic large muscle mass exercise

in an MR environment (discussed further in Section 5.3). However, recent developments in MR techniques and specialised equipment for use in the MR scanner are beginning to provide the ability to measure cerebral haemodynamic and metabolic response to whole body exercise. MR compatible ergometers (e.g. Lode B.V, Netherlands) and steppers (e.g. Ergospect GmbH, Austria) are now available, allowing for large muscle mass exercise during MR data acquisition.

The purpose of the study presented in this thesis chapter was to assess the feasibility of performing a cycling exercise challenge in the MR environment and to use MRI methods to investigate the cerebral response to exercise and the influence of healthy ageing. It was anticipated that this method would allow assessment of cerebral physiology, function and metabolism, and reveal the underlying mechanisms of the age related cerebral response that other techniques fail to address. To perform this a controlled exercise challenge is required at defined levels of cardiorespiratory fitness, VO_{2max} .

5.2 CARDIORESPIRATORY FITNESS

Cardiorespiratory fitness is a measure of the circulatory and respiratory systems capacity to provide oxygen to tissue (in the main skeletal muscle) during incremental intensity exercise, and the ability of tissues to extract and utilise that oxygen. It is known to be one of the best predictors for all-cause mortality. The aim of this study was to assess how the cerebral haemodynamic response to exercise is influenced by age when volunteers are exposed to matched relative loads of physiological stress. It was therefore important to ensure subjects were exercised at equivalent intensities relative to their cardiorespiratory capabilities.

5.2.1 Maximal Oxygen Consumption (VO_{2max})

An individual's VO_{2max} is generally accepted as the gold standard in measurement of cardiorespiratory fitness [78]. VO_{2max} is the maximum rate at which the body is able to consume oxygen. It is typically measured during an incremental exercise test. Normalised for body mass, VO_{2max} is expressed in

terms of ml/kg/min. Cardiac output has a major impact on VO_{2max} [79], as can be seen from the Fick equation which defines maximal oxygen consumption:

$$VO_{2max} = Q \times (C_aO_2 - C_vO_2) \quad [\text{Eqn. 5.1}]$$

where Q is cardiac output and $(C_aO_2 - C_vO_2)$ is the arteriovenous oxygen difference.

For accurate measurement of VO_{2max} , and hence cardiorespiratory fitness, an exercise protocol of sufficient duration and intensity must be applied to ensure exhausting of the aerobic energy system. VO_{2max} is then defined to be the point at which the oxygen consumption reaches a plateau irrespective of an increased workload. All subjects who took part in the study described in this chapter had their VO_{2max} measured, see Section 5.5.

5.3 OPTIMISING THE PROTOCOL FOR COMBINED EXERCISE STUDIES AND MRI MEASURES

Prior to undertaking the full study, pilot data were collected to assess the challenges associated with performing an exercise challenge in the MR environment. A number of considerations were necessary prior to experimentation to ensure safe and accurate MRI measurements.

5.3.1 The Cycle Ergometer

An MR compatible supine cycle ergometer (Lode B.V, Netherlands), as shown in Figure 5-4, was used to perform the supine acute exercise task during MRI. The device has been designed to impose a physiological stress on a subject safely inside a magnetic field. The workload can be varied using an electromagnetic braking principle specifically designed for use in an MR environment and ensuring that if the pedal cadence frequency changes, the resistance changes accordingly such that the workload is maintained constant. This is useful for exercise testing as it allows excellent experimental consistency. Controllable parameters include the revolutions per minute (rpm) and workload (watts, W) which can be automatically recorded in real-time. The ergometer was remotely controlled from outside of the scanner

room using the Lode Ergometer Manager software, which communicated with the device via optical fibres. In an attempt to minimise subject's head motion, handles were added to the scanner bed for stabilisation (See Figure 5-4).



Figure 5-4: Image showing the Lode MR cycle ergometer positioned supine on a 3 T Philips Achieva patient table.

5.3.2 Pilot Data to Assess Exercise-Induced Motion Artefacts

Patient movement is the most common cause of image artefacts in MRI and can significantly corrupt the data collected. In the case of an acute exercise challenge within the MR scanner, subject movement can lead to two types of motion. Stepwise motion arises from subject movement during a transition in exercise intensity with this being most common form in the z-direction (in the direction of the bore), Figure 5-5A. This type of movement was found to lead to SENSE related artefacts, which can be corrected for by re-running a SENSE reference scan at the beginning of each exercise stage. Subject random motion occurs between image dynamics during a given exercise stage (Figure 5-5B). This can typically be corrected by image realignment, but for large levels of movement it can lead to blurring, and for extreme motion the data set has to be discarded.

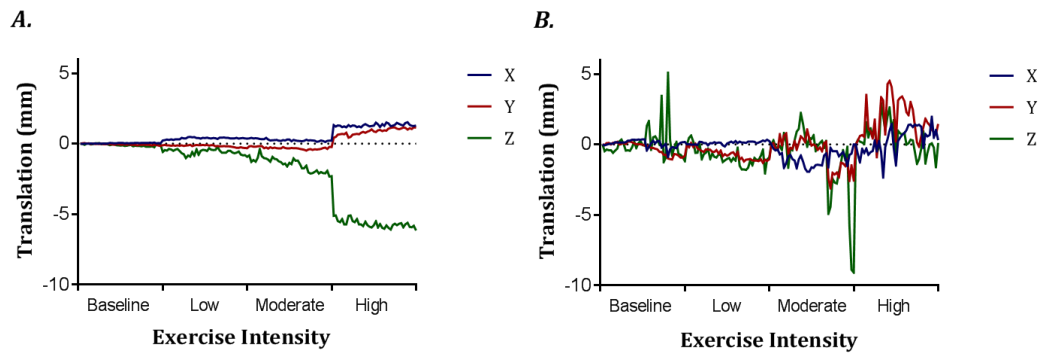


Figure 5-5: A comparison between **A.** stepwise motion and **B.** random motion across increasing exercise intensity. The graphs show the subject's movement in the x, y and z directions. Data collected at 3 mm isotropic.

Since we required participants to perform exercise during MR image acquisition, motion posed a serious challenge. To assess the limitations, pilot data using a combined ASL and fMRI acquisition (see Chapter 4, Section 4.6) were initially collected at baseline and at 10 minutes stages of 30%, 50% and 75% VO_{2max} levels. In an attempt to minimise motion artefacts, the subject's head was padded tightly but comfortably within the head coil, and subjects held handles attached to the scanner bed for stabilisation. The importance of remaining still during the exercise task was also emphasised to the participant.

It was clear that motion was exacerbated at higher exercise intensities, with the majority of data collected at this workload corrupted with image artefacts. After realignment to correct for motion artefact, significant blurring resulted and thus such level of large-scale movement was considered unacceptable. It was therefore decided that the higher intensity 75% VO_{2max} exercise stage should be discarded and replaced with a recovery period following the 50% VO_{2max} level.

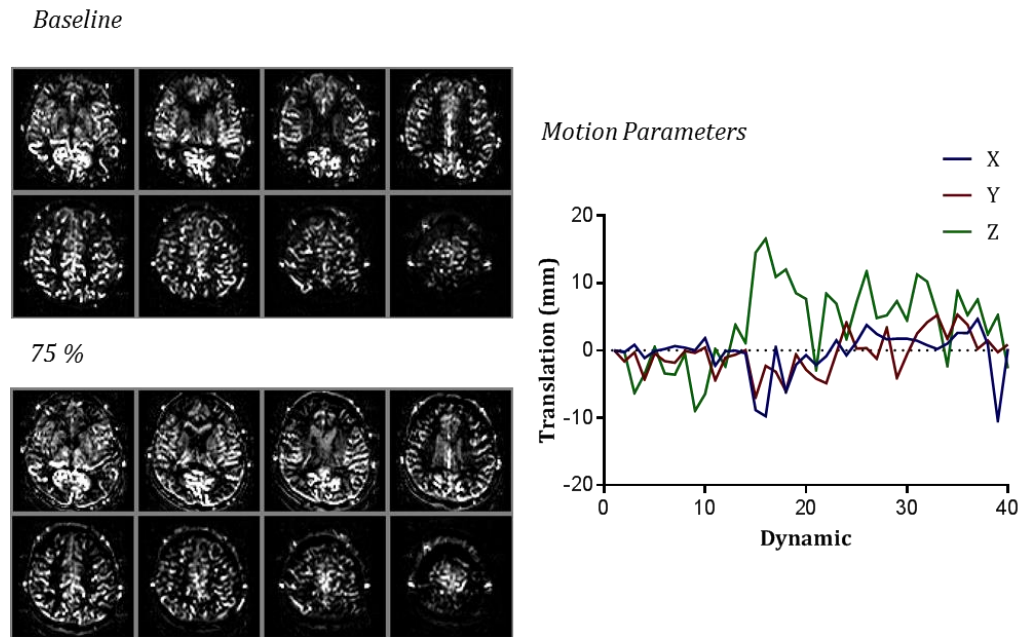


Figure 5-6: Comparison of ASL PW images collected at baseline and at 75 % VO_{2max} exercise intensity, the motion parameters over the course of the 75 % exercise stage are also shown.

5.4 PARTICIPANTS

All subjects gave written informed consent and the study was approved by the University of Nottingham Medical School Ethics Committee. Twenty seven males were recruited to take part in the study. Recruitment was targeted at healthy active individuals, with participants separated into ‘young’ and ‘older’ age groups. All subjects were male and matched for BMI. The resulting subject demographic can be seen in Table 5-2.

Group	Number	Age (years)	BMI
Young	12	21 – 27	23.5 ± 1.8
Older	15	58 – 74	23.3 ± 2.0

Table 5-2: Participant demographics

First, subjects attended a preliminary study visit to ensure they were safe to perform the high intensity exercise protocol in an MR environment. This visit included a questionnaire highlighting any contraindications to MRI and a full medical screening, involving a 12-lead ECG assessment and blood test. Medical tests were performed and assessed by a qualified medical physician. All participants were non-smokers and a medical history was taken prior to the exercise task. Conditions of exclusion included history of cardiovascular or respiratory disease, or abnormal medical screening results.

Subjects were requested to avoid strenuous activity and abstain from alcohol and caffeine 24 hours prior to the start of the study, and to fast for 2 hours prior to each visit.

5.5 MEASURING CARDIORESPIRATORY FITNESS

5.5.1 Cardiorespiratory Fitness Tests

All exercise tests were performed in the School of Life Sciences, Faculty of Medicine and Health Sciences at the University of Nottingham and were supervised by a medical physician. VO_{2max} was assessed for each subject using the same supine cycle ergometer as employed within the MR scanner to determine participant cardiorespiratory fitness. A 12-lead ECG monitored heart rate, and an online gas analysis system (Cosmed, Italy) measured ventilation and oxygen/carbon dioxide concentration of the inhaled and exhaled air. Prior to VO_{2max} testing, the cycle ergometer position was adjusted for subject comfort and to ensure a soft bend in the knee at full extension. This position was recorded for each individual subject and kept constant across all visits. The experimental set up showing the cycle ergometer in the physiology laboratory can be seen in Figure 5-7.



Figure 5-7: *Experimental setup of VO_{2max} tests in the School of Life Sciences at the University of Nottingham. Subjects performed the tests in a supine position using the MR compatible cycle ergometer.*

Subjects performed the VO_{2max} test in a supine position to match the conditions in the MR scanning session. Participants followed a multistage stepwise protocol (Figure 5-8A) beginning at a workload of 25W for 3 minutes. Each subsequent stage duration was three minutes and each workload increment was determined by the subject's rating of perceived exertion (RPE; Modified Borg Scale), scored in the final minute of each stage. Subject cadence was displayed on the cycle ergometer control box and the tester ensured that a consistent rate of 65 rpm was maintained through verbal instruction. VO_{2max} was defined as the point at which VO_2 plateaued irrespective of increasing workload. Tests were terminated at the point of volitional fatigue, when heart rate reached age predicted maximum ($220 - \text{age (years)}$) or due to abnormal ECG readings.

A second VO_{2max} assessment was performed at least 48 hours after the first visit to confirm the previous plateau and account for peripheral fatigue. A similar protocol to the first exercise test was followed, however subjects were permitted an initial warm up at 50W for five minutes before an immediate increase in workload matching their previous maximum workload achieved before exhaustion (Figure 5-8B).

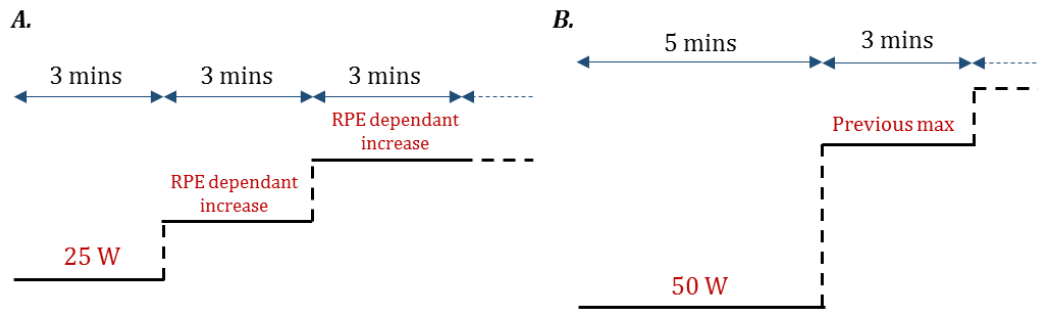


Figure 5-8: The design of the exercise protocol followed to determine each individual's VO_{2max} values. **A.** First study visit. The increase in workload between each stage corresponded to the subject's RPE. **B.** Second study visit. Following an initial warm up period, exercise intensity was increased to the subject's previous maximum workload. The protocol then continued with the same design as the first visit.

VO_2 data were averaged across the two visit assessments to determine the final VO_{2max} value for an individual. Workloads relating to their VO_{2max} level could then be found. An example of a subjects VO_2 data for each visit and the method by which VO_{2max} and corresponding workloads of 30% and 50% VO_{2max} are determined can be seen in Figure 5-9.

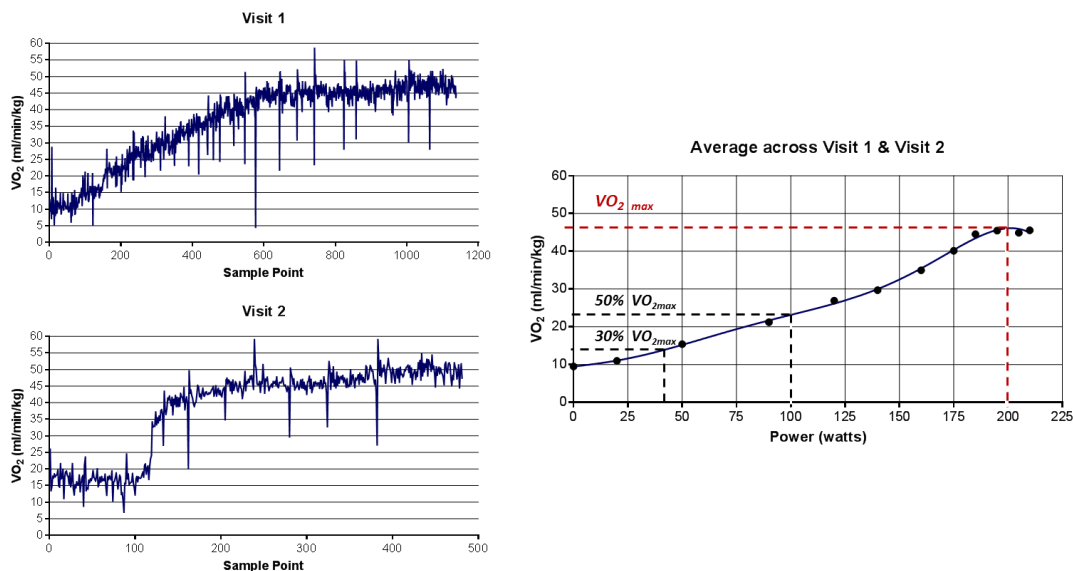


Figure 5-9: Example of an individual subject's VO_2 data for Visit 1 and Visit 2, and the averaged result. The plateau defines the workload at which VO_{2max} is reached, as shown in this example for a VO_{2max} of 46.0 ml/kg/min at a workload of 200 W. Workloads corresponding to individual 30 % and 50 % VO_{2max} levels can then be determined.

5.6 MEASURING CORTICAL HAEMODYNAMICS WITH MRI

5.6.1 MRI Exercise Paradigm

The MRI scan session was performed within 2 weeks of completion of the VO_{2max} tests to ensure minimal variability in cardiorespiratory fitness. A medical physician was present during older adult data acquisition.

The exercise task consisted of four 10 minute stages, the first at baseline. Subjects were then asked to cycle at a workload corresponding to 30% and 50% of their VO_{2max} , followed by a recovery period (Figure 5-10A). A metronome was played throughout to ensure subjects' maintained a cadence of 65 rpm which matched that used in the cardiorespiratory fitness test. The order of the MR data acquisition across the scanning session is shown in Figure 5-10B.

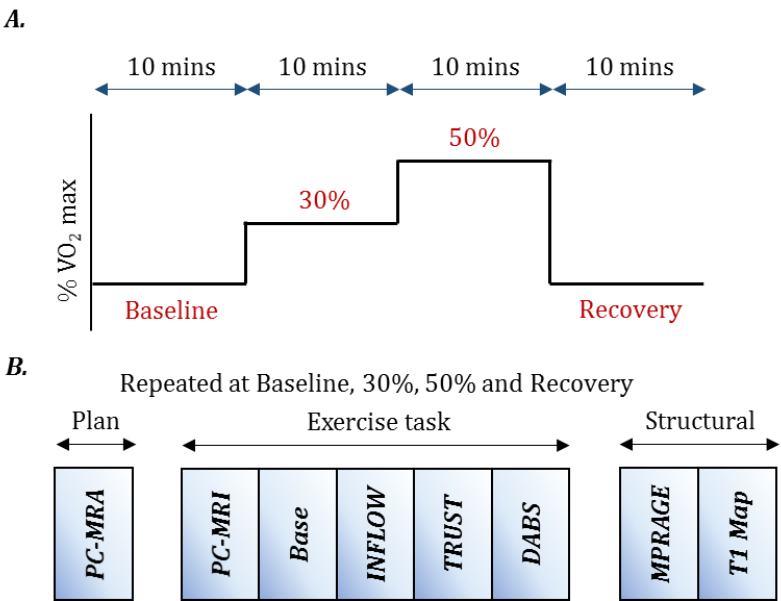


Figure 5-10: **A.** Exercise protocol implemented during the imaging sequence. **B.** Physiological and functional scans repeated for each exercise stage.

5.6.2 MRI Acquisition

Data were acquired on a 3.0 T Philips Achieva whole body MRI scanner using a 32-channel head receive coil. Throughout all scans a vectorcardiogram monitored the subject's heart rate. Planning scans preceded the exercise challenge. These included a survey scout scan, followed by a 2D phase contrast

angiography (2D PC-MRA) which was used to identify the left and right internal carotid arteries, basilar artery and the superior sagittal sinus for planning of PC-MRI scans. The MRI techniques implemented during each stage of the exercise challenge are detailed below.

5.6.2.1 Cerebral blood flow using PC-MRI

Blood flow in the left and right internal carotid arteries, and basilar artery were assessed using a vector cardiogram (VCG) gated phase contrast MRI sequence. Slices were positioned perpendicular to the direction of flow in the vessel of interest (Figure 5-11). Scan parameters were FOV = 280 x 77mm², spatial resolution = 0.75 x 0.75mm², slice thickness 6mm, SENSE factor 3, TE/TR = 6.5/15ms, FA = 25°, V_{ENC} 100cm/s, NSA = 2. The sequence was cardiac triggered and data were acquired at multiple time points across the cardiac cycle. The duration of the scan was dependant on the subject's heart rate.

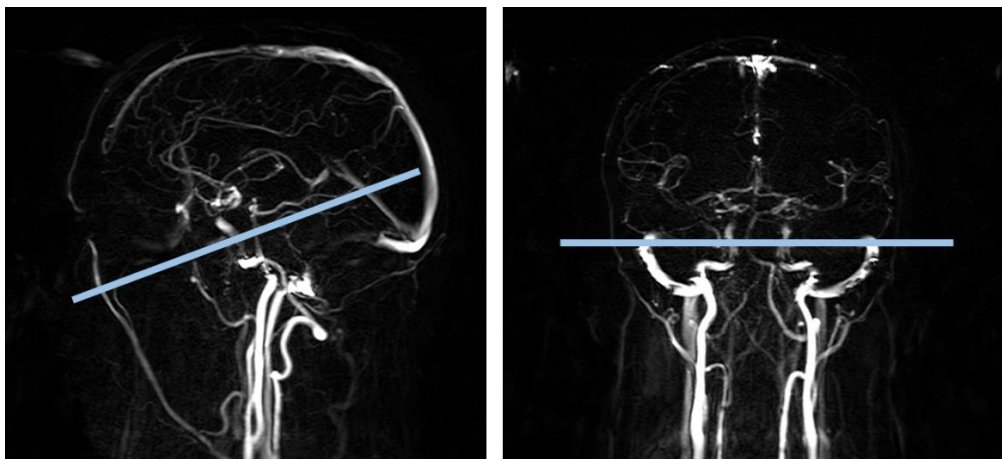


Figure 5-11: Positioning of PC-MRI slices (blue line) in the sagittal and coronal planes, planned off the PC-MRA images acquired prior to commencement of the exercise task. Slices perpendicularly bisect the left and right internal carotid arteries and basilar artery.

5.6.2.2 Oxygen metabolism using a TRUST acquisition

Oxygen metabolism measurements were added to the scanning protocol part way through the study. In total these measurements were assessed in 14 of the 27 subjects recruited to the study. The demographic of subjects in which the oxygen metabolism measurements were collected can be seen in Table 5-3.

<i>Group</i>	<i>Number</i>	<i>Age (years)</i>	<i>BMI</i>
<i>Young</i>	6	21 – 26	23.1 ± 1.7
<i>Older</i>	8	58 – 70	25.1 ± 1.5

Table 5-3: Participant demographic for oxygen metabolism measurements.

Venous oxygenation, Y_v , in the superior sagittal sinus (SSS) was quantified using a T₂ Relaxation Under Spin Tagging (TRUST) sequence [76], as described in Chapter 4, Section 4.4. TRUST images were acquired using a sequence coded in-house which was assessed for reproducibility prior to use in this study. The TRUST imaging slice was positioned parallel to the anterior commissure–posterior commissure line and perpendicular to direction of flow in the SSS vein (Figure 5-12). Scan parameters were as described in [77], with an in-plane field of view (FOV) of 230 mm x 230 mm, spatial resolution 3.44 x 3.44 mm², slice thickness 5 mm, TI = 1022 ms, and TR = 3000 ms per label/control pair. Four eTEs were collected at 1, 40, 80, 160 ms corresponding to 0, 4, 8 and 16 refocussing pulses in the T₂ preparation with an interval $\tau_{\text{cpmg}} = 10$ ms (Carr-Purcell-Meiboom-Gill). The scheme comprised a TILT labelling scheme (see Chapter 4) with labelling thickness of 100 mm and a label gap of 22.5 mm.

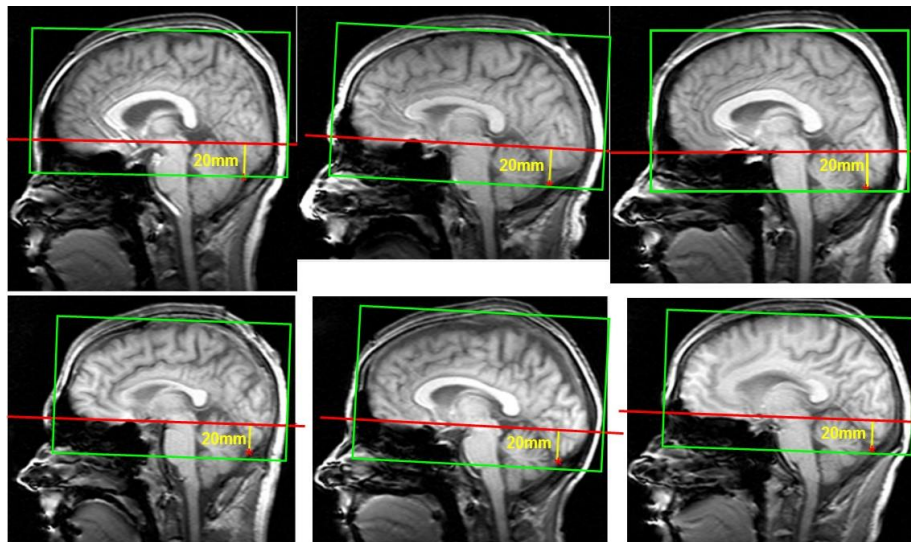
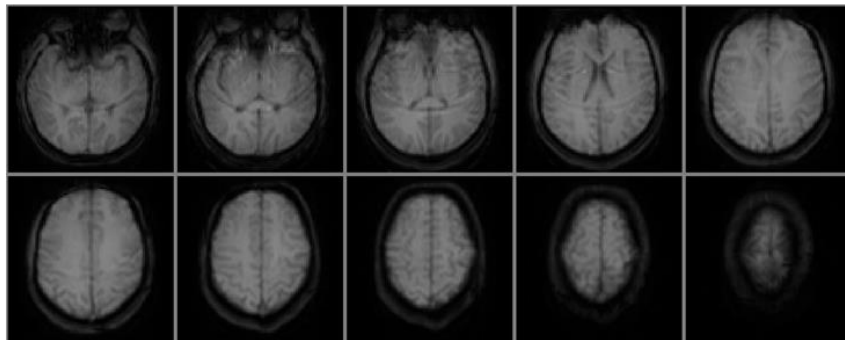


Figure 5-12: Example placement of the imaging slice (red line) shown across six example head sizes. For consistency, the imaging slice is positioned approximately parallel to AC-PC line and 20 mm above the sinus confluence (red asterisk). The shimming box is shown in green and covers the entire cerebrum.

5.6.2.3 Cerebral perfusion and BOLD using a DABS acquisition

Cerebral perfusion and BOLD activation were assessed simultaneously using a Double Acquisition Background Suppression (DABS) [80] ASL sequence, as described in Chapter 4, Section 4.6. Ten slices of ASL and BOLD data were collected with GE-EPI readout with 212mm FOV, spatial resolution 2.33 x 2.36 mm², slice thickness 8 mm, SENSE factor 2.5, ASL data were collected with a short TE of 9 ms to maximise ASL SNR, whilst BOLD data were collected with a TE of 40 ms to maximize BOLD CNR. The DABS sequence comprised two background suppression pulses at 339 ms and 899 ms. Data were collected with a post-label delay of 1500 ms and a TR of 3600 ms was used per label/control pair. 40 ASL label and control pairs were acquired per exercise workload. An example of the ASL control/label image and BOLD images acquired are shown in Figure 5-13A and Figure 5-13B respectively.

A.



B.

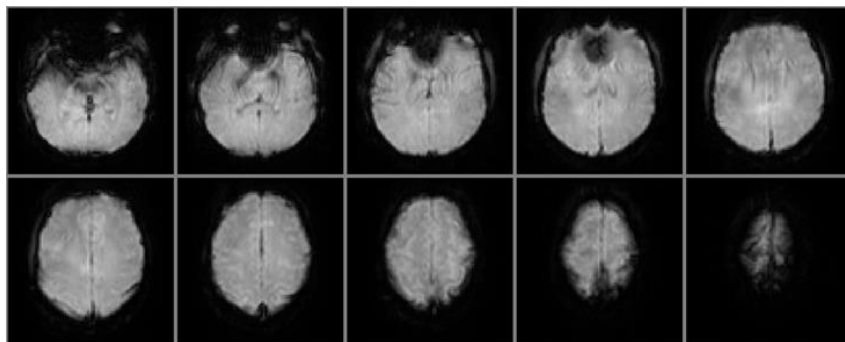


Figure 5-13: Example of **A.** ASL control/label image and **B.** BOLD images acquired simultaneously using a 10-slice double acquisition background suppression (DABS) sequence.

5.6.2.4 Grey matter data

A high resolution whole-brain T₁-weighted MPRAGE sequence was acquired in the sagittal plane to assess grey matter volume. Scan parameters were TR = 8.39ms, TE = 3.86ms, flip angle = 8°, 160 contiguous slices, slice thickness = 1mm, FOV 256 x 256 mm², spatial resolution 1mm isotropic, NSA = 1.

In addition, grey matter segmentation was achieved in DABS space using a T₁-map with matched imaging parameters. This was performed using the same 10 slice GE-EPI readout as the DABS ASL scheme (spatial resolution 2.33 x 2.33 mm², slice thickness 8mm, SENSE factor 2.5, TE = 10ms). Data was collected at increasing inversion times TI of 100, 200, 500, 800, 1000, 1300, 1500, 2000, 3000, 4000 ms using a TR of 5000 ms.

5.6.3 MRI Analysis

5.6.3.1 Global Cerebral blood flow using PC-MRI

PC-MRI data were analysed using the Intellispace software (Philips Medical Systems, Netherlands). The internal carotid arteries and basilar artery were manually identified, and an ROI was drawn around the walls of each vessel based on the magnitude image. This ROI was propagated through all time points across all phase in the cardiac cycle, with the ROI manually inspected and edited if necessary. Blood velocity (mm/s), and vessel cross sectional area (mm²) were directly measured, and the product of velocity and area within the ROI (*velocity* × *area*) computed to calculate flux in each vessel, these were then combined across vessels to compute global cerebral blood flow. A PC-MRI of the brain feeding arteries, example ROI's and corresponding flux waveforms can be seen in Figure 5-14.

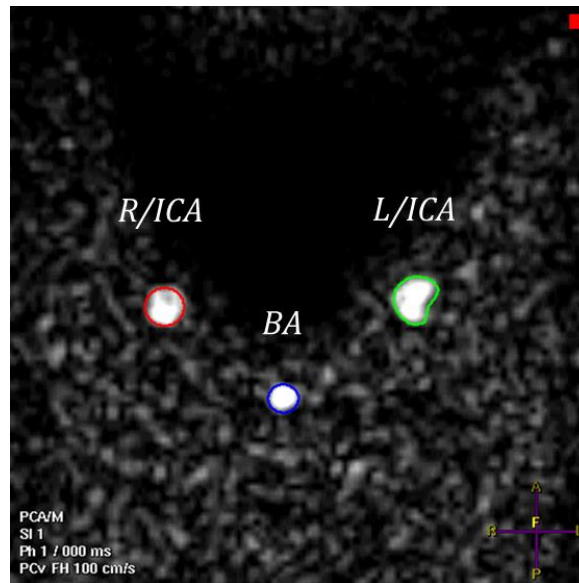


Figure 5-14: Analysis of PC-MRI magnitude data using Intelispace software (Philips Medical Systems, The Netherlands). ROI's drawn around the walls of the right and left internal carotid arteries (R/L ICA) and basilar artery (BA) to estimate blood flow velocity, vessel area and hence cerebral blood flow.

To account for brain atrophy, cerebral blood flow was corrected for grey matter volume, gmCBF. Grey matter volume was calculated using tissue segmentation in FSL (FMRIB, UK) from the high-resolution T₁ MPRAGE image. CBF was then normalised by each individual's GM volume.

5.6.3.2 Global Cerebral Oxygen

TRUST data were processed using dedicated MATLAB code following the methods described in Lu et al [76]. Venous blood signal in the superior sagittal sinus (SSS) was computed from the pairwise subtraction of label and control images to obtain difference images for each eTE. In the resultant difference images, an ROI of four voxels with the highest difference signal created a mask for spatial averaging of the signal. Blood T₂ relaxation was then calculated within the SSS ROI by fitting to the monoexponential signal decay. The TRUST analysis process can be seen in Figure 5-15.

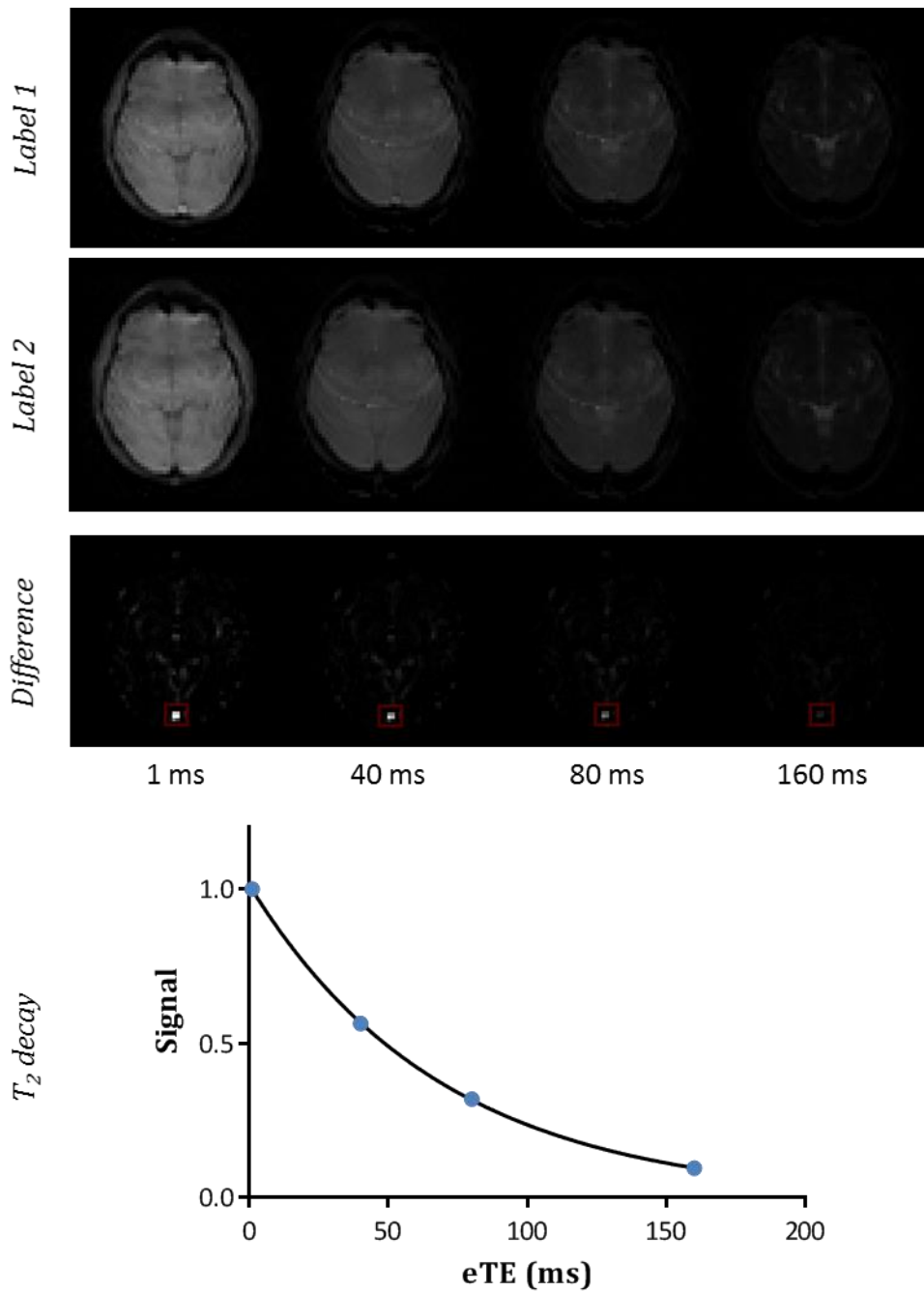


Figure 5-15: Illustration of the TRUST analysis. Label and control images are acquired at 4 TE's. A difference image series is created by the pairwise subtraction of the label and control data at each TE. Four voxels with the highest intensity (highlighted in red) are interrogated to fit the T_2 decay of blood in the superior sagittal sinus.

The blood T_2 relaxation time in the SSS was then converted into venous oxygenation, Y_v , using a calibration plot (Figure 5-16) [81].

