INVESTIGATING CHANGES IN REGIONAL BRAIN TEMPERATURE IN PATIENTS SUFFERING ISCHAEMIC STROKE

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Mar 2017

A thesis submitted for the degree of Doctor of Philosophy of The Australian National University
To Jess, Livia and Thea.

Thanks for your patience and for always making me smile.
This thesis is my own original written work. Experiments were designed and conducted by myself with the following minor contributions from other parties:

The perfusion imaging scans of the stroke patients examined in this thesis were processed by Dr Andrew Bivard of the University of Newcastle.

Dr Murat Tatahli assisted with some lines of code in the macro used to extract data from the finite element simulations performed in Part 5 of the thesis.

Professor Iain Marshall provided a series of prior-knowledge files for use in the JMRUI software package that was examined briefly in Chapter 2.1.

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Thomas Patrick Lillicrap
Acknowledgments:

I would firstly like to thank my supervisory panel, Christian Lueck, Andrew Neely, Peter Stanwell and Rajeev Jyoti for all their help and guidance over the past several years. Christian Lueck’s mentorship has been especially invaluable and working with you has been a great pleasure. Andrew Neely also deserves special mention for giving me the most memorable words of comfort when it came time to begin learning about finite element modelling...

“It’s not rocket science... well, it is rocket science, but it’s not hard rocket science”

I would like to thank the entire stroke research team at John Hunter Hospital in Newcastle, NSW, in particular Dr Andrew Bivard for assistance in acquiring data on cerebral blood flow from stroke patients.

Xiaofei Wang, Murat Tahtali and Lucas Lodi all provided tremendous assistance in learning to use Ansys.

To all the people who have provided baby-sitting over the past 3 years, you are too numerous to mention by name but I couldn’t have done it without you.

To Anna Cowan and Wendy Riley at the ANU CMBE- thank you for your patience and assistance with all the administration involved in doing a PhD.

I would finally like to thank Lauchie Mcomish, Kellie Hargense, Jeremy Michael and the entire team at the Wig and Pen Tavern. The Wig has helped keep the roof over my head for the better part of a decade and you guys have helped keep me sane throughout a number of stressful life events. I could not have wished for a better team to work with.
Abstract:

Stroke is a leading cause of death and disability around the world. Current acute treatments are potentially dangerous and must typically be delivered within a very limited time window to be effective. Current research into ischaemic stroke includes the search for novel therapeutic agents, as well as for techniques that may help identify patients likely to benefit from potentially dangerous therapies such as thrombolysis, or extend the time window within which these therapies are likely to be effective. Brain temperature is potentially significant in all three of these avenues of investigation. Hypothermia may be neuroprotective in its own right and may extend the time window for effective treatment with existing medications. Conversely, elevated temperature may exacerbate ischaemic injury and thus worsen a patient’s prognosis. However, measuring localised brain temperature, as opposed to body temperature, is extremely difficult.

In this thesis two tools are developed with which to investigate regional brain temperature in patients suffering ischaemic stroke. A method of Magnetic Resonance Thermography (MRT), which provides a method for measuring regional brain temperature non-invasively, is developed using readily available medical imaging technology and allows estimation of temperature in healthy volunteers with an accuracy of ±1.3°C. This tool is not yet sensitive enough for routine clinical use but has the potential to be developed further.

A Finite Element Model (FEM) is developed to simulate heat exchange in the stroke-affected brain. This model is validated against experimental data from the literature and is found to be valid for normal tissue, but underestimate temperature changes in ischaemic tissue. The potential implications of this finding are discussed; in particular, it appears that the changes to the process of heat exchange in ischaemic brain tissue are more complex than previously thought.
Abbreviations used in this thesis

ACA: Anterior Cerebral Artery
AMARES: Advanced Method for Robust and Efficient Spectral Fitting (Spanish Acronym)
BA: Bland-Altman plot
CBF: Cerebral Blood Flow
CHESS: CHEmical Shift Selective water suppression
Cho: Choline
CMR: Cerebral Metabolic Rate
Cr: Creatine
CSF: CerebroSpinal Fluid
CT: Computed Tomography imaging
CTP: Computed Tomography Perfusion
DWI: Diffusion-Weighted Magnetic Resonance Imaging
ECI: External Carotid Artery
FEM: Finite Element Modelling
FID: Free Induction Decay
FLAIR: FLuid Attenuated Inversion Recovery
GM: Grey Matter
IC: Internal Carotid Artery
jMRUI: java Magnetic Resonance User Interface
MCA: Middle Cerebral Artery
MRI: Magentic Resonance Imaging
MRS: Magnetic Resonance Spectroscopy
MRSI: Magnetic Resonance Spectroscopy Imaging, another term for CSI
MRT: Magnetic Resonance Thermography
NAA: N-acetylaspartate
NAAG: N-acetylaspartyl glutamate
NHG: Net Heat Generation
NIHSS: National Institutes of Health Stroke Scale
NSF: National Stroke Foundation of Australia
PCA: Posterior Cerebral Artery
PET: Positron Emission Tomography
PRESS: Point RESolved Spectroscopy
PWI: Perfusion-Weighted Magnetic Resonance Imaging
rCBF: Relative Cerebral Blood Flow
RF: Radio Frequency radiation
ROI: Region of Interest
rtPA: Recombinant Tissue Plasminogen Activator
SNR: Signal to Noise Ratio
STAIR: Stroke Trials Academic Industry Roundtable
TCH: The Canberra Hospital
TE: Echo Time
TR: Repetition Time
WM: White Matter
Δ: Chemical Shift e.g. ΔNAA = Chemical Shift of NAA.
# Table of Contents

INVESTIGATING CHANGES IN REGIONAL BRAIN TEMPERATURE IN PATIENTS SUFFERING ISCHAEMIC STROKE

## Chapter 1.1: An Introduction to Stroke

1.1.1 Definition, epidemiology and cost ................................................................. 2

1.1.2 Pathophysiology ............................................................................................. 2

1.1.2.1 Introduction ................................................................................................ 2

1.1.2.2 Ion gradients across the neuron membrane ............................................ 3

1.1.2.3 Initial effects of hypoperfusion ................................................................. 4

1.1.2.4 Secondary effects of hypoperfusion ......................................................... 5

1.1.2.5 Effects of reperfusion .............................................................................. 5

1.1.3 Current management of ischaemic stroke ................................................... 6

1.1.3.1 Introduction to current management ...................................................... 6

1.1.3.2 Imaging acute stroke .............................................................................. 7

1.1.3.3 Treatments for Acute Stroke ................................................................. 9

1.1.3.4 Subacute and Long-term Management ................................................ 10

1.1.3.5 Challenges of Current Management .................................................... 11

1.1.4 Upcoming stroke treatments ........................................................................ 11

1.1.4.1 New methods for generating reperfusion ............................................. 12

1.1.4.2 Neuroprotection .................................................................................... 12

1.2 Body Temperature and Ischaemic Stroke: .................................................... 15

1.2.1 The association between elevated body temperature and stroke outcome. .... 15

1.2.2 The association between hypothermia and stroke outcome ....................... 17

1.2.2.1 Hypothermia has a broad range of effects on neuronal injury ............. 17

1.2.2.2 Hypothermia after other forms of hypoxia/ischaemia .......................... 17

1.2.2.3 Hypothermia after ischaemic stroke: ................................................... 18

1.2.2.4 Hypothermia is most effective if induced before or during cerebral ischaemia. ................................................................................................................. 19

1.2.2.5 Interactions between hypothermia and other treatments. ..................... 21

1.2.2.6 Methods of inducing hypothermia. ........................................................ 22

1.2.3 Conclusions: ................................................................................................ 24

## Chapter 1.3: Non-invasive Thermography

1.3.1 Problems with invasive temperature measurements ................................. 27
1.4.4 An overview of the vascular system with regard to endovascular cooling for brain ischaemia ................................................................. 66
  1.4.4.1 Background ................................................................. 67
  1.4.4.2 Cooling of the blood................................................. 67
  1.4.4.3 The blood vessels of the head........................................ 68
  1.4.4.4 Blood flow after occlusion of a vessel............................ 71
  1.4.4.5 Implications for use of the Pennes bioheat transfer model ...... 72
  1.4.6 Summary ........................................................................... 73
Chapter 2.1: Magnetic resonance thermography methods ........................................ 76
  2.1.1 Scanning Protocol. ......................................................... 76
    2.1.1.1 Protocol details ...................................................... 76
    2.1.1.2 Justification of protocol ......................................... 76
  2.1.2 Spectral processing ....................................................... 79
    2.1.2.1 Processing details .................................................. 79
    2.1.2.2 Justification of processing methods........................... 80
  2.1.3 Data processing ............................................................. 83
    2.1.3.1 Chemical shift calculations ..................................... 83
    2.1.3.2 Amplitude-weighted temperatures ........................... 84
  2.1.4 Summary ........................................................................... 85
Chapter 2.2 Finite element modelling methods ....................................................... 86
  2.2.1 Simulink Model ............................................................. 86
    2.2.1.1 Software ............................................................... 86
    2.2.1.2 Model construction ............................................... 86
    2.2.1.3 Model execution ................................................... 86
  2.2.2 Ansys Model ................................................................. 90
    2.2.2.1 Software and Geometry ......................................... 90
    2.2.2.2 Geometry ........................................................... 90
    2.2.2.3 Net Heat Generation Equations ............................... 93
    2.2.2.4 Data extraction .................................................... 95
  2.2.3 Model Parameters: ......................................................... 96
Chapter 3.1: in vitro calibration and validation of MR thermography. ......................... 102
  3.1.1 Background ................................................................. 102
3.1.2 Methods: ..................................................................................................................... 103
  3.1.2.1 Scanning protocol .................................................................................................. 103
  3.1.2.2 Data processing..................................................................................................... 104
3.1.3 Results: ........................................................................................................................ 104
  3.1.3.1 Overview ............................................................................................................. 104
  3.1.3.2 1.5T experiment ................................................................................................ 104
  3.1.3.3 3T experiment ................................................................................................... 105
3.1.4 Discussion: .................................................................................................................. 106
  3.1.4.1 Comparison to previous studies ......................................................................... 106
  3.1.4.2 Effect of magnetic field strength on temperature estimations ......................... 107
  3.1.4.3 Effect of reference chemical .............................................................................. 107
  3.1.4.4 Choline ............................................................................................................... 108
  3.1.4.5 Potential pitfalls in this experimental design...................................................... 108
  3.1.4.6 Potential pitfalls with regard to applying this technique in stroke patients ....... 109
3.1.5 Summary ................................................................................................................... 109

Chapter 3.2: *in vivo* validation of MR thermography technique ........................................ 111
3.2.1 Introduction: ............................................................................................................. 111
3.2.2 Methods: .................................................................................................................. 112
  3.2.2.1 Data collection: .................................................................................................. 112
  3.2.2.2 Data processing.................................................................................................. 113
  3.2.2.3 Statistical analysis ............................................................................................ 113
3.2.3 Results ......................................................................................................................... 115
  3.2.3.1 Example Bland-Altman analysis of a single data subset ..................................... 115
  3.2.3.3 Total variance analysis of a single subset of data ................................................ 116
  3.2.3.4 Comparison of results between data subsets...................................................... 117
3.2.4 Discussion .................................................................................................................. 119
  3.2.4.1 Summary of results .......................................................................................... 119
  3.2.4.2 Correction of intercepts for each equation from section 3.1 ............................... 119
  3.2.4.3 Best MRT-method for ongoing use .................................................................... 120
  3.2.4.4 Other questions/issues .................................................................................... 121
  3.2.4.5 Summary .......................................................................................................... 121

Chapter 3.3 Regional and temporal variation in healthy brain temperature ...................... 123
3.3.1 Background ................................................................. 123
3.3.2 Methods........................................................................... 123
  3.3.2.1 Data collection ............................................................. 123
  3.3.2.2 Data processing ......................................................... 123
3.3.3 Results ........................................................................... 124
  3.3.3.1 Quality of Spectra ....................................................... 124
  3.3.3.2 Average brain temperature ....................................... 124
  3.3.3.3 Mixed model analysis of brain temperature ............... 125
  3.3.3.4 Analysis of reference PRF amplitude .......................... 126
3.3.4 Discussion ..................................................................... 127
  3.3.4.1 Overview of results .................................................... 127
  3.3.4.2 Temperature variation within the brain ....................... 128
  3.3.4.3 Variation in reference peak amplitude ....................... 129
  3.3.4.4 Implications for ongoing MR Thermography experiments ........................................................................ 129
  3.3.4.5 Summary .................................................................. 130
Chapter 4.1 Application of MR Thermography to Ischaemic Stroke Patients ........ 132
4.1.1 Background .................................................................... 132
4.1.2 Methods ........................................................................ 133
  4.1.2.1 Patient recruitment ................................................... 133
  4.1.2.2 Imaging ..................................................................... 133
  4.1.2.3 Other data collection .................................................. 134
  4.1.2.4 Data processing ......................................................... 135
4.1.3 Results ........................................................................... 135
  4.1.3.1 Recruitment ............................................................... 135
  4.1.3.2 MR Spectra ............................................................... 136
  4.1.3.3 Spatial and temporal variation in temperature ........... 136
  4.1.3.4 Temperature and cerebral blood flow ....................... 141
4.1.4 Discussion ..................................................................... 143
  4.1.4.1 Summary of results ..................................................... 143
  4.1.4.2 Comparison to previous studies ................................. 144
  4.1.4.3 Cerebral Blood Flow .................................................. 144
  4.1.4.4 Possible explanations for temperature variation ........ 145
4.1.4.5 Feasibility of MR thermography in stroke patients ......................................... 145
4.1.5 Conclusions ................................................................................................................. 146

Chapter 5.1 Finite element modelling of heat exchange in the stroke-affected brain. ...... 148
5.1.1 Background ................................................................................................................. 148
5.1.2 Methods ...................................................................................................................... 149
   5.1.2.1 Simulink model ................................................................................................. 149
   5.1.2.2 Model geometry .............................................................................................. 149
   5.1.2.3 Model parameters ........................................................................................... 149
   5.1.2.4 Verification ....................................................................................................... 151
   5.1.2.5 Validation ......................................................................................................... 151
5.1.3 Results ......................................................................................................................... 152
   5.1.3.1 Simulink simulation results .............................................................................. 152
   5.1.3.2 Ansys simulation results .................................................................................. 153
5.1.4 Discussion................................................................................................................ 156
   5.1.4.1 Summary of results .......................................................................................... 156
   5.1.4.2 Verification ....................................................................................................... 157
   5.1.4.3 Validation ......................................................................................................... 158
   5.1.4.4 Conclusions and follow-up ............................................................................... 163

Chapter 5.2 Finite element modelling of stroke-affected brain tissue during therapeutic hypothermia ................................................................. 165
5.2.1 Introduction ................................................................................................................ 165
5.2.2 Methods ...................................................................................................................... 165
   5.2.2.1 Model geometry .............................................................................................. 165
   5.2.2.2 Model parameters ........................................................................................... 166
   5.2.2.3 CMR and CBF variations ............................................................................... 166
   5.2.2.4 Data analysis .................................................................................................... 167
5.2.3 Results ......................................................................................................................... 168
   5.2.3.1 Spatial variation in temperature ...................................................................... 168
   5.2.3.2 Time-course of temperature changes ............................................................ 171
5.2.4 Discussion................................................................................................................ 173
   5.2.4.1 Summary of results within the conceptual model ........................................... 173
5.2.4.2 Interpretation of results with respect to potential failings of the conceptual model ........................................................................................................................... 174
5.2.5 Conclusions ................................................................................................................. 175
Chapter 6 Thesis Discussion and Conclusions .................................................................. 178
6.1 Objectives of this thesis ................................................................................................. 178
6.2 Overall suitability of the magnetic resonance thermography technique described in this thesis .................................................................................................................... 178
6.3 Overall utility of the finite element model developed in this thesis ......................... 181
6.4 The next step ................................................................................................................. 182
6.5 Concluding remarks ...................................................................................................... 184
References ........................................................................................................................... 187
Part I:

Introduction
Chapter 1.1: An Introduction to Stroke

1.1.1 Definition, epidemiology and cost.

Stroke is the second leading cause of death in Australia and a leading cause of disability [1]. The National Stroke Foundation of Australia estimates that there are between fifty and sixty thousand new strokes in Australia every year, with a cost to the nation of over two billion dollars per annum[2].

There are two main types of stroke: approximately 85% are ischaemic strokes, in which a blood clot or similar body obstructs a blood vessel in the brain, thereby restricting blood flow to the brain tissue. The other 15% of strokes are haemorrhages, in which a bleed occurs within the brain tissue[1]. This thesis will focus on ischaemic strokes as they make up the vast majority of acute stroke admissions. Ischaemic stroke can be further divided into embolic stroke and thrombotic stroke. The former occurs when a clot forms somewhere else in the body and is then transported through the arteries to a vessel supplying the brain which is small enough to hold up the clot and hence be occluded by it. A thrombotic stroke occurs when a clot forms in situ within a blood vessel supplying the brain.

1.1.2 Pathophysiology.

1.1.2.1 Introduction

Neurons are highly sensitive to ischaemic injury [3]. This is partly because they have very limited capacity for nutrient and energy storage. After occlusion of a cerebral blood vessel, the cells that are exclusively reliant on that vessel die very quickly from lack of nutrients and oxygen. In the surrounding tissue, a number of secondary injury mechanisms are initiated that will kill many of the surrounding partially injured cells over a period of 24-72 hours, even if blood flow is restored. The region of tissue that has already died is referred to as the infarct core. The tissue surrounding the core, which receives less-than-
normal blood flow but is still alive, is traditionally referred to as the penumbra, from the Latin word for shadow. This term originally referred specifically to tissue suffering a level of hypoperfusion such that neurons were no longer functioning but had not suffered immediate structural damage. In clinical usage this term has evolved and is often used to refer only to hypoperfused tissue that is still alive but which will die unless blood flow is rapidly restored [4, 5]. I note this difference only because the traditional definition of penumbra includes tissue which is mildly hypoperfused but is not at risk of infarction [4]. Throughout this thesis, the term penumbra will refer to hypoperfused tissue that is at risk of infarction. Mildly hypoperfused tissue that is not at risk will be referred to as experiencing benign oligaemia. Most, if not all, acute stroke treatment is directed at salvaging as much of the penumbra as possible.

The function of neurons depends on a meticulously maintained set of ion gradients across their cell membranes [6]. A reduction in blood flow deprives neurons of the energy they require to maintain these ion gradients. As this energy deprivation continues neurons resort to anaerobic respiration which lowers the pH within each cell. This reduction in pH, in effect an increase in the Hydrogen ion concentration, disrupts the normal hydrogen ion concentration gradient across the cell membranes, which in turn disrupts a cascade of ion channels, antiporters and pumps that rely on the normal ion gradients to function properly [3, 7]. The most important outcome of this cascade is an increase in the intracellular calcium concentration. This calcium affects many internal cellular processes leading to uncontrolled neurotransmitter release, disruption of protein synthesis and the production of free oxygen radicals [3, 7]. Paradoxically, when blood flow returns it can exacerbate some of these processes, especially the production of free oxygen radicals, causing what is known as reperfusion injury [7]. Reperfusion injury will be discussed further below.

1.1.2.2 Ion gradients across the neuron membrane
All neurons have a resting membrane potential of approximately -70mV [6]. This membrane potential is essential for the generation and propagation of action potentials, and is formed primarily by gradients in the concentration of sodium (Na⁺) and potassium (K⁺) across the cell membrane. Several other concentration gradients are similarly maintained, in particular the concentrations of calcium (Ca²⁺) and hydrogen (H⁺) ions. The Na⁺ and K⁺ gradients are maintained by a Na/K pump, a membrane protein which, as the
name suggests, pumps sodium out of the cell and potassium into the cell using energy from ATP. Action potentials in neurons involve an influx of sodium into the neuron, followed by an efflux of potassium [6]. Thus, the Na/K pump must work constantly to maintain the sodium and potassium gradients, and a steady supply of ATP is required for this purpose. In fact, some studies have found that the vast majority of a neuron’s normal energy usage is expended purely on maintaining these ion gradients [8]. There are also a number of symporters and antiporters in the neuronal membrane [6]. Symporters transport two different types of particle, such as chloride and potassium, in the same direction across the membrane. Conversely, an antiporter transports two different types of particle, such as sodium and hydrogen ions, in opposite directions across the cell membrane. In both cases, one particle moves ‘down’ its concentration gradient, and this provides the energy to move the other particle against its concentration gradient. These transporters play an essential role in maintaining normal concentrations of various chemicals across neuronal membranes, and the particle that provides the energy (by moving with its concentration gradient) is frequently sodium. Thus, when one concentration gradient is disrupted, such as by a failure of the Na/K pump, it can affect the gradients of many other ions [6].

1.1.2.3 Initial effects of hypoperfusion

The cells of the brain, like other cells in the body, do not have any method of storing oxygen. Unlike many other tissues, such as muscle, the brain also has very limited stores of energy reserves such as phosphocreatine, or even glucose. Therefore, disruption of their blood supply causes neurons to initiate anaerobic respiration very rapidly [6]. Anaerobic respiration is much less efficient than aerobic respiration (resulting in approximately two molecules of ATP for each molecule of glucose consumed, compared to thirty six molecules of ATP for each molecule of glucose through aerobic respiration) and produces lactic acid as a waste product [9]. This process quickly depletes the glucose reserves and lowers the pH within the cells. The effects of the pH change are most devastating in neurons, but also affect astrocytes and other ‘support’ cells within the brain. The loss of energy impairs functioning of the Na/K pump, resulting in depolarisation of the cell and the loss of the normal sodium and potassium gradients. This prevents normal neuronal signalling (which depends on the cell being polarised by sodium and potassium gradients), resulting in electrical silence. The accumulation of lactic acid in the cytoplasm, meanwhile, creates a hydrogen gradient across the cell membrane. These disruptions to the ion gradients affect
many of the symporters and antiporters of the cell membrane resulting, amongst other effects, in an influx of calcium into the cell [7]. Even if these initial effects are mild enough that the cell has survived so far, the secondary effects of ischaemia may be initiated by this increase in calcium concentration.

1.1.2.4 Secondary effects of hypoperfusion
Calcium ions play a key role in a number of normal cell functions, and the uncontrolled influx of calcium initiates a cascade of damaging processes [6, 9]. Some of the proteins activated by calcium ions, particularly phospholipases, damage the cell membrane causing the release of fatty acids into the cytoplasm and the generation of oxidative radicals [7]. Other processes activated by the calcium influx inhibit further protein synthesis and initiate pathways leading to apoptosis [7]. Release of neurotransmitters is also controlled by calcium concentration, and the influx of calcium often causes neurons to ‘dump’ their readily-releasable pool of neurotransmitters [6]. Perhaps most devastatingly, some of these processes reinforce the influx of calcium. Oxidative radicals cause the release of calcium from the endoplasmic reticulum into the cytoplasm, and the release of glutamate, the primary excitatory neurotransmitter in the brain, causes further depolarisation and calcium influx in neighbouring cells [9, 10].

The blood-brain barrier is also frequently damaged by ischaemia, allowing toxic chemicals such as haem to reach to neurons from the bloodstream. Each of these effects can individually lead to cell death, so reducing any one pathway in ischaemic neurons, as many neuroprotective drugs are designed to do, will typically not prevent death in most neurons [3, 7]. Current acute stroke interventions are therefore aimed primarily at reperfusing the ischaemic tissue as quickly as possible [11, 12].

Perversely, while timely reperfusion may allow cells to compensate for their existing injury before they have reached the ‘point of no return’, reperfusion itself can initially accelerate the very processes that cause cell death.

1.1.2.5 Effects of reperfusion
Reperfusion restores the supply of oxygen and glucose, and restores chemical concentrations outside the cell to their normal levels. Unfortunately, removing of hydrogen ions from the extracellular fluid without removing them from the cytoplasm, as occurs during reperfusion, maintains and even amplifies the damaging concentration
gradient of hydrogen ions across the cell, allowing further influx of calcium into the cell. Furthermore, while the restoration of oxygen and glucose supplies allows the cell to generate much-needed ATP, at least some of this ATP will contribute to the damaging phosphorylation reactions initiated during ischaemia. Reperfusion is also associated with an increase in the generation of oxidative radicals which cause further damage to the cell membrane. The combination of deleterious processes that are associated with reperfusion is collectively known as reperfusion injury, and this may be responsible for the majority of neuronal damage in some stroke patients [7]. Furthermore, occlusion can result in damage to the walls of the blood vessel itself. If this occurs, reperfusion can actually result in bleeding into the brain, a phenomenon known as haemorrhagic transformation. Despite this, early reperfusion remains the best tool in the clinician’s arsenal against acute ischaemic stroke [11, 12].

1.1.3 Current management of ischaemic stroke

1.1.3.1 Introduction to current management

Management of ischaemic stroke essentially revolves around three aims. First to salvage as much of the penumbra as possible before it progresses to infarction; second, to prevent further strokes and, finally, to rehabilitate the patient to restore the highest quality of life possible following the patient’s individual neurological injury [13]. Salvaging the penumbra is particularly difficult because of the complex pathophysiology of ischaemia and reperfusion injury. As mentioned previously, the most effective tools currently available are those that restore blood flow rapidly. Unfortunately, each comes with its own set of risks and challenges. Other treatments designed to protect the brain against ischaemic injury or extend the time-window within which reperfusion will be beneficial have met with limited success but are the focus of a great deal of ongoing research[14, 15]. Before any treatment can begin, however, it is necessary to identify the type of stroke (ischaemic or haemorrhagic) and to determine, as accurately as possible, which patients will benefit from potentially dangerous interventions. Medical imaging plays a significant role in these processes.
1.1.3.2 Imaging acute stroke

The first goal of imaging in acute stroke is to identify the type of stroke, either ischaemic or haemorrhagic. The second aim is to identify the amount of tissue that is at risk but still salvageable, i.e. the penumbra [16, 17]. The modality used most often for identifying the type of stroke is computed tomography (CT) imaging. CT uses X-rays emitted and received in a spiral pattern, and the signal from these X-rays is then processed by a computer into three dimensional images. CT is best for imaging hard tissues such as bone and only has limited capabilities with regard to soft tissues[18]. However, because of the iron content of haemoglobin, CT can detect collection of blood, as occurs in a haemorrhage. CT may also be used to detect infarcted tissue in the long term [18]. The key advantage of CT imaging for acute stroke is that the scanning itself is extremely rapid and does not require any pre-screening of the patient. This is an important consideration during the acute phase of a stroke when ‘time is brain’, as CT allows clinicians to rule out haemorrhage and proceed with thrombolytic therapies if appropriate. CT can also be used to determine the presence of an old infarction, since an older infarct may appear on CT, while an acute infarction will not [18].

The capabilities of CT have been further advanced by the use of contrast-enhanced CT imaging. This involves injection of an iodine-based contrast agent into the patient and allows imaging of blood vessels and even of blood flow itself (the latter is often referred to as perfusion CT imaging). Imaging the blood vessels allows clinicians to identify stenosis (narrowing of a vessel) or occlusion (blockage of a vessel) in arteries, and perfusion imaging can be used to identify the location and extent of a perfusion deficit within a patient’s brain [19]. There is growing evidence that perfusion CT can be used to detect infarcted tissue acutely, and even to identify penumbral tissue [16, 20]. The thresholds used to identify infarct and penumbra using perfusion CT are very sensitive to the choice of scanning paradigms and the software used to collect and process the perfusion CT data. This has limited the standardisation of this technique to date, and most stroke centres in Australia only conduct standard CT imaging. Neurologists then make clinical decisions based on standard CT and clinical examination alone. For the purposes of indentifying penumbra, the gold standard is Positron Emission Tomography (PET). However, PET is extremely time-consuming and relies on equipment that most hospitals do not have access to as well as radioactive isotopes which must be prepared in advance within a nuclear reactor. It is thus
not used in clinical stroke management although it does play a role in stroke research. The
other imaging modality that is commonly used in stroke management is Magnetic
Resonance Imaging.

Magnetic Resonance Imaging (MRI) exploits the phenomenon of nuclear magnetic
resonance (NMR), specifically that of hydrogen nuclei [21]. This phenomenon is discussed
in more detail in Appendix 1. MRI involves placing the patient in the bore of a strong
magnet (typically 1.5-3 Tesla) and using radio-frequency (RF) radiation to excite the
hydrogen nuclei in the patient’s tissues. This excitation produces a variety of radio-
frequency responses that can be detected by the MRI scanner and interpolated into a
series of images. MRI has a number of advantages over CT scanning [18]. Radio-frequency
radiation is non-ionising. This means that, unlike X-rays, the RF involved in MRI scans does
not cause damage to DNA or other cellular structures and is not associated with the
increased risk of cancer or other conditions associated with repeated exposure to X-rays.
However, the strong magnetic field involved in MRI can interfere with metallic devices such
as cardiac pacemakers, and patients with such devices are therefore considered unsuitable
for the procedure. It should also be noted in passing that a single CT scan involves levels of
X-rays that are quite safe, but there is a limit on the number of repeated CT scans that can
be performed on a single patient [18].

More importantly, MRI can produce clearer images of soft tissue than CT. It can also detect
areas of restricted diffusion and tissue damage which are used to diagnose infarction in the
early hours of an ischaemic stroke (long before infarction is detectable on CT) [18].
Diffusion-weighted MRI (DWI) is generally regarded as the most reliable method of
detecting acute cerebral infarction in the absence of PET data [22]. In addition, contrast-
enhanced MRI, much like contrast-enhanced CT, can provide information on the state of
blood vessels and perfusion levels in the brain [23]. Newer MRI techniques even allow
perfusion data to be collected without injection of a contrast agent [24]. The mismatch
between the area of the brain that demonstrates hypoperfusion (the perfusion lesion) and
the area that demonstrates restricted diffusion of water molecules (the diffusion lesion) is
widely believed to represent the penumbra [25]. Thus, MRI allows clinicians to estimate
the volume of at-risk brain tissue that may be salvaged if reperfusion occurs quickly
enough, an important piece of information for clinical decision-making.
However, there are some disadvantages to MRI [18]. MRI scanners are typically much more expensive than CT scanners, and are therefore not as widely available. MRI scans themselves take longer than CT scans, and require extensive safety screening before a patient can be scanned [18] (See Appendix 2 for an example MRI safety screening form). Reperfusion therapies for acute stroke, which will be discussed further below, typically need to be administered in the first 4.5 hours after stroke onset, which makes the extra time required to screen a patient for MRI suitability and to perform the MRI itself a significant drawback [11]. For these reasons, CT remains the standard acute stroke imaging while MRI is often performed later in the patient’s care (often 24-48 hours after presentation at the hospital) when time is less critical. At this time, MRI can still provide useful information about the location and size of the stroke, and can still detect infarction more reliably than CT.

1.1.3.3 Treatments for Acute Stroke
Acute treatment of stroke is focused on reperfusing the affected tissue as quickly as possible [11]. The most common method for achieving this is thrombolytic therapy, that is, drugs that dissolve blood clots. The only thrombolytic drug currently approved for use in stroke in Australia is recombinant Tissue Plasminogen Activator (rtPA), although there are several other thrombolytic drugs approved for use in myocardial infarction (which involves a clot blocking a blood vessel that supplies heart muscle)[26]. rtPA is currently approved for use up to 4.5 hours after onset of ischaemic stroke symptoms, as early clinical trials did not show any benefit to the therapy beyond this point [26, 27]. At least one subsequent trial has suggested that thrombolysis may be beneficial over a longer time-window, especially if newer thrombolytic drugs are used and patients are selected for the presence of penumbra (as opposed to selection based on time) [17, 28]. However, these methods have yet to achieve widespread clinical use.