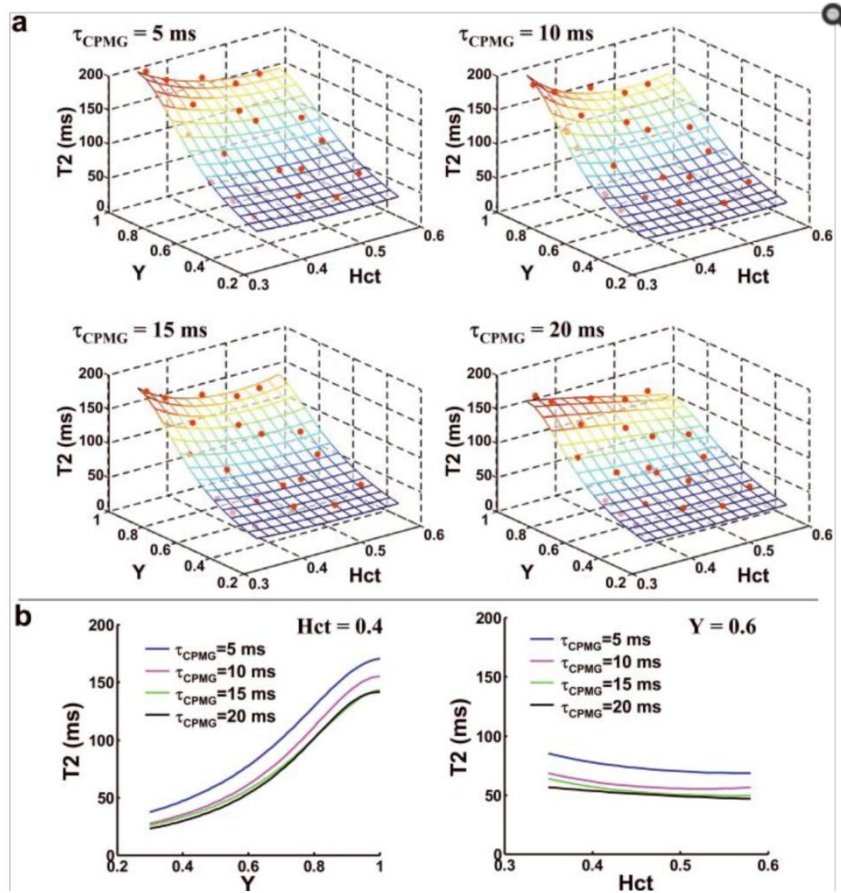


Figure 5-16: Calibration plot as described by Zhao et al. [81] to convert blood T_2 relaxation into venous oxygenation, Y_v .

Metabolic physiology was calculated following the methods outlined in Peng et al. [77]. Arterial oxygenation, Y_a , is close to unity and was estimated accounting for the age and sex [82] of the individual subjects:

$$Y_a = 99.77 - 0.036 \times age - 1.235 \times sex + 0.021 \times age \times sex \quad [\text{Eqn.5.2}]$$

where *age* is in years and *sex* is defined as 0 or 1 for females and males respectively.

Oxygen extraction fraction was then calculated from,

$$OEF = Y_a - Y_v \quad [\text{Eqn. 5.3}]$$

Quantification of global $CMRO_2$ was performed following techniques based on the Fick principle [83] as described by Liu et al. [84]:

$$gCMRO_2 = gCBF \cdot (Y_a - Y_v) \cdot C_h \quad [\text{Eqn. 5.4}]$$

where $gCMRO_2$ and $gCBF$ are global cerebral metabolic rate of oxygen and global cerebral blood flow (gCBF) respectively and C_h is the capacity of blood to carry oxygen. Individual C_h values were calculated using haematocrit data obtained during the medical screening blood tests and were corrected for age assuming a decline rate of $0.0079\mu\text{mol O}_2/\text{ml}$ per year [85].

As with CBF, $CMRO_2$ was also normalised for brain atrophy accounting for grey and white matter loss with age [77]:

$$gmCMRO_2 = \frac{tCMRO_2}{V_{gm} + r \cdot V_{wm}} \cdot \frac{1}{\rho} \quad [\text{Eqn. 5.5}]$$

where $gmCMRO_2$ is the grey matter corrected $CMRO_2$, V_{gm} and V_{wm} are grey and white matter volumes respectively, r is the ratio between white and grey matter and ρ is a constant relating to the mass density of tissue, assumed to be 1.06 g/ml [86].

5.6.3.3 Cerebral perfusion

DABS data were analysed using dedicated in-house MATLAB code (MathWorks, USA). The following was performed for data collected at each of the exercise workloads. First, images were separated to extract the ASL data (perfusion (CBF)-weighted label 1 (label) and label 2 (control) images acquired at $TE=9\text{ms}$) and BOLD data (BOLD-weighted images acquired at $TE=40\text{ms}$). ASL imaging volumes were then inspected for motion and corrected accordingly. Motion correction was performed using the FSL FLIRT algorithm, whereby both label 1 and label 2 are concatenated in the 4th dimension and the central dynamic of label 1 is used as the reference. Despite the slightly different contrast of label 1 and label 2 due to the blood label, this method was found to outperform motion correcting each label individually. The transformation matrices to motion correct each repeat were decomposed to determine the amount of translation and rotation which was applied. If the translation required to motion correct a volume exceeded one voxel (3mm), the dynamic was discarded. The image was split back into label 1 and label 2 formats. A perfusion weighted image was then created by computing the subtraction between label 1 and label 2 using simple pairwise subtraction.

Using the method described by Tan et al., (2009) [87] each dynamic of the PW images was assessed to determine if it was an outlier. The PW images were then averaged over all dynamics to create a final PW image at each exercise workload.

Label 1 of the non-selective condition for each PLD was averaged over all dynamics. These were then co-registered to the T₁-map. From the T₁-map a grey matter mask was formed. The transformation matrices describing the co-registration of each PLD to the T₁map were then applied in inverse such that the grey matter mask was applied to each exercise level. This was performed since each application of a realignment transformation causes the image to blur due to interpolation effects, by moving the mask rather than the data this omits any alteration to the perfusion data.

5.6.3.4 BOLD activation

The BOLD data were separated from the DABS data (BOLD-weighted images acquired at TE=40ms for label1 and label 2). BOLD-weighted image pairs (those images acquired at TE=40ms), collected following each of the label and control data, were averaged to produce a mean BOLD-weighted data. The mean BOLD-weighted data time series at each of the exercise level was then realigned using MCFLIRT (FSL, U.K), and the motion parameters stored. To identify brain areas associated with the exercise task, a GLM analysis was then performed to identify BOLD activation in response to the exercise.

5.7 RESULTS

5.7.1 Cardiorespiratory Response to an Incremental Exercise Task

All subjects completed the exercise task during the MR acquisition, however two subjects' data from the older group were discarded due to excessive motion artefacts. Mean and SEM cardiorespiratory responses to the incremental exercise challenge between the two age groups can be seen in Table 5-4.

	Young				Older		
	30%	50%	100%		30%	50%	100%
VO_2 (ml/kg/min)	12.71 ± 0.29	20.33 ± 0.60	39.69 ± 1.51		9.46 ± 0.41	15.41 ± 0.54	29.00 ± 1.20
VE (l/kg/min)	0.306 ± 0.01	0.530 ± 0.02	1.584 ± 0.07		0.247 ± 0.01	0.394 ± 0.02	1.224 ± 0.05
VCO_2 (ml/kg/min)	11.57 ± 0.70	19.37 ± 1.13	52.55 ± 2.07		7.844 ± 0.20	12.43 ± 0.65	34.29 ± 1.81

Table 5-4: Table showing mean ± SEM for oxygen consumption (VO_2), ventilation (VE) and expired carbon dioxide volume (VCO_2) at 30%, 50% and 100% VO_{2max} for the young and older group.

Overall, supine VO_{2max} was lower in older than young subjects (29.0 ± 1.2 vs. 39.7 ± 1.5 ml/kg/min) as was absolute maximum exercise workload achieved (131 ± 18 vs. 171 ± 23 W) (Figure 5-17A).

There was no significant difference in minute ventilation (VE) between the two age groups at baseline ($p = 0.28$), however at exercise intensities of 30% VO_{2max} and above, ventilation was significantly lower in the older age group (30% VO_{2max} ; $p < 0.05$, 50% VO_{2max} ; $p < 0.001$) (Figure 5-17B). Expired volume of carbon dioxide (VCO_2) was significantly lower in the older age group at baseline and across the exercise task ($p < 0.001$) (Figure 5-17C). All values were normalised for subject body mass. Refer to Appendix for individual subject data.

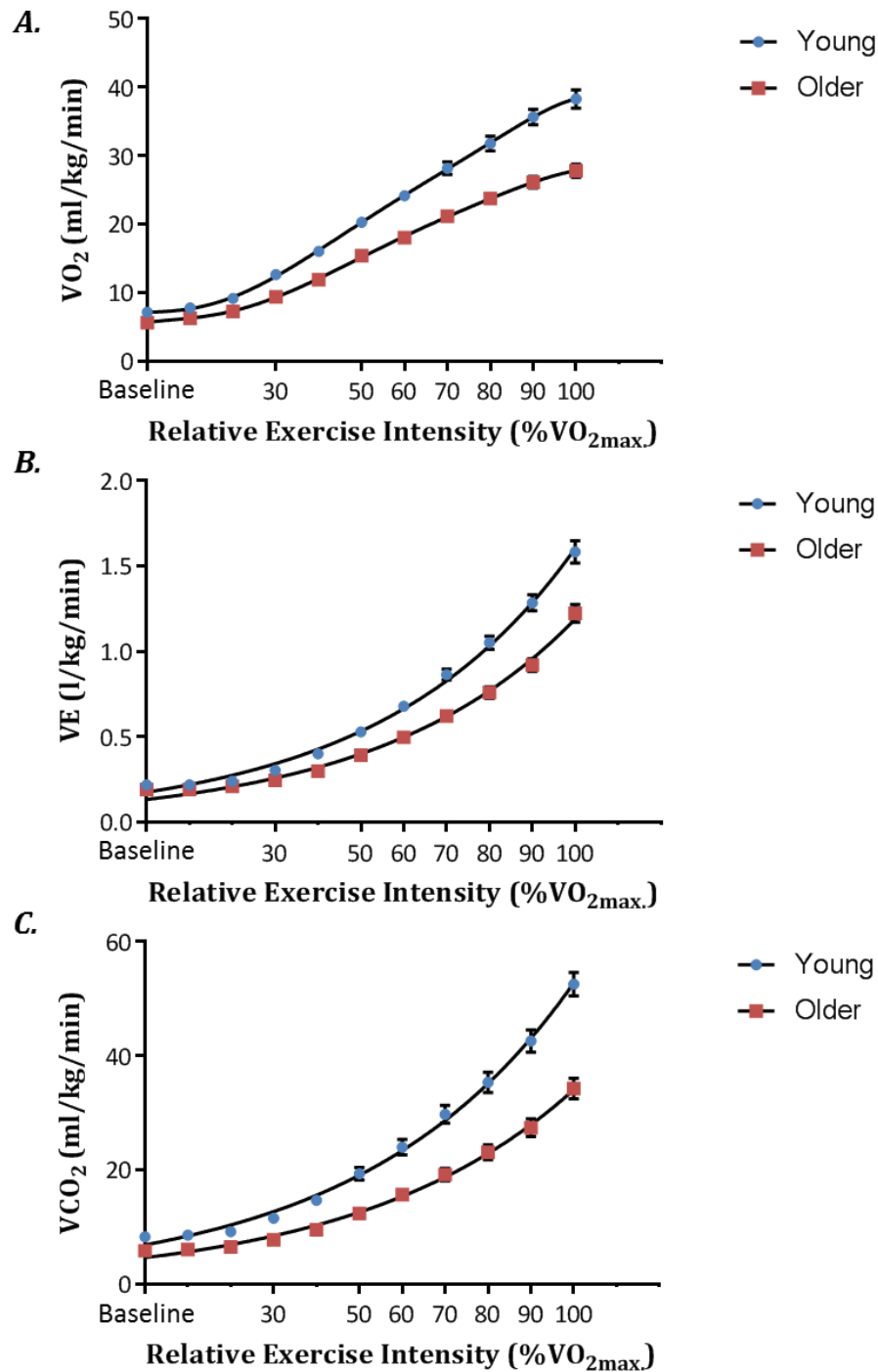


Figure 5-17: Comparison of **A.** maximum oxygen consumption (VO_{2max}), **B.** ventilation (VE) and **C.** expired CO_2 volume (VCO_2) between the young and older age groups assessed during the cardiorespiratory fitness test.

5.7.2 Global Cerebral Blood Flow

Cerebral blood flow was assessed in the ICA and BA by the product of velocity and vessel area and summed to give total cerebral brain blood flow. Figure 5-18 shows PCA data of total CBF at rest, during low to moderate exercise and on recovery for both age groups. There was a significant blunting in the CBF response to exercise in older volunteers. No significant difference in CBF was found between the two groups at rest or on recovery ($p = 0.18$ and $p = 0.19$ respectively).

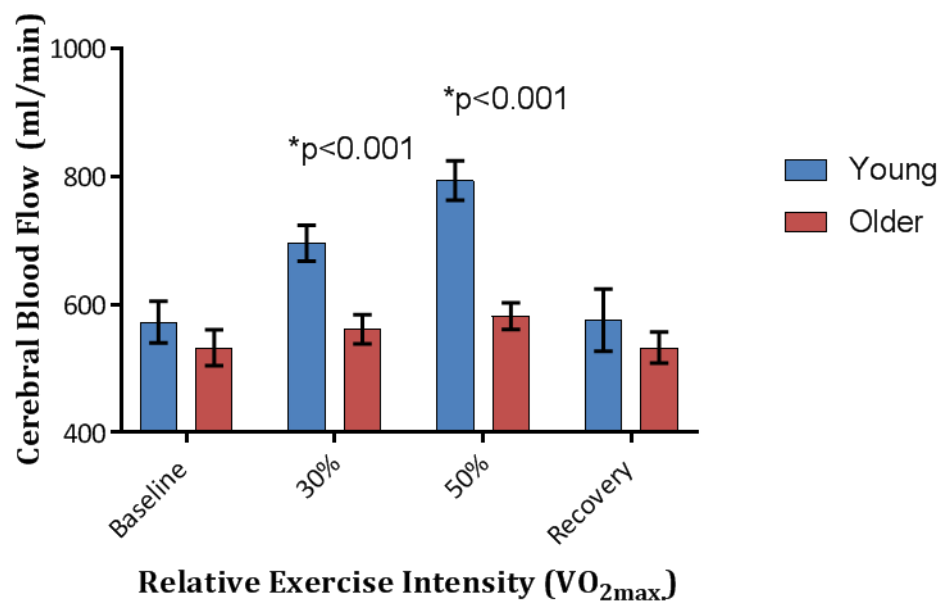


Figure 5-18: Total cerebral blood flow (ICA+BA) across the exercise task. Data shows a significant deficit in CBF for the older group at low and moderate intensity exercise.

The major advantage of using MRI over TCD when assessing the cerebral response to exercise is the ability to account for age related brain atrophy and correct CBF values for grey matter (GM) volume (Figure 5-19). The CBF differences between the young and older group during low to moderate exercise intensity remained significant. Again, there was no significant difference in CBF between young and older subjects at rest or on recovery ($p = 0.34$ and $p = 0.41$ respectively).

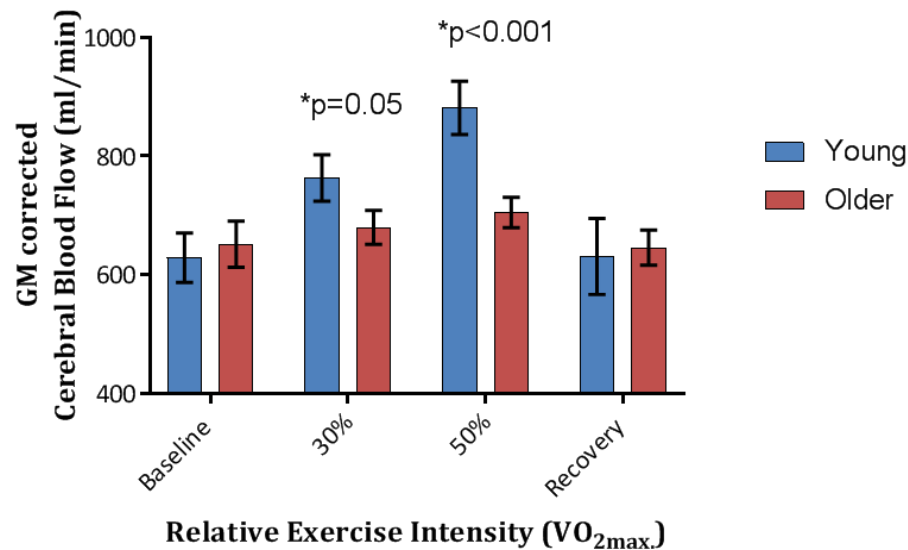


Figure 5-19: Grey matter corrected total cerebral blood flow across the exercise task. This CBF deficit seen in the older group remains after age related atrophy has been accounted for.

5.7.3 Cerebral Perfusion and Activation

Figure 5-20 shows the percentage change in perfusion from baseline as measured by ASL. Concomitant with CBF, perfusion was also found to be blunted in older adults compared to young at both exercise intensities (30 %: $p=0.007$, 50 %: $p=0.002$), while there were no significant differences between the two age groups at rest or on recovery.

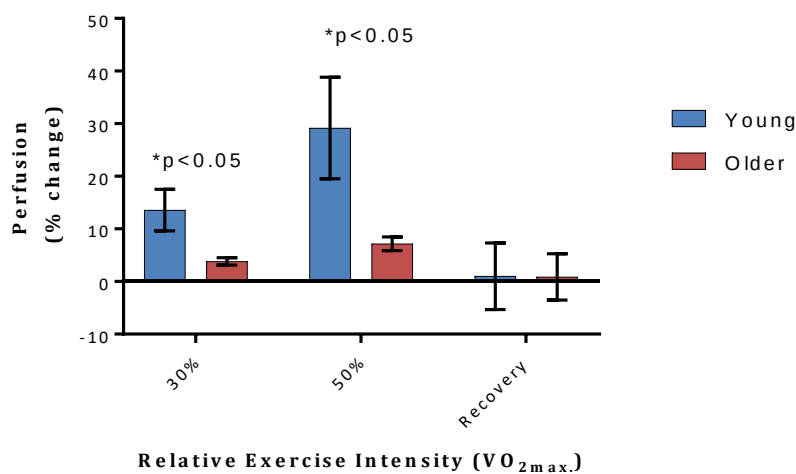


Figure 5-20: Percentage change in perfusion from baseline on exercise between young and older adults.

Similarly, an increase in BOLD response was seen in the young on exercise, however no significant response was found associated with the task in older adults (Table 5-5).

Young	30%	50%
BOLD response (% change from baseline)	6.9 ± 2.1	9.5 ± 2.1

Older	30%	50%
BOLD response (% change from baseline)	No significant response associated with task	No significant response associated with task

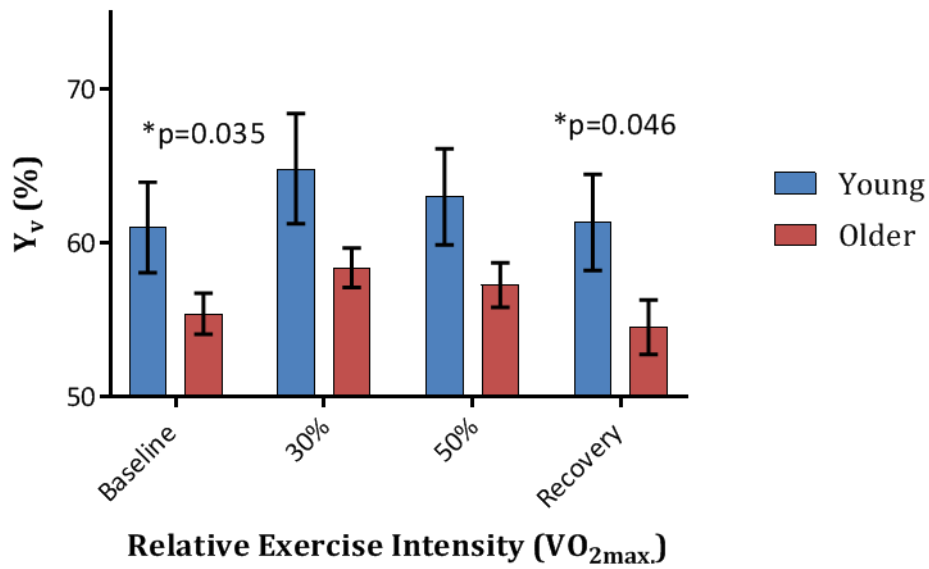
Table 5-5: Percentage change in BOLD in response to the exercise task between young and older adults.

5.7.4 Oxygen Extraction and Utilisation

It was hypothesised that the ageing brain may compensate for the deficit in cerebral blood flow on exercise through increased oxygen uptake and utilisation. Oxygen metabolism measurements were obtained on 14 of the 27 subjects (Table 5-3). Using a TRUST sequence, venous oxygenation in the SSS was assessed across the exercise task (Figure 5-21A). The corresponding oxygen extraction fraction was calculated using Eqn. [5.3] and is shown in Figure 5-21B.

Venous oxygenation was significantly reduced in the older group compared to the young group at rest and on recovery which corresponded to a significant increase in oxygen extraction fraction at these stages. The trend for increased oxygen extraction in the older group remained across the task but was not found to be significant during the exercise stages (30%; $p = 0.114$, 50%; $p = 0.134$). The overall age effect across the exercise task was $p = 0.0018$.

A.



B.

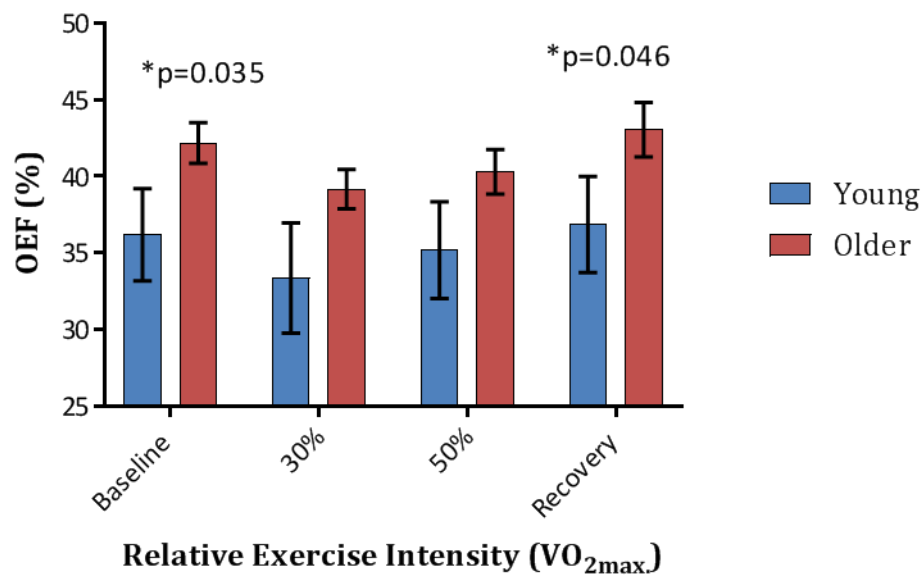


Figure 5-21: Oxygen metabolism measures across the exercise task in young and older subjects. **A.** venous oxygenation and **B.** corresponding oxygen extraction fraction.

The global cerebral metabolic rate of oxygen was calculated using Eqn. [5.4] and corrected for GM volume to account for age -related atrophy using Eqn. [5.5] (Figure 5-22).

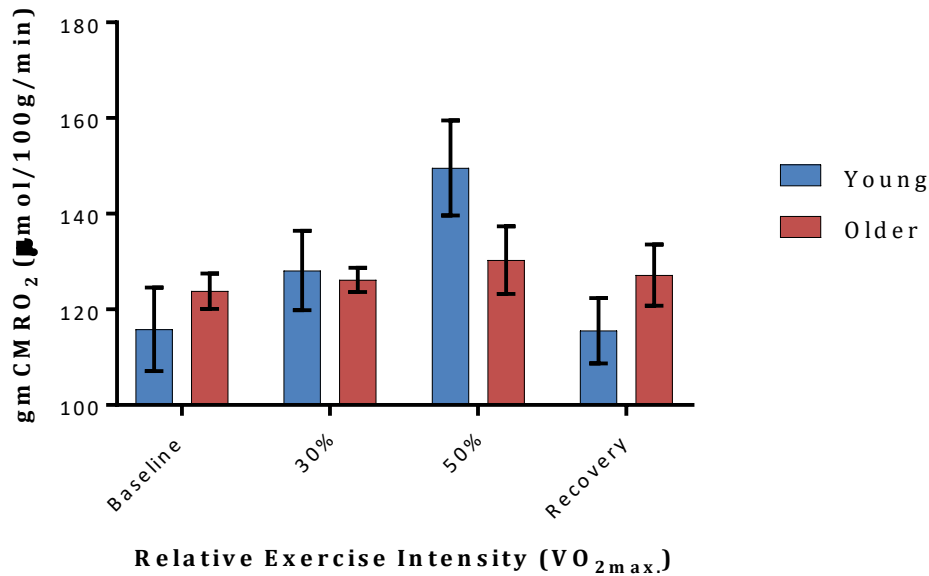


Figure 5-22: Grey matter corrected global cerebral metabolic rate of oxygen ($gmCMRO_2$) in young and older subjects across the exercise task. No significant differences were found between the two groups.

No significant differences in $gmCMRO_2$ were observed between the young and older groups at any stage over the exercise task.

5.8 DISCUSSION

This study has, for the first time, used MRI as a tool to investigate age-related changes in the cerebral response to an exercise challenge involving recruitment of a large muscle mass.

5.8.1 Cerebral Perfusion, Age and Exercise

We have demonstrated a clear deficit in CBF in older adults compared to young during submaximal exercise (30 and 50% VO_{2max}). CBF increased from baseline in both age groups, but remained lower in the older group across the task. The CBF deficit on exercise was matched by a reduction in global brain perfusion as measured by ASL. These data confirm the observations of TCD studies examining $MCA V_{mean}$ in populations of a similar age at similar exercise intensities [7, 47-49]. Previous literature has suggested that the age-related reductions in $MCA V_{mean}$ may reflect global brain atrophy. Importantly, the advance made with the work presented in this chapter is that MRI allows for

normalisation of CBF values to account for grey matter volume where the CBF deficit on exercise remains. This suggests that the observed age-related attenuation in CBF on exercise is independent of brain atrophy.

The decreased *MCA V_{mean}* found in previous TCD studies has been attributed to a hyperventilation-induced, age-dependent reduction in P_{aCO_2} during exercise, lessening the vasodilatory drive [8]. Ventilation (VE) and expired CO₂ (VCO₂) response data was collected during the cardiorespiratory fitness tests. No difference can be seen in absolute VE between the two age groups at baseline, and VE in the older group is significantly less than in the young adults at 30% and 50% VO_{2max}. This demonstrates that the blunted CBF response in older adults observed in this study cannot be caused by a hyperventilation-mediated reduction in P_{aCO_2} .

The significantly lower VO₂ consumption and expired VCO₂ seen in the older group at 30 and 50% VO_{2max} exercise intensities suggests that P_{aCO_2} may also have been lower in the older volunteers. It is well documented that a lowering of P_{aCO_2} constricts blood vessels and reduces blood flow to the brain, which may therefore account for the decreased perfusion observed in this study. However, it is widely accepted that P_{aCO_2} is maintained constant during exercise by increasing expired VCO₂, which necessitates that the lower absolute workload performed by the older volunteers is the most likely explanation for their lower expired VCO₂, pointing to another reason for the lower brain haemodynamic response to exercise in older volunteers.

A second possible mechanism behind the CBF deficit in older adults on exercise is a reduced cardiac output. The influence of cardiac output on cerebrovascular regulation has been suggested since it has been found that when moving from a supine to standing position, *MCA V_{mean}*, S_cO₂ and CBF decrease along with a reduction in cardiac output [88, 89]. These findings cannot be explained by the concomitant decrease in P_{aCO_2} [90, 91]. Cardiac output follows a linear relationship with VO₂ (Eqn. [5.1]) which can be seen to be less in the older adults. The reduction in cardiac output together with the increased demand of blood from the muscle on exercise may compromise

brain perfusion [10]. This suggests that the decline in CBF generally attributed to ageing may, in part, be a consequence of detraining. The implication of this is that a proportion of the CBF deficit observed with age may be attenuated by higher cardiorespiratory fitness.

Contrary to the majority of TCD studies, no significant difference in CBF values were found between the two age groups at rest or on recovery. Crucially, this suggests that age-related deficits in brain cortical haemodynamics only become apparent on exercise, and return to baseline values after a short recovery period. This highlights the importance of acquiring data directly during an exercise challenge when investigating changes in cerebral regulation with ageing.

One factor that must be highlighted with respect to global CBF values presented in this chapter is that, in contrast to typically stated resting CBF values of ~750 ml/min for a healthy adult, baseline measures acquired in this study were significantly lower in both age groups. One potential source of error arises from the delicacy of planning a PC-MRI acquisition. For accurate quantification of blood velocity through a given cross-sectional area (and hence blood flow), the acquisition slice must bisect the vessel perpendicularly. Here, three vessels were interrogated for global CBF measures, the left and right internal carotid arteries and the basilar artery. Ideally, each vessel would be assessed individually to ensure optimum positioning of the acquisition slice, however, due to time constraints associated with performing a strict exercise task, all three vessels were interrogated simultaneously. Natural variation in the cerebrovascular structure across individuals therefore resulted in imperfect planning in the basilar artery for some subjects. Further, the importance of precise positioning of the imaging slice renders PC-MRI highly susceptible to motion. PC-MRI data for each exercise stage were planned using a PC-MRA image, acquired at the beginning of the scanning session. This method relies on the subject remaining in the same position throughout the exercise task. Excessive movement in any direction would result in the mispositioning of the imaging slice, however, evidence of this

would be clear during analysis and the subject data could be excluded accordingly.

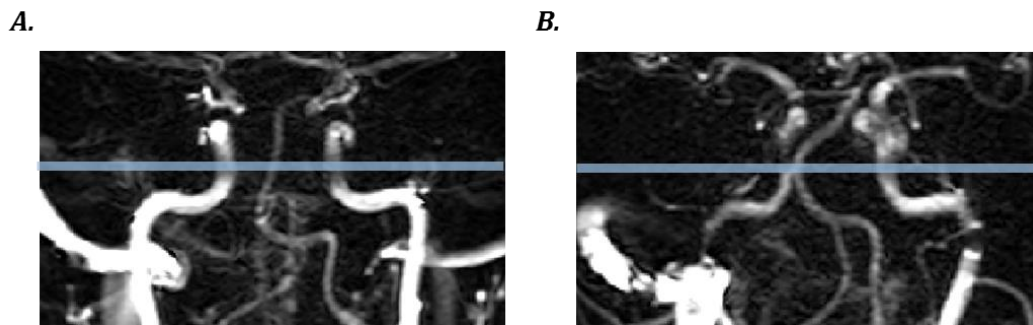


Figure 5-23: *A. Optimum and B. imperfect planning of a slice (blue line) for assessment of cerebral blood flow in the left and right carotid arteries and basilar artery using a PC-MRI acquisition.*

It should be noted that the combination of the measurement of blood flow in the ICAs and the BA does not truly represent total brain blood flow, but rather blood flow to the cerebrum, since the positioning of the BA planning did not include the contribution of blood flow feeding the cerebellum. At the time of performing this study, the PC-MRI methods available were constrained to assessing single-directional flow in two spatial dimensions (2D). However, recent developments allow the comprehensive analysis of blood flow dynamics with complete volumetric coverage [92]. 4D flow MRI refers to time-resolved data acquired using velocity encoding gradients in three spatial directions. This would allow for quantification of blood flow in all vessels in the brain, providing a significantly more robust measure of ‘global CBF’. Limitations of 4D flow MRI include acquisition time, with current sequences requiring ~10mins to complete, and so global CBF have to be the sole measure in an incremental exercise study.

5.8.2 Cerebral Oxygenation, Age and Exercise

At rest and on recovery, oxygen extraction was significantly greater in older subjects compared to young. This age related increase remained on exercise (combined 30/50% $\text{VO}_{2\text{max}}$ stages; $p = 0.044$). The results suggest that the ageing brain attempts to compensate for the deficit in cerebral perfusion by

extracting a higher proportion of oxygen from the blood. However, no significant difference was found in grey matter corrected CMRO₂ between the two age groups suggesting a decreased metabolic efficiency with age. Caution must be taken when interrogating the cerebral oxygenation measures. Results showed high variance in the young age group and given the reduced subject size there is low statistical power.

5.9 CONCLUSION

A number of technical challenges must be overcome to allow physiological measures in response to an acute exercise task to be assessed in an MR environment. Having overcome these, this Chapter provides a body of work on the comprehensive assessment of the cerebral haemodynamic response to exercise and the impact of normative ageing. This set-up and quantitative MR measures developed, now provides a model in which other exercise based studies can be performed to study the underlying cerebral mechanisms of exercise directly. Recent developments in 4D PC-MRI enable the acquisition temporal cerebral blood flow in three spatial dimensions. Further, new scanner hardware now facilitates the study of brain haemodynamics and cardiac output in the same scan session, this will allow direct associations between brain haemodynamics and cardiac output to be studied. Future studies will therefore add to our findings, using these techniques to investigate the influence of cardiac output on cerebral blood flow in direct response to exercise on a cohort of subjects with a larger range of age and VO_{2max} values.

Chapter 6 of this thesis continues the theme of exercise and ageing and aims to investigate the impact of cardiorespiratory fitness and age on grey matter volume and cortical thickness.