The other method of reperfusion that is increasingly being used is mechanical clot retrieval, also known as thrombectomy [29]. This procedure involves feeding a catheter into the artery that has been occluded and physically removing the clot via the catheter. While early studies associated thrombectomy with a greater risk of intracerebral haemorrhage and did not find evidence that it improved the likelihood of a good clinical outcome [11, 29], recent studies have found a significant advantage in using a combination of
thrombolysis and thrombectomy over thrombolysis alone [30-32]. However, thrombectomy is still not as widely used as thrombolysis, in part for logistical reasons. The primary limiting factors are the cost of the equipment and the expertise required to perform the procedure. The expertise, in particular, requires constant practice to maintain and therefore this procedure is only practical in hospitals with a consistently high number of stroke patients [33].

1.1.3.4 Subacute and Long-term Management

Subacute management of ischaemic stroke involves strategies to prevent, or at least limit, complications of stroke and to reduce the risk of secondary stroke. Careful management of temperature, blood pressure and blood sugar levels is recommended as these factors can exacerbate the damage caused by a stroke and increase the risk of complications such as haemorrhagic transformation [27]. Other complications, such as an increase in intracranial pressure caused by swelling of the affected brain may require surgery to manage [34]. Aspirin is the primary treatment used to reduce the risk of a secondary event in the first two weeks after a stroke [27].

During this period, investigations into the cause of the stroke are conducted. These investigations will typically involve investigation of narrowed carotid arteries, irregular heart rhythm (such as atrial fibrillation), serum cholesterol levels and, occasionally, unusual blood-clotting tendencies. The results of these investigations guide long-term prevention strategies. Low-dose aspirin is often recommended long term and anticoagulants may be prescribed for patients with atrial fibrillation (abnormal beating of the heart which allows large clots to form within the chambers of the heart). Drugs such as statins may be used to reduce blood lipid levels, and lifestyle changes such as exercise and smoking cessation are widely recommended [27].

Up to fifty percent of stroke patients experience depression in the two weeks after a stroke, and up to twenty-five percent are diagnosed with major depression [34]. This requires careful management by the entire stroke team, as does management of the long-term disabilities resulting from the stroke. Neurologists, rehabilitation physicians, nurses, physiotherapists and occupational therapists and others, must all collaborate to manage the early symptoms of stroke and to maximise the patient’s long-term quality of life. It has been demonstrated that care in a dedicated stroke unit, where all of the aforementioned
staff specialise in stroke care, significantly improves the likelihood of a positive outcome for the patient [27, 35].

1.1.3.5 Challenges of Current Management
Perhaps the greatest challenge of stroke management is the limited time window in which acute treatments can be administered effectively. While recent research indicates that reperfusion may be beneficial in some patients up to six hours after stroke onset [17, 28], current clinical guidelines only recommend thrombolysis within 4.5 hours of onset, and the efficacy tends to be higher the earlier treatment is administered [12, 27]. Within this time window, the patient or witnesses must recognise the symptoms of a stroke, arrive at hospital (typically by ambulance), be diagnosed by their physician and undergo, at least, a standard CT scan to rule out haemorrhage. Many regional hospitals still do not have the equipment or expertise to utilise thrombolysis, and most major hospitals in Australia do not have the capability to perform thrombectomy. Therefore, even after diagnosis a patient may need to be transported from one hospital to another before reperfusion strategies can be implemented. Furthermore, reperfusion therapy carries a significant risk of causing a haemorrhage (approximately 4% for rtPA and almost 10% for thrombectomy [11]). Therefore, there is a significant need for treatments that can extend the time window for reperfusion, preferably treatments that can be implemented in regional hospitals or even in an ambulance while the patient is in transit. There are a number of such treatments currently under investigation, as will be discussed below.

1.1.4 Upcoming stroke treatments
Research on acute stroke treatment focuses on two main areas, reperfusion (restoring blood flow) and neuroprotection (protecting neurons from ischaemic damage). The key difference between neuroprotective therapy and reperfusion therapy is that reperfusion reduces the length or severity of the ischaemic period itself, whereas neuroprotection reduces the damage caused by ischaemia. More simply, reperfusion targets the cause of ischaemia, whereas neuroprotection seeks to ameliorate its effects. Neuroprotective therapy has met with limited success in clinical use, particularly in cases of permanent ischaemia, and is often combined with reperfusion therapy for this reason.
1.1.4.1 New methods for generating reperfusion

Reperfusion is an essential step in acute stroke treatment, and methods are constantly sought to improve the efficacy and safety of reperfusion therapies. Newer thrombolytic drugs, from a slight improvement on existing rtPA (Tenecteplase) [17] to novel drugs derived from vampire-bat saliva (Desmoteplase) [36] are currently under investigation for their safety and efficacy compared to existing treatments. Novel methods of delivering thrombolytic drugs directly into the affected artery, rather than into a vein, are also being tested. This may allow smaller doses of thrombolytic drug to be administered (since the drug does not get diluted through the entire blood-stream before reaching its target) and also speed up the process of dissolving the clot. However, the procedures required to deliver intra-arterial thrombolysis are quite complicated, being very similar to those for mechanical thrombectomy.

Ultrasound-enhanced thrombolysis (sonothrombolysis) is another method currently under investigation. This is a process of directing ultrasound waves at a clot through the patient’s skin during thrombolytic therapy. It stems from the early observation that ultrasound application (used for measuring blood flow through an artery) increased rates of reperfusion even in the absence of thrombolytic therapy [37, 38]. Sonothrombolysis using high-frequency ultrasound and has been shown to increase the rate of reperfusion dramatically without an accompanying increase in the risk of haemorrhage [39], although at least one study has found that the risk of haemorrhage may be increased if the low frequency ultrasound is used [40]. It may also be possible to improve the efficacy of sonothrombolysis further by injecting gas-filled microspheres, which essentially explode under ultrasound stimulation, helping to break down the clot mechanically within the ultrasound beam [11].

Reperfusion therapy will undoubtedly improve over the coming years. However, reperfusion alone will only ever be effective within a limited time-window, and this will remain a key limitation of acute stroke treatment. Neuroprotective therapies may extend this time-window, amongst other benefits discussed below.

1.1.4.2 Neuroprotection

Neuroprotection refers to treatments that protect neurons, and other cells of the nervous system, from damage. The most common form of neuroprotection is chemical-based [15].
Various agents have been tested for their ability to impede the processes that lead to cell death. Some drugs reduce the influx of calcium into the cell, either by blocking calcium channels or by chelating calcium ions within cells [15]. The former approach has an advantage in that there are a number of such drugs already approved to use in the treatment of epilepsy [41]. Antioxidants have also been trialled in the hope that they would reduce the damaging effects of oxidative radicals formed during ischaemia and reperfusion [42]. Other drugs have been found to be neuroprotective in animal models although the mechanism for this protection is not yet understood. This is the case for some anaesthetic gases such as halothane and desflurane, barbiturates, and caffeinol. The anaesthetic gases and barbiturates may simply work by reducing the metabolic demands of the neurons, although it has also been suggested that they work by reducing the temperature of the brain [43] [44]. A reduction in temperature may itself be neuroprotective, and this will be discussed in detail below. Caffeinol is a very poorly understood neuroprotectant. It consists, as the name suggests, of a mixture of caffeine and ethanol, and has been shown to be an effective neuroprotectant in animal studies despite the fact that caffeine and ethanol individually may be harmful [45].

Unfortunately, despite the large range of drugs that have shown promise as neuroprotectants in animal studies, none has so far been applied successfully in humans with stroke [15]. This is, in part, because animal studies of neuroprotection for stroke are often poorly designed with regard to human applications [15]. These studies frequently involve administering the drug immediately after, or sometimes even before, the onset of ischaemia. It has been shown that the effectiveness of thrombolysis is dependent on the time between onset of ischaemia and application of the treatment, and the same is likely true of neuroprotective therapies [46]. Thus, a drug that is effective if administered within minutes, as is often tested in the laboratory, will not necessarily be effective if administered after 3-6 hours, as is typically the case in the emergency department.

There are also biological variations that complicate the translation of any treatment from animals to humans. Even within the human brain some neurons are more resistant to ischaemia than others, meaning that a treatment may protect the majority of the brain from injury and still not prevent severe disability or death if some crucial neurons are damaged [46]. This produces a particular challenge when it comes to translating stroke
treatments from animals to humans. In animals, the effectiveness of stroke treatment is typically measured by the effect on final infarct size, i.e. the volume of brain tissue that dies. Conversely, clinical trials in humans typically measure the effectiveness of a treatment in terms of the level of disability (or death) suffered by the patient. However, strokes in humans are extremely heterogeneous. It is possible to suffer a large stroke that causes minimal disability, while a very small stroke can still be fatal. Thus, a treatment that reduces infarct size will not necessarily improve the clinical outcome of the patient [15, 47]. The Stroke Trials Academic Industry Roundtable (STAIR) released a series of guidelines for stroke therapy trials, including animal trials [46] in an effort to maximise the clinical relevance of animal trials. At present, there are still no neuroprotective drugs that have been applied successfully in the clinic for ischaemic stroke, but this remains an important area of research [15].

The neuroprotective therapy that arguably shows the most promise is not a drug at all. Hypothermia has been shown to be effective in both animals and humans after certain types of hypoxia and ischaemia, including drowning and cardiac arrest. A number of trials are currently underway around the world to examine the role of hypothermia in the management of acute ischaemic stroke. As these trials are a key focus of this thesis, hypothermia will be discussed thoroughly in the next chapter.
## 1.2 Body Temperature and Ischaemic Stroke:

### 1.2.1 The association between elevated body temperature and stroke outcome.

Elevated temperature is known to increase the rate of metabolism, and also to increase the effects of cytotoxic compounds. This is currently used to a patient’s advantage in hyperthermia therapy for tumours, in which a tumour is selectively heated by 3-8°C by a laser or ultrasound probe [48]. Once heated, a dose of chemotherapy which is ineffective at normal temperatures (and hence does not affect any tissues in the patient which are not being heated), becomes toxic, selectively killing the tumour. Unfortunately, pathological processes affecting healthy tissue are also enhanced by higher temperature.

It has been demonstrated that artificially elevating the temperature of test animals increases the size of an infarct after MCA occlusion [49]. Importantly, temperature elevation has a greater effect following temporary ischaemia than following permanent occlusion [49]. Following permanent MCA occlusion, infarct size was found to be quite large even at normal temperatures since most or all of the territory supplied by the MCA died in the absence of rapid restoration of blood flow. In cases of temporary MCA occlusion, some of the tissue on the periphery of the MCA territory could survive if recanalisation occurred quickly enough [49].

If the temperature of the infarct was elevated, however, the peripheral tissue (the ischaemic penumbra) infarcted more rapidly, meaning that recanalisation had to occur sooner in order to salvage the affected tissue. This model is relevant to clinical practice since acute stroke management revolves around restoring blood flow and salvaging as much of the penumbra as possible [13]. These animal studies suggest that an elevated temperature shortens the ‘therapeutic time window’ in which to restore blood flow if the penumbra is to be preserved. Furthermore, there appeared to be a dose dependent
relationship: the greater the elevation in temperature and the longer the temperature elevation was maintained, the more damage was inflicted [49].

Observational studies in humans have confirmed that elevated core body temperature is associated with more severe strokes and a worse clinical outcome after a stroke [50, 51]. However, it has yet to be proven that temperature elevation increases stroke severity as it may be that a severe stroke causes the temperature elevation [52]. The timing of temperature measurements varies widely between different studies, but overall the studies suggest there may be a causal relationship. Some studies only tested admission temperature and found this to be an important prognostic factor [53, 54]. Other investigators recorded only the maximum temperature measured during the first 7 days after a stroke and also observed that a high temperature was correlated with a poor outcome [50, 55]. Studies that recorded multiple temperature measurements, however, found admission temperature to be a poorer indicator of prognosis than the temperature taken 6-8 hours [52] or 24 hours [56] after symptom onset. One study measured the ‘temperature burden’ after admission by plotting multiple temperature measurements against time and measuring the area under this curve (hence taking into account not just the maximum temperature, but the duration of temperature elevation) over the first 24 hours after admission. This study found that temperature burden was strongly associated with a poor outcome after thrombolysis [57].

While the changes in temperature detected in most of these studies are relatively small - especially since antipyretic therapy is routinely used if a patient’s temperature rises above 37.5°C, even minor temperature elevations were found to be significant. A 1°C increase in temperature is associated with a 30% increase in mortality [58] and more than double the risk of a bad outcome (death or severe disability at discharge) [53]. Thus, even small changes in temperature may have a significant effect on outcome.

Few of these studies clarify the cause of temperature elevation, i.e. separate pyrexia caused by infection or environmental factors from hyperthermia caused by the stroke itself. Hyperthermia is common after both global and focal ischaemia, although it is usually only transient unless the hypothalamus is damaged [49]. Prolonged hyperthermia caused by infection is also common amongst stroke patients and is associated with both higher
temperatures than those associated with “stroke-induced” hyperthermia, and worse outcomes [49].

In summary, it is not possible to determine whether elevated temperatures cause severe strokes or the other way around. In practice, it is likely to be a mixture of both. An individual patient’s temperature may be elevated because of the underlying stroke, and/or because of external factors but, in either case, the elevated temperature exacerbates the ischaemic damage in the penumbra, and reduces the therapeutic time window in which to salvage this tissue. Therefore, reducing the temperature in the penumbra may lengthen the therapeutic window in stroke patients.

1.2.2 The association between hypothermia and stroke outcome.

1.2.2.1 Hypothermia has a broad range of effects on neuronal injury.
Of the countless neuroprotectants that have been trialled for cerebral ischaemia, few have shown as much promise as hypothermia. This is most likely because hypothermia acts on the full breadth of damage mechanisms that are set in motion by ischaemia-reperfusion injury. This involves a number of parallel processes, any one of which can lead to cell death [59, 60] (See section 1.1.2, pathophysiology of stroke). Most neuroprotective drugs can only impede one, or a few, cytotoxic processes [3]. Hypothermia, on the other hand, reduces glucose and ATP usage, reduces the formation of oxidative radicals and the release of excitotoxic neurotransmitters, inhibits calcium influx and preserves mitochondrial and blood-brain-barrier integrity [59, 60]. By inhibiting, or at least delaying, such a broad range of deleterious effects, hypothermia may extend the time-window in which to administer other treatments, especially reperfusion strategies. The evidence for this has been accumulated over countless studies involving both animals and humans, in stroke and other forms of ischaemia or hypoxia.

1.2.2.2 Hypothermia after other forms of hypoxia/ischaemia
Hypothermia is associated with a better outcome after global cerebral hypoxia and ischaemia of various causes. Early evidence came from victims of drowning in near-
freezing water. Many of these patients survived periods of immersion which would be regarded as fatal under normal circumstances [59].

Subsequent studies have demonstrated that induced hypothermia significantly reduced the rates of mortality and severe neurological injury resulting from neonatal hypoxic/ischaemic injury [61] [62].

In 2002, two randomised controlled trials demonstrated the effects of hypothermia on cardiac arrest victims [63, 64]. Bernard et al chilled unconscious patients to 33°C within 2 hours after return of spontaneous circulation (ROSC). Patients were maintained at this temperature for 12 hours, and then rewarmed over 12 hours. The percentage of patients who received the treatment and experienced a ‘favourable outcome’ (defined as a neurological deficit minor enough that the patient could be discharged home or to a rehabilitation facility) was 46%, compared to 29% in the control group. The Hypothermia After Cardiac Arrest Study Group (THACSG) instigated a similar protocol. A target temperature (32-34°C) was achieved within 8 hours of ROSC and maintained for 24 hours before rewarming over approximately 8 hours (although rewarming was not monitored as closely at it was in the Bernard study). This group demonstrated a ‘favourable outcome’ (this time defined as no, or only moderate, disability at 6 months) in 55% of hypothermic patients compared to 39% of controls. The different definitions of outcome, and the differing times-to-treatment and duration of treatment make direct comparison of these two studies difficult, but both demonstrated a significant clinical effect. Unfortunately, there is far less evidence attesting to the effectiveness of hypothermia in cases of focal cerebral ischaemia.

1.2.2.3 Hypothermia after ischaemic stroke:
Animal studies of hypothermia for ischaemic stroke have been quite promising, although there is a great deal of heterogeneity between different studies. One study found hypothermia halved the risk of mortality in mice treated with late thrombolysis but this study was conducted on a small sample and did not reach statistical significance [65]. Other studies have found that hypothermia attenuates reperfusion injury and may even accelerate recanalisation in the absence of thrombolytics by interfering with normal clotting processes [66]. Both of these scenarios are relevant to clinical practice, where the majority of ischaemic stroke patients either cannot receive thrombolysis within the 4.5
hour window, or cannot receive it at all. For an excellent review and meta-analysis of animal experiments, see [60]. Overall, hypothermia was found to reduce infarct size by an average of 43.5% and improved neurobehavioral scores by an average of 45.7% across a variety of animal models. This suggests hypothermia is a very promising treatment; however, the substantial differences between study paradigms limit direct comparison of different studies.

The optimum depth and duration of hypothermia remain unknown. It is likely that these factors will vary between different species, making generalisations from animal experiments extremely difficult. Furthermore, even within a given species, the optimum ‘dosage’ of hypothermia will probably vary with the type and severity of insult, the treatment delay (both time to admission and time to target temperature), and when (or if) the occluded vessel is recanalised. The best longitudinal study of different temperatures found 34°C to be the most effective temperature in rats [67]. However, this study involved inducing hypothermia immediately after occlusion, and only followed the rats for 5 days after treatment (at which time they were sacrificed for histological analysis). Thus, this study is not clinically very relevant (all patients will experience some delay between stroke onset and treatment), and it does not tell us anything about the optimum duration of hypothermia. Unfortunately, there have been no other systematic longitudinal studies to test these parameters in animals, let alone in humans. While most studies have demonstrated some level of neuroprotection, mild to moderate hypothermia (32-35°C) is associated with fewer side-effects and better recovery in all animal models [68], and it is this temperature range that has been favoured for clinical trials.

1.2.2.4 Hypothermia is most effective if induced before or during cerebral ischaemia.

Many experiments testing neuroprotective agents start by introducing the agent before the onset of ischaemia, and hypothermia studies are no exception. Unfortunately, studies that have looked at hypothermia induced after an ischaemic insult have shown that this paradigm is not as effective as inducing hypothermia before or during ischaemia [59, 60, 69, 70]. Unfortunately, applying a therapy before the onset of symptoms has little or no clinical application, yet this is the scenario which the vast majority of animal studies have tested. The few studies which have been done on delayed induction of hypothermia have typically involved a delay of less than 3 hours. Those that did test induction beyond this
time-window included comparatively few animals, so it is impossible to draw any firm conclusions about the time interval between stroke onset and induction of hypothermia and the effectiveness of hypothermia therapy [60]. It may be that the longer the delay between insult and treatment, the greater the dose of hypothermia needs to be, either in terms of depth, duration, or both. There have been few studies to test this relationship systematically, but it has been found that a given level of hypothermia is neuroprotective if induced within 30 or 60 minutes, but it was ineffective after 90 minutes [71]. Several studies involving hypothermia for 2-6 hours found it to be ineffective if induction was delayed by more than 3 hours from stroke onset. On the other hand, in studies where animals were cooled for 24 hours, hypothermia showed a significant benefit even if delayed for up to 6 hours [59]. This suggests that if treatment is delayed, hypothermia must either be deeper, or be maintained for longer to be clinically effective.

Evidence is mounting that the rate of rewarming after hypothermia also has a significant effect on patient outcome. Rapid rewarming can be associated with a dangerous rise in intra-cranial pressure [70, 72]. This has also been noted in studies of hypothermia for traumatic brain injury (TBI), which is particularly important as one of the key benefits of hypothermia in TBI is that it manages intracranial pressure [73]. There is also some evidence from rat studies that rapid rewarming can cause or exacerbate anomalies in microvasculature after TBI, and can damage mitochondria leading to the formation of oxidising radicals [73]. This may also be the case in ischaemic damage, though less research has been done into this aspect. It has been demonstrated that rapid rewarming after hypothermia can cause microvascular dysfunction in rats even in the absence of brain injury [73]. Since vascular anomalies and free radical formation are both key components of ischaemia reperfusion injury, the effect of rewarming on these processes would be difficult to separate from the effects of ischaemia and reperfusion [3], and these studies are all but impossible to conduct on humans.

The ideal rate of rewarming therefore remains unknown. Rewarming from 33°C to 37°C within 16 hours has been associated with a dangerous rise in intracranial pressure in early trials of hypothermia for acute ischaemic stroke, but beyond this no comprehensive longitudinal studies have been done [74]. Once again, the problem arises that ideal parameters established in animal models do not translate to humans (rewarming over 90
minutes is considered slow in rats, and is associated with a good outcome, but this would be considered extremely fast rewarming in humans). While rewarming as slowly as possible may appear to be a good option in the absence of comprehensive studies, this would place a significant cost burden on the hospital treating the patient, as the patient must typically remain in intensive care for the duration of the hypothermia therapy. Furthermore, even mild hypothermia maintained over a long time period (72 hours) is associated with a significant rise in the risk of complications such as pneumonia [75]. Some institutions have opted to adjust the rewarming rate in stroke patients according to the individual patient’s intracranial pressure [76]. This may be the best option based on the current knowledge base, but does not account for effects such as mitochondrial perturbation, which cannot be monitored in a clinical setting.

1.2.2.5 Interactions between hypothermia and other treatments.

In addition to being neuroprotective in its own right, hypothermia may increase the efficacy of other neuroprotectants, or prolong the time window within which these treatments can be effectively administered [77]. Alternatively, combination with other neuroprotective agents may also reduce the dose of hypothermia that is required to be effective [45]. A number of drugs have been tested in conjunction with hypothermia, the most promising of which have been caffeinol (a mixture of caffeine and ethanol) and tirilazad (combined with Magnesium Chloride). Both combinations have been shown to reduce infarct volume more than either component in isolation, and a clinical trial of caffeinol with hypothermia is currently underway in ischaemic stroke patients [45].

Hypothermia can also interact with the standard treatments used in ischaemic stroke, though these interactions are not always beneficial. The most effective treatment currently available for ischaemic stroke is thrombolysis. Unfortunately, current thrombolytic drugs are invariably enzymes (rtPA and Urokinase are the two currently used, Tenecteplase, an improved version of rtPA may come into use in the near future) [11]. As such, thrombolytic activity is temperature-dependent. In vitro studies have shown that rtPA does not dissolve clots as quickly at lower temperatures [66, 78]. One observational study in humans found that lower body temperature (within 6 hours of onset) was associated with a better outcome in the absence of thrombolysis, while the trend was reversed in patients who received thrombolysis [79]. This difference was attributed to a
higher rate of recanalisation at higher temperatures. The validity of this study is difficult to assess, as temperature measurements within 6 hours have previously been found to be less prognostic than temperatures taken more than 6-8 hours after onset [52]. However, the temperature at the time of administration of rtPA is likely to be the most important in terms of thrombolytic efficacy.

It should also be noted that all of the studies mentioned above that involved humans measured core body temperature or tympanic temperature, not brain temperature per se. While tympanic temperature is believed to be a reasonable correlate of average brain temperature under normal circumstances [80], it does not provide any data regarding regional brain temperature.

1.2.2.6 Methods of inducing hypothermia.

There is a variety of methods available to induce cerebral hypothermia. Some aim to reduce brain temperature specifically without causing systemic hypothermia. Others induce systemic hypothermia and rely on cooled blood to lower the temperature of the brain. Surface cooling of the head and neck [81] and nasopharyngeal cooling [82] have met with limited success, possibly due to the low thermal conductivity of the brain itself [83]. Boller et al found that 24 hours of cooling applied to the exposed dura of monkeys delivered a 5-9°C temperature reduction to the tissue 10mm under the surface, but 15mm under the surface this temperature reduction was only 2-3 degrees [82]. This suggests that cooling the brain surface produces a steep temperature gradient within the brain, and so fails to cool deeper tissue. One study did find a cooling helmet to be effective at reducing brain temperature in comatose patients [84]. However, this study also only measured brain temperature 7.5mm below the cortical surface (using a surgically implanted temperature probe). Furthermore, the helmet also cooled the neck extensively, and may have cooled the blood flowing into the brain, as well as the surface of the head itself. Since the normal brain is highly perfused at all levels, cooling the blood can reduce temperature of the entire brain under normal circumstances [85, 86].

For this reason, systemic hypothermia appears to be the most effective method of reducing brain temperature [76, 77], although it is associated with more side-effects than localised head cooling, so the latter remains an important avenue of research [87]. There are several of ways to induce systemic hypothermia, each with advantages and disadvantages. The
most rapid and precise method is through cardio-pulmonary bypass surgery, during which a heat exchanger can directly control the temperature of the blood as it re-enters the patient [88]. The bypass can even be designed so that the blood entering the brain is cooled, while the blood returning to the body remains at normal temperature [88]. Importantly, it has been demonstrated that his technique reduces brain temperature as well as blood temperature [89]. However, cardio-pulmonary bypass is extremely invasive, places a tremendous burden on hospital services, and is therefore not suitable for most stroke patients. A cooling catheter has been developed which can be inserted into a patient’s inferior vena-cava and cool the blood directly without necessitating cardio-pulmonary bypass [90]. This method has several advantages over comparable techniques. First, the skin surface, and hence the temperature receptors within it, can be kept warm. This reduces the sensation of cold experienced by the patient and allows moderate hypothermia to be maintained without the need for general anaesthesia and intubation, although patients still need to be sedated [75, 76, 91]. This is important as it reduces the burden on staffing (since it does not require an anaesthetist) and reduces the risk of iatrogenic complications. Endovascular cooling has also been proven to be more reliable and more precise than surface cooling in terms of maintaining a target core body temperature [92]. It has also been proven to reduce brain temperature at a similar rate to core body temperature in non-human primates with surgically implanted parenchymal temperature probes [90]. An infusion of ice-cold fluid into a patient’s veins can also reduce body and brain temperature quite rapidly [93]. However, there is a limit to how much fluid can be infused into a patient before causing injury, so this technique can only be used to lower temperature by around 1.6° before other cooling techniques must take over [93]. Infusion of ice-cold fluids does have an advantage in that it requires minimal expertise or equipment to initiate so it can, and has, been used to initiate hypothermia in cardiac arrest patients before they arrive at hospital, after which other methods can be used to continue hypothermia induction and maintenance [69, 93, 94].

Traditional methods of inducing hypothermia, such as ice-packs and ethanol bathing have largely been abandoned as new technologies have evolved that allow more rapid temperature reduction and more precise temperature maintenance with considerably less effort by the nursing staff [76, 92]. Some of these technologies still involve surface cooling which is commonly applied to patients who are already comatose or who require a general
anaesthetic such as those suffering cardiac arrest or traumatic brain injury [92]. In these cases, the advantages of endovascular cooling (not requiring a general anaesthetic or intubation) are largely negated, and thermostatically-controlled cooling pads applied to the skin can provide a similar rate of cooling to endovascular devices without the need for catheter placement which carries its own risks [95]. Endovascular cooling is still more reliable than surface cooling, however, with more temperature fluctuation occurring in patients cooled using surface techniques than those exposed to endovascular cooling [92].

Whatever cooling method is used, pharmaceutical intervention is required to complement the physical cooling, and this may have a differential impact of the patient’s brain temperature. Most commonly, a sedative or anaesthetic is required (depending on the cooling technique) and a drug to suppress shivering is also used [68, 75]. Anaesthetics and sedatives, in particular, are known to influence brain metabolism and temperature, and may be neuroprotective in their own right. Barbiturates, for example, have a dramatic effect on brain temperature [44]. Chloral Hydrate and Pentobarbital have been shown to increase the effectiveness of hypothermia in rats [60]. Trials testing the effectiveness of hypothermia, therefore, must attempt to separate the effects of drugs from physical cooling techniques, both on the temperature of the brain and the level of neuroprotection achieved. Even minor differences in the pharmaceutical regime used in different studies may explain some of the differences in effectiveness reported by different studies.

1.2.3 Conclusions:

All observational studies reporting on the effects of temperature on stroke outcome have focused on core body temperature or tympanic temperature (which is considered a reasonable surrogate of brain temperature in healthy individuals, but not in disease conditions) [80]. However, implanted temperature probes have demonstrated that brain temperature in animals can vary dramatically in the absence of any changes in core body temperature [86], and these changes may have more prognostic value, or a greater effect on outcome (or both), than body temperature. Similarly, the vast majority of clinical trials of hypothermia only monitor core body temperature [63, 64, 77, 96]. Of those that have monitored brain temperature in both humans [84, 97] and animals [90], all have monitored only normal or random segments of brain tissue. There are simple reasons for this: invasive temperature probes only allow monitoring of one, or at most a few, regions at a
time. Furthermore any surgical implant can cause damage, particularly in a delicate organ like the brain, and alter the results of the experiment. This is even more pertinent in human studies. Ischaemic stroke patients do not typically require neurosurgery, so implanting temperature probes is needlessly dangerous and unethical. Core body temperature is monitored as a surrogate, since studies in both animals and humans have shown that reducing blood temperature does reduce brain temperature in normally-perfused brain tissue [72, 90]. However, ischaemic tissue is by definition not normally perfused. This means that cooled blood cannot deliver the same temperature reduction to ischaemic tissue as it does to normal tissue. However, it is within the ischaemic tissue, particularly the ischaemic penumbra, that neuroprotection (and hence temperature reduction) is most necessary. The level of temperature reduction that can be achieved in ischaemic tissue is not actually known, and is likely to vary from patient to patient. Even within the ischaemic core blood flow is rarely reduced to zero, so cooling the blood is likely to reduce the temperature of the core to a certain degree, depending on the absolute level of residual blood flow. Simple conduction might be helpful: cooling the normal brain tissue surrounding an ischaemic region might permit the removal of a certain amount of heat away from the ischaemic tissue. However, the amount of heat removed in this way will depend on the size of the ischaemic region and its location within the brain. The thermal conductivity of brain tissue is quite limited [98], so in patients with a very large perfusion deficit it may be necessary to cool blood to a lower temperature and/or for a longer time to achieve an adequate temperature reduction in the target tissue. In some patients, such a temperature reduction may be impossible without generating unacceptable side-effects. The ability to recognise such patients is necessary if therapeutic hypothermic regimens are to be tested effectively.

There are two main techniques that will be explored in this thesis which may help to answer some of these questions. Magnetic resonance thermography (MRT) allows non-invasive measurement of the temperature of specific regions of interest within a patient’s brain[99]. This can be used to measure the temperature within the ischaemic core, the penumbra and surrounding normal tissue in patients with ischaemic stroke. Such data regarding regional brain temperature could prove to be significantly more useful in prognostic terms than measurements of body temperature. In addition, when applied to patients receiving therapeutic hypothermia, MR thermography could be used to determine
whether the tissue that actually requires cooling, i.e. the penumbra, does reach target temperature. Regional brain temperature measurements could also provide data on the effects of drugs such as barbiturates, which are believed to reduce overall brain temperature [44], and be used to compare the efficacy of different pharmaceutical regimes used to aid the induction of cerebral hypothermia.

The second technique is thermal modelling. Amongst other applications, thermal modelling has been used by biomedical engineers to predict the relative effectiveness of surface cooling compared to blood or CSF cooling. However, thermal modelling of focal ischaemia in brain tissue has been extremely limited thus far, particularly in regard to cooling ischaemic brain tissue. The data needed to build a model including an infarct with a perfusion deficit can be drawn from previous experiments and from CT perfusion maps of individual patients. When fully developed, this model should allow us to determine the optimum method and duration of cooling an individual patient needed to induce a therapeutic temperature reduction throughout the ischaemic penumbra.

Both these techniques will be discussed in detail in the following chapters.
Chapter 1.3:
Non-invasive Thermography

1.3.1 Problems with invasive temperature measurements

Brain temperature can be monitored by conventional means, through the insertion of temperature probes into the parenchyma. However, this technique has several drawbacks, particularly where ischaemic stroke patients are concerned. Invasive probes can only monitor one, or a few locations at once, and the placement of the probes is often decided by the ease of surgical access and safety concerns, rather than the relevance of the locations to a clinician or researcher. Furthermore, the placement of these probes requires a neurosurgeon and anaesthetic support, both of which are exceedingly expensive and not normally required for the management of ischaemic stroke. Finally, and most importantly, such procedures are inconvenient and potentially risky from the patient’s point of view.