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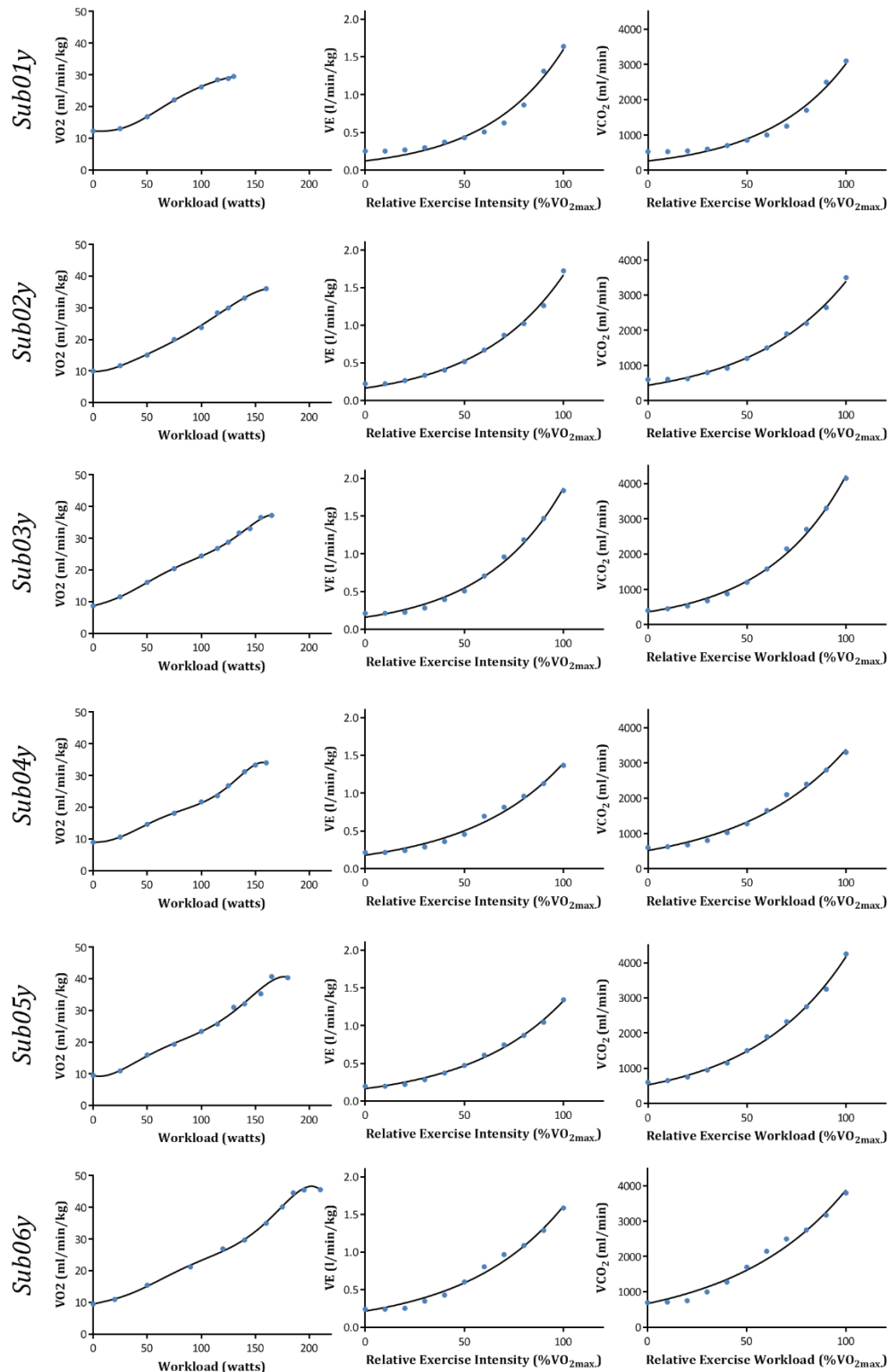
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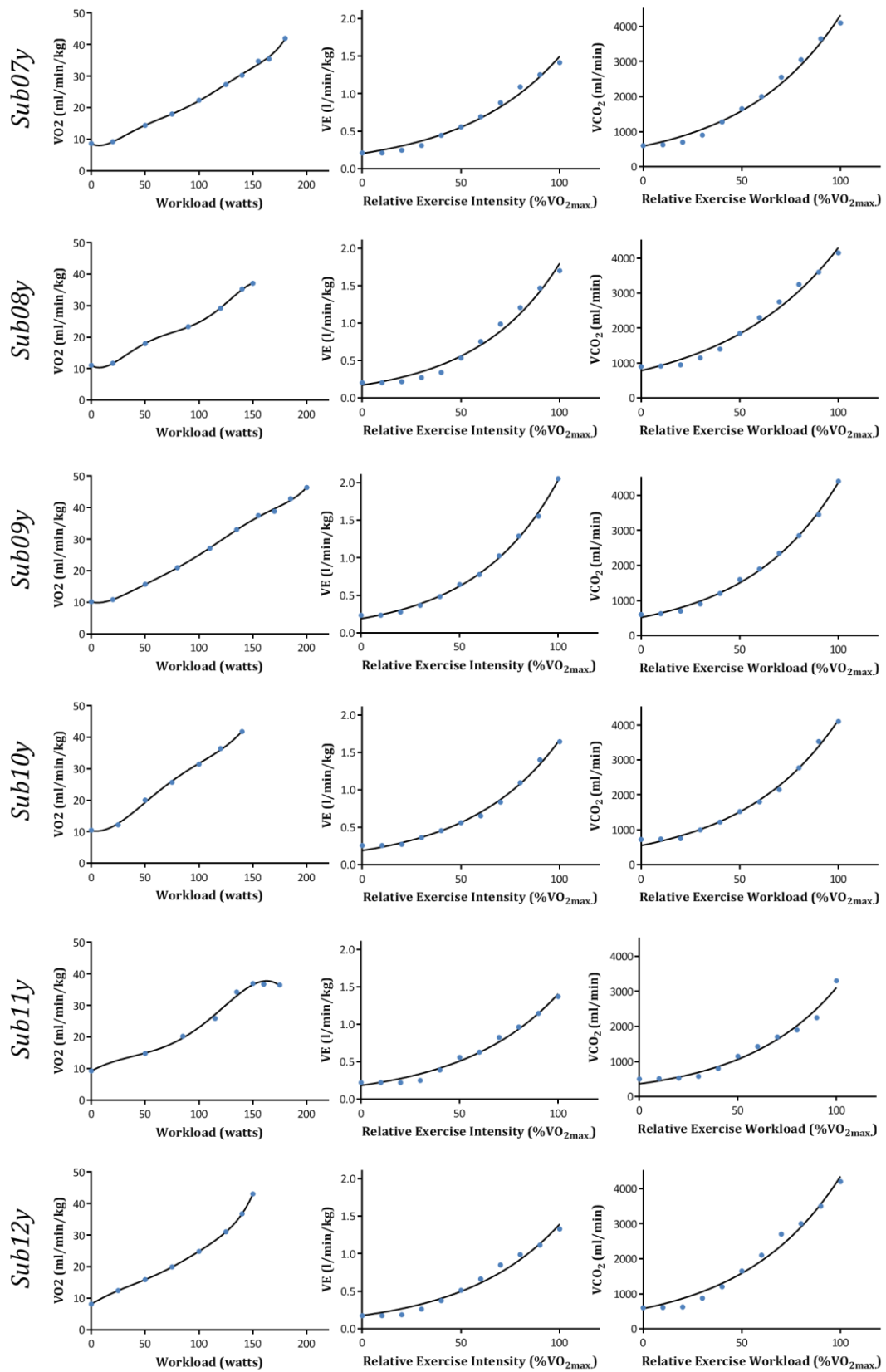
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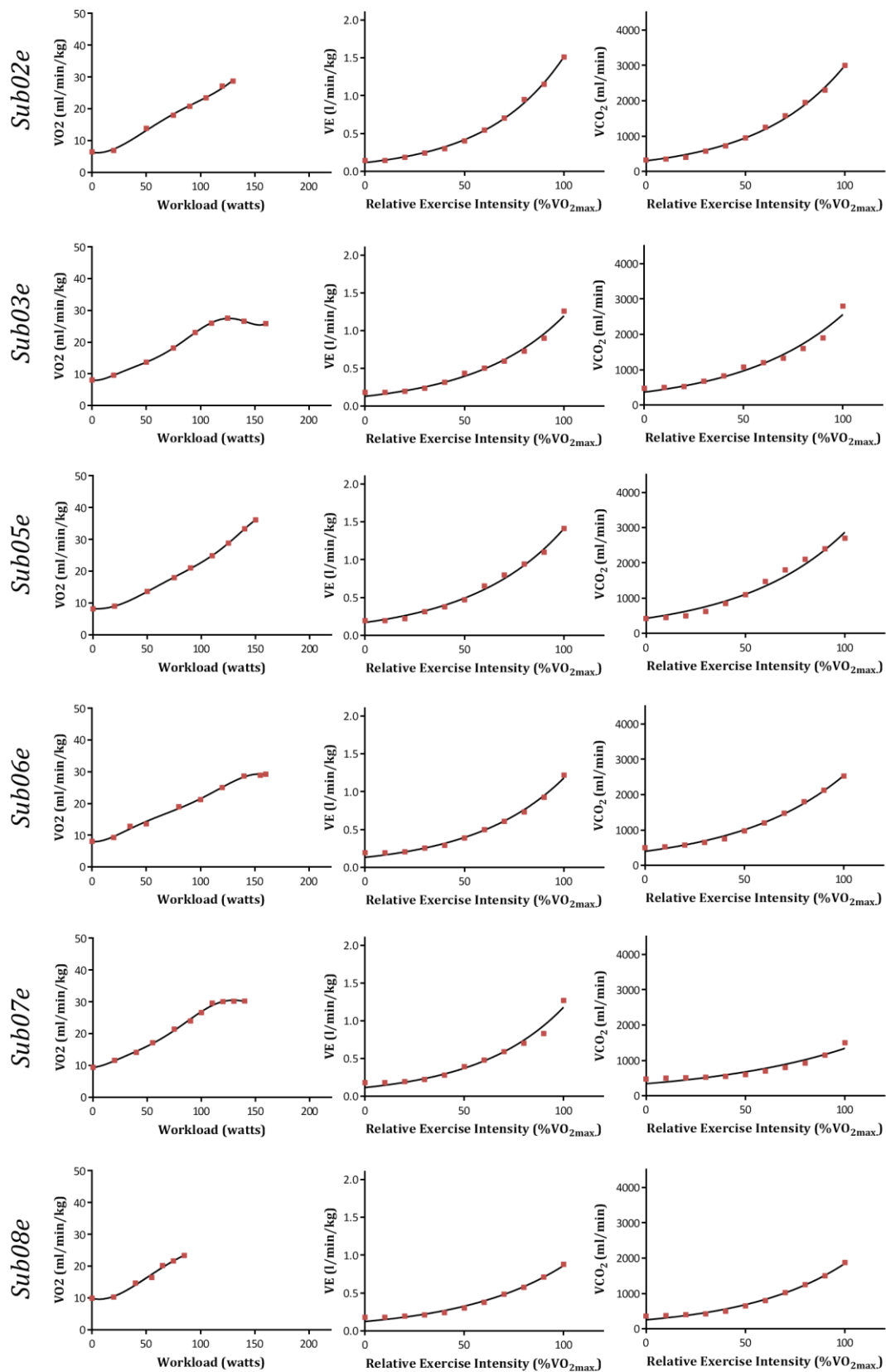
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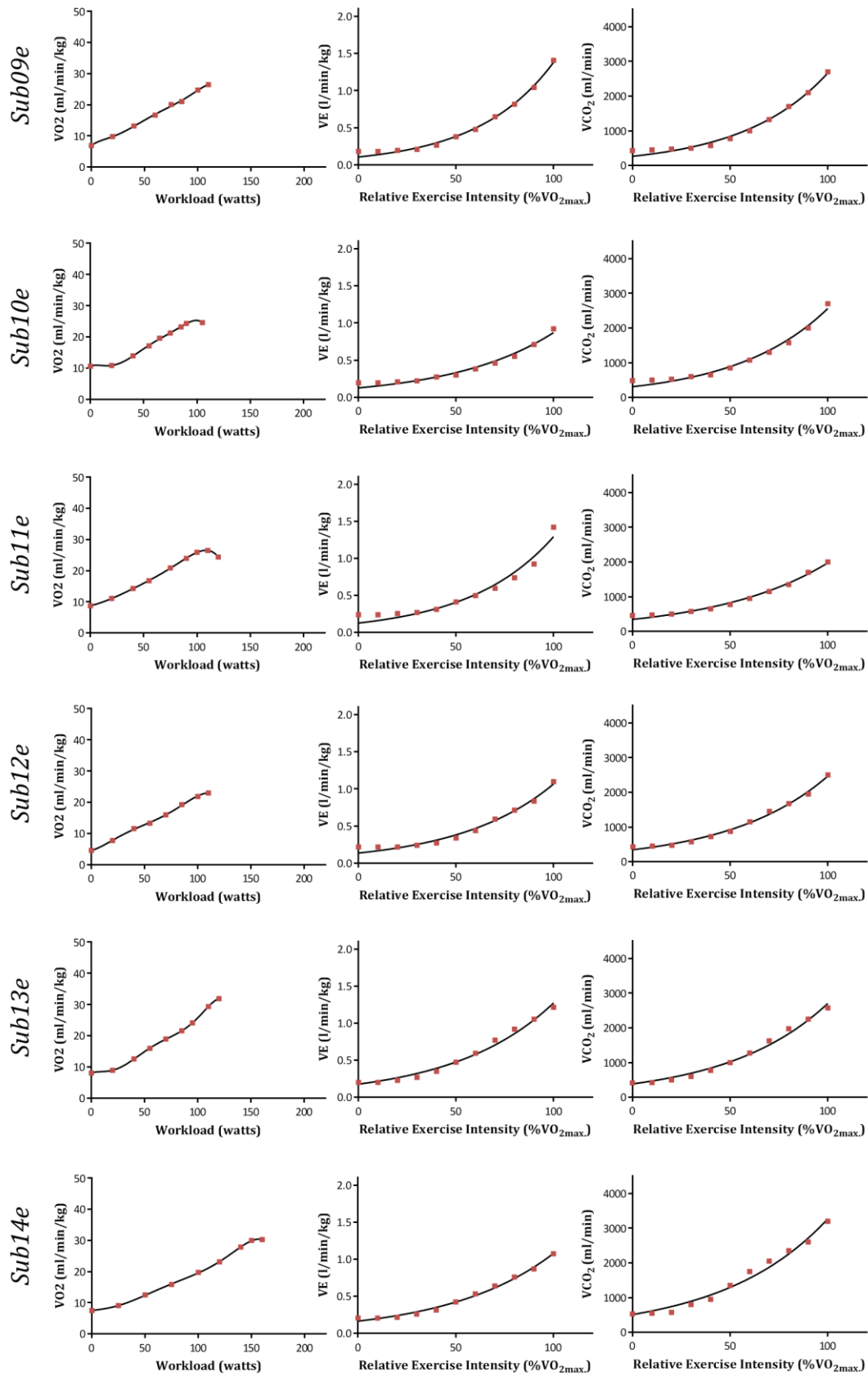
5.10 APPENDIX

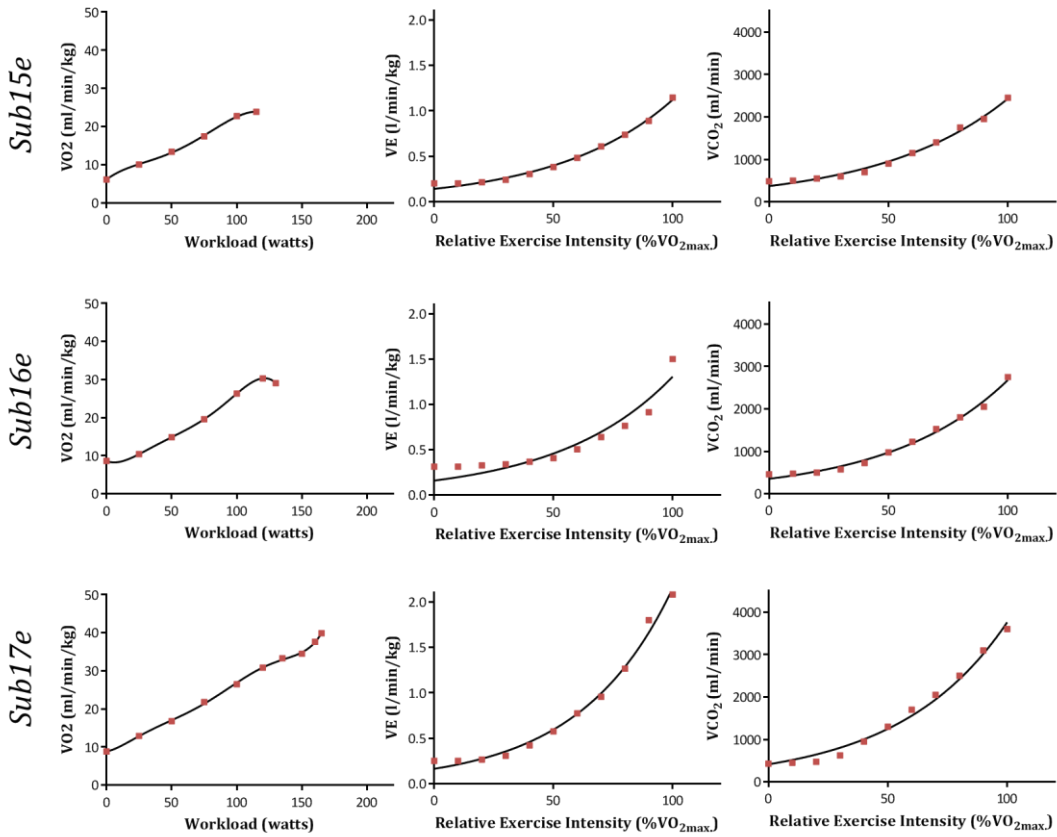
The following graphs show oxygen consumption (VO_2), ventilation (VE) and carbon dioxide consumption for each subject collected during the cardiorespiratory fitness tests.











6. The association between cardiorespiratory fitness and the ageing brain

Ageing is associated with a reduction in grey matter volume and cortical thickness. It has recently been suggested that lifestyle factors, such as physical inactivity underlie the decline in cognitive function associated with ageing.

The purpose of the study described in this chapter was to use structural MRI to investigate global and regional grey matter volume and cortical thickness in young and older adults, and to assess their association with cardiorespiratory fitness. Importantly, here we use the gold standard assessment of cardiorespiratory fitness by measurement of maximal oxygen (VO_{2max}) uptake rather than self-reported physical activity scores or estimated VO_{2max} .

6.1 INTRODUCTION

Grey matter atrophy and cortical thinning are factors associated with the natural ageing process [1], with ageing considered to be the single most important risk factor for cognitive decline [2]. An increasing number of studies have suggested that lifestyle factors have a significant impact on how well we age. In 2004, a Lancet paper by Fratiglioni et al. [3] suggested that three lifestyle factors play a significant role in slowing the rate of cognitive decline and preventing dementia: a socially integrated network; cognitive leisure activity; and regular physical activity, with physical activity providing the greatest protective effect against the deleterious effects of age on health and cognition [4, 5].

Physical activity is defined as the movement of skeletal muscles, resulting in energy expenditure exceeding the resting rate, and can encompass both aerobic and non-aerobic activity. You can generally relate the volume of oxygen consumption at maximum intensity exercise (VO_{2max}) to physical activity levels, with the very inactive at one end of the spectrum having a low VO_{2max} , and the highly active having a greater VO_{2max} . Indeed, cardiorespiratory fitness is a sensitive index of physical activity levels and is a very good predictor of all-cause mortality rates.

6.1.1 Effects of Ageing on Grey Matter

A large number of MRI studies have assessed the cross-sectional effects of age on grey matter volume or cortical thickness of various brain structures [1, 6-12]. In general, studies have assessed one of these measures, rather than investigate the effects of change in both grey matter volume and cortical thickness.

It is widely accepted that age influences total brain volume, and that age-related atrophy does not occur uniformly across the brain, but instead the pattern of changes is highly heterogeneous with some structures being shown to decline substantially in old age, while others appear better preserved [13]. A number of investigations into age-effects on brain structure have been cross-sectional studies in cohorts of subjects across a range of ages, from which the

consensus is that the reduction in grey matter volume begins early in life. Age-related declines in grey matter volume have been widely reported in the prefrontal, superior parietal, and middle/inferior temporal cortices [10, 11, 14, 15].

More recently longitudinal studies of age-related changes have been performed, suggesting that the rate of cortical atrophy increases with age. In a report by Fjell et al. [16] of cortical thickness, age trajectories of cortical thickness were delineated both cross-sectionally ($n = 1100$) and longitudinally ($n = 207$). It was shown that in healthy adults aged 69+ years cortical thinning occurs at a rate of 0.59% per year. More recently, Shaw et al. [17] performed a similar investigation, collecting T₁-weighted structural MRI data to assess longitudinal estimates of cortical thinning over a 12 year period in a large ($n = 396$) cohort of healthy adults of age 60-66 years. The authors reported an age-related cortical thinning of around 0.3% per year in this time frame across the majority of the cortices, with the most significant thinning being found in the inferior and superior temporal cortex and inferior frontal cortex as shown in Figure 6-1. In addition, the mean cortical thinning was found to be greater in men compared to women. The brain regions most susceptible to age-related cortical thinning have been shown to largely coincide with areas that support higher-level processing, such as the lateral temporal, inferior parietal and frontal regions [16-19].

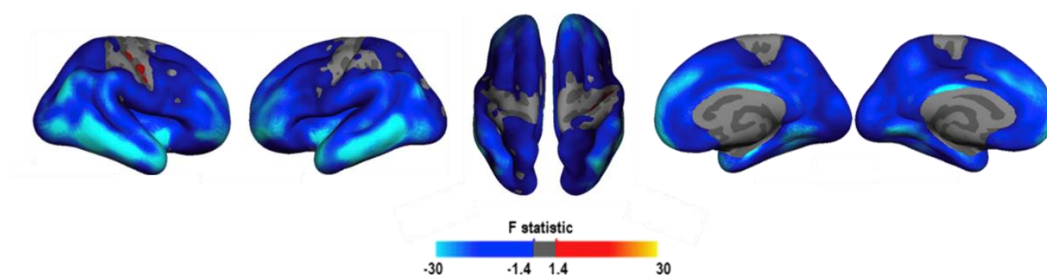


Figure 6-1: Results reported by Shaw et al. [17] showing an association between change in cortical thickness and age across a 12 year longitudinal study in 60-66 year old adults. Blue (red) shows areas with a significant decrease (increase) in cortical thickness with age ($p < 0.05$, FDR corrected).

However, across these studies it has also been shown that atrophy is highly individualistic with respect to rate, extent and region [13, 20]. This has led to

considerable interest in 'healthy ageing', describing those individuals who exhibit less atrophy and cognitive impairment in late adulthood than their age-matched counterparts [21]. Identifying factors that contribute to healthy ageing, such as the suggested influences of behaviour and lifestyle, may aid in the development of new treatments and prevention methods to reduce the risk of age-related atrophy, cognitive decline and neurodegenerative diseases [22].

6.1.2 Physical Activity and Cognitive Decline

Much research has been undertaken to assess whether physical activity reduces cognitive decline and prevents dementia, though findings from prospective studies have shown mixed results. Aarsland et al. 2010 [23] showed that in five correlations of physical activity and cognitive decline, two reported a significant relationship for both sexes, two reported a significant relationship for females only, and one showed no association. Similarly, mixed results were found for comparisons with dementia, with Aarsland et al. 2010 [23] reporting that of 12 studies examining physical activity and dementia, over half did not find statistically significant effects. However, despite individual variation between findings, systematic reviews and meta-analyses of longitudinal studies do lend support to the association between physical activity, cognitive decline and dementia. Sofi et al. 2011[24] undertook a systematic review and meta-analysis of prospective studies exploring physical activity and risk of cognitive decline in non-demented individuals. Participants with high and low-to-moderate levels of physical activity were compared to those who were sedentary in a meta-analyses of 15 cohorts. This demonstrated that both low-to-moderate level and high level physical activity were shown to be protective of cognitive decline.

6.1.3 Associations between Physical Activity and Brain Grey Matter Volume and Cortical Thickness

Examination of the link between cardiovascular exercise and brain health in animal models has shown positive effects of aerobic fitness on a wide range of brain health markers, including increased levels of brain-derived neurotrophic factor, serotonin, capillary density, and neurogenesis. These changes have

been suggested to result in a well-preserved brain that is more plastic and adaptive to change.

In humans, cross-sectional investigations into the association between physical activity and grey matter volume using self-reported physical activity questionnaires have been inconsistent. In 2010, Flöel et al. [25] assessed 75 healthy older individuals aged 50–78 years for levels of self-reported physical activity and cerebral grey matter volume using voxel-based morphometry (VBM) of structural MRI data (see Section 6.2.1 for a more detailed description of VBM). They reported that higher physical activity levels were predictive of greater grey matter volume in regions comprising the right prefrontal and cingulate cortex, and left prefrontal cortex, cingulate cortex, bilateral occipito-temporal regions, and cerebellum. The highest association with physical activity levels was in the right anterior frontal cortex. No regions were found to show a significant negative correlation between grey matter volume and physical activity level. Colcombe et al. [26] examined the association between cardiorespiratory fitness levels and grey matter volume in 55 healthy older adults (55 – 79 years). In this study they used an estimated cardiorespiratory fitness score derived from a number of self-reported measures. Regional changes in grey matter volumes were obtained from MRI data and analysed using VBM. It was found that, in line with previous literature [13, 17-19, 27], tissue density declined as a function of age in the frontal, parietal and temporal cortices. Crucially, however, the grey matter loss was substantially attenuated in participants with higher estimated fitness levels. However, it should be stressed that the use of self-reported activity is not a reliable method by which to determine habitual physical activity, nor a gold standard method by which to assess cardiorespiratory fitness.

Other studies have stated that the relationship between grey matter volume and physical activity levels is multifactorial [28-30]. Ho et al. [30] mapped the 3D profile of brain volume differences in 226 healthy older adults (aged 77.9 ± 3.6 SD years) controlling for age, sex, physical activity and higher educational level. They concluded that independent contributions to brain structure could not be separated as all factors were highly correlated with one another.

Conversely, when comparing a group of active and sedentary elderly individuals ($n = 20$, mean age = 81.5; $n = 10$, mean age = 80.8 respectively), Rosano et al. [31] found no association between self-reported physical activity levels and grey matter volume ($p = 0.1$). Despite these inconsistent findings, several reviews have concluded that while more work is needed to understand how it may be used as an effective prevention method, it is becoming more apparent that physical activity can have a direct effect on brain health [32-39].

Of note is that the majority of studies to date have used self-reported physical activity questionnaires [40], likely chosen since they can be easily implemented on large sample populations at low cost. However, a self-report questionnaire is a highly subjective method and semi-quantitative at best, exposing the measurement to a high degree of risk of underestimating or overestimating ratings, and introducing variance between individual assessments. This issue can be especially exacerbated in studies of older people due to recall bias and memory degradation. This may underlie the reason why these self-reported physical activity studies have led to inconsistent results.

Recently, methods have been introduced to reduce the subjective nature of physical activity ratings. Devices such as pedometers and accelerometers are becoming more prevalent in physical activity studies [41, 42]. However, these methods also have a number of challenges, such as noncompliance and modification of behaviour due to the awareness of being monitored.

A more objective validation of physical health is to assess cardiorespiratory fitness, most commonly determined by a measure of peak volume of oxygen consumption at maximum intensity exercise, VO_{2max} test (for full details of this method, see Section 5.5 of Chapter 5).

In a very recent 2017 study, Williams et al. [43] used surface-based morphometry (SBM, described in more detail in Section 6.2.2) to measure cortical thickness and assess the relation with cardiorespiratory fitness, as measured by maximal oxygen uptake, in 32 young and 29 older adults (aged 64 ± 7 years). A between-group age comparison showed the older adults had

widespread cortical thinning compared to young adults in ventrolateral, dorsolateral and dorsomedial prefrontal cortex, lateral and inferior temporal cortex, and inferior parietal regions. When older adults were categorized into groups of high or low fitness, a stepwise pattern was observed whereby the cortex was thickest in young adults, intermediate in high fit older adults and thinnest in low fit older adults. Assessment of the effects of cardiorespiratory fitness revealed a positive association between cardiorespiratory fitness and cortical thickness in regions overlapping with areas that showed significant age-related cortical thinning. The authors suggested this provides strong evidence to support the premise that cardiorespiratory fitness influences brain plasticity [44-48].

To date, only this one study has investigated the relationship between brain plasticity and true measure cardiorespiratory fitness [43]. The purpose of the work described in this chapter is to investigate both global and regional grey matter volume and cortical thickness in healthy young and older adults, and to assess its association with cardiorespiratory fitness using gold standard measures of VO_{2max} . This provides an objective and reliable indicator of CRF with physical activity levels, compared to self-reported measures of physical activity.

6.2 ANALYSIS METHODS TO ASSESS GREY MATTER VOLUME AND CORTICAL THICKNESS USING MRI STRUCTURAL SCANS

MRI provides high spatial resolution information of the brain structure that can be segmented into tissue type (white matter, grey matter, CSF) and interrogated to study changes in the size and shape of the brain (brain morphometry), and alterations in cortical thickness. Traditionally, methods of analysing atrophy in MRI data involved visual assessment by experienced radiologists. For quantitative assessment, ROI measurements of the volume of structures of interest can be used [49], for example in this Chapter the single-time-point SIENAX package which is part of the FSL software package was adopted (FSL, UK). Alternatively, mapping based techniques such as voxel based morphometry (VBM) to assess grey matter volume, and surface based

morphometry (SBM) and other packages to assess cortical thickness have been developed to reduce the intensive manual workload associated with structural analysis, especially when studying data from larger subject groups. Grey matter volume and cortical thickness analyses are both assessed in data shown later in this Chapter.

6.2.1 Voxel Based Morphometry (VBM) Assessment of Grey Matter Volume

Voxel based morphometry [50] is an automated technique which applies statistical parametric mapping to provide voxel-wide estimation of a specific tissue volume. VBM is typically applied on a T₁-weighted image by performing a statistical test on a voxel-by-voxel basis to identify volumetric differences between groups. Regression analysis can also be performed to identify correlates of the change in volume, e.g. with age or cardiorespiratory fitness.

For successful VBM analysis, high quality data and accurate pre-processing are essential. The T₁-weighted images are first DARTEL registered [51] to a group template space for normalisation to ensure that the statistical analysis compares homologous structures across all subjects. This is a non-linear registration which allows the image to be stretched and compressed, generating a deformation field with information on the distance each voxel is moved to match with the group template. Grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) are then classified and segmented from the deformed image based upon voxel intensity and tissue class priors of the likelihood of a tissue type being in a given location. The resulting image is a non-binary probability map with each voxel containing the probability of a given tissue class. The image can then be modulated using the Jacobian determinants of the deformation used to initially register the brain to the group template. The final pre-processing step is to spatially smooth the data with a low-pass filter. This is typically achieved by convolution with an isotropic Gaussian kernel, see Figure 6-2 for VBM processing steps.

Once pre-processing is complete, voxel-based analyses are performed by fitting a general linear model on a voxel-by-voxel basis [52] to the smoothed

data, for example comparing between groups, or correlating with a variable such as age or cardiorespiratory fitness.

In VBM, there are two quantities used to measure morphometric properties: volume and concentration. A volume study assesses the number of grey matter voxels in the brain, and then compares between groups/with covariates. However, subjects with larger bodies tend to have bigger heads and correspondingly bigger brains, and thus a larger grey matter volume. To overcome this confound, the grey matter volume can be divided by the volume inside the cranium to account for head size – yielding a concentration that it is the amount of grey matter per unit of intracranial volume. This analysis is applied to the data shown in the Chapter.

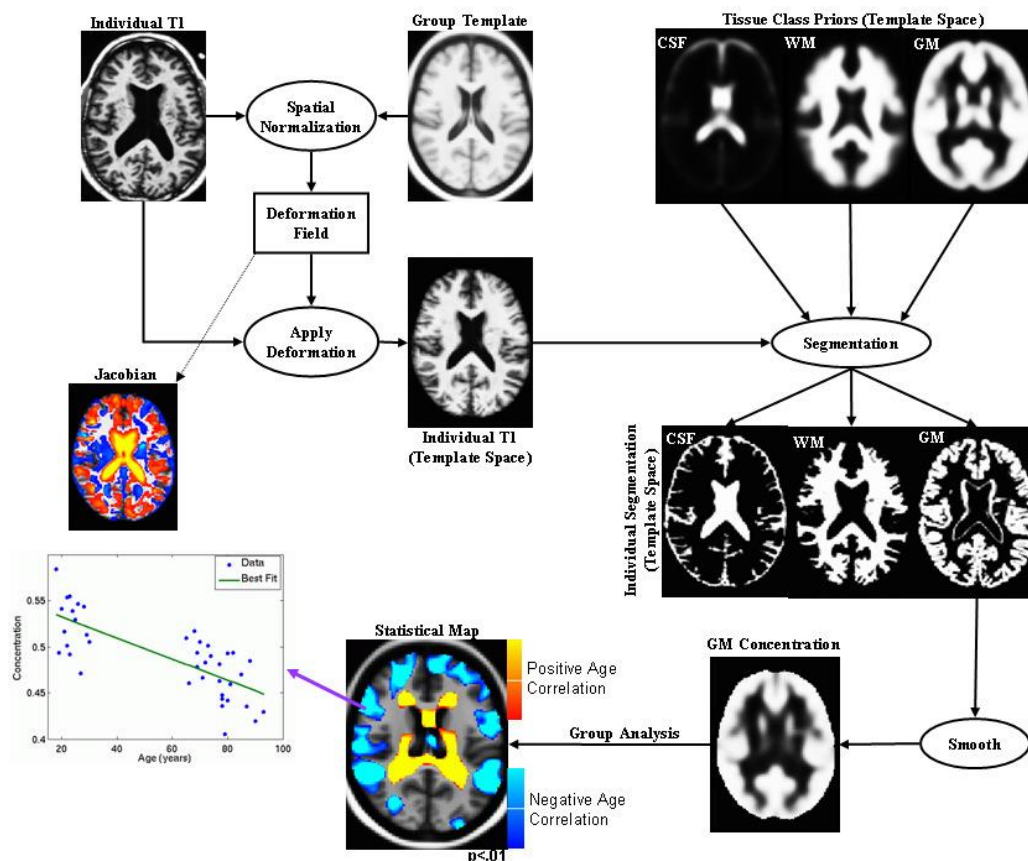


Figure 6-2: Schematic of voxel-based image analysis for the assessment of global and regional grey matter volume reproduced from Greve et al. [53].

Standard software for VBM includes the Statistical Parametric Mapping (SPM, www.fil.ion.ucl.ac.uk/spm) software with the Computational Anatomy Toolbox 12 (CAT), an extension to SPM12 (Wellcome Department of Cognitive

Neurology, U.K) which is used this Chapter. Additionally there is the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl). A number of reviews have investigated the validity of VBM, comparing the automated approach with the more traditional visual and manual methods. The authors reported a good correspondence between the techniques [54-57].

6.2.2 Surface Based Morphometry (SBM) and Cortical Thickness Assessment

Surface based morphometry derives morphometric measures from geometric models of the cortical surface. Rather than analysing the image properties at the voxel level, as is the basis of VBM, SBM uses projection-based thickness assessment for cortical analyses, and subsequent investigation of group differences or influences of age-related atrophy and cardiorespiratory fitness.

The first step in SBM is to segment the high resolution T₁-weighted images such that the corresponding cortical surface is extracted. This is performed by defining first the cortex as the outer boundary of the brain. A surface boundary is also defined between cortical white matter and cortical grey matter, this is known as the white surface and defines the inner boundary of cortex. A boundary between the grey matter and the dura and/or CSF is then defined, and this is referred to as the pial surface. The cortex is then constructed as a surface model in the form of a mesh of triangles, which can then be rendered in 3D.

Information on the coordinates within the meshed image provides a measure of cortical thickness (the distance between the white and grey matter surfaces). The surface can be inflated and unfolded so there is no area hidden behind a fold (Figure 6-3).

To perform group analysis, a reference surface is required that is normalised in size. The surface image is manipulated to show the curvature of the brain. This is a measure of how sharply the cortex is folded at each point in the mesh. The curvature surface is then mapped onto a unit sphere. To perform spatial normalisation, the sphere of each individual is adjusted to best match the curvature across the group. The spatial normalisation used in SBM is non-

linear, meaning that parts of the surface may be compressed or stretched in order to match the curvature better. Once registration is complete, it is applied to the cortical thickness data and the results are mapped back on a reference brain surface where it is smoothed before performing statistical analysis across the group. Similar to the assessment of grey matter volume and concentration in VBM, cortical thickness can be used in a group analysis to track changes associated with age and cardiovascular fitness.

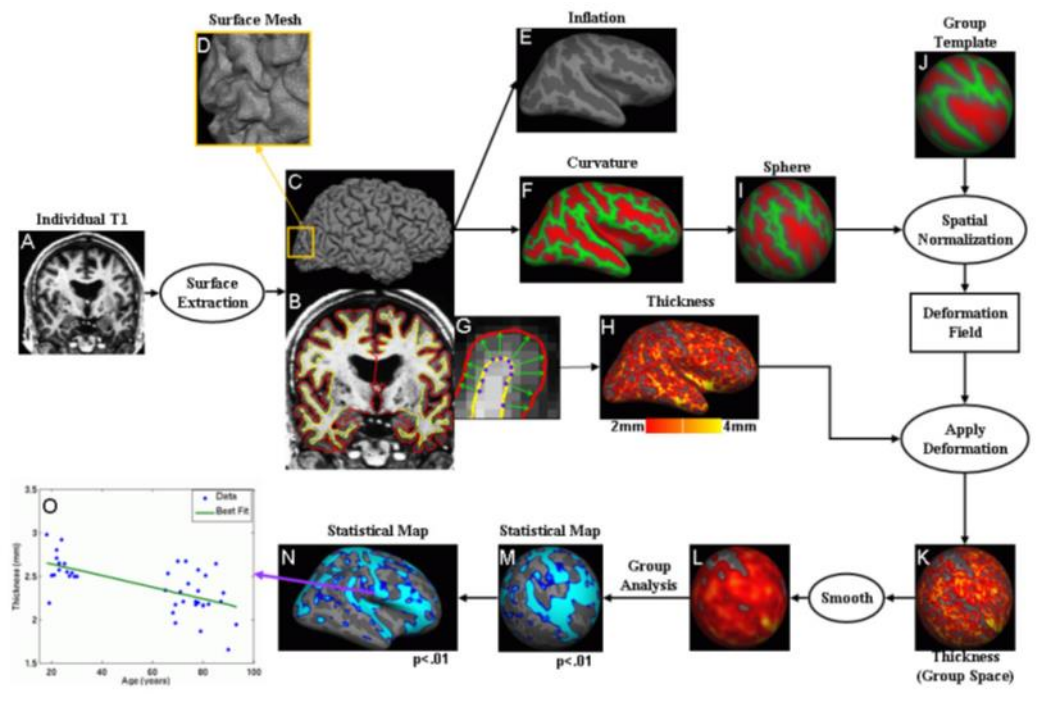


Figure 6-3: Schematic of surface-based morphometry for the assessment of cortical thickness measures reproduced from Greve et al. [53].

The software available for SBM includes FreeSurfer (surfer.nmr.mgh.harvard.edu) [58], Brain Visa (brainvisa.info), CARET (brainvis.wustl.edu/wiki/index.php/Main_Page), and Brain Voyager (brainvoyager.com). More recently, the Computational Anatomy Toolbox (CAT) under SPM12 has provided an option for cortical thickness analyses, which operates on a projection-based thickness principle. This uses tissue segmentation to estimate the white matter distance, then projects the local maxima (which is equal to the cortical thickness) to other grey matter voxels by using a neighbour relationship described by the white matter distance. The surface pipeline uses topology correction, spherical mapping, estimation of

local surface complexity and local gyrification. CAT provides the advantage of reducing the computational time compared to FreeSurfer which can take up to 23 hours per subject. CAT has been validated against FreeSurfer in [59] and is used to analyse the data shown in this chapter.

6.3 PARTICIPANTS

This study formed an extension of the work described in Chapter 5. The study procedure was approved by the local Ethics Committee and written informed consent was obtained from all participants. Recruitment was targeted at high functioning, habitually active healthy young (18-30 years) and older (55-75 years) adults. Cardiorespiratory fitness was measured with a multistage stepwise protocol to determine VO_{2max} , as described in Section 5.5.1 of Chapter 5. The subject demographics and the different fitness levels between the two age groups can be seen in the table below.

Group	Number	Age	VO_{2max} (ml/kg body mass/min)	Workload_{max} (watts)
<i>Young</i>	12	23.3 ± 1.8	30.8 – 46.3	177 ± 24
<i>Older</i>	15	68.7 ± 4.1	23.0 – 39.9	145 ± 18

Table 6-1: Demographic characteristics of young and older adults for age and maximum workload achieved (Mean $\pm$ SD) and cardiorespiratory fitness levels as range across each group.

6.4 DATA ACQUISITION

T_1 -weighted structural MRI scans were collected on a 3.0 T Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) using a 32-channel head receive coil. A high resolution whole-brain T_1 -weighted MPRAGE sequence was used to acquire data in the sagittal plane. Scan parameters were TR = 8.39ms, TE = 3.86ms, flip angle = 8° , 160 contiguous slices, slice thickness of 1mm, with a field of view (FOV) of 256 mm x 256 mm, and 1mm isotropic spatial resolution. These data were collected as part of the scan session

described in detail in Chapter 5, see Figure 5-10. All T₁-weighted MR-images showed no morphological abnormalities or artefacts.

6.5 IMAGE ANALYSIS

Images were analysed to estimate global and regional grey matter volume in each participant using ROI analysis to map grey matter volume using VBM, and assess cortical thickness using CAT.

6.5.1 Global Grey Matter Volume

Global grey matter volume was determined using the single-time-point SIENAX package [60, 61] (FSL, UK). This cross-sectional analysis estimates brain tissue volumes from a single image and normalises it for subject head size. The pipeline for this is shown in Figure 6-4.

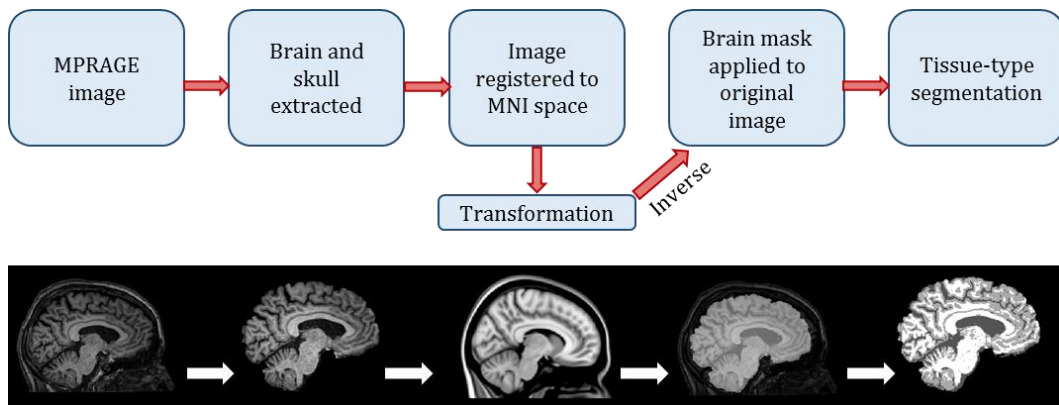


Figure 6-4: Pipeline of global brain tissue segmentation performed in SIENAX (FSL)

First the brain and skull is extracted from the whole-head MPRAGE image. To normalise brain volume to skull size, the image is then affine registered to the MNI152 standard brain [62, 63] using the skull to determine the scaling factor. To avoid issues with resampling, such as voxel blurring, the scaling factor is transformed back into native space and applied to the original image. A dilated MNI152 standard brain mask is also converted into native space using the previously found transform. This is then applied to the original image to remove any artefacts arising from the original brain extraction, such as residual eyeball signal. Lastly, tissue types (grey matter, white matter, peripheral grey matter and CSF) are separated using contrast based

segmentation with partial volume estimation [64]. An example of outputted grey matter segmentation overlay can be seen in Figure 6-5.

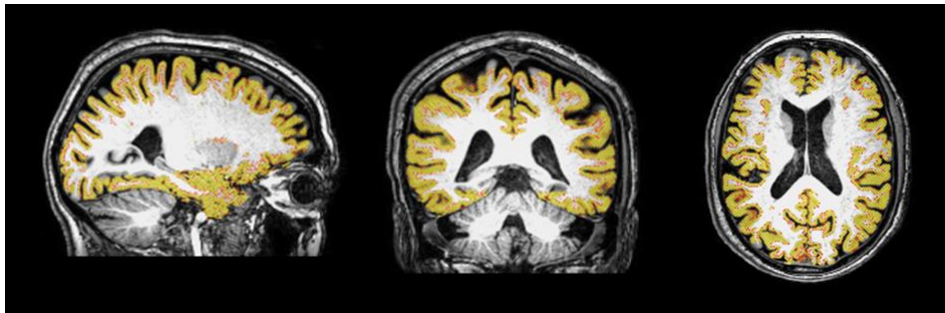


Figure 6-5: Grey matter segmentation (highlighted in yellow) performed by SIENAX.

White matter and ventricular cerebral spinal fluid can also be segmented.

Final volume estimates of each tissue type of grey matter, white matter, peripheral grey matter and ventricular cerebral spinal fluid (CSF) are then reported relative to a normalised skull size.

6.5.2 Regional Grey Matter Volume

Regional grey matter measures were assessed using a dedicated in-house MATLAB code. An MNI152 standard brain was first segmented to extract the brain and then registered to the native space of the MPRAGE image. The resulting transform was applied to a dilated MNI structural atlas [65, 66] using FLIRT (FSL). The 9 anatomical structural regions of the atlas were then separated and binarised into regional masks. These masks were applied to the grey matter partial volume map output by the original SIENAX process, and the voxels summed to compute the grey matter volume for each regional mask. Analysis was performed on the frontal, occipital, parietal, and temporal lobes and putamen, cerebellum, insula, thalamus and caudate. The schematic showing the pipeline of regional grey matter volume analysis is shown in Figure 6-6.

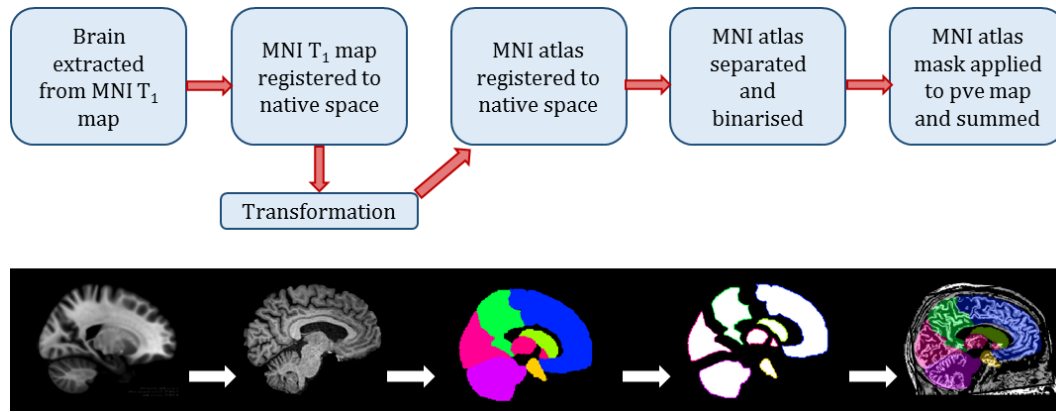


Figure 6-6: Pipeline of regional grey matter volume assessment performed using in-house MATLAB code.

6.5.3 VBM Grey Matter Volume Analyses

Since differences in the shape and neuroanatomical configuration of individual brains may be lost by the assessment of a ROI analysis alone, a VBM approach that it is not biased to one particular structure and gives a comprehensive assessment of anatomical differences throughout the brain was performed using SPM12. The processing pipelines of this method is detailed in Section 6.2.1.

VBM analysis results in an estimate of grey matter volume, white matter volume, CSF volume and total intracranial volume (TIV).

First, VBM analysis was implemented to compare the group responses between the young and older group. A linear regression with VO_{2max} accounting for age interactions was then performed. All analyses were normalised for brain size by inputting total intracranial volume as a statistical covariate.

6.5.3 Cortical Thickness Analyses

Volumetric approaches have been shown to be less sensitive to age-related cortical changes compared to cortical thickness [15] so in this chapter both measures are assessed. Cortical thickness was determined using the fully automated Computational Anatomy Toolbox 12 (CAT), an extension to SPM12 (Wellcome Department of Cognitive Neurology, UK). The processing pipelines of this methods is detailed in Section 6.2.2.

For cortical thickness analysis, average values were first computed for each individual subject in the young and older age group. Group differences in whole brain average cortical thickness was then assessed between the young and older subjects using a two sample t-test. A correlation analysis was performed between cortical thickness and VO_{2max} accounting for age. A multiple regression analysis was then performed on cortical thickness measures using age and VO_{2max} as factors.

Statistical analysis was extended to cortical thickness maps within the CAT. First, maps of mean cortical thickness were computed for the young and older group. Statistical maps showing clusters of significant cortical thinning associated with age were then identified (as expected, no areas showed significant age-related thickening). Significant changes in cortical thickness associated with VO_{2max} were assessed accounting for interactions with age.

6.6 RESULTS

6.6.1 Global Grey Matter Volume

Figure 6-7 shows each individual subjects' global grey matter volume as a function of age. As expected, a significant difference in volume was found between the older and young group (older; $700816 \pm 7854 \text{ mm}^3$, young: $787067 \pm 11059 \text{ mm}^3$, unpaired t-test $p < 0.001$).

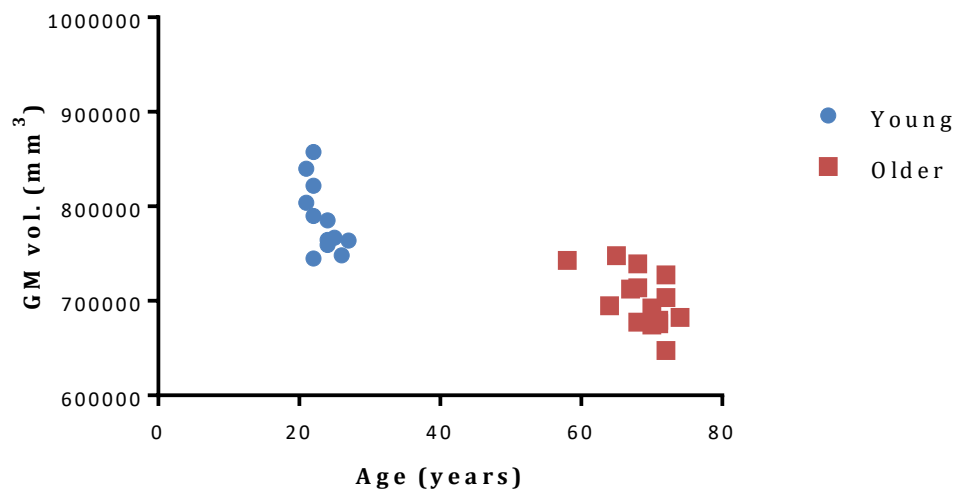


Figure 6-7: Global grey matter volume as a function of age, showing the reduction in the older group.

Figure 6-8 shows each individual subjects' global grey matter volume as a function of VO_{2max} , a significant linear correlation was found (Pearson $R=0.76$, $P<0.001$).

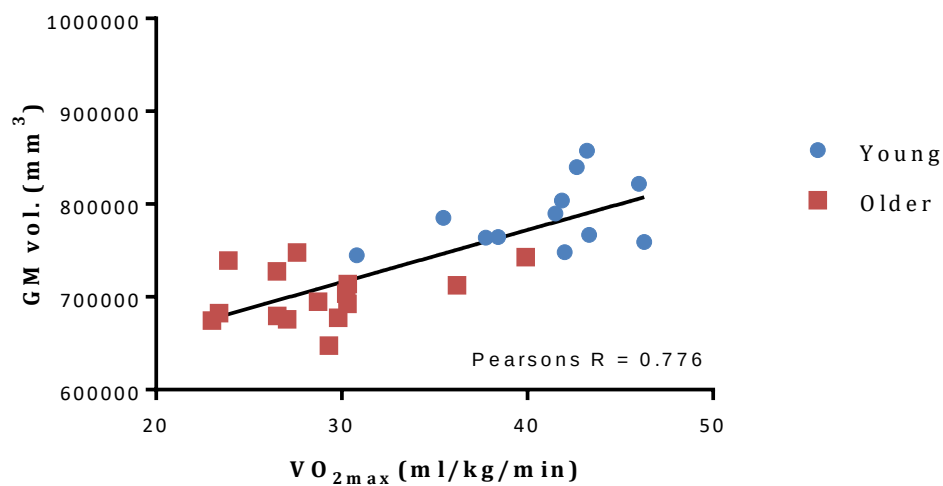


Figure 6-8: Global grey matter volume as a function of age, showing the reduction in the older group.

A multiple regression analysis gave

$$\text{Global grey matter volume} = -1606 \cdot \text{age} + 1371 \cdot VO_{2max} + 770106$$

with significance of $p = 0.0017$ for age and $p = 0.34$ for VO_{2max} .

6.6.2 Regional Grey Matter Volume

To further investigate age-related differences, ROI analysis of GM volume versus age (Figure 6-9) and VO_{2max} (Figure 6-10) are shown for the nine regions in the MNI structural atlas of the brain. It can be seen from Figure 6-9 that the most prominent changes appear in the frontal lobe. Across all regions except the temporal lobe and caudate there was a significant reduction in grey matter volume for the older cohort compared to the young cohort.

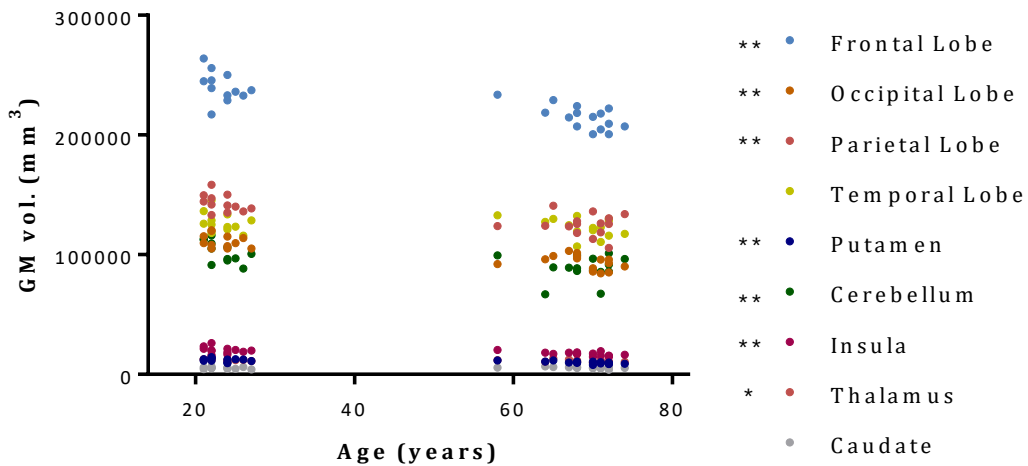


Figure 6-9: Grey matter volume within the nine regions of the MNI structural atlas as a function of age. Those regions with a significant difference between groups are shown as * $P < 0.05$ and ** $P < 0.001$.

Figure 6-10 shows GM volume versus VO_{2max} , with a significant correlation in the frontal lobe ($R^2 = 0.53$), occipital lobe ($R^2 = 0.59$), and insula ($R^2 = 0.44$).

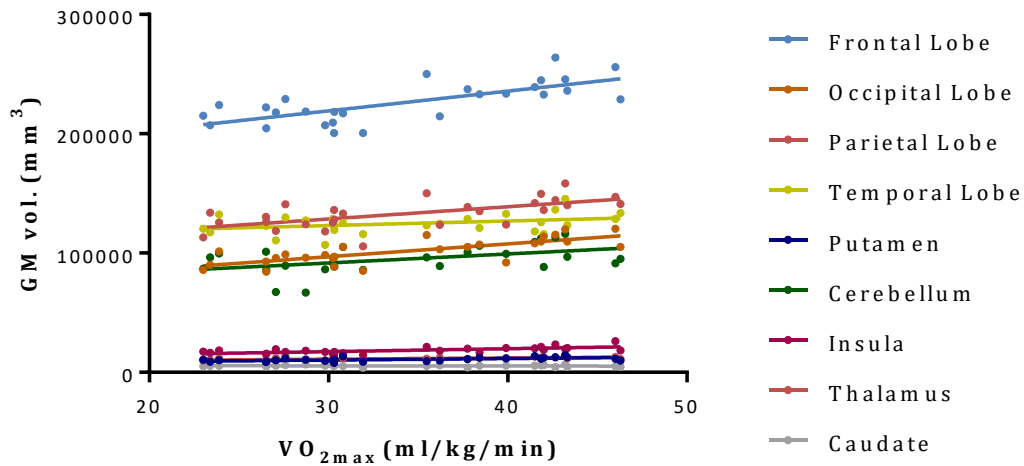


Figure 6-10: Global grey matter volume within the nine regions of the MNI structural atlas as a function of VO_{2max} .

6.6.3 VBM Grey Matter Volume Analyses

Figure 6-11 illustrates those areas which show an age-related reduction in grey matter volume (FWE corrected < 0.05 , cluster size $k > 10$). A significant reduction in grey matter volume in the older compared to younger group was found in the inferior, middle and superior frontal gyrus, insula, posterior cingulate, putamen and precentral gyrus, and cerebellum.

Figure 6-12 shows that a significant positive correlation between grey matter volume and cardiorespiratory fitness ($p < 0.001$, cluster size $k > 10$), accounting for age effects, was found in the precentral cortex, putamen, thalamus, and cerebellum.

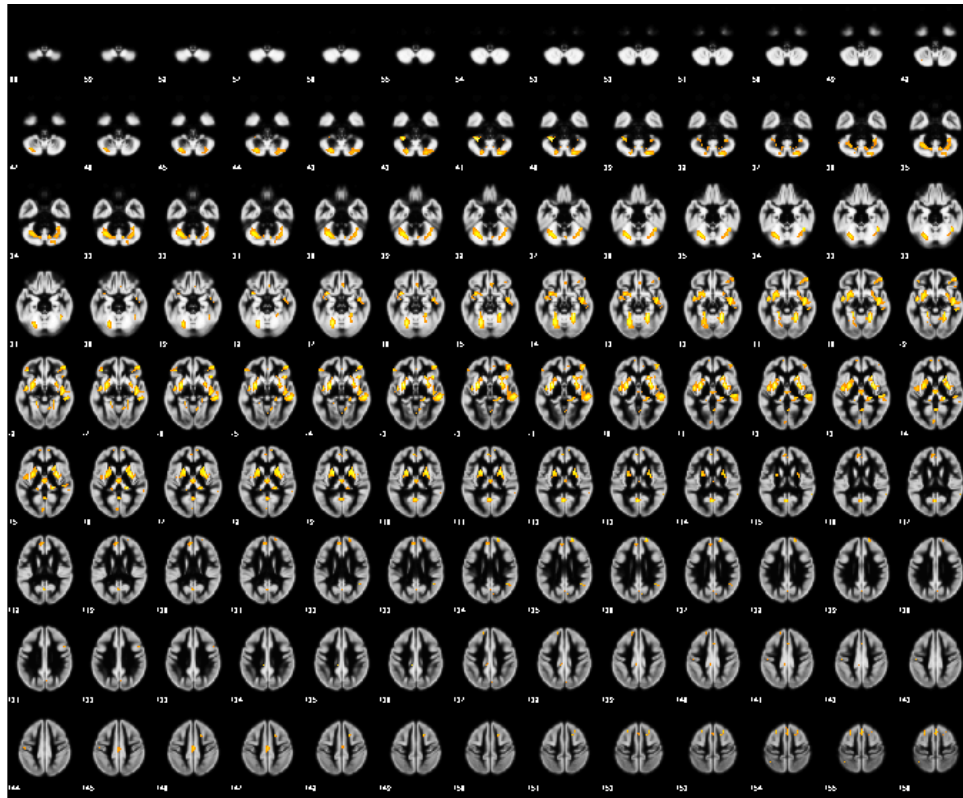


Figure 6-11: VBM maps showing areas with a significant reduction in volume with ageing (FWE-corrected p 0.05, $k = 10$).

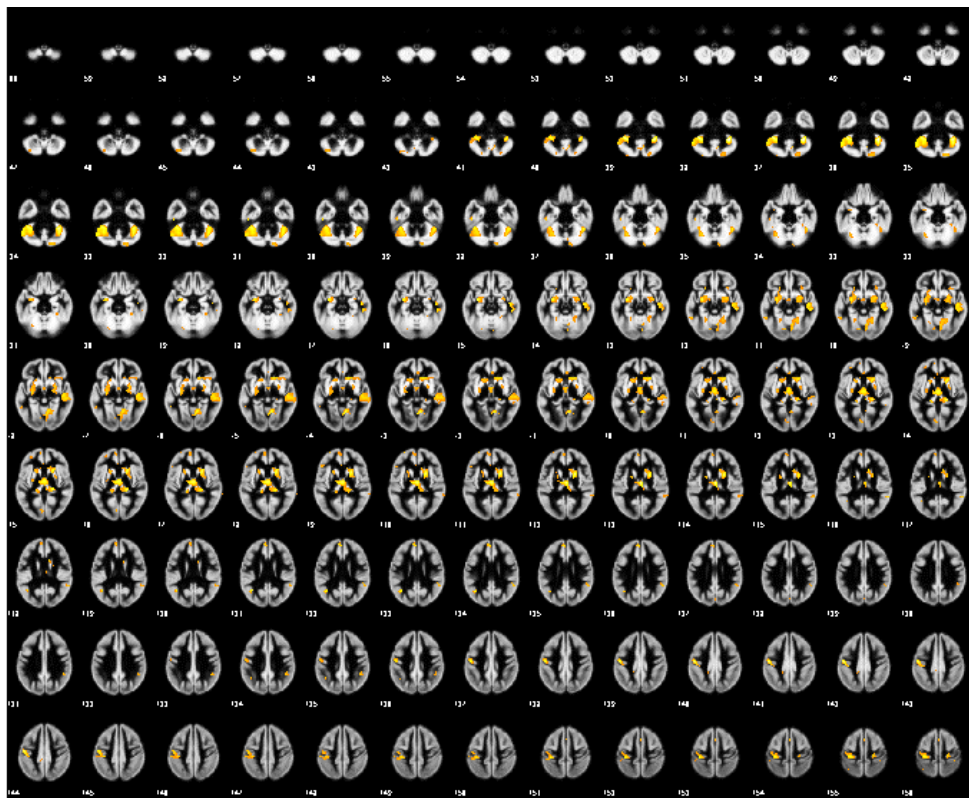


Figure 6-12: VBM maps showing areas with a significant positive correlation between grey matter volume and cardiovascular fitness, $p < 0.001$ (uncorrected).

Figure 6-13 shows the ROI analysis of the VBM results within an MNI atlas, illustrating those areas with (A) a significant decrease in volume with age, and (B) a significant positive association between grey matter volume and VO_{2max} .

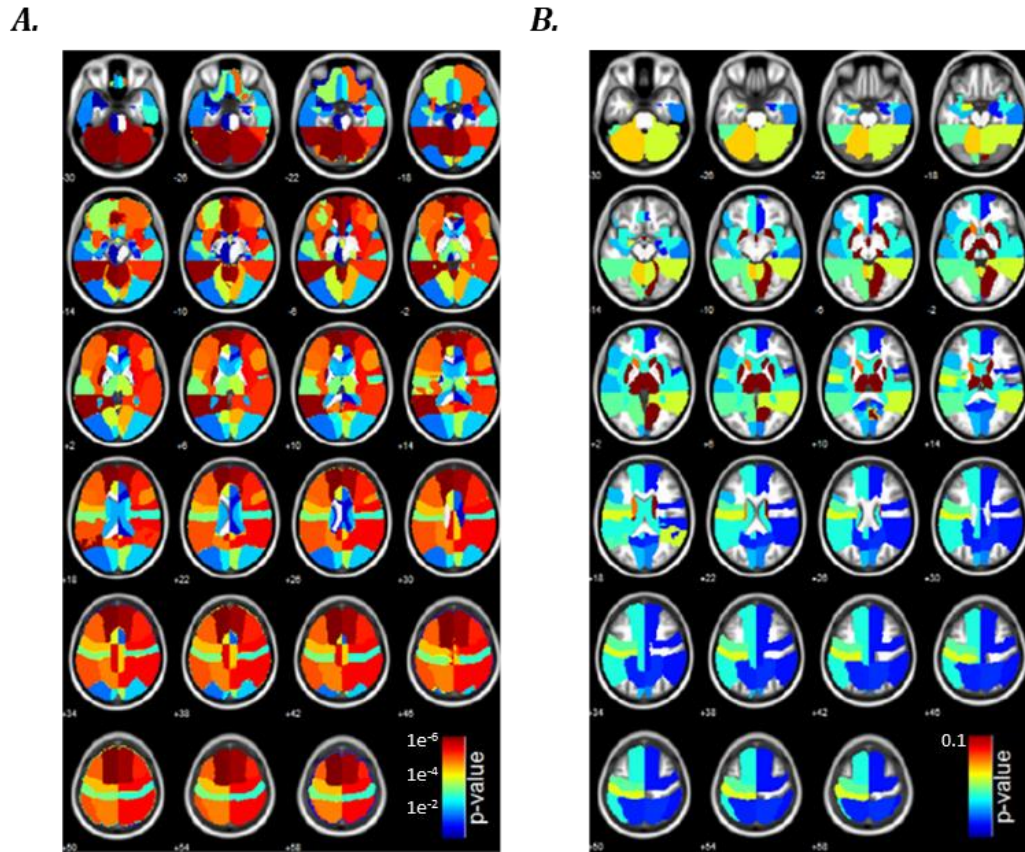


Figure 6-13: ROI analysis of those areas showing **A.** a significant negative correlation between GM volume and age and **B.** a positive association between GM volume and VO_{2max} accounting for age. False Discovery Rate (FDR) corrected $p < 0.05$.

6.6.4 Cortical Thickness Analyses

The surface maps of cortical thickness for the young and older group are shown in Figure 6-14. The expected spatial distribution is seen across the brain, with the cortex being thinner in visual regions and thicker in the temporal and frontal lobes, and the insula.

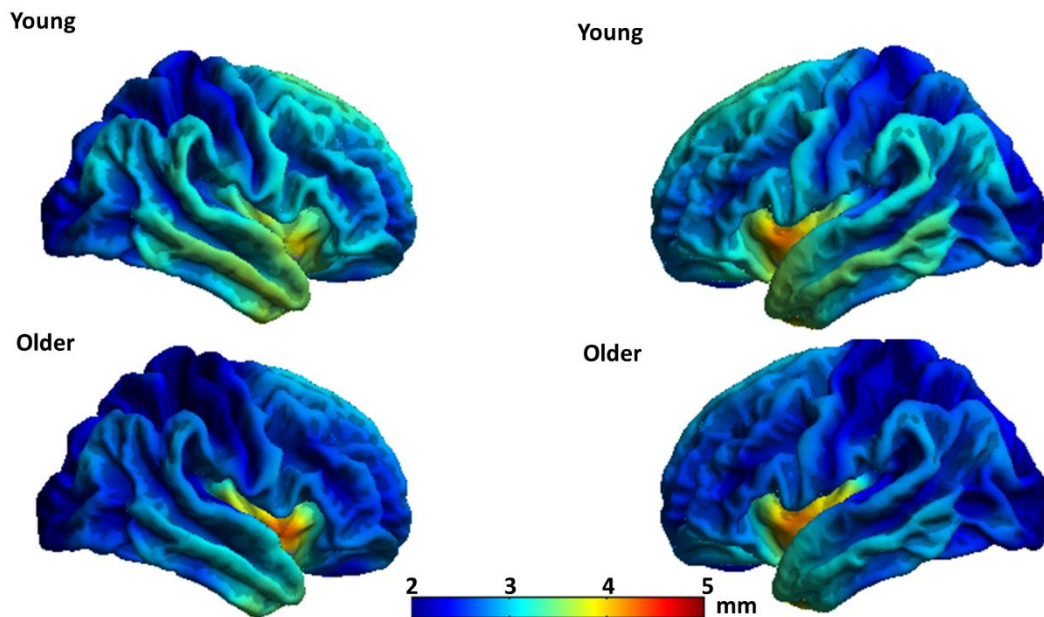


Figure 6-14: Surface maps of mean cortical thickness (mm) in the young and older groups.

Comparing the individual global cortical thickness between the young and older groups revealed that it was significantly lower in the older group (older; 2.52 ± 0.02 mm, younger; 2.72 ± 0.03 mm, unpaired t-test $p < 0.001$), Figure 6-15. The variance in global cortical thickness was similar between the older (0.72 ± 0.01 mm) and young group (0.76 ± 0.01 mm).

A significant positive correlation was found between cortical thickness and VO_{2max} of $R^2 = +0.74$, $p < 0.001$, i.e. thicker cortex associated with a higher level of cardiovascular fitness, VO_{2max} , Figure 6-16.

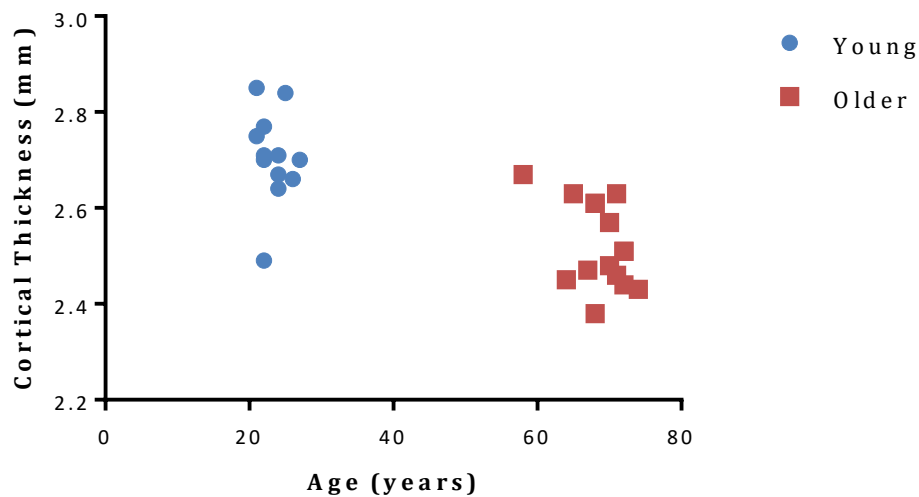


Figure 6-15: Grey matter cortical thickness as a function of age, showing the reduction in cortical thickness of the older group.

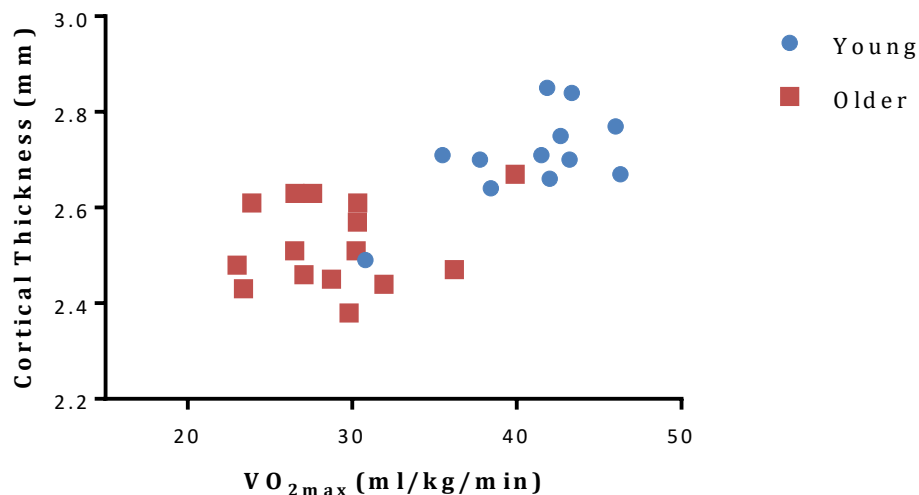


Figure 6-16: Grey matter cortical thickness as a function of VO₂max, showing the reduction in the older group.

A multiple regression analysis for cortical thickness gave

$$\text{Global grey matter cortical thickness} = -0.00237 \cdot \text{age} + 0.00675 \cdot \text{VO}_{2\text{max}} + 2.4889$$

with significance of $p = 0.08$ for age and $p = 0.11$ for VO₂max.

Statistical maps were derived of those areas showing a significant decrease in cortical thickness due to age as shown in Figure 6-17. Significant age-related cortical thinning was found in the precentral cortex, postcentral cortex,

inferior- medial- and superior frontal cortex, posterior cingulate, superior parietal cortex, inferior and middle temporal cortex. This is also illustrated in Figure 6-18 which shows the ROI analysis of the age-related cortical thinning.

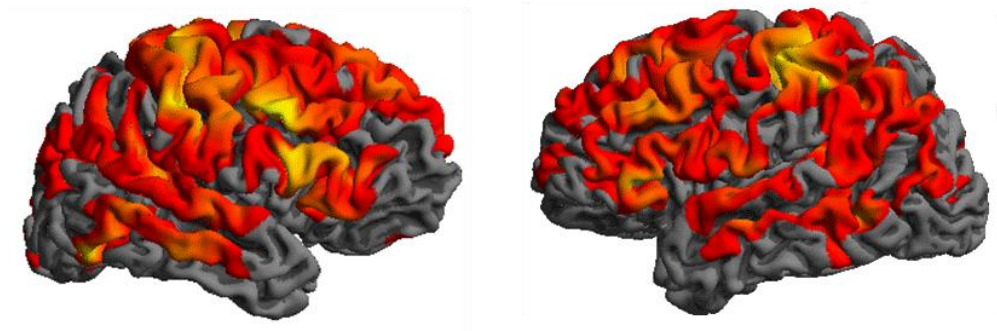


Figure 6-17: Statistical maps showing clusters of significant cortical thinning with age, $p < 0.001$ (uncorrected).

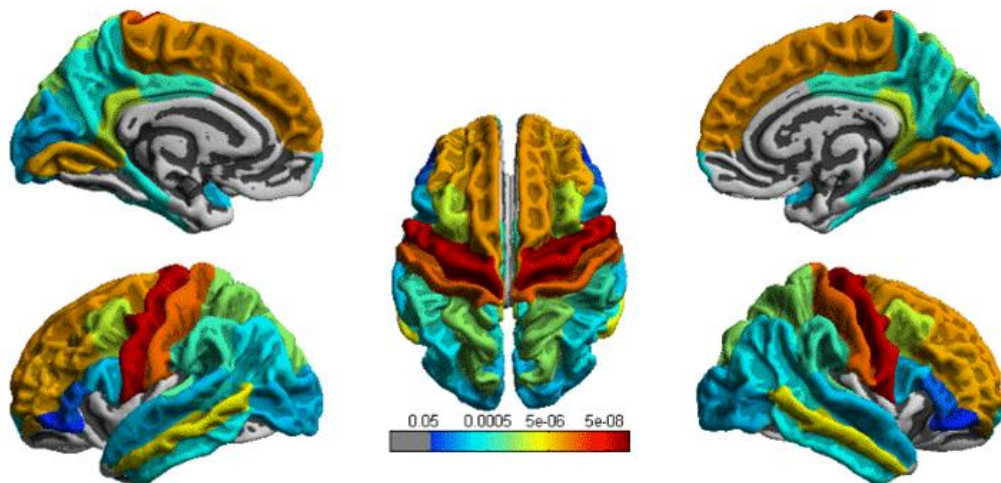


Figure 6-18: ROI analysis highlighting regions showing a significant cortical thinning derived by a negative linear correlation between cortical thickness and age. False Discovery Rate (FDR) corrected $p < 0.05$.

Figure 6-19 shows statistical maps illustrating those areas with a significant positive association between cortical thickness and VO_{2max} , and Figure 6-20 shows the corresponding ROI analysis of this association. A significantly greater cortical thickness can be seen in the precentral and postcentral cortex, middle temporal cortex, middle and superior frontal cortex, and superior parietal cortex when normalised to VO_{2max} .