A non-invasive temperature measurement is theoretically safer, potentially less traumatic and probably cheaper (since it does not require the expensive neurosurgery or anaesthetic support) than invasive techniques. Ideally, temperatures could be localised to the regions of most interest to the clinician or researcher, using medical imaging techniques to identify such regions. Various applications of Magnetic Resonance Imaging (MRI) have the potential to identify regions of interest and measure their temperature.

1.3.2 MR parameters dependent on temperature

Several of the parameters that can be measured by MRI, such as the T1 and T2 relaxation times and the proton density signal all vary with temperature [99-102]. The apparent diffusion coefficient (ADC) value is temperature dependent as a result of Brownian motion of water molecules, and has been applied to measure temperature in vitro, or even in vivo in bodies of liquid such as the cerebral ventricles [100-102]. However, each of these parameters is also significantly affected by tissue type, injury and variations between individuals[101]. The ADC value, in particular, can be used to measure changes in
temperature from a baseline scan, but none of the above can be used to detect absolute temperature [99].

The resonance frequency of protons (PRF) of certain molecules is also dependent on temperature, and can be measured by proton (1H) MR Spectroscopy (or simply MRS). MRS can be used to detect signals from a number of different nuclei, with hydrogen (1H-MRS, commonly referred to as proton MRS), phosphorus (31P-MRS) and carbon (13C-MRS) being the most commonly examined in medicine[103]. For the purposes of this thesis, all subsequent references to MRS refer to 1H-MRS. All other MRI techniques measure the amplitude of the MR signal; MRS is unique in that it measures the resonant frequency of the signals. This is important because the frequency of these signals (measured in Hz) is a function of the chemical identity, and the local strength of the magnetic field. Because both these factors are largely independent of tissue-type or injury, MRS measurements can be calibrated in vitro, and still applied in vivo, allowing absolute temperature measurements to be derived [99]. Furthermore, like most MR techniques, localisation of the MRS temperature measurements can be guided by standard MR images without any of the risks associated with invasive probe placement. The subtle changes in the PRF of water as a result of temperature cause small changes in the phase of the water signal detected by the MRI scanner and this can be used to detect changes in temperature using a technique called ‘phase-mapping’[101]. Like the parameters described above, this technique can measure only relative changes in temperature from a baseline value, however, the scans are significantly faster than MRS scans.

Monitoring cerebral hypothermia therapy, or temperature changes after a stroke, will require measuring temperatures several hours apart. A baseline scan conducted long before the follow-up scan would be of little use, since parameters such as the ADC or the signal phase will change for reasons unrelated to temperature. It is also impossible to collect a baseline scan before a patient has a stroke. Therefore, MR Spectroscopy techniques are more suited to such applications, particularly since the need for temporal resolution in these circumstances is not high. In the management of stroke patients, temporal resolution in the order of minutes (as opposed to seconds) is quite sufficient. Alternate methods of MR thermography have found other clinical applications, as outlined in the next section.
1.3.3 Other applications of MR Thermography

The features of MR thermography make it ideal for monitoring therapies that involve altering the temperature in a localised volume of tissue, as the effects of the device instigating the temperature change can be monitored non-invasively. The most common application has been monitoring thermal therapy of tumours. There are two broad types of thermal therapy, the first being thermal ablation and the second being what is known as hyperthermia therapy. Thermal ablation involves heating a tumour to a high enough temperature to kill it directly. Hyperthermia therapy involves heating a tumour to 43-45°C in order to increase the local effectiveness of other therapeutic agents, such as chemotherapy or radiation [48]. In either therapy, identified tissue is heated by means of a laser or ultrasound probe and the temperature must be monitored to ensure that the target tissue reaches the desired temperature, while the surrounding healthy tissue is not heated excessively. In such applications, the temporal resolution of the temperature measurements is more important than the ability to measure absolute temperature (assuming temperature changes can be accurately monitored). Furthermore, it is always possible to perform a ‘baseline’ scan before applying the heating device, providing a reference from which to measure temperature changes. For these reasons, the MR thermography techniques used tend to be those based on the ADC or ‘phase-mapping’ of the PRF, which allow temperature data to be collected in a matter of seconds rather than a matter of minutes, but this is done at the expense of the ability to measure absolute temperature [99, 100].

1.3.4 The physics underlying absolute temperature measurement using MR Spectroscopy

The resonance frequency of protons in a given chemical environment is primarily a function of the local magnetic field. This, in turn, is dependent on both the base magnetic field strength (i.e. the strength of the MRI scanner) and local magnetic shielding. The local magnetic shielding is the result of moving electrons producing a magnetic field (according to Faraday’s law) [99]. The motion of the electrons depends on the bonds they are involved in. Hence, the chemical environment (i.e. what other atoms the hydrogen atoms are bonded to) plays the greatest role in determining the proton resonance frequency. However, in molecules which readily form hydrogen bonds, such as water, the PRF will also
be dependent on the extent of hydrogen bonding. A hydrogen bond reduces an electron’s effective range of motion to the area between the two nuclei forming the bond. The more readily individual hydrogen bonds form, and the longer they last, the less the electrons surrounding hydrogen nuclei are free to move. Thus, electrons involved in hydrogen bonding generate less magnetic shielding than those that are moving freely [99] (See Figure 1.3.1).

Hydrogen bonding, in turn, is dependent on temperature. At low temperatures, molecules are moving relatively slowly and hydrogen bonds can form readily and persist for a relatively long time. As the temperature rises, the individual molecules begin moving faster and hydrogen bonds tend to form and break much more rapidly. This permits an increase in the magnetic shielding generated by the electrons, causing a change in PRF that can be used to determine temperature [99]. Just as importantly, hydrogen nuclei in non-polar bonds, which are not subject to hydrogen bonding, have a PRF that is not dependent on temperature.

There are many other factors that can subtly alter the resonance frequency of a given hydrogen nuclei, such as the magnetic susceptibility of the tissue and minor inhomogeneity of the static magnetic field. However, by comparing a PRF that is known to be temperature dependent, such as that of water, to a PRF that is known to be temperature independent, such as that of N-acetylaspartate (NAA), the effects of these other factors can be mitigated, since they will affect both frequencies to the same degree. Using this method, it is possible to single out the change in PRF due to temperature from other sources of error, and thus derive absolute temperature measurements.
Figure 1.3.1 The effects of hydrogen bonding on magnetic shielding by electrons orbiting a hydrogen nucleus.

While electrons orbiting hydrogen nuclei are involved in hydrogen bonding their movement is restricted. As temperature increases, less hydrogen bonding occurs which allows the electrons to move more freely. Since every moving charge generates a magnetic field, the increased motion of the electrons generates a stronger localised magnetic field, which shields the nucleus in question from the base magnetic field of the MRI scanner. Adapted from [99].
Figure 1.3.2 The molecular structures of metabolites examined in MR thermography experiments.

The hydrogen nuclei that produce the MR signals associated with these metabolites are highlighted by blue circles.

Note the difference in electronegativity between the hydrogen nuclei and oxygen nucleus in the water molecule (with the resulting charges denoted by $\delta^+$ and $\delta^-$, respectively). It is this slight charge that allows the formation of hydrogen bonds as described in Figure 1.3.1. Carbon atoms possess electronegativity levels much closer to that of hydrogen, so the hydrogen nuclei that produce the MR signals associated with the other metabolites do not experience the weak charge that a hydrogen nucleus in a water molecule experiences, and thus are not subject to hydrogen bonding.
1.3.5 Scanning paradigms

Figure 1.3.3: A typical MR Spectrum collected from a human brain

(A) (Top) Full spectrum showing the water peak and Choline (Cho), Creatine (Cr) and N-acetylaspartate (NAA) peaks to the right. The anatomical location of the voxel can be seen in the localising images on the right hand side.

(B) (Bottom) A magnified view of the Cho, Cre and NAA peaks, including the amplitude (A) and line-width at half maximum height (LW) of NAA.
Factors affecting choice of scanning paradigm

There are different scanning sequences that can be used to collect MR spectroscopy data and they will all produce spectra that look more-or-less like the one in Figure 1.3.3. The spectrum is plotted as a graph with frequency on the horizontal (x) axis, and amplitude on the vertical (y) axis. The horizontal position of a peak is therefore defined by its PRF (and hence its chemical identity). The area under the peak is related to the concentration of the chemical. The choice of sequence and parameters will always be an attempt to balance several aims. The spectra must be of sufficient quality to allow accurate PRF measurements. The quality of an MR spectrum is measured by the evenness of the baseline, the signal to noise ratio (SNR) and the line-width at half maximum height (typically of the non-suppressed water peak or a reference peak, such as NAA). The signal to noise ratio is the ratio of the magnitude of signals from individual metabolites to the background noise, which is manifest as random fluctuations of the baseline not attributable to any recognised metabolite signals. The line-width at half-maximum height (also simply called line-width, LW) is a measure of the range of frequencies covered by a given peak.

Perfect spectra collected from pure samples in a laboratory MRS instrument consist of lines rather than peaks, with each peak being less than 0.01Hz wide. Such spectra, unfortunately, cannot be produced from in vivo samples with clinical MRI scanners, partly because the metabolites themselves are not pure, and are bound up in cellular compartments instead of being in solution, and partly because it is much more difficult to create a perfectly homogenous magnetic field the size of a human head (for example) than it is to create one the size of a test-tube, especially if structures such as the skull and scalp contribute additional magnetic interference[104, 105]. Thus each peak within a spectrum acquired in vivo covers a small range of frequencies. The greater the line-width, the more difficult it is to identify the horizontal position of the peak, that is, to attribute a single frequency to the peak. This is particularly important here since minor changes in the PRF, thus minor shifts in the horizontal position of one of the peaks are used to derive temperature.

New sequences are being developed constantly, and one choice that researchers must make is whether to use standard MRS sequences available on their MRI scanner, or to develop their own. This will usually be decided by the expertise of the researchers themselves (e.g. MR physicists are more likely to develop their own sequences than...
clinicians). There are some important advantages to using the standard sequences. First, they can be applied without the possibility of voiding the manufacturer’s warranty on the scanner itself (development of new sequences typically requires a research agreement be signed with the manufacturer before development begins). Secondly, the techniques are more widely applicable, since radiographers at other facilities can apply the standard sequences without requiring any special training or modification of their scanner software. A number of improved sequences tailored to MR thermography have been published in recent years [106-109]. However, most of these sequences are not yet widely available, and there is little evidence that they provide enough of an advantage over the standard MRS sequences. Some of the new techniques are notably faster, but this is more relevant to monitoring tumour thermal therapy than the monitoring of stroke patients.

1.3.5.2 Single-voxel versus multi-voxel techniques

The methods of MRS acquisition can be broadly divided into single voxel and multi-voxel techniques. A voxel is essentially a 3 dimensional pixel, and 3 dimensional MRI scans are divided up into a number of voxels in the same way a 2 dimensional photograph is broken up into pixels.

Single voxel techniques, as the name suggests, collect MRS data from a single voxel in each scan, while multi-voxel techniques, often called MR Spectroscopic Imaging (MRSI) or Chemical Shift Imaging (CSI), simultaneously sample a grid of voxels with localised spectroscopic data from each voxel. There are several advantages and disadvantages to both techniques, but the most important for thermography purposes is simply that multi-voxel techniques can collect data from a large volume (almost the entire brain) simultaneously [104], thus allowing the analysis of different regions of interest (such as infarct, penumbra and healthy tissue) from a single scan [103, 110, 111]. However, the spectra from each individual voxel are not as reproducible as those collected via single-voxel methods[103, 104]. A single voxel scan can be conducted much more rapidly than an MRSI scan, though if multiple voxels are to be collected the total scan time may be longer. For diagnostic purposes of conditions such as Alzheimer’s disease, the spectra from specific regions of the brain are compared to a ‘normative database’[103] which, at the time or writing, does not exist for MRSI spectra[104]. This is referred to as ‘global spectroscopy’[104]. Conversely in pathologies such as tumours, spectra from the outer
edge of the tumour are often compared to those from healthy appearing tissue and from the centre of the tumour to provide insight into how aggressive the tumour may be[104]. This is referred to as ‘focal spectroscopy’ and for these purposes, a normative database is not required and MRSI is often more appropriate[104]. Therefore, the choice of single-or multi-voxel sequences will often be decided by the number of voxels that need to be examined and the type of pathology being examined. If significant changes in the level of metabolites such as NAA, or the presence of metabolite resonances such as lactate were the primary focus of the MRS acquisition, MRSI would probably be the more appropriate technique in the case of ischaemic stroke. However, given that MR thermography relies on detecting very minor changes in spectra, the higher quality of single voxel spectroscopy techniques offers a definite advantage. Furthermore, the individual voxels from an MRSI sequence typically need to be post-processed individually so, while scanning time may be reduced compared to single voxel techniques, data processing may take longer. However, new multi-voxel techniques are currently being developed [109] that may increase the quality of MRSI spectra to a point where they become the sequence of choice for MR thermography.

1.3.5.3 Voxel size and scanning time
MRI typically focuses those protons in water and fat, both of which produce strong signals. MRS aims to distinguish much weaker signals from chemicals that are found in concentrations no more than 1/10000 that of water in the brain and therefore must make use of larger voxels. The voxel size can be adjusted for each scan but will always be a compromise. Inevitably, the larger the voxel, the higher the SNR of the spectrum that will be collected from that voxel [21, 104]. However, the spectrum collected (and the temperature derived from that spectrum) will represent an average value across the whole voxel. Therefore, a voxel size must be chosen that achieves the spatial resolution needed for a particular study, while also achieving the SNR needed to derive accurate temperature measurements.

Scanning time requires a similar compromise. The scanning sequences can be tweaked to adjust the total scanning time for each study. In general, the longer the scanning time, the higher the SNR. The temperature measurements will necessarily represent an average value across the scanning period. This is not overly important for studies of stroke or
hypothermia induction, as the maximum scanning time is typically not more than 5 minutes, but it does become important when monitoring tumour thermal therapy [101]. More relevant to stroke studies is the issue of patient movement. Stroke patients frequently find it difficult to remain perfectly still for any length of time, especially if their ability to understand instructions has been affected by the stroke. The longer an MRS scan takes, the more likely it is that the patient will move, and any movement will reduce spectral quality and interfere with spatial localisation of the temperature measurement. Thus, a scanning time must be selected that is long enough to collect a good quality spectrum, but short enough to ensure patient comfort throughout the scan. In this respect, single-voxel techniques can have another advantage. Three single-voxel scans which are each 1-2 minutes long, but are separated by breaks can be easier for a patient to tolerate than a single MRSI scan which takes 4-5 minutes (personal experiments for honours thesis, unpublished data).

1.3.5.4 Water suppression

In the human brain, water is ten thousand times as concentrated as the next most concentrated metabolite (NAA) [21]. Thus the water peak is typically much wider, as well as higher than the other peaks in an MR spectrum acquired in vivo. In many cases, the water peak will actually overlap some of the other peaks, making it impossible to quantify them accurately [105]. There are a number of techniques available to suppress the water signal and allow accurate measurement of the smaller peaks. All sequences involve a number of RF pulses and signal collections (the number of such collections is referred to as the ‘number of averages’, and can be varied along with other parameters). Some sequences collect a number of unsuppressed ‘water reference’ averages, and then use a series of RF pulses to suppress the water peak completely (this is called a Chemical Shift Selective suppression, or CHESS sequence, as it selectively suppresses signals with a PRF, or chemical shift, close to that of water), allowing data on the other peaks to be collected accurately. Other sequences use a CHESS pulse that only partially suppresses the water signal, and collect data on all the peaks for every average. Different manufacturers tend to favour different approaches for historical, rather than scientific reasons, and in this thesis a partial water suppression pulse was chosen.
1.3.6 Spectral processing

1.3.6.1 Basic processing
The signal detected by an MRI scanner is actually a series of electric currents induced in the receiving coil, all of which oscillate and decay at different rates[21]. The raw electrical data collected by the MRI scanner is known as ‘free induction decay’ and is illustrated in Figure 1.3.4 below[21]. There are a number of different software packages and methods available to process the raw FID into a spectrum, and then derive clinically useful data from the spectrum itself[103, 104, 112]. Some processes are applied universally, such as Fourier transformation which separates the FID into a spectrum of frequencies like that seen in Figure 1.3.3). Others will depend on the preferences of the operator and the software they have access to. In general, besides Fourier transformation, the signal will be phase-corrected (either manually or automatically, depending on the software) and a filter (also known as apodization) will be applied to improve the quality of the spectra, and various techniques are applied to accurately determine the frequency of each peak[112]. Some of these techniques are explored in more detail below.