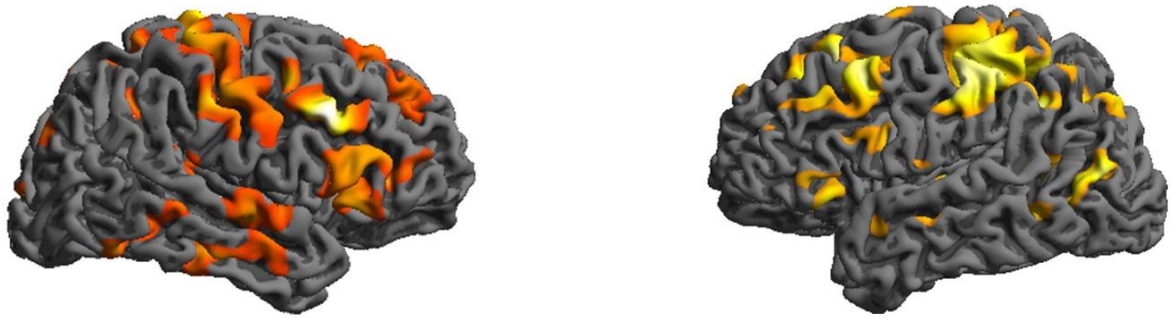


Figure 6-19: Statistical maps showing clusters with a significant positive association with cardiovascular fitness (VO_{2max}), $p < 0.001$ (uncorrected).

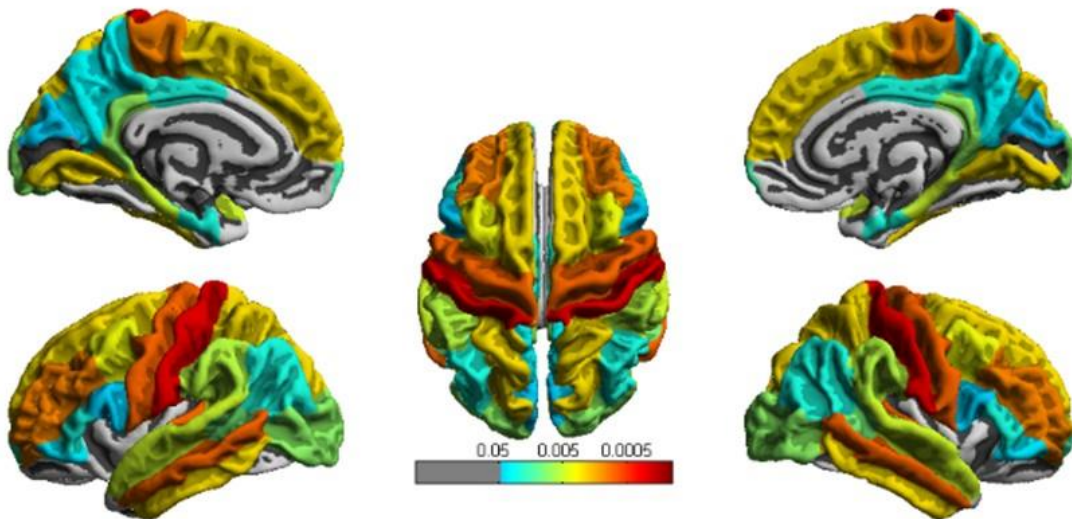


Figure 6-20: ROI analysis showing regions with a significant positive correlation between cortical thickness and VO_{2max} . False Discovery Rate (FDR) corrected $p < 0.05$.

Figure 6-21 shows the association between cortical thickness and VO_{2max} within the four significant ROI's highlighted (orange to red) in Figure 6-20.

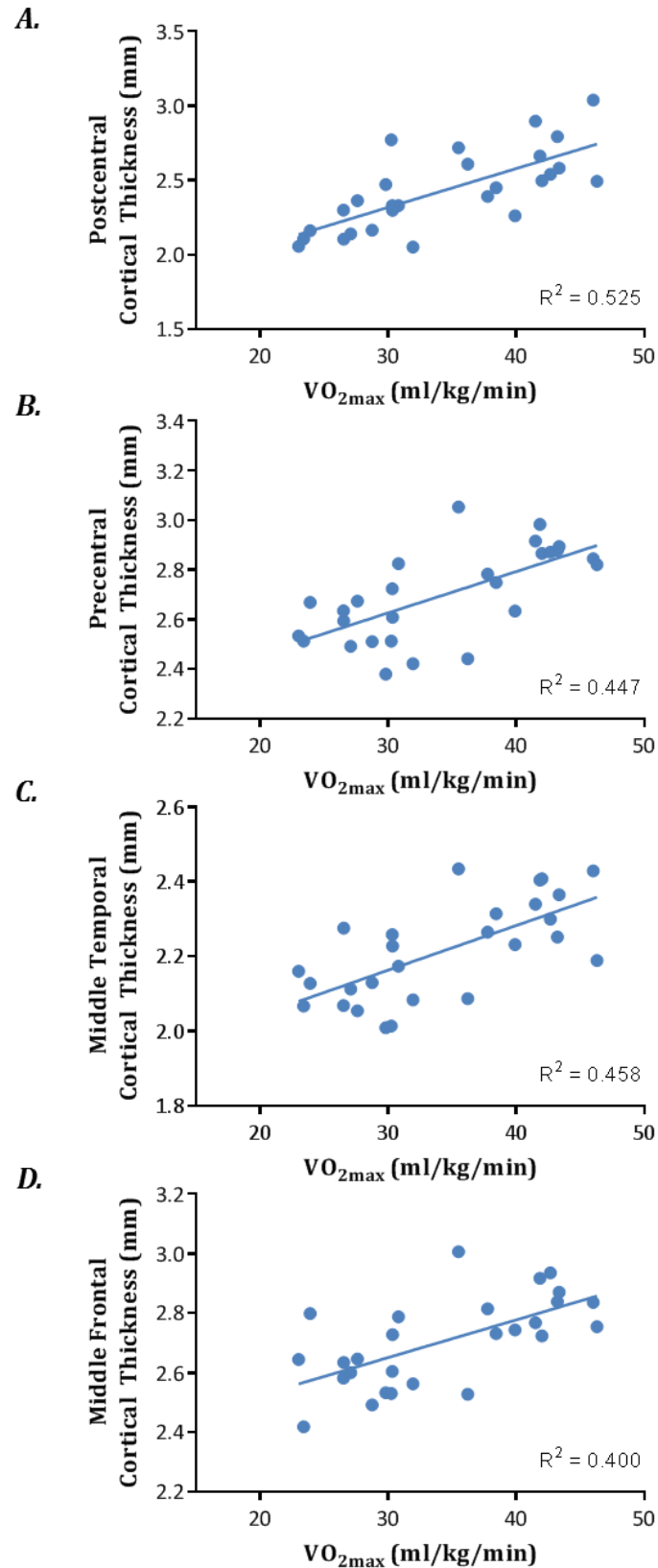


Figure 6-21: Association between cortical thickness and cardiorespiratory fitness as measured by VO₂max in the **A.** postcentral gyrus, **B.** precentral gyrus, **C.** middle temporal lobe and **D.** middle frontal lobe.

6.7 DISCUSSION

In this current study, differences in grey matter volume and cortical thickness between healthy young and older volunteers was assessed, and both measures were associated with cardiovascular fitness (determined using the gold standard measure of VO_{2max}). The major findings were as follows:

Firstly, ROI analysis revealed a significant reduction in global grey matter volume in the older compared to young group, and a significant positive linear correlation between global grey matter volume and VO_{2max} . Regional assessment revealed significant ($P<0.001$) grey matter volume loss in the frontal, occipital, and parietal lobes, and putamen, cerebellum and insula. A significant positive association between global grey matter volume and VO_{2max} was found in the frontal and occipital lobes, and insula.

Secondly, voxel based morphometry provided spatial maps of this grey matter volume loss, showing an age-related volume loss in the inferior, middle and superior frontal gyrus, insula, posterior cingulate, putamen and precentral gyrus, and cerebellum, and a significant positive correlation between grey matter volume and cardiorespiratory fitness, accounting for age effects, in the precentral cortex, putamen, thalamus, and cerebellum.

Thirdly, cortical thickness measures showed a significantly lower global cortical thickness in the older group compared to younger group, and a significant positive correlation between cortical thickness and VO_{2max} . Maps of cortical thickness revealed a significant age-related cortical thinning in the precentral cortex, postcentral cortex, inferior- medial- and superior frontal cortex, posterior cingulate, superior parietal cortex, inferior and middle temporal cortex. Areas which showed a positive association between cortical thickness and VO_{2max} were the precentral and postcentral cortex, middle temporal cortex, middle and superior frontal cortex, and superior parietal cortex.

An age-related reduction in both grey matter volume and cortical thinning was found. The significant reduction in grey matter volume observed in the middle and inferior frontal gyrus, anterior insula, temporal gyrus, SMA, and mid-

cingulate gyrus is in agreement with previous studies [1, 6-12]. Significant age-related cortical thinning was shown in similar regions of the precentral cortex, postcentral cortex, inferior- medial- and superior frontal cortex, posterior cingulate, superior parietal cortex, inferior and middle temporal cortex. This pattern of change with ageing is in agreement with the meta-analysis of cortical thinning by Fjell et al [19].

Maximal oxygen uptake was positively associated with global grey matter volume and global cortical thickness. Regional grey matter volume, as assessed by Sienax ROI analysis, revealed a positive trend with VO_{2max} in the frontal and parietal cortex. Exploring this association further, VBM analysis showed a significant positive association between VO_{2max} and grey matter volume in the precentral cortex, putamen, thalamus, and cerebellum. Assessment of cortical thickness revealed regions with a stronger positive correlation with VO_{2max} than VBM analysis, with positive associations between cortical thickness and VO_{2max} found in the precentral and postcentral cortex, middle temporal cortex, middle and superior frontal cortex, and superior parietal cortex.

Additionally, ROI analysis of the VBM and cortical thickness mapping data provided visual probability maps illustrating spatial overlap in regions which show a decline with ageing, and those which demonstrate a positive association with VO_{2max} . In Figure 6-13, the strongest overlap of these effects was within the precentral and postcentral gyrus, which showed a significant reduction in grey matter volume in the older compared to young group, and a positive correlation with cardiorespiratory fitness. When assessing cortical thickness changes, contrasting the data shown in Figures 6-18 and 6-20, there was a strong correspondence of age-related thinning in areas of precentral and postcentral cortex, and the superior frontal cortex, areas which were also shown to have greater thickness with higher cardiorespiratory fitness. Surface-based cortical thickness techniques have been suggested to yield more sensitive estimates of age-associated cortical atrophy when compared to volumetric approaches [15]. The findings presented here support that premise, with the multi-regression analysis of average individual cortical

thickness showing a stronger association with VO_{2max} as well as the results of the cortical thickness mapping.

These spatial maps revealing opposing effects of ageing and VO_{2max} suggest that there is plasticity in brain regions which are shown to reduce in volume and cortical thickness with age. Previous ageing studies of functional brain correlates of cardiorespiratory fitness or exercise have examined the functional response to performing tasks using executive functions [31, 67-69] such as semantic memory [70], and motor function [71]. In general, these studies have shown that exercise or higher cardiorespiratory fitness is associated with greater brain activity in both executive areas comprising frontal and parietal regions. Thus it can be hypothesized that brain regions used for executive functions are most at risk of age-related decline, and that these areas will gain most from an active lifestyle with associated structural changes. The results shown here support this finding, since positive associations were found between grey matter volume/cortical thickness and VO_{2max} in the middle/superior frontal cortex and superior parietal cortex, areas associated with executive function. In addition, positive associations were found in precentral and postcentral cortex. These regions are associated with sensorimotor motor function and believed to expand with greater activity, supported by rehabilitation studies showing plasticity in these regions with enlargement of cortical representation [72]. Ageing is known to lead to vascular changes and enhanced cardiovascular fitness levels may help to preserve the integrity of these brain regions, not only in primary motor and sensory cortex but other areas of the brain which are susceptible to cortical thinning and volume loss, such as the frontal and parietal regions.

In summary, this Chapter has demonstrated the differing methodologies which can be used to explore brain structure including both grey matter volume and cortical thickness changes. Prior studies have used only one of these methods rather than the synergy of both to provide complementary measures. Importantly, here the gold standard assessment of VO_{2max} was performed rather than the use of self-reported questionnaires or estimated VO_{2max} , a measure that has only been used in one previous study [43].

However, the findings reported here have some limitations. Firstly, the data set comprised a small sample size. Future studies should be performed on a larger cohort, in particular by recruiting an active elderly group. This will provide a greater range of VO_{2max} values for improved assessment of the independent effects of age and cardiorespiratory fitness. Secondly, in this study no data on cognitive function was collected and in the future these measures should be investigated. It will also be of interest to collect self-reported physical activity scores as this will allow a direct comparison between the gold standard VO_{2max} measure and the self-reported methods.

6.8 CONCLUSION

Here, the expected spatial pattern of age-related grey matter volume reduction and cortical thinning has been shown. A positive association has been found in precentral and postcentral cortex, middle temporal cortex, middle and superior frontal cortex, and superior parietal cortex related to the gold standard measure of cardiorespiratory fitness, VO_{2max} , independent of age. This data suggests that cardiorespiratory fitness is associated with structural brain plasticity, and that physical activity may provide a protective effect against the effects of ageing.

6.9 REFERENCES

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72. Stark, A., et al., *Plasticity in cortical motor upper-limb representation following stroke and rehabilitation: two longitudinal multi-joint fMRI case-studies*. Brain topography, 2012. **25**(2): p. 205-219.

7. Effects of Limb Immobilisation on Brain and Muscle Function

The primary aim of the work described in this chapter is to develop methods to study the impact of limb immobilisation and associated loss of muscle strength on brain structure and muscle function using ultra-high field, 7 Tesla MRI. Prior studies on the effect of immobilisation on brain and muscle function are first introduced. This is followed by a description of the development of muscle magnetic resonance spectroscopy (MRS) to interrogate musculoskeletal lipid content. Results of functional magnetic resonance imaging (fMRI), to assess changes in brain motor cortex architecture, and longitudinal quantification of forearm muscle lipid accumulation over a 16-day period of upper limb immobilisation are then shown.

7.1 INTRODUCTION

It is believed that physical inactivity can lead to a progressive decline in musculoskeletal function and the onset of chronic diseases such as type 2 diabetes [1, 2]. Emerging evidence suggests that sedentary behaviour is associated with musculoskeletal deterioration including loss of muscle mass [3], metabolic impairments [4, 5] and lipid accumulation [6-8], all of which strongly contribute to functional decline [9-12]. Models of physical inactivity bring about significant deteriorations in muscle morphology, the most apparent adaptation of which is the progressive loss of skeletal muscle mass, termed muscle atrophy. Whilst the rate of muscle atrophy is dependent on a number of factors, studies investigating lower limb immobilisation suggest that the majority of muscle mass loss occurs in the early stages of inactivity. When assessing the impact of bed rest on the quadriceps, White et al. [13] found a 5 % loss in muscle mass after 7 days, whilst a further reduction of only 2-5 % has been reported after 21 days [14, 15]. Conversely, upper limb immobilisation studies have provided conflicting results. Miles et al. [16] evaluated the effects of 9 days of forearm immobilisation on twelve subjects, reporting a 4 % loss in forearm muscle mass, while Kitahara et al. [17] reported no change to forearm muscle cross sectional area following 21 days of immobilisation. Inconsistencies in these findings are likely due to the differing models of physical inactivity used, and the fact that the magnitude of habitual loading of the legs is greater than that of the arms.

Muscle cross sectional area is proportional to muscular strength and so muscle atrophy is concomitant to weakness. This can have a major impact on day-to-day routine and quality of life. Similar to muscle atrophy, the early stages of physical inactivity are crucial in the loss of muscle strength [18, 19]. However, the reduction of muscle strength is often proportionally greater than the relative reduction in muscle mass and therefore cannot be accounted for by atrophy alone. Lower limb immobilisation induces a major decline in maximal strength which far outweighs any decrease in muscle mass. Jones et al. [20] investigated changes in muscle mass and function after 2 weeks lower limb immobilisation in 9 healthy young males. The authors found a far greater

reduction in the quadriceps isometric strength (27 ± 3 %) compared to its reduction in skeletal muscle mass (4.7 ± 0.9 %).

The mechanisms behind the disproportionate reduction in strength are unknown. However, it has been suggested that the aetiology underlying reduced muscle strength post immobilisation may, in fact, reside in the peripheral and/or central motor system. Langer et al. [21] attempted to address this by conducting a longitudinal study to investigate whether upper limb immobilisation was reflected in structural alterations in grey matter cortical thickness. The authors investigated grey matter volume using MRI before and after 16 days of immobilisation in 10 healthy adults who were fitted with an above-the-elbow cast. Surface based morphometry (SBM) analysis of cortical thickness revealed a decrease in thickness in regions responsible for the sensorimotor control of the upper extremity immobilised (primary motor cortex $p = 0.00015$, and primary somatosensory area, $p = 0.002$), as shown in Figure 7-1. The authors concluded that limb immobilisation induces a rapid reorganisation of the sensorimotor system, which warrants further investigation.

A growing number of both structural and functional MRI studies of the human brain have revealed a link between variations in brain architecture and an individual's experience. For instance, BOLD fMRI has been used to investigate functional lateralisation as a function of handedness. Right-handed individuals have shown larger BOLD responses to hand movements in the dominant motor cortex compared with the non-dominant area, suggesting a greater hyperaemic response to neuronal activation in the dominant motor cortex. It is unclear, however, whether this lateralisation of the BOLD response reflects an asymmetry in the neuronal response, or whether it is at least partly due to an asymmetry in vascular structure or reactivity between the dominant and non-dominant motor cortices.

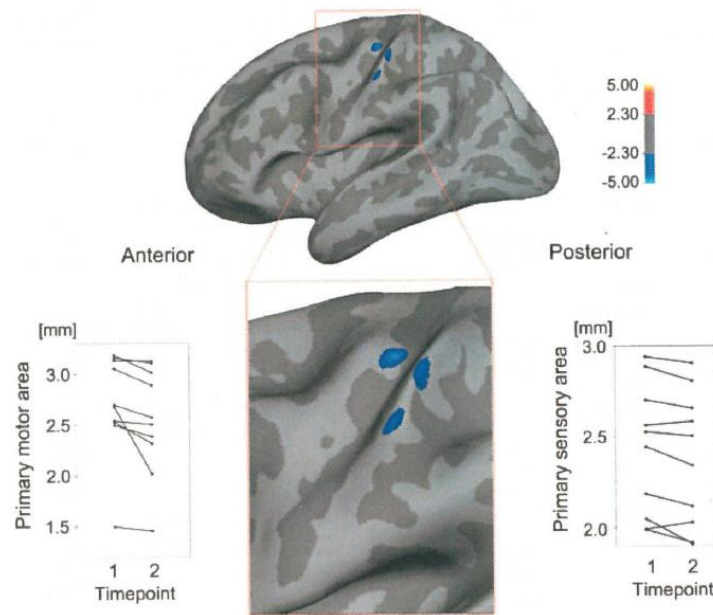


Figure 7-1: Results reported by Langer et al. [21]. Post right-arm immobilisation, a significant decrease in cortical thickness was found in the precentral gyrus, superior postcentral gyrus and the inferior postcentral gyrus in the contralateral hemisphere. Additional trend plots represent cortical thickness for each subject and time point in the primary motor area and primary sensory area.

To separate these effects and to study the impact of immobilisation on the underlying vascular structure, the BOLD response to an increase in arterial carbon dioxide tension (P_{aCO_2}), termed hypercapnia, can be assessed, which causes vasodilation and an increase in CBF and CBV. Hypercapnia can be used to assess the capacity of local cerebrovasculature to dilate and constrict, referred to as cerebrovascular reactivity (CVR). BOLD CVR is defined as the change in %BOLD signal per mmHg change in P_{aCO_2} .

Physical inactivity is also a risk factor associated with muscle insulin resistance, which has been linked to the development of Type 2 diabetes [22-24]. Mikines et al. [25] were the first to report an onset of whole body and leg insulin resistance in healthy young volunteers after seven days bed rest. Several lines of evidence point to a possible association between the development of inactivity induced insulin resistance and increased intra-myocellular and/or extra-myocellular lipid content. Cree et al. [26] investigated the effects of 28-days bed rest on intra-myocellular lipid (IMCL)

content and insulin sensitivity in the leg in healthy young adults. IMCL was quantified using MRS and a 76% increase in IMCL content was found post bed rest which correlated to insulin sensitivity ($p = 0.035$). Similarly Manini et al. [27] investigated the effects of a 4-week reduction in lower limb activity on extra-myocellular lipid (EMCL) content using MRI in healthy young adults. The authors reported a marked increase (20%) in EMCL accumulation in the calf after reduced activity. Conversely, an absence of skeletal muscle lipid accumulation in the face of physical inactivity induced insulin resistance was reported by Dirks et al. [28] over a shorter seven day period bed rest. In this study, IMCL was quantified histochemically on air dried muscle cryosections using oil red O (ORO) staining. This technique has however been reported to provide inconsistent results, and has faced criticism [29]. Importantly, no literature has quantified muscle lipid (IMCL or EMCL) in a longitudinal manner during a period of limb immobilisation. Such an approach is essential in determining the role of muscle lipid accumulation in the aetiology of immobilisation induced muscle insulin resistance, and in particular whether muscle lipid accumulation is a driver of immobilisation induced insulin resistance or simply a consequence of immobilisation per se.

The aims of this study were twofold:

1. To quantify any changes in brain sensorimotor cortex architecture and activation during muscle contraction over the 16 days of upper limb immobilisation, and assess whether any change observed associates with the loss of voluntary handgrip strength over the same time period. Forearm muscle cross sectional area was also measured to allow determination of immobilisation induced muscle atrophy and confirm whether the magnitude of any loss of grip strength exceeds the magnitude of decline in forearm muscle cross sectional area during immobilisation.
2. To develop magnetic resonance spectroscopy (MRS) methodology at ultra-high field MRI (7.0 T) to quantify forearm muscle IMCL and EMCL content over the course of 16 days of forearm immobilisation in healthy, young volunteers.

This study was run jointly between the School of Life Sciences and the Sir Peter Mansfield Imaging Centre at the University of Nottingham. It formed a pilot study to assess the feasibility of performing muscle and brain measures at 7T. Since muscle MRS at 7 T is novel, development was first required to set-up this sequence. In the following section, the development of MRS protocols is outlined, and followed by the study protocol, measures collected and findings.

7.2 DEVELOPMENT OF MUSCLE SPECTROSCOPY MEASURES

Prior to commencement of the study a series of development scans were performed to optimise the spectroscopy protocols on the 7.0 T Philips scanner.

Quantification of skeletal muscle lipid content by ^1H MRS has attracted wide spread interest due to its non-invasive nature. The lipids are stored in both intra- and extra-myocellular spaces to accommodate the body's energy requirements. An example of a musculoskeletal metabolite spectrum can be seen in Figure 7-2.

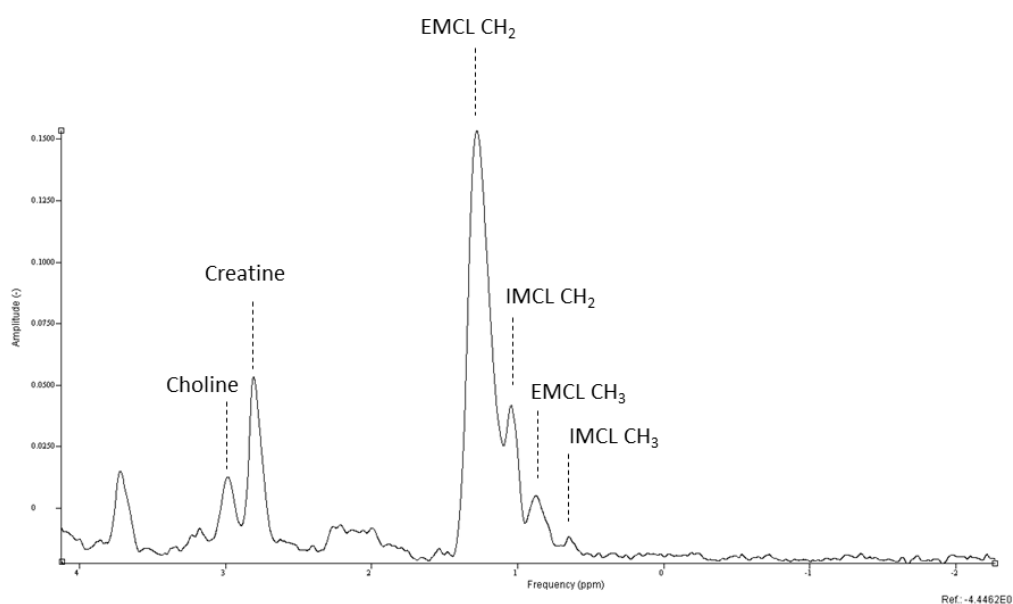


Figure 7-2: A typical ^1H spectra demonstrating the chemical shift between metabolites within the muscle.

The chemical shift resolution between IMCL and EMCL peaks in ^1H spectra arise due to the magnetic field dependency on shape, orientation and magnetic susceptibility. It is known that extra-myocellular lipids can be modelled as long

strands of fat nestled between muscle fibre bundles, while intra-myocellular lipids can be modelled as spherical fat droplets within the fibres and close to mitochondria. This assumption has been confirmed in phantoms [30] and in vivo [31, 32]. The dominant signal from the CH₂ chain is used to measure IMCL content, however the EMCL and IMCL signals commonly overlap, and therefore both peaks must be fit to a symmetrical line shape such as a Gaussian or Voigt function. While this method is widely accepted, investigators report the difficulty of successfully fitting spectra acquired from muscle [31, 33]. It is therefore important to optimise the protocol to achieve maximum SNR and resolution between the peaks to aid in spectral fitting.

7.2.1. Optimising Acquisition

While higher magnetic fields promise improved resolution and sensitivity, susceptibility-induced static field inhomogeneities may contribute to further line broadening. The following section outlines the methods by which SNR and chemical shift resolution may be improved.

7.2.1.1 Voxel size

The simplest method to achieve greater SNR is to increase the size of the voxel from which the signal is detected. The majority of previous studies investigating myocellular lipid content have focussed on the large muscles within the leg, most commonly the soleus muscle. However, this study was constrained to upper limb immobilisation because an arterio-venous balance technique to assess forearm glucose uptake repeatedly over the course of immobilisation had been developed in the School of Life Sciences, and so the flexor digitorum profundus (FDP) muscle (Figure 7-3) was chosen due to its size and depth within the forearm. This allowed for the largest voxel to be used whilst ensuring that it remained within the muscle of interest.

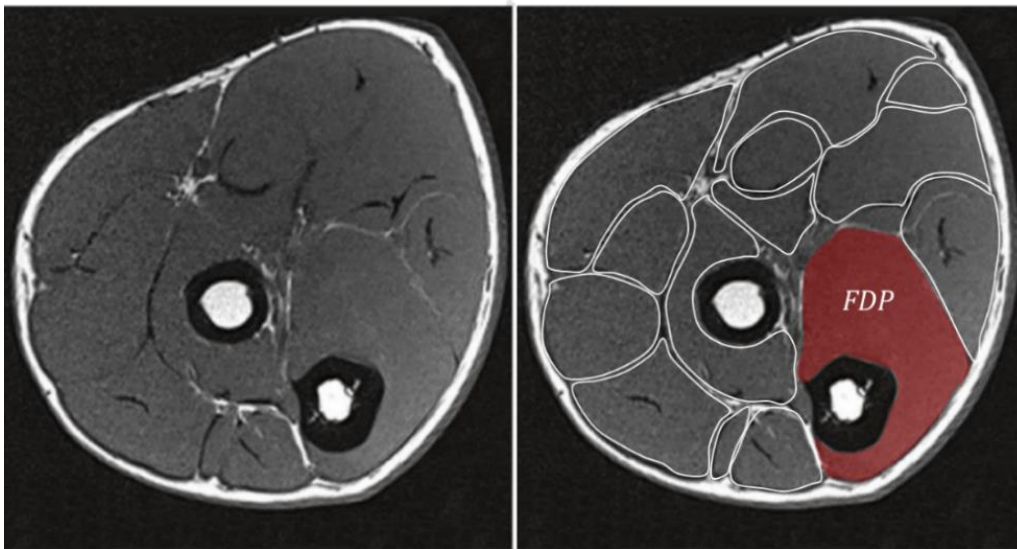


Figure 7-3: Illustration of the muscle groups within the forearm. The flexor digitorum profundus (FDP) (highlighted) was chosen to be interrogated due to its size.

Although typically an isotropic voxel is used in MRS experiments, the long shape of the FDP muscle allowed for further manipulation of the voxel size. By increasing the length of the voxel running parallel to the muscle fibres, a larger volume from which signal can be collected is achieved. However, care must be taken to remain within the muscle of interest otherwise the signal will be contaminated by adipose tissue or artefacts from the bone/muscle interface.

A preliminary experiment was conducted to find the optimum voxel size when quantifying metabolites within the FDP. One subject was scanned using a STEAM sequence (see Chapter 3, Section 3.8.1 for more details). Scan parameters were TR = 2000 ms, spectral bandwidth of 4 kHz, 4096 samples and 32 averages. Keeping all parameters constant, and ensuring consistent voxel placement, three voxel volumes were tested; 10 mm³ isotropic, 10 x 10 x 25 mm³ and 10 x 10 x 50 mm³. The resulting spectra can be seen in Figure 7-4.

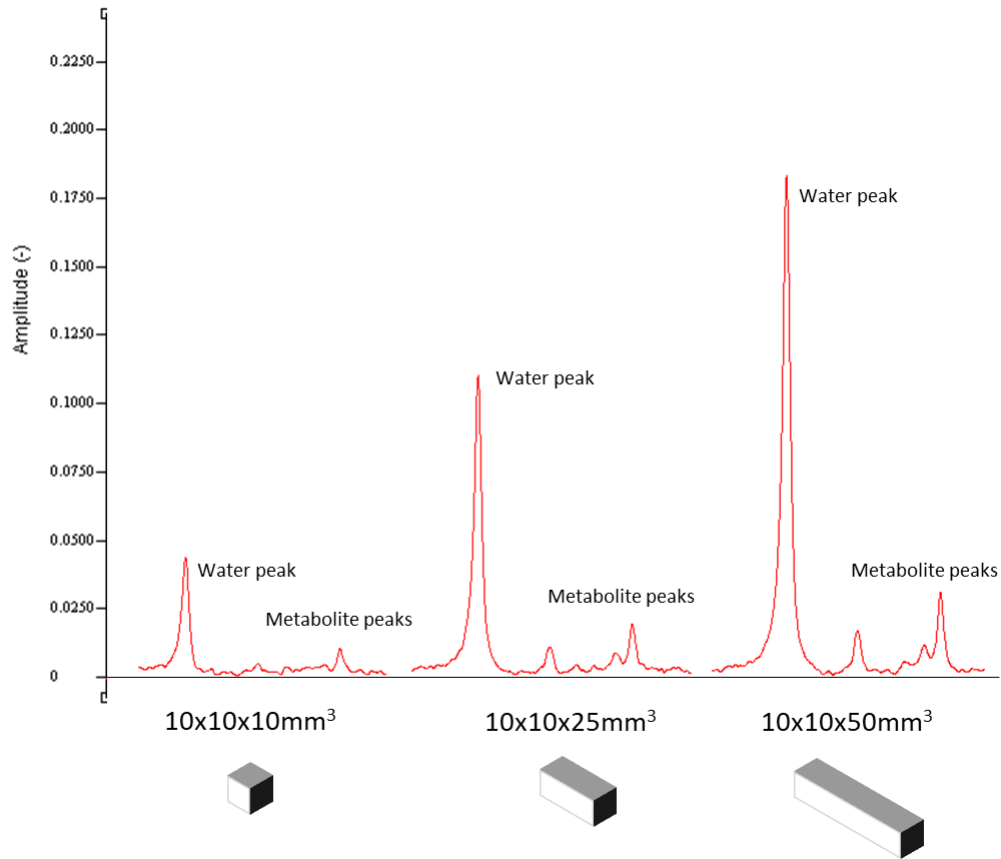


Figure 7-4: Resulting spectra showing the change in signal with increasing volume, performed when optimising voxel size for lipid quantification in the flexor digitorum profundus (FDP) muscle.

Although the water signal was not fully suppressed during this sequence, it can clearly be seen that, as expected, the largest volume provided the greatest SNR between the peaks without apparent line broadening or leakage into extracellular compartments, and therefore all following experiments were performed using a voxel with dimensions of 10 x 10 x 50 mm³.

7.2.1.2 Orientation

In addition to shape, in an ordered tissue such as muscle, chemical shift resolution is also dependent on orientation relative to the main magnetic field. Since IMCL is modelled as a spherical droplet, chemical shift remains constant independent of its angle to B_0 . However, the anisotropy of the EMCL strands cause field perturbations when non-parallel to the main magnetic field, resulting in asymmetry of its signal. As the angle of the lipids relative to B_0 increases, the chemical shift of the EMCL signal broadens while the chemical

shift of the IMCL signal remains unchanged, resulting in the two peaks merging and resolution deteriorates. To investigate the impact of lipid orientation on the metabolite spectra, data was collected in one subject using identical parameters to the previous optimisation experiment. The resulting spectra can be seen in Figure 7-5.

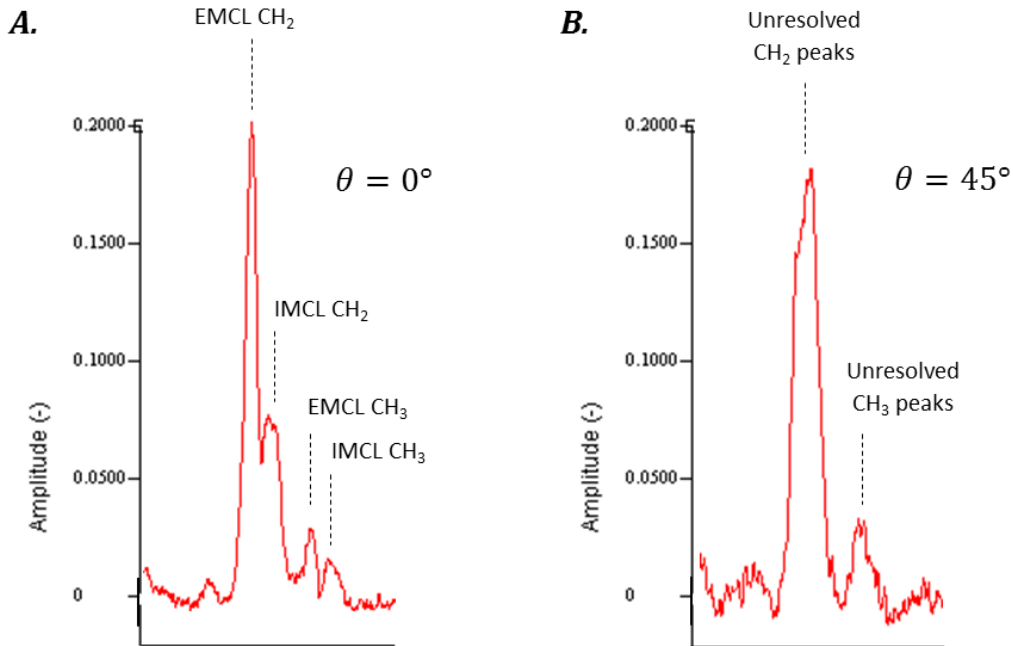


Figure 7-5: Illustration of the effect of forearm orientation on lipid spectra. Spectra acquired with muscle fibres running **A.** parallel and **B.** at an angle of 45° to the B_0 field.

The results showed that with increasing angle relative to B_0 there is a reduction in spectral resolution and, as predicted, the conditions are optimal when EMCL strands run parallel to B_0 . This highlights the importance of keeping the forearm aligned with the magnetic field during lipid spectroscopy. As a result of these findings, a simple device was created to ensure that the forearm remained stationary and parallel to the magnetic field throughout the study. A wooden base was tailored to fit within the transmit coil of the 7.0 T Philips MRI scanner. Two Velcro straps were attached to the base and looped around to fix the subject's arm in position. A schematic of the device can be seen in Figure 7-6.

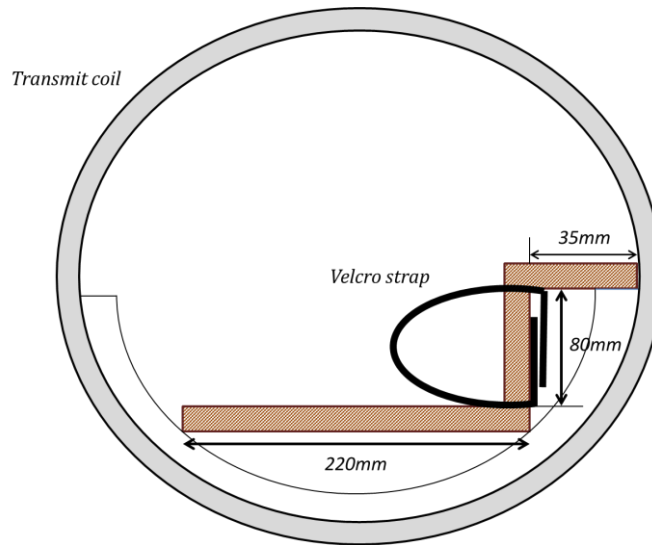


Figure 7-6: Simple device created to ensure the subject's arm remained stationary and parallel to the B_0 field throughout the scanning session for optimised SNR and metabolite resolution.

Clearly, not all fibres within a muscle group run parallel to one another [34] and hence not all EMCL strands are aligned. The angular dispersion of muscle fibres within a muscle group contributes further to the asymmetric lineshape of the EMCL signal rendering conventional line fitting methods insufficient.

Khuu et al. [35] developed a new spectral analysis approach to account for lipid orientation and dispersion, termed fibre orientation modelling (FOM). Assuming that an EMCL strand could be represented as a cylinder, the influence of B_0 on the strand can be described by $3\cos^2(\theta) - 1$. A mathematical model was then devised to examine the influence of variable orientation and dispersion on chemical shift and peak broadening. The number of protons in a lipid strand at an angle θ to the B_0 field can be described as a Gaussian distribution; $N(\theta) = e^{-\left(\frac{\theta-\alpha}{\beta}\right)^2}$ where α is the dominant angle and β is the width of the distribution, which also characterises the dispersion of the cylindrical strands within the voxel. By summing each resonance Gaussian lineshape at varying θ , and converting orientation into chemical shift by $\delta = 3\cos^2(\theta) - 1$, EMCL spectra can be simulated. An example of the FOM can be seen in Figure 7-7.

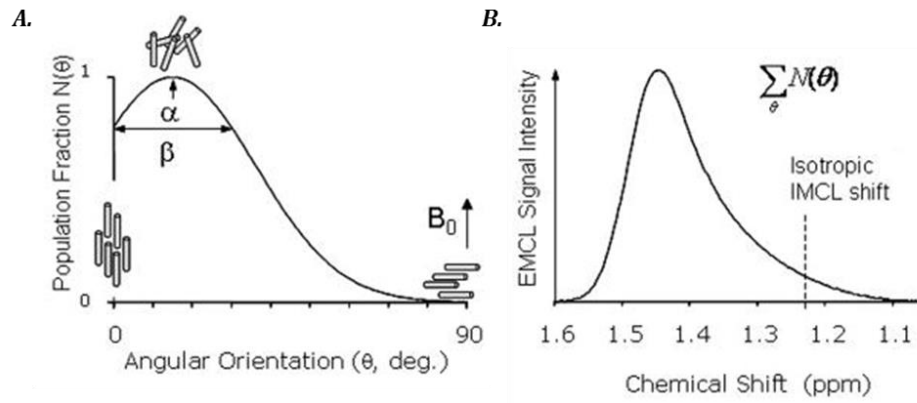


Figure 7-7: Illustration of Fibre Orientation Modelling (FOM) as reported by Khuu et al. [35]. Angular orientation is varied while α and β are fixed ($\alpha=15$ and $\beta=30$ in this example). **A.** Example showing fibre orientation. **B.** The final simulated EMCL signal intensity

Using their modelled EMCL signal, the authors simulated the influence of orientation relative to the B_0 field from 0° to 30° and increasing dispersion from 0° to 45° . Their findings can be seen in Figure 7-8. With increasing orientation, it can be seen that the chemical shift resolution is reduced, while increasing dispersion results in the EMCL signal broadening asymmetrically.

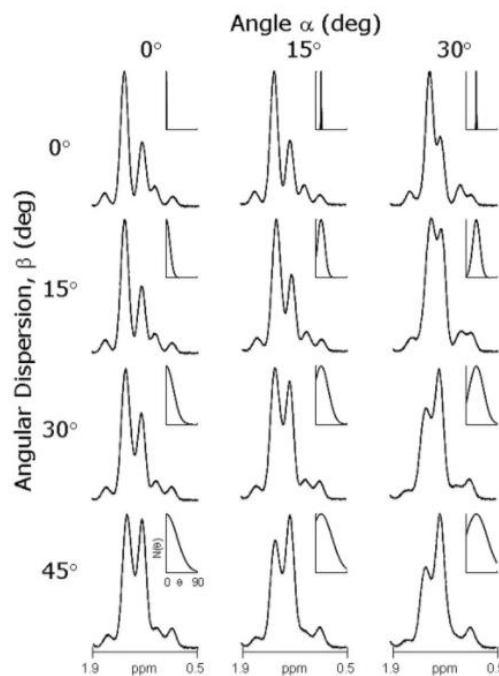


Figure 7-8: Report by Khuu et al. [35] illustrating the effect of variable orientation of muscle relative to the B_0 field, and dispersion of muscle fibres within the muscle group on chemical shift resolution and lineshape of lipid signal acquired by ^1H MRS.

These results highlight the importance careful positioning of the subject and precise planning of the voxel when performing muscle lipid quantification using ^1H MRS. Although the fibres of the FDP predominantly run parallel to the direction of the arm, an FOM following the method of Khuu et al. [36] created by Olivier Mougín (SPMIC, United Kingdom) was used to ensure robust fitting.

7.2.1.3 Optimising Parameters

Acquisition parameters have a large effect on the appearance of a spectrum and therefore care must be taken when planning a sequence. The most important parameter to consider is echo time (TE). In the time between signal excitation and readout, each metabolite peak relaxes to thermal equilibrium with its characteristic T_2 -relaxation time. In addition, the signal amplitude is modulated. TE can therefore be used to manipulate spectra and provide greatest information on the metabolite of interest.

Concentrations of metabolites within muscle are $\sim 10^4$ times smaller than the water content, and so their signal is drowned out by the dominating water peak. To allow for lipid assessment, the water signal must be suppressed. Typically a sophisticated water suppression technique is applied at the beginning of the pulse sequence, however, when saturating the water peak these methods unavoidably perturb nearby signals which may in turn distort information about the lipid content. One method to achieve saturation of the water signal without the confounds of water suppression is to combine a long TE with large localisation voxels. This method relies on the faster T_2 -decay of water compared to fat. Using a long echo time allows the water signal to completely decay to baseline while the fat signal remains. While resolution of the lipid peaks is thought to increase using this technique due to the differing T_2 -decay rates between EMCL and IMCL, the long TE also partially reduces the fat signal and therefore multiple averages have to be performed. Ren et al. [37] compared the effect of short (20ms) and long (280ms) TE on lipid quantification in the soleus muscle. Using large volume voxels ($\sim 10\text{-}15\text{ml}$), a STEAM sequence was performed with 128 averages. Water suppression was applied for the short TE studies. The authors' findings can be seen in Figure 7-9. Results showed that the spectrum collected using a TE of 280ms yielded a

set of well separated peaks from CH₂ and CH₃ resonances for both EMCL and IMCL.

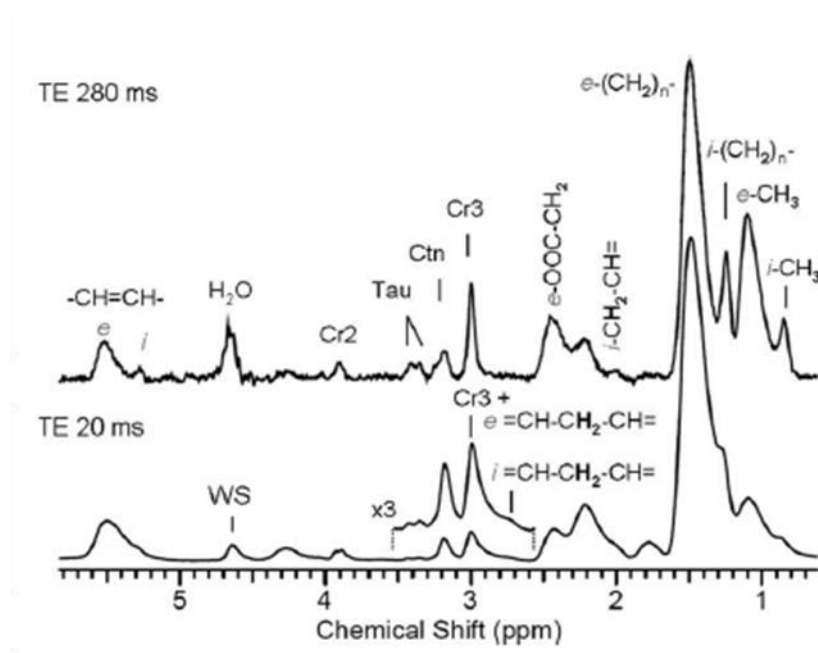


Figure 7-9: *¹H MR spectra reported by Ren et al. [37] comparing spatial resolution of soleus lipid signal between long (280ms) and short (20ms) TE.*

Taking into account these observations, an experiment was performed to investigate whether the advantages of using a long TE remained when interrogating muscle lipid in the forearm. One subject was scanned using a STEAM sequence with a TE of 20 ms and 280 ms, collecting 128 averages. A water suppression enhanced through T₁ effects (WET) method was used to saturate the water signal at low TE. Upon analysis, no fat signal remained at the long echo time. And so the long TE experiment was repeated at a TE of 100 ms. A comparison in lipid spectra between short and revised long TE acquisitions can be seen in Figure 7-10.

Results show that a short TE provided good SNR (~0.7 AU) and distinction of IMCL and EMCL peaks. However, there was poor resolution downfield of the main lipid signals and other metabolites were lost in the remaining fat peaks. There was similar distinction of the lipid peaks in the long TE study, and other metabolite signals were preserved. However, the longer echo time allowed the T₂ of the lipid signal to decay further, and therefore SNR was reduced to less than a third of the short TE signal (~0.17 AU).

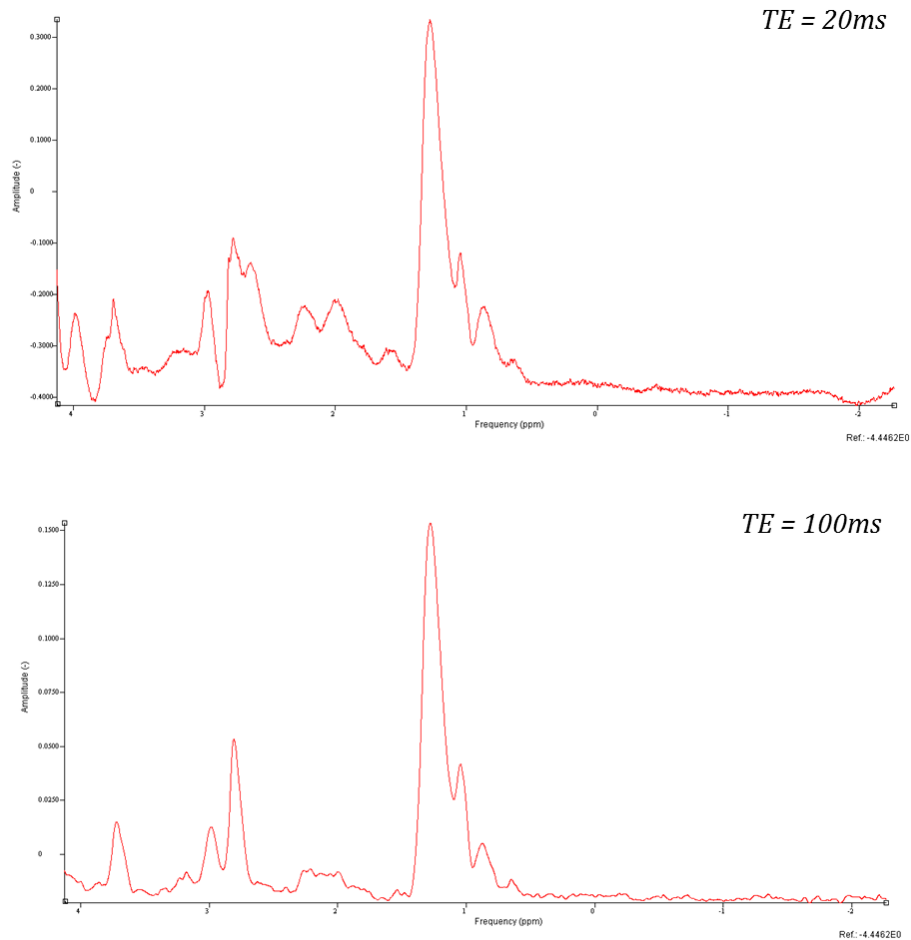


Figure 7-10: A comparison of lipid spectra acquired at short (20ms) and long (100ms) echo times (TE).

The complete loss of signal at long TE in this study was likely due to the constraints that arise when focusing on upper limb muscle groups. The soleus muscle is significantly larger than the FDP and the voxel size used in the lower limb study was $\sim 3 - 5$ times greater than the maximum volume available for forearm assessment, thus there was an inherently greater SNR.

A major disadvantage to water signal reduction by long TE methods is that it is more difficult to correct for T_2 -relaxation. For absolute quantification of metabolite concentrations a reference signal of known quantity is needed. Here we use water as a physiological standard. Ideally, the area under the peaks of an MR spectrum would directly reflect concentration. However, signal decay from T_1 and T_2 processes are inherent to MR signals and can cause inconsistencies in both the reference peak and the signal of interest, and so

must be corrected. This can be achieved by varying TE across the acquisition and fitting the curve of the signal of each metabolite to find their respective T_2 .

Another important parameter for MRS acquisition is the time between each excitation, termed repetition time (TR). Typically, for absolute quantification of metabolites a long TR is used. This ensures that the magnetisation of each metabolite is fully recovered, and therefore prior knowledge of individual T_1 -relaxation times for metabolite peaks is nonessential. However, for spectra that are acquired with a TR substantially longer than the T_1 -relaxation times, SNR is compromised and acquisition time is unnecessarily increased. Optimising TR maximises SNR per unit time.

The T_1 of water is greater than that of lipids and therefore to find the optimum TR for this study, an experiment was performed to assess the recovery time of the water peak. A voxel (size 10x10x50 mm³) was positioned within the FDP of the forearm of one subject. Using a STEAM sequence with varying TR, water signal recovery was investigated. The results can be seen in Figure 7-11.

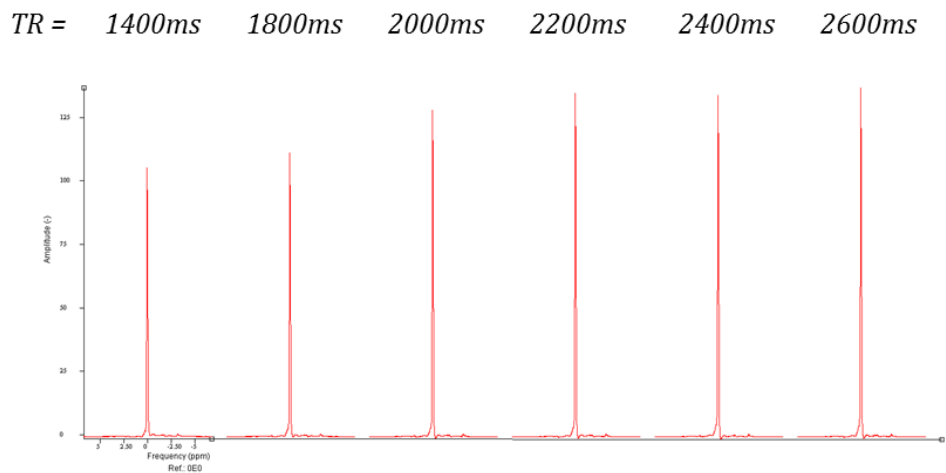


Figure 7-11: Spectra collected using varying repetition time (TR) to assess water signal recovery.

Results showed that the water signal had recovered sufficiently by 2000 ms.

Taking into account findings from the developmental stages of the study, an optimised ¹H MRS protocol for lipid quantification in the forearm was created. Scan parameters used for all following spectra acquisitions were; TR

= 2000 ms, water suppression by WET, varying TE = 20 (x2), 50, 80, 150 ms, 148 averages, total scan time of 12 minutes.

Varying TE's were used to measure T₂ of water and lipid peaks for signal relaxation correction. Two spectra were collected at 20ms to improve SNR.

7.3 PARTICIPANTS

The study was approved by the University of Nottingham Medical School Ethics Committee. Recruitment was targeted at 10 right-handed, healthy, young males with potential participants screened with reference to the inclusion and exclusion criteria described in Table 7-1. All subjects gave written informed consent prior to taking part in the study.

<i>Inclusion</i>	<i>Exclusion</i>
Male	Any metabolic or endocrine abnormalities
Age 18 – 35 years	Clinically significant abnormalities on screening
Right-hand dominant	History of varicose veins, deep vein thrombosis or clotting abnormalities
Non-obese (20 – 25)	Taking routine medication
Non-smoker	Contraindications to MRI including claustrophobia
Able to give informed consent	Incapacity to consent

Table 7-1: List of inclusion and exclusion criteria for participants.

In total nine male volunteers were recruited to the study. However, of these subjects recruited, three volunteers failed to complete the protocol due to scanner failure and adverse effects (see discussion). Additionally, one volunteer failed to complete the muscle assessment and one volunteer failed to complete the brain assessment, resulting in n = 5 for both MRS and fMRI studies.

7.4 THE 7T PROTOCOL

Prior to experimental visits, subjects underwent a health screening session during which measurements of heart rate, blood pressure, height and weight (and hence BMI) measurements were collected. Subjects were familiarised with the laboratory and asked if they were willing to attend a further 9 experimental visits. A 10 ml blood sample was taken from a forearm vein by venepuncture to screen for full blood count (FBC), urea and electrolytes (U&Es), and clotting time assessment, to ensure the subject was eligible to take part in the study. Participants completed a Safety Screening Form to highlight any contraindications to MRI, and a Study Consent Form.

The study was completed over a sixteen day period, as was performed by Langer et al. [21], during which the subject's lower arm was immobilised using a below-the-elbow cast (Figure 7-12). One subject had their dominant arm immobilised while the remaining 5 subjects had their non-dominant arm immobilised.



Figure 7-12: Below the elbow cast used for forearm immobilisation. Note that the subject was asked to wear a sling and the thumb was also immobilised to further restrict any muscular activity over the study protocol.

The full study timeline can be seen in Figure 7-13, indicating the time at which forearm muscle measures and brain measures were taken. Forearm glucose uptake (FGU) measures formed part of a second study, the results of which will not be reported in this thesis. Baseline muscle measures and brain assessment were taken within 3 days prior to the arm cast being fitted. Subjects were required to fast overnight before each study visit.

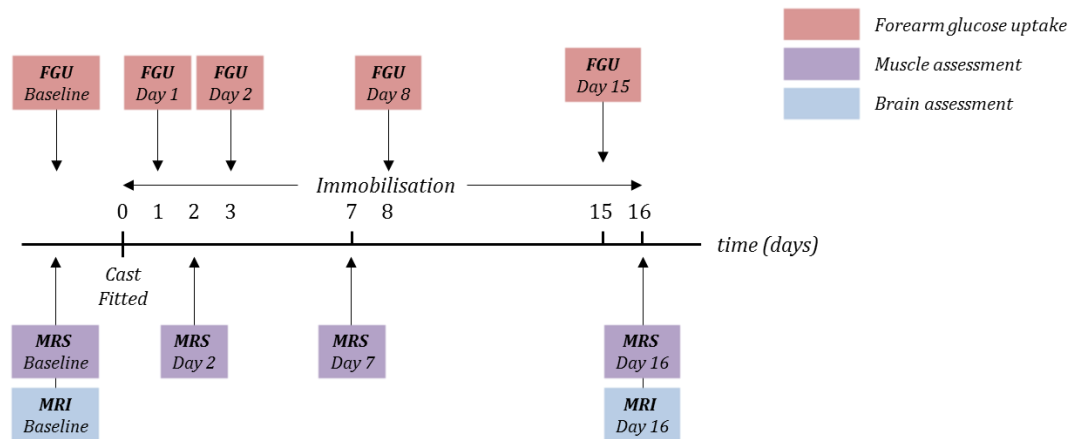


Figure 7-13: Summary of the full study timeline.

7.5 ASSESSING MUSCLE FUNCTION WITH MRI AND MRS

Handgrip isometric strength was assessed for both arms at four time points throughout the study - baseline measures were acquired before the cast was fitted, and repeat measures were collected on Day 2, Day 7 and Day 16 of immobilisation.

At the start of each scan session, an anatomical marker was placed on the cast to ensure consistency between time points. During the first visit, the participants forearm was measured from the styloid process of the ulna to the crease of the antecubital fossa. The marker was then placed at the position corresponding to 30% of the forearm length from the antecubital fossa (Figure 7-14). This length was recorded and reused for all proceeding scans. Participants were also asked to wear a dummy cast for baseline scans to simulate the same conditions as during immobilisation.

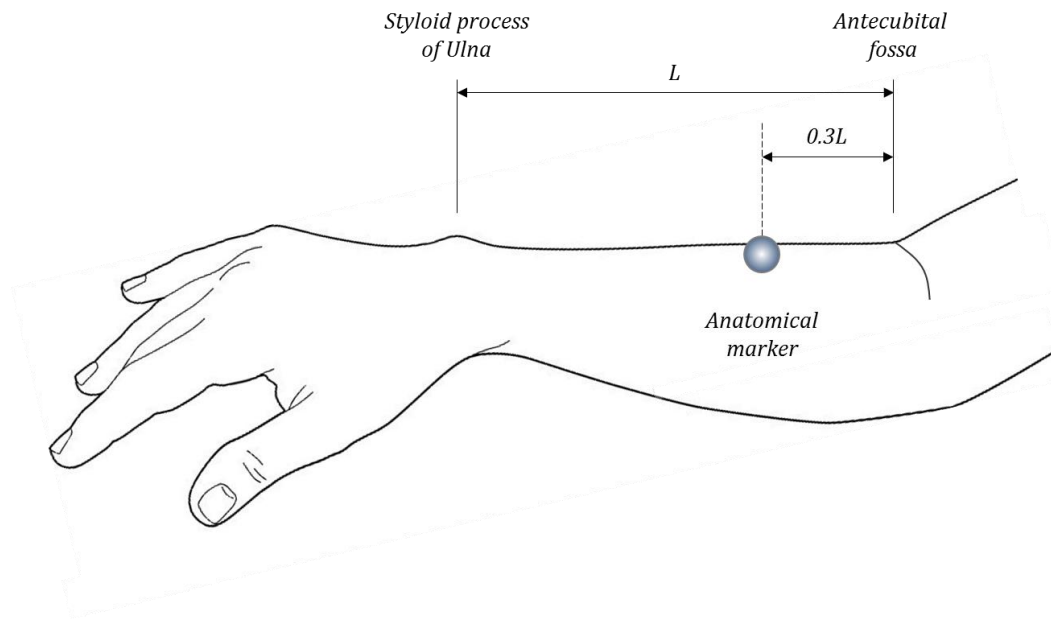


Figure 7-14: Illustration showing the method for placement of the anatomical marker. The marker ensured consistency of voxel positioning across the four study visits.

Data were acquired on a 7.0 T Philips Achieva MRI scanner using a head volume transmit coil and a pair of 16-channel surface receive coils (MRCoils, Netherlands). The subject was positioned head-first and prone with the forearm of interest outstretched and parallel to the magnetic field. Two Velcro straps were used to secure the forearm and ensure it remained in position for the duration of the scan. The subject remained immobilised during data acquisition and a dummy cast was used for the non-immobilised arm to match conditions across the protocol. The 16-channel surface receive coils were placed above and below the forearm and centred on the anatomical marker.

First a scout image was acquired of the arm, using a coarse resolution 1 mm isotropic MPAGE sequence. This was reconstructed on the scanner into 3 orthogonal directions to locate the anatomical marker and plan subsequent scans.

7.5.1 Muscle Cross-Sectional Area (CSA)

An in-phase and out-of-phase gradient echo sequence was used to interrogate muscle cross-sectional area. Care was taken to centre the imaging volume on the anatomical marker. Scan parameters were FOV 122 x 130mm², in-plane

spatial resolution $0.9 \times 0.9\text{mm}^2$, slice thickness 1mm, 80 contiguous slices, TE/TR = 5/20ms, NSA = 1 and acquisition time 2.8 minutes.

7.5.2 MRS: EMCL and IMCL Quantification

A high resolution T_1 -weighted turbo-spin-echo (TSE) sequence was first acquired for voxel planning. Scan parameters were FOV $120 \times 120\text{mm}^2$, in-plane spatial resolution $0.5 \times 0.5\text{mm}^2$, slice thickness 2mm, 25 slices, TE/TR = 7.45/1200ms, NSA = 1 and acquisition time 5.2 minutes. A B_0 -map was then collected over the volume of interest to allow subsequent image-based (IB) volume shimming of the MRS acquisition. ^1H MR spectra were collected using a STEAM sequence from a voxel of $10 \times 10 \times 50 \text{mm}^3$ centred on the FDP muscle. Care was taken to ensure the entire voxel was contained within the FDP muscle group and aligned parallel with the muscle fibres. The spectral acquisition parameters were TR = 2000 ms, varying TE = 20, 80, 100, 150 ms, spectral bandwidth of 4 kHz, and 1024 samples. Two averages were taken at the shortest TE for higher SNR and the long repetition time allowed for full longitudinal relaxation.

7.6 ASSESSING BRAIN FUNCTION

Brain MRI measures were collected at two time points during the study – Day 0, prior to the cast being fitted, and on Day 16 of immobilisation, the day on which the cast was removed. At each time point, data were collected to quantify changes in brain motor cortex activation during a handgrip task of forearm contraction to ascertain whether changes in brain activation occur during the 16 days of arm immobilisation. In addition, MRI data were collected in response to a hypercapnic challenge to assess any changes in cerebrovascular reactivity (CVR).

7.6.1 The Handgrip Task

A handgrip exercise task was employed using a 500 Newton MR compatible handgrip dynamometer (Current Designs, U.S.A), as shown in Figure 7-15. The device is non-magnetic and non-electronic to minimise noise and avoid safety concerns in the MR environment.

Scripts were coded in Presentation (Neurobehavioral Systems, Inc., CA) to calibrate the handgrips and perform the handgrip task. First, grip strength was calculated on the handgrip, by asking each participant to perform a maximum voluntary contraction.



Figure 7-15: The handgrip dynamometer used in this fMRI study. The device is MR compatible and communicates with an interface box via an optical fibre cable. (Images sourced from '<http://www.curdes.com>').

Handgrip tasks were then performed during fMRI in which volunteers were required to squeeze the handgrip to a predetermined intensity. The fMRI sessions comprised two handgrip protocols – a handgrip localiser and the handgrip task.

The handgrip localiser provided wide spatial coverage and allowed the sensorimotor cortex to be identified for planning of the slices for the handgrip task. The localiser protocol was performed on the subject's right hand and comprised an initial 7 s OFF period, followed by four repeats of 35 s ON period where the subject performed a handgrip contraction at 30 % of their individual maximum voluntary contraction (MVC). Each ON period was followed by a 56 s OFF period.

The main handgrip task comprised subjects' squeezing the handgrip at 15% and 30% of their individual MVC. These tasks were chosen to avoid substantial muscle fatigue and the development of occlusion mediated ischaemia that occurs at isometric forces > 30% MVC. The task consisted of six 35 s periods of handgrip contraction, alternating between 30% and 15% MVC, with a 56 rest period between each contraction period, as shown in Figure 7-16A. Using real-

time visual feedback during the handgrip contraction, subjects were asked to move a red box into a blue box, the position of which corresponded to the correct level (15% or 30% MVC) of contraction (Figure 7-16B). Throughout the handgrip task the level at which the contraction reached on the VAS scale was continuously recorded, thus providing a measure of the ability of the subject to perform the task. This handgrip task was performed on both the left and right hand.

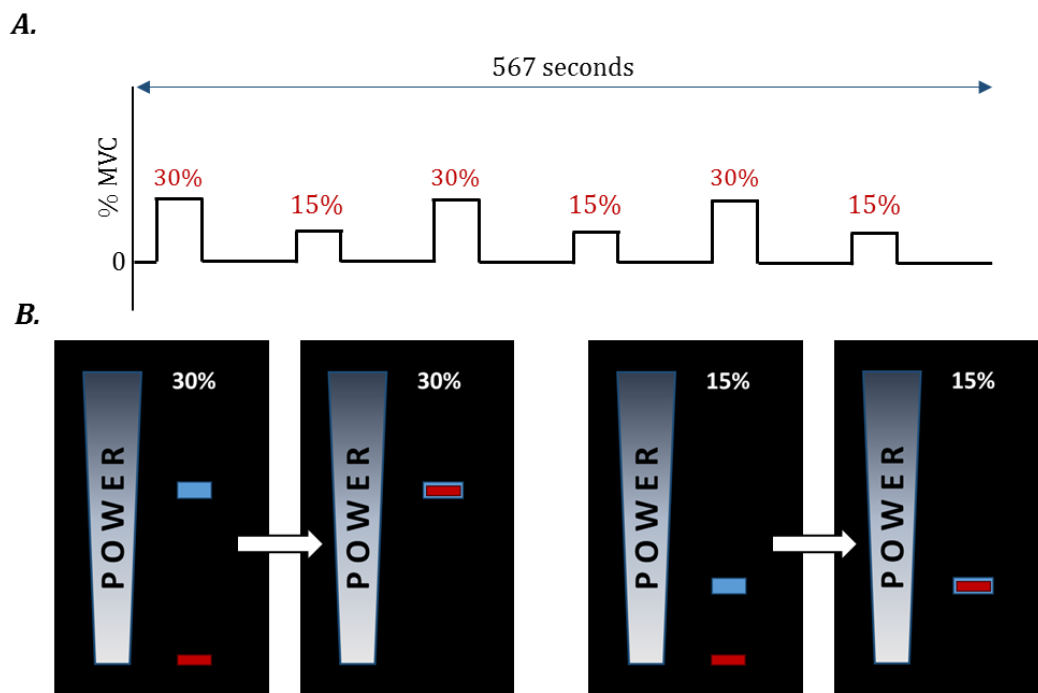


Figure 7-16: *A. Schematic of the handgrip protocol performed during brain imaging.*

B. An example of the visual feedback provided to subjects during the protocol.

Surface electromyography (EMG) data was also recorded during the handgrip task to monitor the muscle activation in the forearm extensor digitorum used to perform the contraction, thus potentially providing a method to assess the direct correlation between motor output and brain activation. Four electrodes were used: two active electrodes plus, a reference and a ground electrode. The two active EMG electrodes were placed on the surface of the extensor digitorum. EMG recording was performed using a Brain Vision Recorder and a MR compatible BrainAmp amplifier (Brain Products, Munich, Germany). Data were collected with a sampling rate of 5000 Hz and a scanner trigger recorded to allow gradient artefact correction of the EMG trace.

At the end of their second brain session visit, on Day 16 of immobilisation, the subject was asked to perform another MVC with each arm using the handgrip dynamometer to measure changes in forearm strength over the immobilisation period.

7.6.2 The RespirAct System to deliver Hypercapnia

To assess cerebrovascular reactivity (CVR) it is important to have a precise and accurate method of increasing the arterial pressure of carbon dioxide (P_aCO_2). This is typically achieved by inducing hypercapnia. A number of different methods have been developed to induce hypercapnia, and the effect of each is typically monitored by taking the end-tidal (end exhaled) PCO_2 ($P_{ET}CO_2$) as an index for P_aCO_2 [38-41]. The most common method of controlling levels of carbon dioxide in the blood is the administration of fixed concentration gases [38, 42]. However, this technique can stimulate hyperventilation and gives variable results within and between subjects. An ideal system would uncouple the effects of CO_2 and O_2 by modulating the levels of carbon dioxide while ensuring the concentration of oxygen remains unchanged.

The RespirAct (Thornhill Research Inc., Canada) is an MR compatible computer-controlled gas blender that supplies precise gas flows and concentrations into a sequential gas delivery (SGD) breathing circuit. The system targets $P_{ET}CO_2$ and end-tidal oxygen ($P_{ET}O_2$) separately allowing it to achieve rapid transitions to predictable $P_{ET}CO_2$ levels while constraining $P_{ET}O_2$ to normoxic levels.

The RespirAct circuit is supplied by four input gases, (1) oxygen (100% O_2), (2) medical air (21% O_2 , 79% N_2), (3) Gas A (10% O_2 , 90% N_2) and (4) Gas B (10% O_2 , 20% CO_2 , 70% N_2). All supplies have a minimum of 10% O_2 and a maximum of 20% CO_2 for safety. Further safety precautions include an automatic switch to the 100% O_2 supply in case of software / power failure. An emergency button is also available for immediate switch to the 100% O_2 supply. The software validates the gases and calibrates the system prior to experimentation to ensure correct setup and connections. The calibration gas

is a mixture of 9% CO₂ and 91% N₂ therefore the cylinder must be removed before the mask is connected. Finally, a removable plug on the mask allows the seal of the SGD to be broken, releasing the gases into the atmosphere and allowing the subject to manually end the gas challenge. The location and purpose of this plug is emphasised to the participant prior to any gas challenge experiment.

The SGD breathing circuit (Figure 7-17) comprises of a novel 3-way valve system and two gas reservoirs. The RespirAct computer fills the inspiratory gas reservoir with the calculated gas mixture for the subject to inhale. On exhalation, the valve system ensures that the exhaled gas travels to the expiratory gas reservoir. Once full, expired gas will be expelled through a hole in the bottom of the expiratory gas reservoir. Ideally the subject will collapse the inspiratory bag on each breath, ensuring that the computer calculated amount of gas mixture is inhaled. If this happens, another valve opens allowing previously exhaled gas to be drawn in. This is known as *rebreathing*. Exhaled gas is considered to be neutral as it has already equilibrated with the blood and therefore it does not contribute to gas exchange.

The face mask has two connections that feedback information on the subjects P_{ET}CO₂ and P_{ET}O₂, and pressure measures to detect periods of inspiration and expiration.

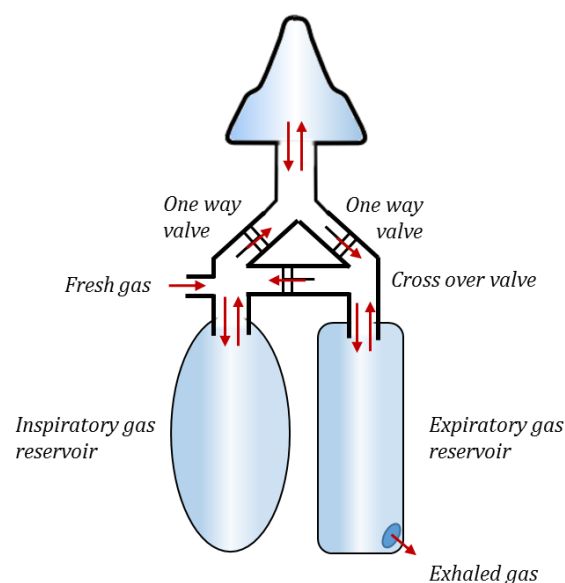


Figure 7-17: Schematic of the RespirAct sequential gas delivery breathing circuit.

Gas challenge sequences to induce hypercapnia are individually designed for each subject based on their physical characteristics (age, height and gender), and expired gas composition (basal $P_{ET}CO_2$ and $P_{ET}O_2$ approximately 35 mmHg and 110 mmHg respectively), typical flow rate and maximum flow rate. Basal $P_{ET}CO_2$ and $P_{ET}O_2$ are measured on the day of experimentation. The typical flow rate is based upon the subject's ability to comfortably collapse the inspiratory gas reservoir with each breath and the maximum flow rate is largest flow rate permitted to induce transitions.

The gas challenge used in this study was a simple box-car design commencing of 2 minutes at a subject-specific baseline $P_{ET}CO_2$, followed by 3 minutes of induced hypercapnia (targeted at baseline + 8 mmHg $P_{ET}CO_2$) and a final 2 minutes at baseline $P_{ET}CO_2$, as shown in Figure 7-18. $P_{ET}O_2$ was targeted to remain at baseline throughout the challenge.

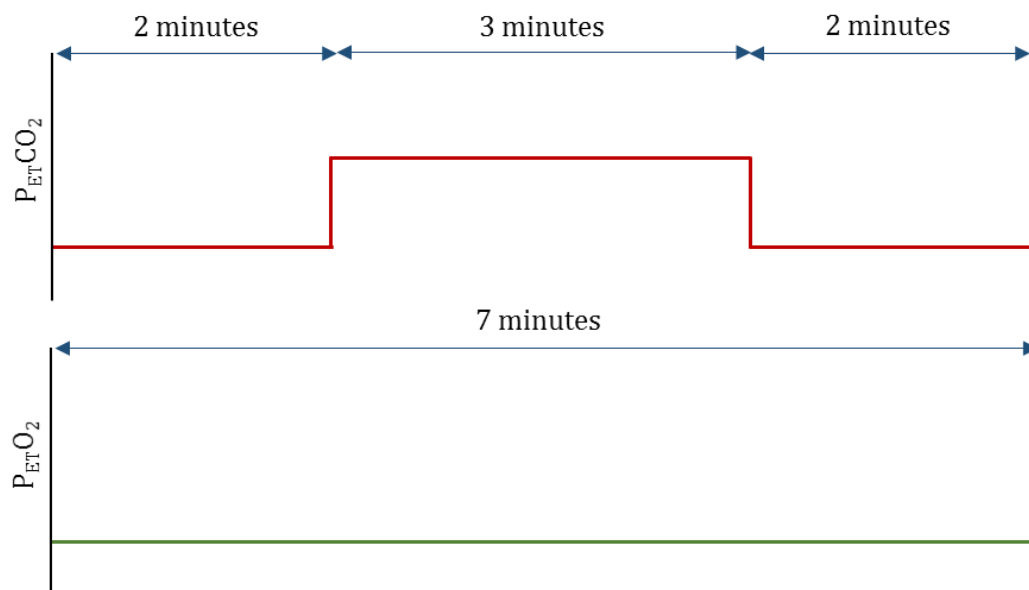


Figure 7-18: Schematic of the gas challenge. $P_{ET}CO_2$ was targeted at 8mmHg above the subject specific baseline for the period of induced hypercapnia, while $P_{ET}O_2$ was targeted to remain constant.