MR spectroscopy frequency can be measured in either hertz (Hz) or parts-per-million (ppm). The two units are interchangeable, although ppm has the benefit of not being dependent on magnetic field strength[112]. If the operator is measuring frequency in hertz they must remember to adjust any calculations for the magnetic field strength of the scanner.

1.3.6.2 Filters and Zero-filling
A filter is applied to the signal before Fourier transformation, and is a well established method to improve the quality of the final spectrum. The two most commonly used filters are Lorentzian and Gaussian filters. The mathematical processes used for each are slightly different, but both work to reduce the amount of ‘noise’ that appears in the final spectrum, and increase the contribution of metabolite signals. Lorentzian filters produce better SNR than Gaussian filters, and are often favoured for this reason[104, 112]. However, Gaussian filters produce better spectral resolution, particularly of overlapping peaks [21], and may therefore be better suited to MR thermography, where extremely accurate frequency identification is required[104].
Zero-filling is likewise applied almost universally[112]. This technique involves adding a number of zero-points to the end of the FID signal after the point at which the signal itself has decayed to zero. This simulates continuing to collect the signal over a longer time, but does so without collecting the noise that would be detected if the receiver were to actually continue collecting data over this time[112] (See Figure 1.3.4). Zero-filling makes little difference to the signal itself, as it does not add any new data, but it does improve the digital resolution of the spectrum. The digital resolution will determine how precisely the operator can determine the frequency of each peak, and hence determine the precision of any temperature measurements made from the spectrum [21, 111]. To minimise the time required by the computer to perform Fourier transformation, the total number of data-points collected should be a power of 2[112]. Therefore, the number of zeros added to a signal during zero-filling should ideally make the final number of data-points a power of 2[104].

![Figure 1.3.4 Effects of zero-filling on the FID (Left) and the resulting spectrum after Fourier Transformation (Right). From [112], reproduced with permission.](image-url)
1.3.6.3 Line-fitting software

As mentioned previously, it can be difficult to attribute one specific frequency to a peak that appears to span a range of frequencies. To overcome this, many researchers use software which attempts to fit a line-of-best fit (typically Lorentzian-shaped[105]) to each peak, and use this line to identify the centre frequency of the peak. This same line-fitting technique is sometimes used to remove the water peak from the spectrum mathematically [110] rather than (or as well as) suppressing the water signal during the scanning process[104]. Each MRI scanner will have its own software for performing line-fitting, and there are also several off-line software packages available. Some researchers develop proprietary software for the purposes of MR thermography [111, 113]. Thus, researchers analysing clinical MR spectra have a number of options with regard to software, a relevant selection of which will be discussed below.

Utilising the standard software supplied with the MRI scanner provides a number of benefits. This would simplify the processing as spectral data would not need to be transferred to another computer for analysis. Any methods using scanner software will be easily generalizable to other hospitals using the same type of MRI scanner and do not add to the cost of the experiment (in contrast to purchasing third party software).

Unfortunately, not all scanner packages are suitable for this purpose. The MRI scanners used for the purposes of this thesis were both Siemens Scanners using the Siemens Syngo software. While the line-fitting algorithms included with this software are excellent, they only report the resonance frequency of individual peaks to a precision of 0.01ppm or 1Hz which is not sufficient for temperature estimation (0.01ppm being equivalent to approximately 1°C [100]). This may be why researchers conducting MR thermography do not typically use standard scanner software despite the logistical benefits of doing so.

There are a number of third-party software packages that can be used to process MR spectra. Some, such as LCModel, provide excellent processing algorithms but are prohibitively expensive. One package, the java Magnetic User Interface (jMRUI) is also well-regarded by researchers and has the advantage of being free for academic use [114]. Unfortunately early experiments using jMRUI on spectra collected for this series of experiments were unsuccessful and this software package was abandoned. It was later determined that the line-fitting algorithm in jMRUI assumes that a separate unsuppressed
water reference has been collected, and does not produce accurate results when this is not the case. To collect such a reference on the Syngo software being used at the time would have required conducting a separate scan of each voxel after conducting the initial scan. It was decided during the initial design of the study that the extra time and complexity of this deviation from the standard scanning protocol would discourage the clinical application of the protocol in an acute stroke setting. However, the lack of an unsuppressed water reference is a key weakness of the thermography paradigm tested in this thesis. At the time of submission, a new version of Siemens Syngo is in use which does collect a separate unsuppressed water reference as part of the standard single-voxel MRS protocol, which could have a positive impact on the accuracy of MR thermography based on the standard MRS protocol.

Slight differences in the line-fitting algorithms used by different software packages can affect the calibration curve of PRF against temperature, in particular the intercept of the PRF/temperature graph [105]. It is worthwhile, therefore, for each researcher to calibrate their own technique, including their method of data processing, rather than relying on calibration equations published by other researchers, unless the previous scanning and post-processing paradigm is being replicated exactly.

There is another pitfall with regards to line-fitting software. Line-fitting algorithms typically rely on ‘prior knowledge’ regarding the relative locations of different peaks. By relying on this prior knowledge, the software may fit the curve where its files state the peak should be, rather than determining where it actually is, especially if the peak is shifting as a result of temperature changes.

1.3.6.4 Temperature-insensitive reference
There are several metabolites with temperature-insensitive PRFs found in the brain. Many of these metabolites produce signals which decay quite quickly, so a short echo time (TE) is needed to detect the signals[104] (See Appendix 1 for an explanation of echo time). However, longer TE s tend to produce cleaner baselines and therefore spectra that are simpler to analyse with regard to certain metabolites, at the expense of not detecting other metabolites at all [21, 104]. Complex baselines are particularly prevalent in spectra acquired by MRSI, but can be present in spectra acquired using SVS. Most MR thermography studies make use of only 1 reference peak and often use MRSI sequences,
therefore longer TEs have been favoured in order to simplify the spectral baseline for this single reference peak [110, 111, 115].

The three resonance peaks that are easiest to identify in the brain at long TE (or short TE, for that matter), are the CH₃ resonance of Choline, the CH₃ resonance of Creatine and the Acetyl moiety of N-Acetylaspartate (NAA) at approximately 3.2, 3.0 and 2.0ppm, respectively[103, 104]. Each of these metabolites also produce other, smaller resonances (in particular the CH₂ group on creatine, labelled ‘Cr2’ by Siemens Syngo software, is frequently discernible even at longer echo times). For the purposes of this thesis, all references to choline or ‘Cho’ creatine or ‘Cr’ and NAA refer to the prominent resonances described above, unless otherwise indicated. NAA is the most widely used as a temperature reference[116], in part because it is present in higher concentrations in the brain than the other metabolites, and is well separated from other resonances[104]. Choline and creatine are quite close to each other and the resonance peaks may overlap if the voxel has been poorly shimmed, making them less widely used [105] although careful post-processing can mitigate this problem to a certain extent (see section 1.3.6.2). Injuries such as ischaemic stroke may alter the concentrations of certain metabolites making the choline and creatine peaks difficult to identify. The few studies that have compared temperature measurements made using different reference peaks have typically found no significant difference between them, and it has been demonstrated that more accurate results can be obtained by averaging the temperature measurements from more than one reference chemical [117]. This is a very convenient technique for increasing the accuracy of the temperature measurements made using MRS, although it remains to be fully validated. In ischaemic brain tissue, it may not be possible to identify all three reference peaks readily. Furthermore, one study did find significantly different results by using two chemical references (choline and NAA) in stroke-affected dogs[118], even though they had previously found that this was not the case in pigs, before, or after death[105].

Some validation studies have used fat as a reference chemical. This is relevant to MR thermography of fatty tissue such as the human breast, but is of little use in the brain [99].
1.3.7 Potential pitfalls of MRS thermography

The accuracy with which MRS can detect temperature is affected first and foremost by the accuracy with which an operator can determine the frequencies of relevant metabolite peaks. The three peaks most often examined for MR thermography are typically treated as resonances from single chemical species. However, each of the peaks is in fact made up of several resonances which are similar enough that they cannot be differentiated in vivo but are not, in fact, identical. Even the water peak contains a second (unidentified) resonance slightly offset from the main peak [119]. The NAA peak is, in fact, made up of NAA and N-acetylaspartylglutamate (NAAG). The creatine peak represents both creatine and phosphocreatine. The choline peak represents glycerophosphorylcholine and phosphorylcholine as well as choline. The PRFs of these additional components are invariably slightly offset from the ‘prime’ resonance, that is, the PRF of the most concentrated metabolite [99, 119]. Thus, if the relative concentrations of the components that make up a resonance peak change (as may happen during tissue injury or necrosis), the centre of the peak may change, which would in turn alter any temperature measurements made from that peak.

Due to the complex nature of living tissue, PRF can never be determined as accurately in vivo as it can in vitro. It has been demonstrated that the relationship between the PRF of water and temperature is independent of tissue type [101, 115, 120]. However, the quality of spectra that can be collected is affected by minor variations in chemical make-up, patient movement and a multitude of other factors. Hence, while the relationship between PRF and temperature measured in vitro is the same as that measured in vivo, the level of accuracy of any temperature measurements derived from this relationship is not. This must be taken into account when using a model validated in vitro to measure temperature in vivo.

Hydrogen bonding, and hence the PRF of water, is affected by pH [21]. This is of particular concern since the pH of ischaemic tissue tends to be lower than normal due to the production of lactic acid by ischaemic cells. It has been previously shown, however, that pH changes within a physiological range (pH>5.5) have minimal effect on the MRS spectra[115], although this effect likely contributes to the overall margin of error found in MRS-based temperature measurements in vivo.
As mentioned previously, differences in post-processing software can cause slight differences in the slope and the intercept of the PRF/temperature graph. Even the same software may produce different results if different ‘prior knowledge’ files are utilised. It was therefore considered worthwhile calibrating temperature estimations using the exact scanning and spectral-processing paradigm that was to be implemented in this thesis, rather than relying on figures from the literature.

1.3.8 Previous validation studies

Cady et al [115] tested MRS thermography in a phantom, and in a piglet using a 7T research scanner. They found that reducing the pH in the phantom to 2.8 did not alter the slope of the PRF/temperature model, but did shift the intercept by the equivalent of approximately 3°C. This would obviously affect the accuracy of any absolute temperature estimations that did not account for changes of this magnitude in the pH of the sample. However, the pH changes in the piglet resulting from induced cerebral ischaemia did not affect temperature measurements. Overall, MRS-based thermography was found to be accurate to within ±0.75°C in vivo using this technique. It should be noted that clinical (non-research) scanners are currently limited to 3T, which reduces the level of accuracy that can be achieved in the clinic. Cady et al also found in a subsequent study that the accuracy and reproducibility of temperature estimations could be improved by calculating an amplitude-weighted average of the temperatures from each reference metabolite (i.e. Choline, Creatine and NAA) [116].

Corbett et al tested a single-voxel technique in healthy pigs [105] and then in stroke-affected dogs [118] using a 1.5T clinical MRI scanner. They found that their MR thermography measurements were accurate to within approximately ±1.0°C in vivo. Importantly, they found the relationship between PRF and temperature was independent of tissue injury (24 hours after stroke onset) or scanner parameters such as echo time (TE). However, they did report a significant difference in the temperature measured using choline as a reference compared to NAA in the stroke-affected animals (whereas in the non stroke-affected pigs the measurements were similar enough that they could be averaged). The reasons for this are unknown, but should be considered before the practice of averaging the temperatures from different metabolites is globally applied to improve the accuracy of MR thermography.
Marshall et al [110] tested an MRSI sequence in a homogenous phantom, and in healthy volunteers using a 1.5T clinical scanner. The calibration of this technique relied on using the figure 0.01ppm/°C from the literature as the slope of the PRF/temperature graph. They derived the intercept of the graph by scanning 20 healthy volunteers, and assumed that their average brain temperature was 37°C. This is not ideal as a method of calibration, but did allow the authors to compare differences in average temperature across different tissue-types when they applied the technique to stroke patients [121, 122]. The authors also found that the quality and reliability of the MRS spectra collected in vivo were significantly degraded compared to in vitro measurements, and many voxels had to be discarded as being too poor to process. The authors were therefore restricted to making group comparisons, rather than deriving data that might influence treatment in individual patients. These group comparisons were put to good use in a number of subsequent studies [123-125].

Weis et al tested another MRSI technique which provided much better spatial resolution (using voxel sizes of less than 7 cubic mm) at the expense of spectral information. This study was conducted in pigs with brain temperature being monitored by MR-compatible fibre-optic probes which provided a reference temperature that the authors could be quite confident in, but produced spectra that did not allow the analysis of reference metabolites such as NAA (only the water PRF could be determined). Thus, the technique tested would only be useful for monitoring relative changes in temperature. The authors found similar problems to Marshall et al in terms of failed voxels [126], with fifty percent of all spectra collected in vivo being rejected by the authors for poor quality or unrealistic temperatures.

Thrippleton et al compared the reproducibility of MRSI-derived temperature maps at 1.5T and 3T [127]. This study found that the biggest source of variation was between voxels within the same examination, rather than between patients or between days. Reproducibility was found to be better at 3T than at 1.5T, with residual variation at 3T approximately half that at 1.5T (0.14°C vs 0.36°C).

Childs et al [111] tested both an SVS and an MRSI technique in a phantom and in healthy volunteers. They found spectra collected by SVS to be significantly more reliable than those collected by MRSI. They went on to perform temperature measurements in healthy volunteers, but used the PRF/temperature equation published by Corbett et al, rather than
calibrating their own technique, which may impede the accuracy of their absolute temperature measurements.

Other authors [109, 128] have published improved sequences for MRSI, which may make this technique more useful in the near future. For the moment, however, the data available on the reliability or accuracy of temperature measurements made using these sequences are very limited, and the sequences themselves are not widely available for further experimentation.

Zhu et al [119] tested a single voxel technique in an 11.74T, small-bore research scanner. This technique demonstrated an accuracy of ±0.5°C in a mouse, using NAA as the single reference. This result is extremely encouraging although clinical scanners which use much lower field strengths are unlikely to replicate this level of accuracy.

Covaciu et al [117] validated temperature measurements from MRS in a phantom using NAA, choline and creatine. Their results were similar to those of previous studies. However they were the first to demonstrate that that accuracy could be improved by taking the average of these 3 temperatures for any given scan.

1.3.9 Previous applications of MRS thermography in the human brain

1.3.9.1 Applications in healthy volunteers
MRS thermography has previously been used to examine variations in regional brain temperature, the brain-body temperature gradient and changes in brain temperature associated with pathology.

Corbett et al [105] applied their technique to measure regional temperature variations within the healthy brain. They found a statistically significant temperature difference between the thalamus and superficial frontal lobe tissue, but did not examine any of the other factors that affect brain temperature (such as metabolic rate and blood flow).

Childs et al applied their single voxel technique [111] to measure four different voxels within a single cerebral hemisphere in healthy volunteers. They did not find any significant temperature differences between the different regions, but did report that all brain
temperatures recorded were significantly lower than core body temperature. This contradicts many previous studies on the brain-body temperature, but may be explained by the fact that the authors did not calibrate their own scanning and post-processing technique, thereby reducing the reliability of their absolute temperature measurements.

Kauppinen et al [129] used SVS at 3T to measure changes in brain temperature associated with changes in cerebral blood flow and cerebral metabolism. The study found that neuronal stimulation (which increases both CBF and metabolism) did not alter local temperature. However, hypercapnia (which increases CBF without altering metabolism) resulted in a decrease in brain temperature. The authors claim that their temperature measurements were accurate to within ±0.2°C. However, this figure was based on the fact that the mean standard deviation of seven consecutive MR thermography acquisitions within each of a series of healthy volunteers was 0.2°C. However, this figure represents the reproducibility of the measurements, rather than the accuracy. No other study that has performed a more thorough validation has reported this level of accuracy for MRT conducted in vivo at 3T, or even at 7T [115].