7.6.3 Measuring Brain Function: MRI acquisition

Data were acquired on a 7.0 T Philips Achieva MRI scanner using a Nova 32-channel head receive coil and transmit coil. Before the subject entered the scanner, their maximum grip strength was measured for each hand using the

handgrip dynamometer and the EMG electrodes were also placed on the surface of extensor digitorum muscle of each arm. In addition, for each subject their baseline $P_{ET}CO_2$ and $P_{ET}O_2$ were recorded by the RespirAct system. This entire procedure took approximately 45 minutes to complete. A pulse oximeter monitored the subject's heart rate throughout the scanning protocol.

7.6.3.1 Assessing the functional response to the handgrip challenge

Initially a handgrip localiser scan was performed comprising a baseline of 7 s OFF period followed by four repeats of a 35 s ON period during which subjects underwent a 30 % MVC and a 56 s OFF period on their right hand. During this 2 mm isotropic axial-oblique GE-EPI BOLD fMRI scans were acquired (TE = 25 ms, TR = 2.5 s, 96 x 96 matrix, 30 slices) with spatial coverage of 60 mm in the foot-head direction to identify the sensorimotor cortex activated in response to the handgrip task using real-time IViewBOLD analysis.

Subjects then performed the main handgrip task, as described in Section 7.6.1, during which cerebral perfusion and BOLD activation were simultaneously assessed using a Look Locker-DABS (LL-DABS) sequence [43]. This is a 'patched' scan which was developed at 7T for the simultaneous assessment of ASL perfusion and BOLD data (see Chapter 4 Section 4.6). The LL-DABS acquisition had limited slice coverage (7 slices) and so was planned based on the active sensorimotor region identified from the handgrip localiser task. The LL-DABS acquisition comprised ASL and BOLD data collected using a GE-EPI readout, with 7 slices of a FOV of 192 x 192 mm², in-plane spatial resolution of 2 mm, and a slice thickness of 3 mm, and a SENSE factor of 2.5. The sequence comprised 6 phases, with an initial delay of 400 ms and spacing of 350 ms. The first 5 phases were used to generate ASL images whilst the final 6th phase provided BOLD contrast. A TR of 3.5 s was used per label/control pair. Throughout the handgrip protocol 78 ASL label and control pairs were acquired. The handgrip challenge and acquisition of LL-DABS data was performed on both left and right hands.

7.6.3.2 Assessing the functional response to the hypercapnic challenge

Subjects then performed a hypercapnic challenge using the RespirAct system. During the hypercapnic challenge, an identical LL-DABS data acquisition to that used in the handgrip task was used to collect ASL perfusion and BOLD data, thus providing a measure of Cerebral Vascular Reactivity (CVR). During the hypercapnia protocol, 60 dynamics of the LL-DABS sequence were collected.

7.7 IMAGE ANALYSIS

7.7.1 Muscle Measures

7.7.1.1 Muscle CSA

Muscle cross-sectional area was assessed using data acquired by an in-phase and out-of-phase gradient echo sequence. A complex subtraction was performed to yield contrast between fat and water. ROI interrogation was then performed using NeuRoi, a free software package available from <http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx>. Care was taken to ensure analysis was performed in a consistent slice across all four visits using the anatomical marker as a reference. Example ROI analysis for one subject can be seen in Figure 7-19.

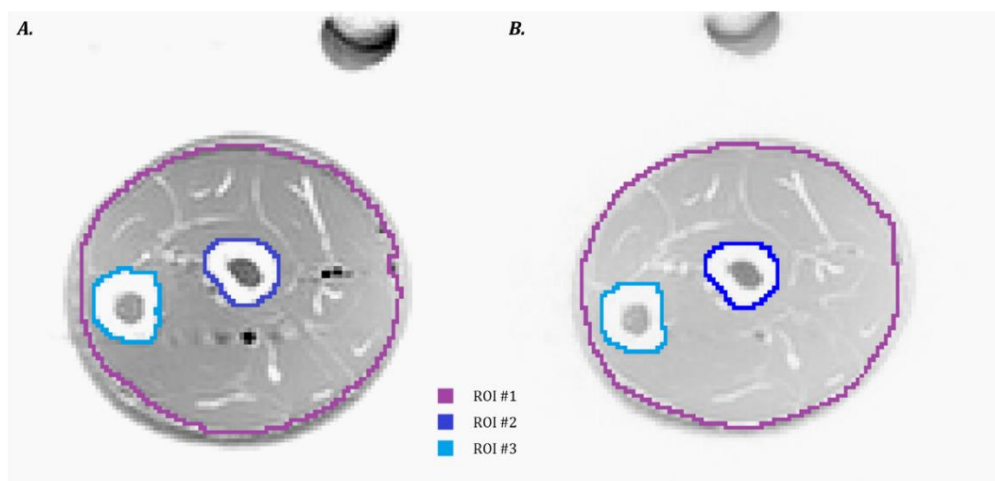


Figure 7-19: An example of ROI interrogation performed using the NeuRoi software package. ROI #2 and ROI #3 were subtracted from ROI #1 to account for the radius and ulna bones. The anatomical marker ensured consistent positioning of CSA measures across the study. A comparison between **A.** Visit 1 and **B.** Visit 3 can be seen.

7.7.1.2 MRS: EMCL and IMCL quantification

Forearm lipid spectroscopy data were processed using the fibre orientation model outlined in Section 7.2.1.3. Prior to fitting, phase correction and frequency alignment were applied manually. Zero order phase was corrected until all peaks were positive, judged visually by the experimenter (Figure 7-20). Shifts in resonance frequency between successive spectra were also manually corrected by aligning the dominant EMCL peak before averaging repeated TE's.

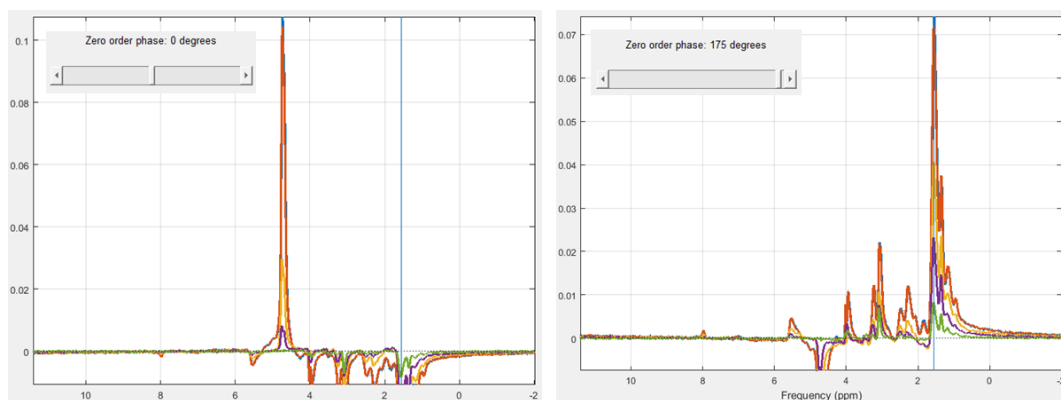


Figure 7-20: Zero order phase correction on ^1H MRS lipid spectra.

Following phase correction and frequency alignment, the EMCL and IMCL CH_2 signals were selected and the left and right extremes of the peaks of interest were defined to aid the fitting algorithm.

7.7.2 Behavioural Measures: Isometric Contraction and EMG analysis

On each scan day isometric grip strength was determined during a maximal voluntary contraction (MVC) manoeuvre. During the repeated hand grip fMRI task force generation during contraction at 15% and 30% MVC was continuously recorded on a VAS scale. These measures were compared between baseline and Day 16.

In addition, EMG data were collected during the handgrip acquisition, with the aim to assess muscle activity. EMG data was processed using Brain Vision Analyzer software (Version 2). The EMG difference signal between each pair of active electrodes were corrected for scanner artefacts using the average gradient artefact correction method [44] and applying a high-pass filter with a

40 Hz cut off to remove movement artefacts, and then rectified (Brain Vision Analyzer version 2.0). The three 15% and three 30% VCs resulted in six EMG responses per scan for each hand. For each scan, the average EMG response to the 15% and 30% MVCs was calculated. The amplitude, area (calculated from the integral of the EMG response over its duration) and time-to-peak of the EMG response for each cycle was calculated. However, due to scanner artefacts it was found that the EMG data collected in the 7T scanner during the task was not a reliable measure, this will be outlined in the Section 7.8.

7.7.3 Analysis of the Response to Handgrip Contraction

fMRI data analysis was performed using MATLAB and Statistical Parametric Mapping Software (SPM5; Wellcome Department of Imaging Neuroscience, London, UK) software.

The handgrip localiser task was spatially realigned, spatially smoothed with a 4 mm full-width at half-maximum (FWHM), and temporally filtered with a cut-off of 182 s (twice the cycle length). A general linear model (GLM) was then used to model the sensorimotor response to the task, including motion parameters as covariates of no interest.

The handgrip task LL-DABS ASL data was first separated into the BOLD (phase 6) and ASL data (phases 1-5) for the label and control pairs. The label and control pairs of the BOLD data was then linearly interpolated (Interp function, Matlab, Mathworks USA) to an effective TR of 3.5 s. The BOLD time series was then analysed using SPM5. Data was spatially realigned, spatially smoothed using a Gaussian filter with FWHM of 4 mm and temporally filtered.

For each subject, a general linear model (GLM) statistical analysis was performed using the handgrip trace convolved with a canonical haemodynamic response function (HRF) as an input in the GLM. Statistical parametric maps of the pattern of brain activity in response to the handgrip were formed for each of hands with results displayed at FWE corrected probability $P < 0.05$. For the active BOLD region, the mean of beta-value parameters estimates (indicating the strength of the BOLD response, see Chapter 4) was assessed in the sensorimotor cortex, and a paired t-test

performed to assess any significant differences in response between Day 0 and Day 16.

Label-control pairs of ASL data were subtracted to create a time series of perfusion weighted images for each phase. For the active BOLD region (FWE corrected $P < 0.05$), the mean perfusion value was assessed by fitting the mean ASL data at each of the phases at rest and during the handgrip task to a multi-compartmental model (see Chapter 4 for details).

7.7.4 Analysis of the Response to Hypercapnia

Data was restricted to the same seven slices covering the hand sensorimotor area as collected for the handgrip task.

Pre-processing followed a similar analysis path to that described in Section 7.7.3. In brief, data was first separated into the BOLD (phase 6) and ASL (phases 1-5) data for the label and control pairs, and the label and control pairs of the BOLD data linearly interpolated (Interp function, Matlab, Mathworks USA) to an effective TR of 3.5 s. The BOLD time-series was then realigned to the first dynamic volume.

Analysis was then performed on the BOLD data to generate a BOLD CVR map. The time-course of each voxel was first normalized to its mean value and multiplied by 100 to give the voxel's %BOLD time-course. The $P_{ET}CO_2$ timecourse from the RespirAct system was sampled to the same temporal frequency as the MR data and then convolved with a canonical haemodynamic response function (HRF). To calculate each voxel's BOLD CVR, for each voxel a linear regression was performed between the %BOLD signal and the $P_{ET}CO_2$ to form CVR maps. The CVR within the sensorimotor strip was then assessed using a sensorimotor ROI defined from the Harvard-Oxford cortical and subcortical structural atlases MNI atlas (FSL, FMRIB, Oxford, UK), shown in Figure 7-21.

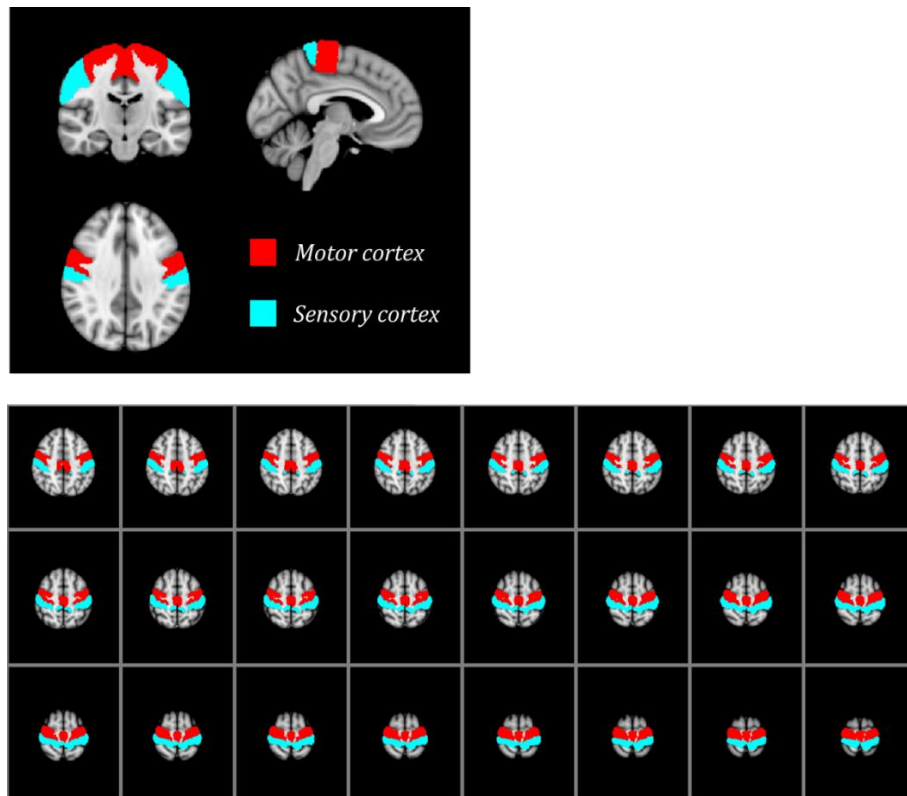


Figure 7-21: The motor and sensory ROIs showed in MNI space.

These motor and sensory ROIs from the MNI space were transformed into the seven slice CVR space using the pipeline shown in Figure 7-22 using FLIRT (FSL, FMRIB, Oxford, UK). The median CVR was then assessed in the sensorimotor cortex for each subject.

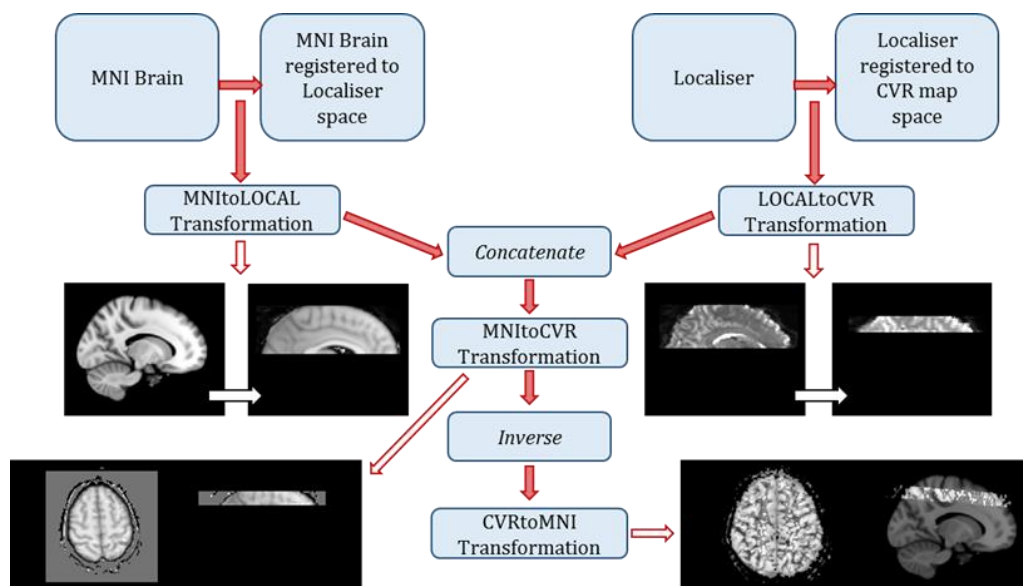


Figure 7-22: Schematic of the analysis pipeline to move between CVR data space and MNI space.

7.8 RESULTS

7.8.1 Assessing Muscle Strength and Cross-Sectional Area

Table 7-2 shows each subjects' MVC force at Day 0 and Day 16 for the 5 volunteers that completed the study. In the immobilised limb there was a significant loss in strength ($-21 \pm 2 \%$, $p = 0.03$), while no significant loss in strength was seen in the non-immobilised limb ($p = 0.43$).

	<i>Non-immobilised arm strength (Nm)</i>		<i>Immobilised arm strength (Nm)</i>	
	<i>Day 0</i>	<i>Day 16</i>	<i>Day 0</i>	<i>Day 16</i>
Subject 1	392.3	399.3	392.3	319.4
Subject 2	433.4	441.8	510.0	391.4
Subject 3	529.6	525.4	525.4	397.6
Subject 4	470.7	474.1	364.0	274.8
Subject 5	500.1	516.0	500.1	420.0
Strength change (%MVC) Average ($\pm$SEM)	<i>1.7 (± 0.5)</i>		<i>-21 (± 2)</i>	

Table 7-2: Muscle strength (Nm), as calculated from maximum voluntary contraction, for the immobilised and non-immobilised arm at Day 0 and Day 16, and percentage change in MVC strength.

Figure 7-23 shows individual forearm CSA measures across the 16 day study and the average change across subjects. Overall, a decrease in muscle CSA can be seen in the immobilised arm ($-3.6 \pm 0.8 \%$, $p = 0.005$) while there was no significant change in the non-immobilised arm ($P = 0.09$).

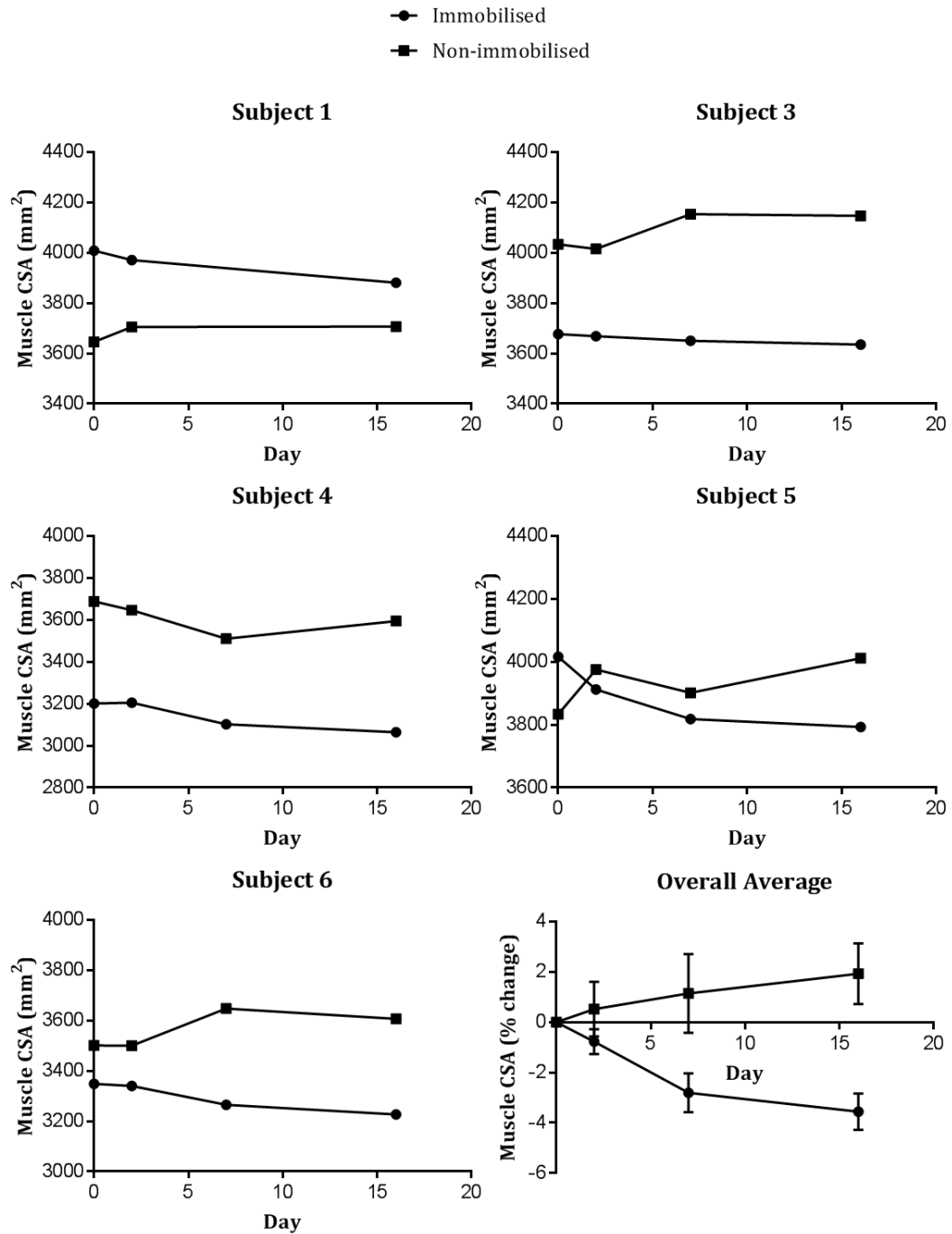


Figure 7-23: Individual forearm muscle cross-sectional areas and the average percentage change across 16 days of upper-limb immobilisation.

7.8.2 Assessing Lipid Accumulation using MRS

^1H MRS lipid spectroscopy data was highly variable across study visits. Example spectra and their associated fit can be seen in Figure 7-24.

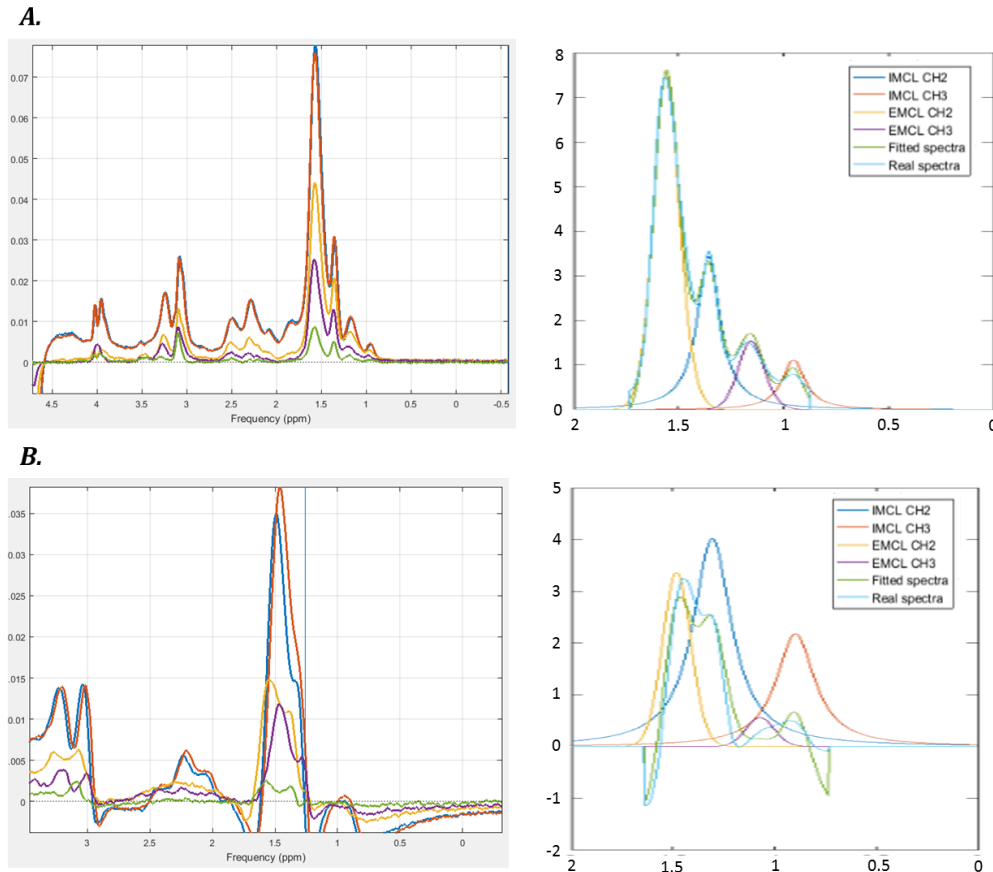


Figure 7-24: Example phase corrected spectra and their associated fit, accounting for orientation and dispersion of EMCL strands, for **A.** well resolved lipid peaks and **B.** poor quality data.

For 4 subjects, insufficient data were available at all time points to allow trends to be determined. Spectra were complete and of good quality for one subject. Figure 7-25 shows longitudinal MRS data of EMCL concentration (A), IMCL concentration (B) and EMCL:IMCL ratio (C) in the FDP across the 16 day immobilisation. After an initial decrease in both EMCL and IMCL concentration in the immobilised arm, IMCL concentration increases. After a small increase in EMCL and IMCL concentration in the non-immobilised, lipid concentration remains relatively constant. Water T_2 could be measured in all subjects and was found to steadily increase in the immobilised arm across the study while there was no significant change in the non-immobilised arm (Figure 7-25D).

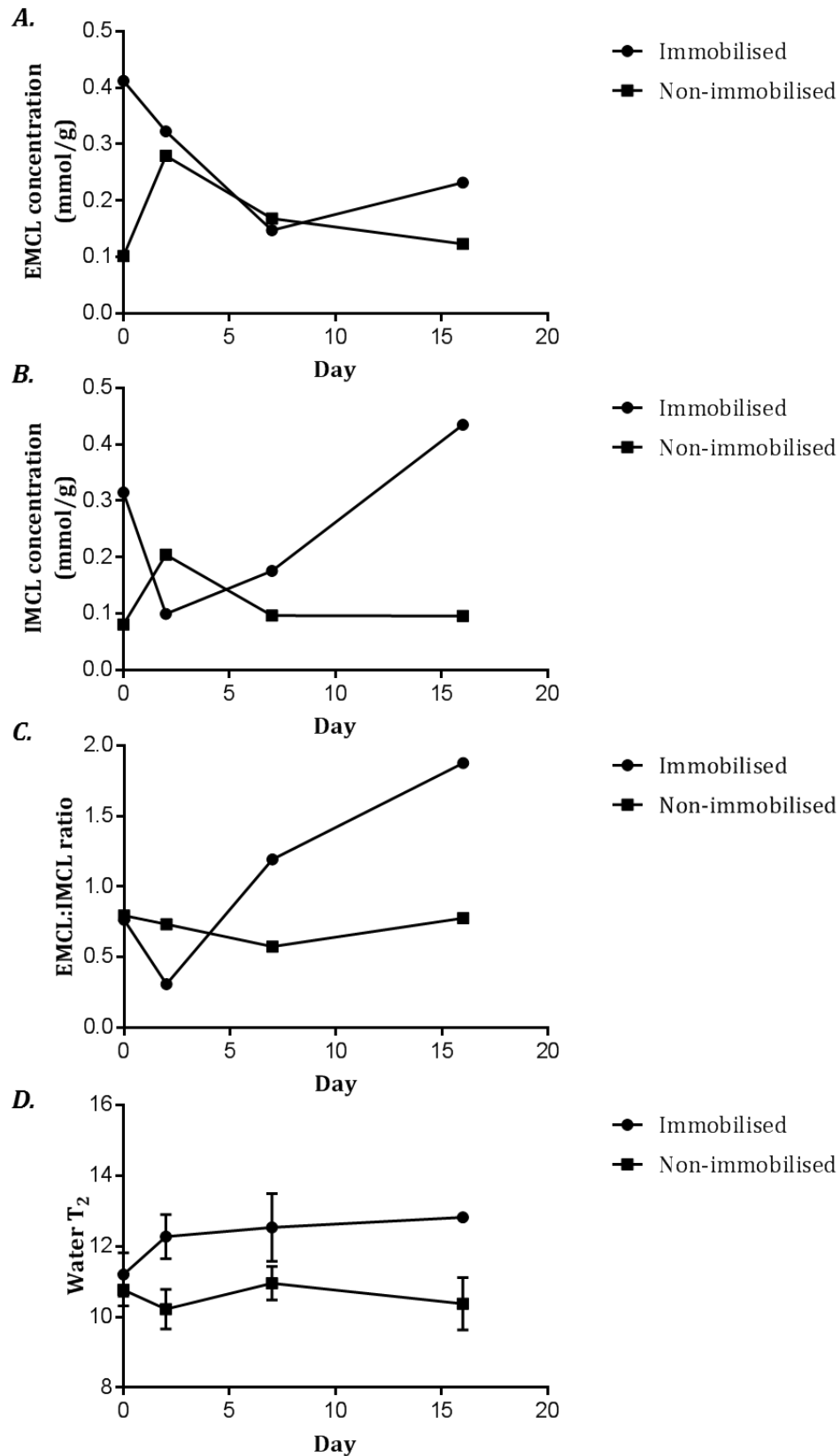


Figure 7-25: *A. EMCL concentration B. IMCL concentration and C. EMCL:IMCL ratio in the FDP of one subject and D. average water T_2 across all subjects over the 16 day study.*

7.8.3 Brain Function on Immobilisation

7.8.3.1 Cortical response to the handgrip task

Figure 7-26 shows the cortical activity to the right hand handgrip localiser at 30% MVC in an example subject. This highlights the strong response in the contralateral (L) sensorimotor cortex, weaker response in ipsilateral (R) sensorimotor cortex, and activity of the supplementary motor area. This was used to plan the coverage of the imaging stack for the LL-DABS acquisition.

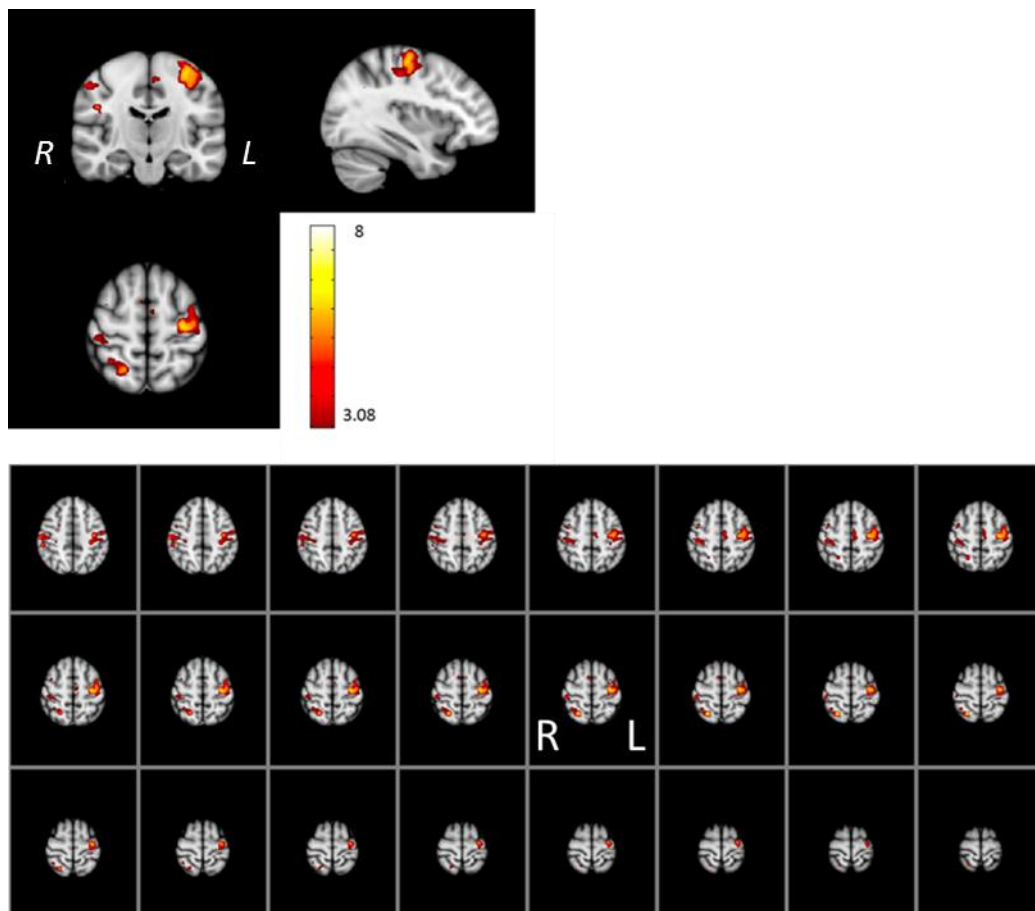


Figure 7-26: Cortical response to the right hand handgrip localiser task at 30% MVC in Subject 5. A strong response in contralateral sensorimotor cortex is seen.

Figure 7-27 shows the task force generation recorded on the VAS scale during the fMRI handgrip task for the non-immobilised and immobilised arm pre- and post- immobilisation (Day 0 and Day 16).

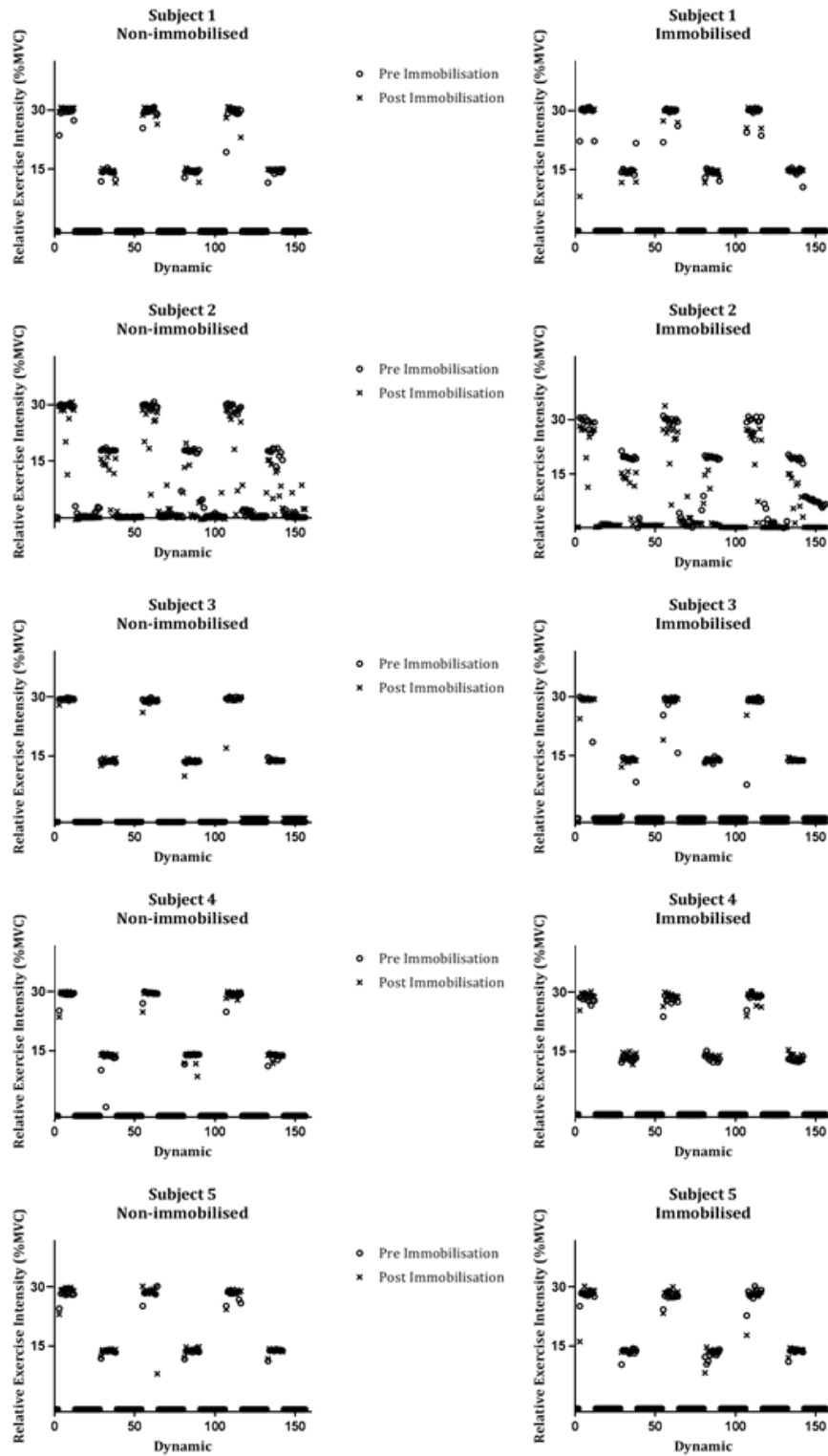
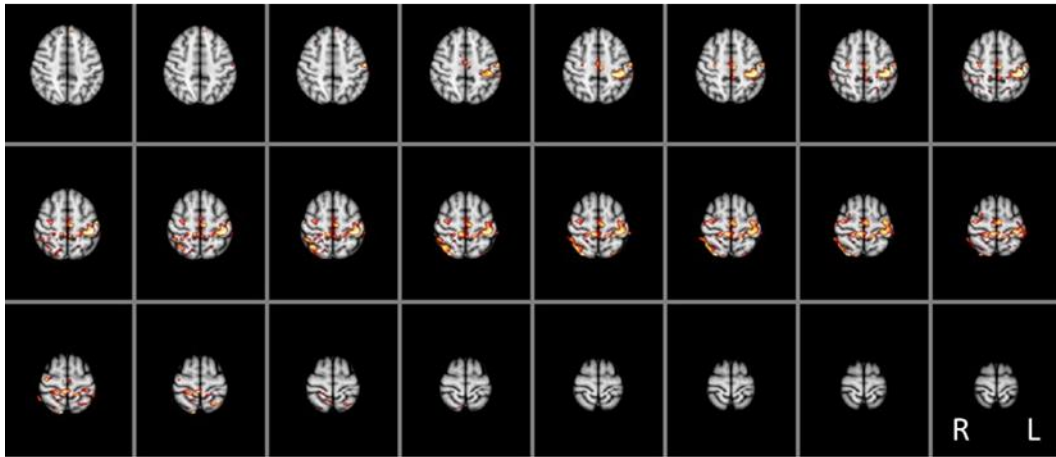


Figure 7-27: Handgrip feedback responses for the non-immobilised and immobilised arm pre- and post-immobilisation, at Day 0 and Day 16.