Harris et al [130] used the MRSI technique published by Marshal [110] to examine the effects of head fanning, and combined head and neck fanning on cerebral temperature. They found that 30 minutes of head cooling resulted in a 0.45° change in average brain temperature. When this was followed by 30 minutes of head and neck cooling, it resulted in a further reduction of 0.37°C. Importantly, the authors found no significant temperature differences between the superficial and deep brain structures, and the core body temperature was reduced to a similar degree to brain temperature, suggesting that the cooling effect on the brain was due to a reduction in blood temperature rather than conduction of heat through the skull and scalp.

Ishigaki et al used single-voxel spectroscopy and the temperature calibration published by Cady et al [115] to compare interhemispheric temperature differences between healthy volunteers and patients experiencing chronic carotid artery stenosis or occlusion [131]. The authors hypothesised that greater levels of hemispheric hypoperfusion (detected by PET scan) would correlate with greater differences in temperature. This study did not find any significant difference between patients and controls. However, the same group subsequently found that greater interhemispheric temperature differences in patients
before treatment by carotid endarterectomy (CEA) were correlated with an increased risk of potentially dangerous hyperperfusion after CEA [132]. Thus, MR thermography could potentially be used to screen patients before CEA to enhance management of hyperperfusion in those patients found to be at higher risk.

1.3.9.2 Applications in stroke patients

The application of MR thermography to stroke patients has thus far been more limited. Karaszewski et al [121] used the same MRSI method published by Marshall [110] to examine temperature in different regions within the brains of ischaemic stroke patients. Each patient was scanned within 26 hours of stroke and the voxels within their scan were classed as “definitely abnormal, possibly abnormal, possibly abnormal plus (grouped together with possibly or only slightly abnormal voxels as “potential penumbra”), ipsilateral normal and contralateral normal”, according to their appearance on diffusion-weighted imaging. The poor quality of individual spectra limited the analysis that could be conducted to relative comparisons between groups of voxels and made analysis based on absolute temperature measurement impossible. Nonetheless, this study produced some interesting results. The “potential penumbra” was found on average to be warmer than “definitely abnormal” tissue, which in turn was warmer than normal tissue on average. However, there was significant variation between individual patients within these average values. In some patients, the definitely abnormal tissue was found to be cooler than normal tissue, and it was found that this was more likely if the level of blood flow in the lesion was normal. In patients with reduced blood flow within the lesion, the abnormal tissue was typically warmer. Higher temperatures in the lesion were also associated with larger infarcts and more severe strokes. Interestingly, the definitely abnormal tissue tended to be cooler than contralateral tissue in patients not found to have a perfusion/diffusion mismatch (a sign of tissue which is hypoperfused but not yet infarcted) but warmer than contralateral tissue in patients who did demonstrate a perfusion diffusion mismatch.

Karaszewski et al extended on this study by conducting two MR thermography examinations (<26hr and 3-5days post stroke onset) on a new cohort of non-thrombolysed patients who also underwent much more frequent tympanic temperature measurements (every 4 hours from admission) [123, 124]. This allowed the authors to collect data on when tympanic temperature peaked and the total “thermal load” (area under the
temperature/time curve) for each patient, and to compare this temperature data to both brain temperature at the two MRI time-points and clinical outcomes. Interestingly, they found that tympanic temperature peaked on average 1.5-2 days after stroke and that temperature in patients with more severe stroke tended to peak later and remain elevated over a longer period[123]. Peak temperature and thermal load did correlate with stroke severity (as measured by admission NIHSS) whereas admission temperature did not. With regard to the MR thermography data, this study found that brain temperature was consistently higher than the corresponding tympanic temperature and that possible penumbra was, on average, warmer than contralateral normal tissue both at admission and follow-up. Contralateral normal tissue temperature during the admission MRI scan was found to be higher on average in patients who experienced subsequent lesion growth than those that did not. The temperature of the contralateral normal tissue at admission was also found to correlate with initial stroke severity where the temperatures of other brain regions did not. Ipsilateral brain temperatures (both normal and abnormal) tended to be higher at admission in those patients whose NIHSS worsened over the subsequent 5 days than those that did not. The authors postulate that the mechanisms of temperature elevation are different in ischaemic tissue to those in healthy brain tissue within a stroke-affected brain. They argue that temperature in ischaemic tissue is probably elevated by the reduction in the amount of heat being removed from the brain tissue by perfusion (which is also one of the key assumptions of the heat exchange simulations described in Chapter 5 of this thesis) whereas temperature in non-ischaemic brain is probably elevated by systemic inflammatory responses to ischaemia [124].

The same group tested their inflammation hypothesis by comparing the recorded inflammatory marker (C-reactive protein and fibrinogen) levels in the same cohort of patients on the same days that the patients underwent MR thermography examination [125]. They found that higher levels of inflammatory markers were correlated with elevated tympanic temperature and normal brain temperature at both time-points, but with ischaemic brain temperature only at the time of the admission MRI. Overall this study did provide some evidence that inflammatory processes may affect brain temperature in the stroke-affected brain but the sample-size was too small to draw any conclusions.
Bainbridge et al successfully applied the amplitude-weighted averaging technique described by Cady [116] to human neonates undergoing hypothermia therapy for neonatal hypoxia-ischaemia [133]. These authors performed temperature estimations from the spectral data using both the calibration published by Cady [116] and that published by Zhu et al [119]. Bainbridge et al found that not only did they derive different absolute temperatures using the different calibrations, but the limits of agreement between temperatures calculated from different reference metabolites also varied noticeably depending on which calibration was used. Nonetheless, the temperatures calculated using the amplitude-weighted average and the Zhu calibration were moderately successful, achieving correlation coefficients (when brain temperature was compared to rectal temperature) of 0.85 and 0.78 in grey matter and white matter respectively. It is also noteworthy that this study was conducted at 1.5T, on a scanner very similar to the one used in this thesis.

1.3.10 The next step
MR thermography is ideally suited to ischaemic stroke research, particularly MR thermography based on MRS. It has already been demonstrated that single-voxel spectroscopy can be used to measure temperature in the brain, in both healthy and infarcted tissue. Further validation is needed, however. As has been stressed previously, it is important to calibrate each scanning and post-processing technique independently, as the subtle differences in software packages may alter the absolute temperature measurements. It is therefore necessary to settle on, and calibrate a scanning and data processing paradigm in vitro. Having done so, it will be necessary to try to determine the level of accuracy this paradigm can achieve in vivo, mindful that it is unlikely to match the level achieved in vitro.
Chapter 1.4 in silico Modelling of Heat Exchange in the Human Brain

1.4.1 Reasons for in silico modelling of heat exchange.

1.4.1.1 Advantages of in silico modelling.
There are a number of advantages of in silico modelling over in vitro and in vivo experiments. Firstly, there is no risk of harm to researchers or experimental subjects, which allows experiments to be conducted that would be unsafe or unethical if they involved human or animal subjects. However, the greatest advantages of in silico experimentation lie in rapidity and thoroughness. A computer model may be developed over a matter of weeks and simulations can be run over a matter of hours, or even minutes. This means that an experiment can be repeated many times in a single day, with changes to individual parameters made in between each run. Computer simulations can be more thorough than in vitro or in vivo experiments because data collection is not limited by the number or type of sensors that can be employed in each experiment. To take temperature as an example, there are practical limits to the number of thermometers that can be implanted in a brain, human or animal, which in turn limits the number of locations at which temperature can be monitored. In a computer simulation, on the other hand, temperature can be monitored continuously in thousands of locations at once, as can pressure, fluid flow, chemical concentration and any number of parameters the experimenter wishes to monitor. Obviously, increasing the level of complexity that is built into a model will often increase the time required to code each simulation and increase the amount of data that must be stored and analysed but, in general, this makes an in silico experiment slower to conduct, not impossible.

1.4.1.2 Limitations of in silico modelling
For simulations to be useful there must be some experimental data available. First, there must be some data available to allow the researcher to assign accurate material properties and appropriate boundary conditions. Second, data regarding the results of the processes
being simulated are required against which to compare the results of the simulation to establish whether the simulation is valid. Thus, modelling cannot replace observation and experimentation entirely, but can supplement it by filling in the gaps between experimental data [134].

1.4.1.3 The process of in silico experimentation

The first step in in silico experimentation is to determine which processes the experimenter wants to simulate, such as chemical reactions, heat transfer or fluid flow. This determines the modelling algorithm to be used. It is seldom necessary or practical to include every minute detail of a system in a computer model, and experimental data are not typically available for every parameter. Therefore, a conceptual model is first formed that imposes a number of simplifying assumptions on the system and allows the model to be populated with whatever data are available. The computer code required to simulate this conceptual model is then generated, a process referred to as ‘coding’ [135].

Once a computer simulation is completed, before the results can guide further experimentation or clinical decision-making, the experimenter must perform the processes of verification and validation [135]. Broadly speaking, verification is the process of ensuring that the simulation matches the conceptual model, while validation is the process of ensuring that the simulation matches reality [136].

The process of verification involves testing for errors in the computer code, and testing the dependence of the simulation results on computer settings that are not part of the model itself [135, 136]. The computer parameters to be tested in verification vary according to the type of model and the software being used, but one important example is time-step. The time-step of a model is the frequency with which the software measures the
parameters of the simulated environment and performs calculations on these parameters. The shorter the time-step, the more accurate and more detailed the results of the simulation but, once again, the time and computing power required to run the simulation will increase. Therefore, an experimenter generating a model will aim to make the time-step as long as possible, while keeping it short enough that shortening the time-step further would not result in any substantial difference in the results of the simulation. This process of ensuring that shortening the time-step does not significantly alter results forms an important part of verification [135, 136]. The process of verification will vary with the type of model being verified and the specific computer settings to be tested, and will therefore be described in detail in the first publication of a given model.

Validation revolves around comparing the results of a simulation to experimental results from a similar *in vivo* or *in vitro* experiment. This tests whether the model does reliably simulate reality within the limits of available knowledge [134, 135]. Validation data may be difficult to obtain for biological simulations, and acquiring such data remains a key challenge to *in silico* biomedical experiments [134]. It is always a risk that even the most carefully constructed model will not produce results that fit experimental data. However, this can be a useful finding in itself as it may indicate that the current understanding of the process being simulated is incorrect, or that factors thought to be irrelevant to the simulation are, in fact, having significant effects.

1.4.2 Bioheat transfer modelling

1.4.2.1 General heat transfer modelling

Bioheat transfer refers to the flow of heat energy within a biological system, typically a tissue or organ, although crude models of entire organisms have been produced [137]. Heat transfer is an important aspect of mechanical engineering, and simulations of heat transfer are regularly performed by mechanical engineers. These simulations are often focussed on one or more of the cooling systems within a machine such as a car or aircraft engine. To take a (grossly oversimplified) aircraft engine as an example, most of the cooling is provided by cool air flowing through the engine. The simplest method to determine if this cooling effect is sufficient is to examine the volume and temperature of the air entering the engine, and exiting the engine. This provides information about the
total amount of heat energy being removed from the engine, and potentially the average
temperature of the engine itself. However, this approach does not provide spatial
information about the engine components. It is possible for some components of the
engine to be overcooled while others overheat without this being manifest in the average
engine temperature. It is seldom practical to insert temperature probes into every
component of an engine while it is running, and this is where heat transfer modelling has
the potential to contribute. By modelling the heat generation (through friction, fuel
combustion and other processes) and conduction between the different components of the
engine and the air, an engineer can anticipate spatial inconsistencies in temperature and
correct for them. The same principle applies to a number of medical techniques that rely
on inducing a temperature change, such as therapeutic hypothermia, cryosurgery or
localised hyperthermia [138, 139]. The latter two techniques are often used in the
treatment of tumours, when the aim of the process is to induce a significant temperature
change in the tumour without overly affecting the surrounding healthy tissue.

1.4.2.2 Complications with bioheat transfer modelling

Compared to standard heat transfer simulations, there are a number of factors that make
bioheat transfer relatively complex [139]. The existence of blood flow is arguably the most
important such factor. Blood can act as a heat sink or heat source (depending on the
temperature difference between the blood and the tissue) and heat exchange between the
blood and the tissue will depend on the path blood takes through the tissue [139]. The
precise anatomy of capillary beds is likely to vary between individuals, and between
different tissue types within an individual. Furthermore, there is currently no practical
method to examine the microstructure of capillary beds in living patients. In addition, the
temperature of the arterial blood may vary along the length of an artery, particularly in
arteries supplying limbs with a large surface area-to-volume ratio (such as arms in humans
or fins in marine mammals) or arteries subject to counter-current heat exchange1 [140].

1 Counter-current heat exchange is a process that occurs when the arteries and veins supplying an
extremity run parallel to each other. Under such circumstances, warm blood in the artery loses heat
to cool blood in the veins, and by the time the arterial blood reaches the capillary beds, where most
of the heat in the blood would be lost to the environment, the blood temperature is very close to
the surrounding temperature. This means that the heat energy in the arterial blood is lost to the
venous blood (and not to the surroundings), which carries the heat energy back into the central
circulation. This mechanism is commonly found in the fins and other extremities of marine
mammals and aids the maintenance of temperature homeostasis in very cold environments.
Blood flow through such regions may also be altered by homeostatic responses such as vasodilation or constriction near the skin surface in response to temperature changes. All of these factors will affect heat exchange between the blood and tissue and may potentially change on a minute-by-minute basis. It is not practical to collect the data on blood flow that would allow an engineer to build all of these variations into a model of bioheat transfer. Therefore, engineers have been forced to find ways to simplify the ‘perfusion’ term of bioheat equations (that is, the term that accounts for heat transfer between the blood and the tissue) [138].

Another complicating factor of bioheat transfer is the heat generated by tissue metabolism. Every cell within the body generates heat and, like blood flow, this heat generation can vary from one cell to the next and from one second to the next within a given cell. It is not possible to measure metabolic heat generation at a cellular level in living patients. Techniques such as Positron Emission Tomography (PET) can measure tissue metabolism with a spatial resolution of 2.4mm [141] but cannot monitor metabolism continuously for any length of time, making it difficult to include changes in tissue metabolism over time in bioheat transfer models. Thus, the majority of simplifying assumptions made in bioheat transfer models are related to blood flow and metabolic heat generation [138].
Table 1.4.1: Various equations that have been proposed for modelling bioheat transfer.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennes</td>
<td>( \rho_b c_b \frac{\partial T_t}{\partial \text{time}} = -k \nabla^2 T + \omega c_b (1 - \kappa)(T_a - T_t) + q_m )</td>
</tr>
<tr>
<td>Wulff</td>
<td>( \rho_t c_t \frac{\partial T_t}{\partial \text{time}} = k \nabla^2 \rho t_b c_b U_h \cdot \nabla T + q_m )</td>
</tr>
<tr>
<td>Chen-Holmes</td>
<td>( q_p = \omega^* (\rho c) b(T_a^* - T_t) )</td>
</tr>
<tr>
<td></td>
<td>( q_c = (\rho c) b u \cdot \nabla T )</td>
</tr>
<tr>
<td></td>
<td>( q_{pc} = -\nabla \cdot k_p \nabla T )</td>
</tr>
<tr>
<td>Weinbaum-Jiji</td>
<td>( \rho c \frac{\partial T}{\partial t} = \frac{\partial}{\partial x} \left( K_{eff} \frac{\partial T}{\partial x} \right) + q_m )</td>
</tr>
</tbody>
</table>

Legend:
- \( \rho_t \): density of tissue (kg/m³)
- \( c_t \): specific heat capacity (J/kg/K)
- \( \rho_b \): density of blood
- \( c_b \): specific heat capacity of blood
- \( T_a \): Temperature of arterial blood (K)
- \( T_t \): Temperature of tissue (K)
- \( k \): conductivity (W/m/K)
- \( K_{eff} \): effective conductivity
- \( \nabla^2 \): conduction of heat in 3 dimensions
- \( \nabla T \): local \( T_t-T_{blood} \).
- \( \omega^* \): perfusion (blood flow) rate (kg/m³/s)
- \( q_m \): metabolic heat output of tissue (W/m³)
- \( q_p \): heat transfer due to perfusion
- \( q_c \): heat transfer due to convection
- \( q_{pc} \): heat transfer due to ‘perfusion convection’.
- \( \kappa \): Constant that accounts for incomplete equalisation between blood and tissue temperature.
- \( U_h \): Enthalpy flux due to volume averaged velocity of blood flow (Darcy velocity).