It can be seen that all subjects completed the task satisfactorily, reaching the targeted 15% and 30% MVC levels for both visits on the immobilised and non-immobilised arms.

Figure 7-28 shows the fMRI response in an example subject (Subject 5) to the handgrip task for the dominant and non-dominant arm at Day 0.

A. Dominant Arm



B. Non-dominant Arm

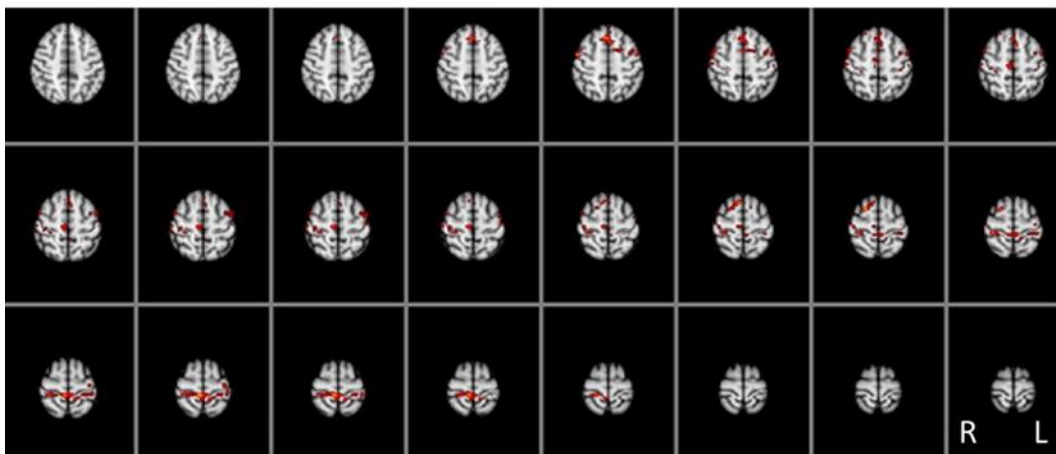


Figure 7-28: fMRI response in an example subject (Subject 5) to the handgrip task for the **A.** dominant and **B.** non-dominant arm.

Figure 7-29 plots the beta values in the contralateral sensorimotor cortex activated in response to the handgrip task for the non-immobilised and immobilised arm at Day 0 and Day 16. It can be seen that on Day 16 the fMRI response is lower for both arms (non-immobilised $P=0.2$, immobilised $P=0.09$).

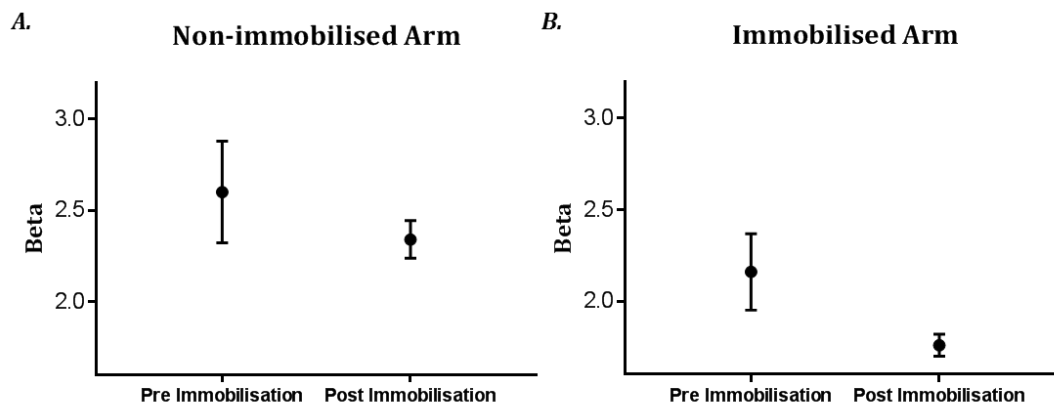


Figure 7-29: Pre- and post-immobilisation beta values in BOLD contralateral sensorimotor cortex in response to the handgrip task for the **A.** Non-immobilised (dominant) and **B.** immobilised (non-dominant) arm.

It can be seen that the beta response is greater in the contralateral hemisphere to the non-immobilised (dominant) arm compared to the immobilised (non-dominant) arm ($P=0.04$). There was no significant difference in the beta value to the hand grip task for the non-immobilised arm between Day 0 and Day 16 ($P=0.2$), for the immobilised arm there was a trend for reduction in beta value post-immobilisation ($P=0.09$).

Figure 7-30 shows example ASL images collected using the LL-DABS sequence across each ASL phases for each of the seven slice collected.

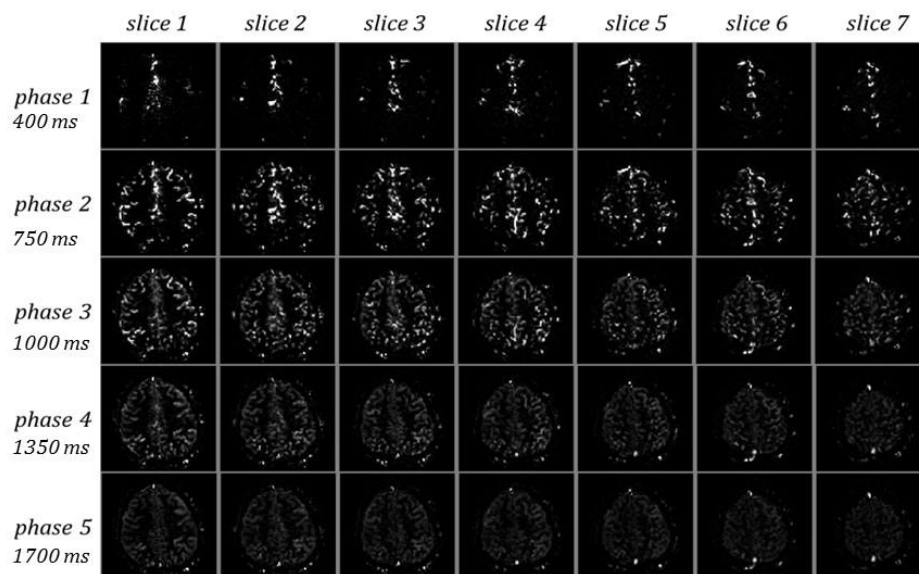


Figure 7-30: Example ASL images collected across a range of post-label delay times using the LL-DABS scheme.

Figure 7-31 plots the perfusion in the contralateral sensorimotor cortex activated in response to the handgrip task for the non-immobilised (dominant) and immobilised (non-dominant) arm at Day 0 and Day 16. There was no significant difference in the change in perfusion to the hand grip task for the immobilised arm between Day 0 ($33 \pm 12 \%$) and Day 16 ($24 \pm 5 \%$). Similarly, no significant difference was found in the non-immobilised arm (Day 0: $43 \pm 14 \%$, Day 16: $43 \pm 14 \%$). It can however be seen that the perfusion response is greater in the contralateral hemisphere to the dominant (non-immobilised) arm compared to the non-dominant (immobilised).

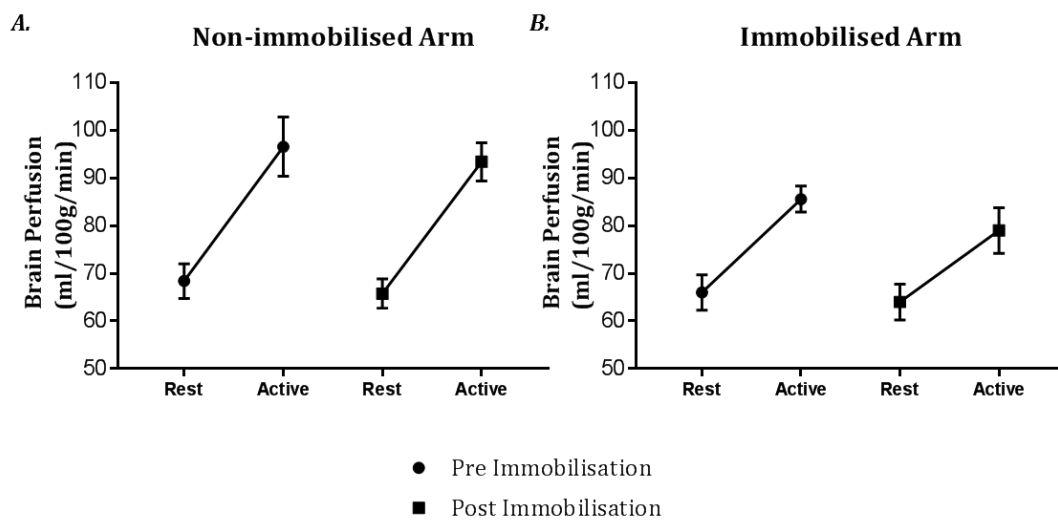


Figure 7-31: The difference in perfusion response during the hand grip task between Day 0 and Day 16 for the **A.** non-immobilised arm and **B.** immobilised arm.

7.8.3.4 Cortical response to hypercapnia

Figure 7-32 shows an individual subjects' $P_{ET}CO_2$ time-course. Table 7-3 provides the baseline $P_{ET}O_2$ values, baseline and hypercapnic $P_{ET}O_2$, and step change in $P_{ET}CO_2$ achieved for each subject at both visits. Individual subjects' $P_{ET}CO_2$ time-course data is shown for Day 0 and Day 16 in the Appendix.

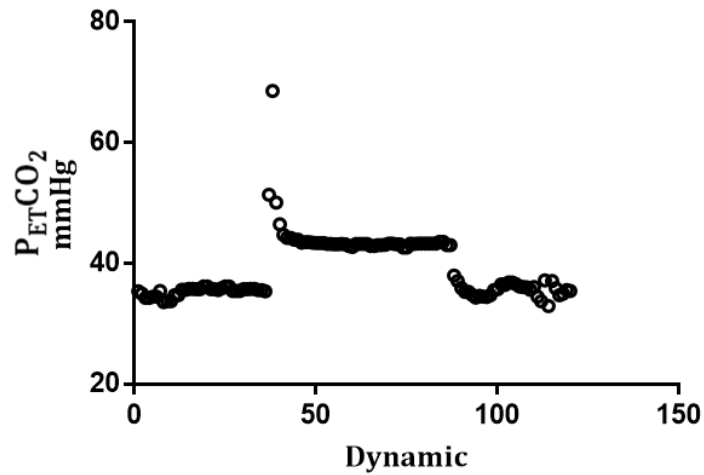


Figure 7-32: An example of an individual subjects' $P_{ET}CO_2$ trace for the hypercapnia challenge.

		$P_{ET}O_2$ (mmhg)	Baseline $P_{ET}CO_2$ (mmhg)	Hypercapnic $P_{ET}CO_2$ (mmhg)	Change in $P_{ET}CO_2$ (mmhg)
Subject 1	Visit 1	110.6	35.2 (0.1)	41.6 (0.3)	6.4
	Visit 4	110.3	31.2 (0.5)	37.4 (0.3)	6.2
Subject 2	Visit 1	110.0	43.5 (0.3)	57.4 (0.1)	13.9
	Visit 4	110.4	46.2 (0.6)	51.9 (0.1)	5.7
Subject 3	Visit 1	110.6	36.7 (0.1)	39.9 (0.2)	3.2
	Visit 4	111.2	41.6 (0.1)	48.9 (0.1)	7.3
Subject 4	Visit 1	109.7	35.3 (0.1)	44.2 (0.5)	8.9
	Visit 4	110.4	36.4 (0.2)	44.1 (0.1)	7.7
Subject 5	Visit 1	110.5	45.5 (0.2)	49.3 (0.3)	3.8
	Visit 4	110.3	42.1 (0.3)	47.9 (0.2)	5.8

Table 7-3: Baseline $P_{ET}O_2$ values, baseline and hypercapnic $P_{ET}O_2$, and step change in $P_{ET}CO_2$ achieved for each subject at each visit (mean and standard error).

An individual subjects' BOLD CVR map is shown in Figure 7-33 (Subject 5). As expected, grey matter CVR is larger than white matter CVR due to higher basal cerebral blood volume.

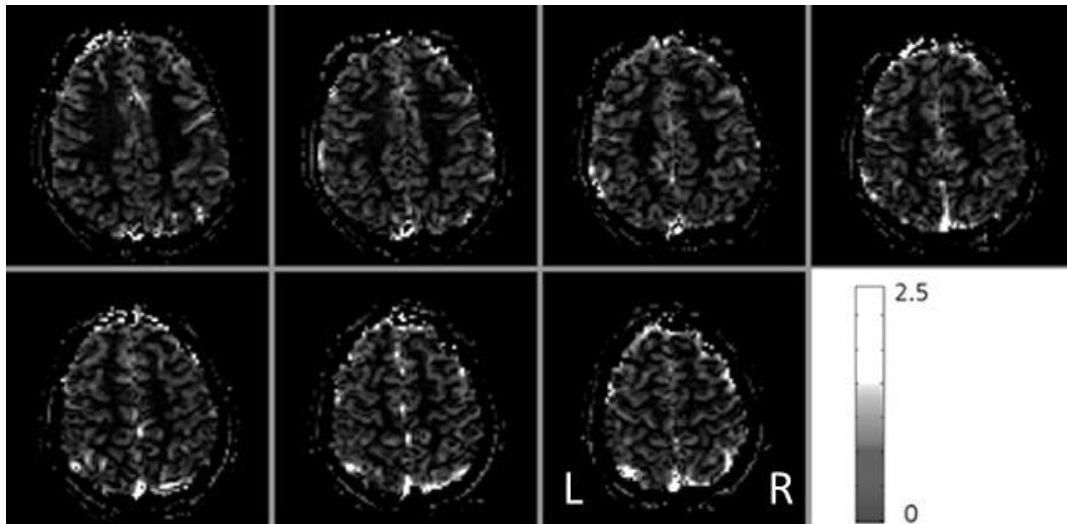


Figure 7-33: Example BOLD CVR map acquired at Day 0 windowed between 0 and 2.5%/mm Hg showing the clear contrast between grey matter and white matter.

Figure 7-34 shows the CVR measured in the sensorimotor cortex at baseline and at Day 16 for the non-immobilised and immobilised arm. No significant difference in CVR was found between Day 0 and Day 16 for either arm. The absolute CVR value can be seen to be greater for the non-immobilised arm compared to the immobilised arm.

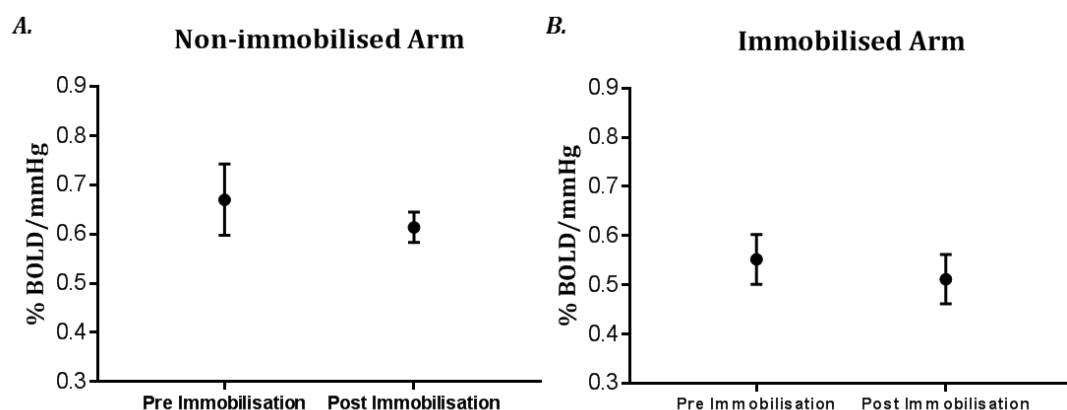


Figure 7-34: CVR values in sensorimotor cortex pre and post-immobilisation for the **A.** immobilised and **B.** non-immobilised arm.

7.9 DISCUSSION

This study has used magnetic resonance spectroscopy and imaging techniques to assess changes in muscle function and brain motor cortex responses after 16 days of upper limb immobilisation. There was a significant loss in strength in the immobilised arm, while no significant change was seen in the non-immobilised arm. Similarly, there was a loss in muscle cross-sectional area between Day 0 and Day 16 in the immobilised arm, with most of the change occurring in the first six days. However the loss in strength far outweighed the loss in area, confirming previous findings [20].

7.9.1 The Impact of Immobilisation on Muscle Function

The T_2 of water increased in the immobilised arm, while no significant changes were found in the non-immobilised arm. This is likely due to changes in oxygenation, microstructure or metabolism. Future work should use magnetic susceptibility, magnetisation transfer or MRS to investigate this further. The separation of IMCL and EMCL was challenging. ^1H MRS data was acquired at four time points across the 16 day study to provide a novel longitudinal insight into inactivity induced lipid accumulation with potential connotations to the onset of insulin resistance. Spectroscopy techniques were developed to optimise SNR and chemical shift resolution, and account for the orientation and dispersion of muscle fibres. However, despite these efforts, multiple data sets had to be discarded due to poor quality spectra. In one subject with good quality data, it could be seen that after an initial rapid decrease in lipid concentration, IMCL content increased while EMCL content remained below baseline. On Day 7, IMCL content remained below baseline, however by Day 16, there was a 38 % increase from Day 0. This highlights the importance of taking longitudinal data when studying the impact of immobilisation on lipid accumulation and may suggest why previous findings have been inconsistent. Of course the results from the MRS data collected in this study have no statistical significance due to the issues with the acquired spectra. Past studies investigating lipid accumulation with MRS have focussed on lower limb muscle groups allowing for large volume acquisition and reducing the risk of contamination of water and adipose signal by misplacement of the voxel or

motion. Due to recruitment issues, this study was constrained to upper limb immobilisation and therefore there was SNR was inherently lower. Moreover, when collecting data in small limbs, susceptibility artefacts can arise from the bone/muscle interface resulting in further limb broadening. Future work should acquire initial diffusion tensor imaging and B_0 maps to select volumes with uniformly orientated fibres and good field homogeneity.

Additionally, when imaging the forearm at ultra-high field, patient comfort is compromised, increasing the risk of motion artefacts. In this study, subjects were positioned headfirst and prone during MRS acquisition. A commonly reported side effect experienced while moving in and out of the bore of an ultra-high field scanner is nausea and dizziness, termed vertigo. It has been hypothesised that this is due to the static magnetic field inducing a Lorentz force which acts on the vestibular system of the inner ear [45]. The extent of vertigo experienced is highly dependent on the subject, however it has been suggested that the maximum vertigo response might be while lying prone with the head pitched upwards [46]. Indeed, 4 out of 9 subjects recruited to this study reported a feeling of dizziness, with two subjects having to be excluded due to extreme nausea. Future work should use specific methods of subject positioning to reduce vertigo based on knowledge on how the field interacts with the inner ear.

7.9.2 The Impact of Immobilisation on Brain Function

fMRI data was collected to assess the impact of immobilisation on brain motor cortex response.

A handgrip task at 15 % and 30% MVC was performed with both arms at Day 0, and repeated after 16 days of immobilisation of one arm, with the functional response assessed across visits. All subjects completed the task satisfactorily reaching the target levels of handgrip for both immobilised and non-immobilised arms.

At Day 0, pre-immobilisation, the fMRI data showed an increased BOLD response in the contralateral sensorimotor cortex to the dominant hand compared to the BOLD response in the contralateral hemisphere to the non-

dominant hand. This is in agreement with previous studies which have showed an increase in BOLD amplitude [47, 48] and/or BOLD spatial extent [36, 48-51] in the contralateral hemisphere to the dominant hand compared to non-dominant hand. Assessing the beta value of the BOLD response at Day 0 and Day 16 for the immobilised and non-immobilised arm, it was shown that for the immobilised arm there was a trend for reduction in beta value post-immobilisation ($p=0.09$). Assessing the perfusion change in the BOLD regions, there was no significant difference in the change in perfusion to the hand grip task for immobilised arm between Day 0 and Day 16. It can however be seen that, in agreement with the BOLD response, the perfusion change in response to the handgrip task was greater in the contralateral hemisphere to the dominant arm compared to the non-dominant.

Data was collected to assess the CVR response. The hypercapnic paradigm did show variability between the scan visits, $P_{ET}CO_2$ was targeted at a 8 mmHg step but the range of change achieved was between 3.2 -13.9 mmHg. However, since the data was analysed using each subjects individual $P_{ET}CO_2$ time-course recorded from the RespirAct system to calculate each voxel's %BOLD/mmHg, this variability was taken into account in this calculation. Comparing CVR responses between Day 0 and Day 16, no significant difference was found pre- and post-immobilisation, though the absolute CVR value can be seen to be greater for the non-immobilised arm compared to the immobilised arm.

There are limitations of this study. Recruitment was targeted at 10 subjects, and nine subjects participated. However, a number of subjects dropped out part-way through the study, 3 subjects due to adverse effects and 2 due to scanner failure. This resulted in data from 5 subjects, limiting the statistical power to detect any changes.

It should be noted that initially it was intended that the dominant arm would be immobilised, as was the case for the first subject. However due to recruitment difficulties, after subject 1 all following subjects had their non-dominant arm immobilised. This may lead to spread in the data. Data from this pilot has demonstrated larger responses to the handgrip task and greater CVR in the contralateral hemisphere to the dominant arm, suggesting that a future

studies should immobilise the dominant arm to allow maximum sensitivity to change. Notably, in the study by Langer et al. [21] all subjects were right handed, and their dominant right arm was immobilised.

In future, if data were collected on a larger subject group it would be of interest to combine CVR assessment with functional analysis of BOLD and ASL data to the handgrip task to provide an estimation of the cerebral metabolic rate of oxygen (CMRO₂) changes associated with the handgrip task. To overcome the intensive scan protocol for the subjects, it would be of interest to collect data on structural measures alone (as described in Chapter 6) and performed by Langer [21], rather than collect muscle and brain functional data together, this would provide a simple protocol to carry out in a large cohort. Further, in the study of Langer et al [21], diffusion tensor imaging (DTI) data was collected in which they showed a reduction in fractional anisotropy (FA) in the contralateral hemisphere to the dominant immobilised arm. They suggested this resulted from a change in the efferent or afferent fibre tract and neuronal firing. The trend of the data collected in this chapter to show a reduction in BOLD response in the immobilised arm supports this finding, together with no change in CVR, suggesting this effect is neuronal rather than associated with haemodynamic remodelling.

7.10 CONCLUSION

Muscle immobilisation causes a loss in muscle mass but a relatively greater loss in muscle function. There was a decrease in the muscle tissue, as assessed by MRI T₂ measurements, across 16 days immobilisation, allied with a trend for a decrease in BOLD response, as assessed by fMRI, while there was no change in CVR. This suggests that the disproportionate loss in muscle function relative to loss in muscle mass manifests itself as a change within the central nervous system, rather than as an adaptation of the cerebrovascular architecture.

7.11 REFERENCES

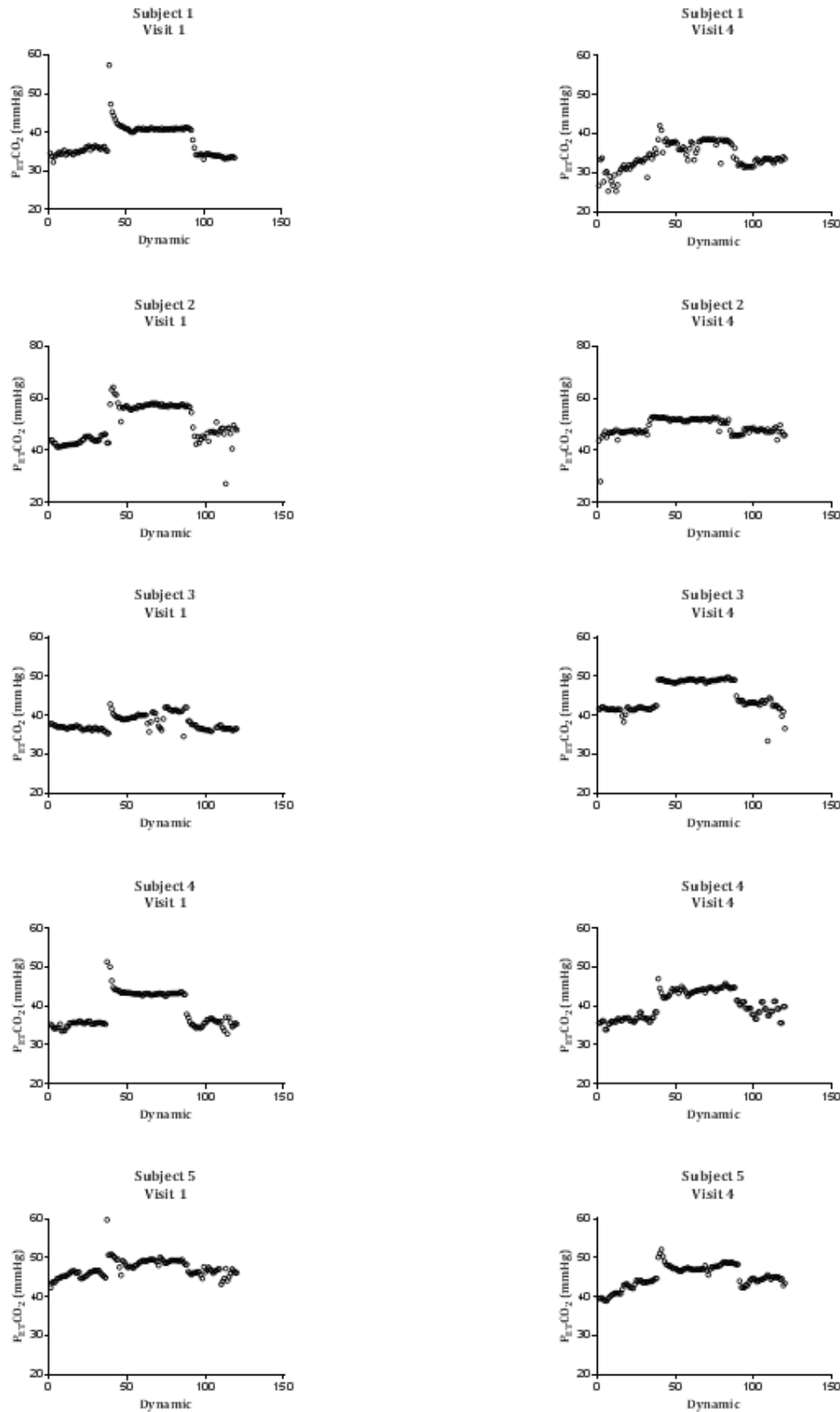
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7.12 APPENDIX

Individual subject hypercapnia response.



8. General Discussion

8.1 OVERVIEW

The work presented in this thesis aimed to provide a more comprehensive understanding of brain structure and haemodynamic and metabolic responses to acute exercise in the context of ageing and limb immobilisation.

In Chapter Five the cortical haemodynamic and metabolic responses to an acute low/moderate intensity exercise challenge within a MRI scanner were assessed to determine how this is influenced by normative ageing in healthy volunteers. The challenges of performing acute exercise within a MR scanner environment were outlined, showing that exercise studies are possible at low to moderate intensities equivalent 30% and 50% of maximal oxygen uptake. Measures of cerebral blood flow, regional perfusion, brain activation and oxygen utilisation were collected at rest, during low to moderate intensity exercise and on recovery in young and older adults. Results demonstrated a clear deficit in CBF in older adults compared to young during submaximal exercise (30 and 50% $\text{VO}_{2\text{max}}$). Cerebral blood flow, which was no different between age groups at rest when correcting for grey matter volume, increased upon exercise but was less in the older group across the exercise task. These data confirmed the observations of TCD studies examining MCA V_{mean} in populations of a similar age at similar exercise intensities. Importantly, the advance made with the work presented here is that MRI allowed for normalisation of CBF values to account for grey matter volume differences with age, and illustrated that after correcting CBF for GM volume the age related CBF deficit on exercise persists. Results from this study suggest that the observed age-related attenuation in CBF on exercise is independent of age-related reductions global brain atrophy. Alternative mechanisms behind the CBF deficit observed in older adults on exercise may, in part, be due to decreased cardiac output with age. A reduction in cardiac output together with increased demand of blood from the muscle on exercise may compromise brain perfusion. Importantly, oxygen extraction was found to be significantly

higher in older adults, suggesting that the ageing brain attempts to compensate for decreased CBF.

Chapter Six described the use of structural MRI to investigate global and regional grey matter volume and cortical thickness in young and older adults, and to assess their associations with cardiorespiratory fitness. Changes in global grey matter volume were assessed with both ROI analysis and voxel-based morphometry, and cortical thickness changes were measured with surface based morphometry. Importantly, these structural data were associated with direct measurement of maximal oxygen uptake (VO_{2max}) during supine exercise, rather than self-reported physical activity scores or estimated VO_{2max} . Data demonstrated a significant reduction in grey matter volume and cortical thinning with age in the inferior, middle and superior frontal cortex, occipital, and parietal lobes, and putamen, cerebellum and insula. But they also revealed a significant positive correlation between global grey matter volume and VO_{2max} in the precentral cortex, putamen, thalamus, and cerebellum, across the study age range. Global cortical thickness was significantly less in older compared to younger volunteers, and a significant positive correlation between VO_{2max} and cortical thickness in the precentral and postcentral cortex, middle temporal cortex, middle and superior frontal cortex, and superior parietal cortex was observed. The positive association revealed between grey matter volume and cortical thickness with VO_{2max} , shown in the spatial maps, suggests that there is plasticity in defined brain regions which were found to be diminished in grey matter volume and cortical thickness with age. This included both sensorimotor areas (precentral and postcentral cortex) involved in motor function, and brain regions involved in executive function, such as the middle/superior frontal cortex and superior parietal cortex. Of importance, these findings clearly suggest that maintaining cardiovascular fitness across the life-course may preserve the integrity of primary motor and sensory brain regions, and also other high order cortical areas of the brain which are susceptible to cortical thinning and volume loss with ageing. Intervention studies to test this suggestion should be a priority for future research.

Chapter Seven described the development of methods to study the effect of immobilisation on skeletal muscle and brain function using ultra-high field, 7T MRI. MR spectroscopy and imaging techniques were developed for 7 Tesla to investigate muscle lipid accumulation and changes in brain motor cortex architecture and activation following a 16-day upper limb immobilisation. For the MRS data, the motivation was to use the high spectral sensitivity that is achieved when collecting data at ultra-high field. However, despite developmental efforts to improve signal-to-noise and chemical shift resolution of the lipid signal, the spectra acquired across the study were highly variable for all subjects and multiple data sets had to be discarded. This highlighted the difficulty of applying ^1H lipid spectroscopy to small, upper-limb muscles.

In accordance with previous literature, it was found that lower limb immobilisation induces a major decline in maximal strength which far outweighs any decrease in muscle cross-sectional area. In the immobilised arm there was a significant loss in strength over the 16 day immobilisation period, while no change in strength seen in the non-immobilised arm. This suggests that the mechanisms underlying reduced strength may reside in the central nervous system. To assess this, functional MRI was performed to assess the changes in brain motor cortex responses to acute muscle contraction and a CO_2 stress before and after the 16 day forearm immobilisation. A handgrip task at 15 % and 30% MVC was performed with both arms at Day 0, and repeated in both arms after 16 days of immobilisation of one arm. fMRI data showed the expected increased BOLD response in the contralateral sensorimotor cortex to the dominant hand compared to the BOLD response in the contralateral hemisphere to the non-dominant hand. There was a trend for reduction in the BOLD response post-immobilisation for the immobilised arm ($p=0.09$). In agreement with the BOLD response, the perfusion change in response to the handgrip task was greater in the contralateral hemisphere to the dominant arm compared to the non-dominant. A hypercapnic mapping was performed to assess the CVR response in the sensorimotor cortex in these subjects at Day 0 and Day 16. No significant difference was found pre- and post-immobilisation, though the absolute CVR value was greater for the non-immobilised arm compared to the immobilised arm. This study was a pilot

study to assess feasibility, and it highlighted a number of issues associated with adverse effects experienced in a number of subjects when performing the muscle protocol, resulting in them withdrawing from the study.

8.2 FUTURE DIRECTIONS

Chapter 5 of this work aimed to assess the response to acute exercise, and this resulted in challenges when performing the acute exercise task within the 3 T Achieva Philips MR system, since this has a limited 60 cm patient bore diameter. This limited subject recruitment and ease of performing the acute exercise task within the scanner. In future, studies can be performed using the 3T Ingenia Philips system, this is a wide bore MR system, providing a 70 cm patient bore diameter which will facilitate ease of performing exercise within the MR environment. In addition, the Ergospect diagnostic pedal is now available, which provides greater ease of use for large muscle mass inside the MR scanner compared to the cycle ergometer.



Figure 8-1: **A.** Exercise set up in the 3T Philips Achieva using the MR compatible supine cycle ergometer (Lode B.V, Netherlands), **B.** Exercise set-up now available in the 3T Philips Ingenia wide bore scanner using the Ergospect diagnostic pedal system.

The 3T Ingenia also provides the advantage of being able to perform both brain and cardiac measures within a single scan session, as the system is a dStream MRI scanner which allows multiple coil arrays to be plugged in and thus used in a single scan session to image multiple organs, such as the brain and heart here. This will allow us the simultaneous observation of interactions between cardiac output, brain blood flow and brain fractional oxygen extraction in young and old volunteers during steady-state submaximal exercise at workloads up to 50% maximal oxygen consumption.

In future studies it will be of interest to study the response to interventions. For example, to use a pharmacological intervention in young volunteers to acutely reduce the increase in the cardiac output during exercise (cardio-selective β_1 adrenergic blockade) [1], or perform a supervised chronic physical activity exercise programme in older volunteers focused on increasing maximal oxygen uptake, and scanning subjects pre- and post-training. In these studies it will be possible to perform a comprehensive assessment of measures of both cardiac output and brain function (CBF, OEF, and vascular CO_2 responsiveness (CVR) at baseline, as well as CO, CBF and OEF at baseline, and during 5 min of exercise at 30% and 50% $\text{VO}_{2\text{max}}$ and at recovery). Alternatively, it would be of interest to study highly trained elderly volunteers who have exercised for all of their adult life, or to assess those volunteers who have chronic inactivity, for example in frail subjects following a fracture, and determine whether opposing effects are seen in this group.

In addition, it will be of interest to measure grey matter volume and cortical thickness changes in all subjects as well as assess their cognitive function with validated questionnaires. Such studies could be performed on any subjects who perform a $\text{VO}_{2\text{max}}$ test and do not have contraindications to MRI, forming a large cohort over which to assess structural changes related to physical activity.

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