1.4.2.3 The Pennes bioheat equation

Arguably the most influential bioheat transfer model is the one published by Pennes in 1948 [142]. This model was originally validated in the human forearm using temperature measurements at various depths beneath the skin of a number of human volunteers. The Pennes model takes the form:

\[
\rho_t c_t \frac{\partial T_t}{\partial \text{time}} = -k \nabla^2 T + \omega c_b (1 - \kappa)(T_a - T_t) + q_m
\]
(See Table 1.4.1 for notations) Pennes set κ to zero for his calibration studies meaning that he assumed the blood temperature fully equalises with the tissue, that is, blood entering a vein will be the same temperature as that of the tissue it has just passed through. Pennes’ experiments validated this assumption [142]. Therefore most subsequent studies using the Pennes equation have also set κ to zero [143], which simplifies the Pennes equation to:

$$\rho_t c_t \frac{\partial T_t}{\partial \text{time}} = -k \nabla^2 T + \omega c_b (T_a - T_t) + q_m$$

The other major simplifying assumptions of the Pennes model are also related to heat exchange between the blood and the tissue and are worth noting separately. Firstly, with κ set to zero it is assumed, as mentioned above, that the temperature of the blood is the same as the temperature of the tissue by the time the blood enters the venous system. Secondly, the perfusion term used by Pennes assumes that virtually all the heat exchange between blood and tissue takes place in the arterioles and capillary beds, and that the distribution of these vessels and the level of flow through them are uniform across the volume of interest. The perfusion term does not account for either the direction of blood flow or the exchange of heat between arterial and venous blood (that is, counter-current heat exchange). Thus, blood is treated as a uniform heat source or sink which instantaneously and continuously either deposits or removes heat energy from all of the tissue within the volume of interest, that is, it treats blood flow as a single, global entity rather than as occurring in discrete vessels. For this reason the Pennes model is referred to as a ‘continuum’ model. These simplifications allowed Pennes to model heat exchange between blood and tissue without having to simulate individual blood vessels themselves. Similarly, metabolic heat generation is assumed to be constant and uniform across the tissue.

1.4.2.4 Limitations of the Pennes equation

A number of papers have questioned the assumptions of the Pennes model, particularly when it is used to model changes in tissue temperature associated with application of a localised heat source [144-146]. This is relevant to modelling thermal therapy for tumours in which a method such as focused ultrasound is used to heat tumour tissue selectively, typically close to the skin of a limb. Under these circumstances, the direction of blood flow can play an important role in dispersing the heat from the tumour to tissue downstream of
the heated region. It is also likely that a certain level of counter-current heat exchange does take place in the circulation of the limbs, where this model is often applied. The central premise of Pennes’ perfusion term, that heat exchange occurs entirely (and uniformly) in the small arterioles and capillary bed, has been the subject of the most intense debate.

Wulff [146] argued with the conceptualisation of the Pennes equation on several levels, though not all of them reasonable. He argued that the simplified Pennes equation treated 3 different media (arterial blood, venous blood and tissue) as occupying the same location in space, which is physically impossible. This is true, but if one considers a given volume of tissue, the volume will almost certainly contain tissue and arterial blood and venous blood; therefore, the three media do effectively occupy the same volume. If one is modelling at a small enough scale to consider individual capillaries within the tissue this is no longer true, but this scale of modelling for heat transfer is not often used. Wulff further argued that the Pennes equation treated the difference in temperature between arterial blood and venous blood as the driving force behind heat exchange between the blood and the tissue when, in fact, the difference between tissue temperature and blood temperature is responsible. This is true of the simplified Pennes equation used by many authors, but only because Pennes assumed that the temperature of blood equalises with tissue temperature before blood enters the venous system. Therefore, the difference in temperature between arterial and venous blood effectively represents the temperature difference between blood and tissue [142]. Wulff himself acknowledged that this is a reasonable assumption, but argued that the blood might equalise in temperature very close to the arteriole, meaning that the blood temperature at the distal end of the capillary would no longer be equal to arterial blood temperature. This is perfectly true but, once again, one would need to be modelling at a small enough scale for the length of individual capillary beds to account for this difference. This scale of modelling is seldom performed, most likely because the data to populate the model are not available.

To examine the usefulness or otherwise of Pennes’ simplifying assumptions, Chen and Holmes [147] devised a parameter known as the equilibrium length ($L_e$), which describes the length along a given blood vessel that blood would need to flow in order for the temperature difference between the blood and the surrounding tissue to be reduced to $1/e$. 
of its original value. Examining a series of typical vessel sizes, Chen and Holmes calculated a parameter they designated ‘xe’, which is the actual length of the vessel divided by its equilibrium length, and postulated that temperature equilibration takes place in blood vessels with an xe value of approximately 1. Importantly, Chen and Holmes’ calculations indicated that the vessels that would be expected to fill this criterion are those with a diameter of approximately 175µm, which correspond to small arteries that feed into precapillary arterioles (and where xe is approximately 9mm). Thus, it is likely that the blood is already at tissue temperature by the time it reaches the capillary bed. How this fact affects the accuracy of the Pennes equation depends on the distribution of these small arteries across the tissue (they may not be as uniformly distributed as capillaries).

A number of more inclusive bioheat transfer models have been described in an effort to improve on the Pennes equation. Some are continuum models, similar to the Pennes equation in that they treat the blood as an isotropic heat source or sink, but more complex in that they scale the magnitude of heat exchange between the blood and tissue according to a number of relevant factors. Others, the so called ‘discrete vessel’ models actually compute the heat exchange between individual blood vessels and their surrounding tissue. However, this type of modelling becomes impractical when dealing with the extremely small size and large number of vessels such as capillaries, so discrete vessel models are typically used in combination with continuum models, applying the discrete vessel models to larger blood vessels and using a continuum model to estimate the effects of small vessels [138].

1.4.2.5 More inclusive (complex models).

Table 1.4.1 lists the various models which have attempted to improve on the Pennes equation. Each of these models has advantages and disadvantages, but most are not relevant to hypothermia modelling in the brain.

The Weinbaum, Jiji and Lemons model [140], and the more simplified Weinbaum and Jiji model [148] derived from it both were both designed only for application in peripheral tissues and assumed that the primary mode of heat exchange in tissue is counter-current heat exchange between pairs of arterioles and venules. As such, these models are not applicable to brain tissue, where counter-current heat exchange is not believed to be relevant [138].
The Wulff model [146] was designed for application to tumour hyperthermia therapy, where blood that has passed a heating implement will convect heat energy to cooler tissue downstream. To account for this convection, Wulff substituted the Darcy velocity of the blood into the Pennes equation (see Table 1.4.1). The Darcy velocity is calculated by dividing the local volume blood-flow by the volume cross section. This model is unlikely to produce more accurate results than the Pennes model unless blood is actually warmer than tissue, or there is a significant temperature difference between ischaemic tissue and normal tissue [100]. In this case, the final localised tissue temperatures would be dependent on convection of heat from ischaemic tissue to adjacent tissue (or vice-versa) as well as conduction between these tissues and the Wulff equation would likely provide more accurate results. However, measuring the Darcy velocity in brain tissue poses problems, especially since the measurement would be heavily dependent on which volume the fluid flow was averaged over. If the flow was averaged over the entire brain the Darcy velocity would effectively be zero unless the cerebral blood volume is increasing or decreasing. One could divide the brain up into smaller volumes, indeed this is a standard part of finite element modelling, and potentially estimate the Darcy velocity for each volume. However, the individual Darcy velocity of a given volume (in terms of direction of blood flow, if not volume or speed) would be heavily dependent on the size and location of the tissue volume being measured. Thus, the results of the model could change significantly even with small changes in the grid that the tissue is divided up into, unless the grid is divided into individual capillary beds, which is likely not feasible. This could make the overall Wulff model less robust than the more simplified Pennes model, even though the results from the Wulff model may be more accurate if the tissue is divided into small enough volumes.

Chen and Holmes proposed a model that separated blood vessels into 2 broad types, large vessels (which covers arteries down to the smallest diameter for which individual analysis of the artery is practical) and small vessels, which must be treated as a continuum. They proposed calculating the effect of large arteries individually, and calculating the temperature of the blood as it exits each large artery as a function of the vessel length, its equilibrium length and the difference between blood and tissue temperature. The small vessels are treated as treated as a continuum, with their effects on heat transfer divided into 3 equations. The first deals with the deposition or removal of heat from the tissue.
caused by blood temperature equilibrating with tissue temperature and the heat energy transferred by this phenomenon was denoted $q_p$ (see Table 1.4.1). The second equation deals with heat transfer between the blood and the tissue when convection is occurring against a temperature gradient and the heat transferred by this phenomenon was denoted $q_c$. The third equation deals with heat transfer along a temperature gradient and is treated as an effective conductivity, since movement of blood in this direction can help transfer heat energy from warm tissue to cooler tissue (hence, effectively increasing the conductivity of the tissue). The heat energy transferred in this way was denoted $q_{pc}$ (for ‘perfusion conductivity’). The total combination of blood flow to the heat transfer equation was assumed to be the sum of the 3 separate equations ($q_p+q_c+q_{pc}$). The Chen and Holmes model is significantly more thorough than the Pennes model, but this comes at the expense of simplicity. Unfortunately since it is difficult, sometimes impossible, to obtain the data on the localised vascular geometry that is needed to populate the Chen and Holmes model, this three equation model has not found widespread use [138]. Thus, until perfusion imaging advances to the point where data regarding the magnitude and direction of blood flow can be collected at the microscopic level, the Pennes equation remains the most appropriate for our purposes. Indeed, the vast majority of studies involving heat transfer modelling in the brain have used the Pennes equation or a minor variation of it. These studies are summarised below.

1.4.3 Previous *in silico* studies of heat transfer in the brain.

Heat transfer modelling has previously been applied in the human brain in a variety of studies. Some studies have used the technique to examine normal fluctuations in brain temperature and the contributions of various factors (such as changes in blood flow) to normal temperature homeostasis [149-153].

Other studies have used heat transfer modelling to examine the risks of tissue heating from radiation associated with medical imaging technology such as MRI (Johansson, Eriksson et al. 2006) and medical devices such as deep brain stimulators [154].

Heat transfer modelling has also previously been applied to the study of cerebral hypothermia, both unintentional hypothermia from events such as cold-water drowning [155] and therapeutic hypothermia have focussed on scenarios such as cardiac arrest,
cardiopulmonary bypass or traumatic brain injury where blood flow has returned to normal levels throughout the brain [83, 156-162]. Few studies have examined heat transfer in localised cerebral ischaemia, and those that have typically did not include an explicit simulation of the penumbra but rather just modelled the infarct [157, 163].

Diao et al [157], used the Pennes equation to model scenarios where cooling is applied to the outside of a stroke patients scalp to induce localised cerebral hypothermia. The cerebral hemisphere was divided into 4 quadrants, and one of these was modelled as ischaemic by reducing blood flow to 20% of its normal value. This model did not include other regions of hypoperfused tissue, such as penumbra or mildly hypoperfused healthy tissue, which are often found in cases of ischaemic stroke. The results of this study matched previous findings that suggest the effects of external head cooling are restricted to shallow brain tissue, but aside from this there are no directly comparable in vivo data against which to validate the in silico results. Indeed, validation has proven to be the most troublesome issue with in silico modelling, as none of the aforementioned studies describe directly comparable validation data.

Slotboom et al [163] conducted a series of simulations and experiments to test the feasibility of infusing cold saline via a micro-catheter directly into the occluded artery, thus cooling the ischaemic brain tissue without inducing systemic hypothermia. Intraarterial microcatheters have already been used to introduce thrombolytic drugs directly into the clot, as these catheters can actually be inserted into or through the clot itself [164] and this method holds the potential to cool the ischaemic tissue effectively without the side-effects caused by inducing systemic hypothermia [163]. The authors conducted a series of experiments to test the magnitude of heat exchange that would occur between the coolant and the surrounding blood stream as the coolant travelled through the microcatheter from the site of insertion (in the femoral artery) to the site of occlusion (in a cerebral artery). After determining the temperature at which coolant could be delivered to the ischaemic tissue, the authors simulated the temperature reduction that could be achieved by infusing coolant at that temperature (approximately 15°C) the authors used a simplified Pennes equation much the same as that used in this thesis to determine the level of tissue cooling that could be accomplished using this technique. They also calculated the potential effect this cooling would have on core temperature. The results of the simulations were
extremely promising, suggesting that the infarcted area could be cooled approximately up to ten times more rapidly than is currently possible by inducing systemic hypothermia, with a change in core temperature of no more than 0.7°C (although this study did not simulate maintaining the cooling for 24 hours, as is the protocol in current use of hypothermia therapy for cardiac arrest patients [93]). However, the authors in this case assumed that the occlusion of the artery was complete, that is, that the only perfusion in the ischaemic zone was that which passed through the microcatheter. They also assumed that metabolism in the ischaemic region was reduced to zero, that is, that the entire ischaemic region was infarct, rather than penumbra. The possibility of cooling the penumbra using the same method was mentioned in the discussion of the paper, but was not examined in the simulations.

Zhu et al [165] used the Pennes equation to test the effects of an intraparenchymal cooling probe (i.e. one that is inserted into the brain tissue to cool it directly). This study is primarily interesting because they performed an animal experiment with a prototype cooling probe and a single implanted thermometer a set distance from the probe, allowing the collection of in vivo data for the purposes of validation. Unfortunately, the authors did not collect real-time perfusion data during the animal experiment, but rather adjusted the perfusion term of their Pennes model until it produced results that matched the temperature data collected from the animal experiment. Encouragingly, the perfusion levels that produced these temperature fields were within the previously published perfusion ranges for the species of monkey involved [165]. Dennis et al [166] produced a finite element model similar to that developed in this thesis, based on the Pennes equation. However, this model used a much more accurate anatomical geometry, including meninges, skull, scalp, air spaces within the head (such as nasal passages) and even facial features. This model was used to simulate cooling the brain using a cooling helmet. While some simulations did model the effects of global ischaemia in the brain, none modelled a region of focal ischaemia, such as an ischaemic stroke. In all cases except for global ischaemia, the simulations in this study indicated that the heat transfer as a result of perfusion dwarfed any heat transfer across the skull and scalp, even when the outer surface of the scalp was set to 0°C [166]. This suggests that in simulating cooling via the arterial blood only (as was performed in this thesis), conduction across the skull and scalp can effectively be ignored without significantly affecting the accuracy of the model.
The vast majority of studies that have applied heat transfer modelling in the brain have used the Pennes equation or a slight modification of it (see Table 1.4.1). The exceptions are the Johansson study [167], two studies by Neimark et al [151, 160] and one by Wang et al [162]. The Johansson study examined the risk of heating in brain tissue as a result of deep brain stimulation, which is performed by an electrode implanted directly into the brain. This study assumed that the key effect of blood flow was in the form of convection and simplified this effect as an ‘effective conductivity’, much like the ‘q_{pc}’ term in the Chen and Holmes model (see above). The Neimark and Wang studies used hybrid models similar to the Chen and Holmes 3 equation model, examining the thermal effects of large vessels individually and using a continuum term to model the effects of small vessels. In the case of the Wang study, this was necessary because the authors were simulating a device which could cool an individual blood vessel in the neck (meaning heat exchange within this vessel needed to be modelled individually do determine the temperature of the blood that would feed into the smaller vessels.

In general, the Pennes equation remains the most popular choice for heat transfer modelling in the human brain for all the reasons mentioned previously in section 1.4.2. The data required to populate the Pennes equation are, comparatively, readily available. The simplicity of the Pennes equation makes coding these models and running them much faster than the more complex multi-equation models (such as the Chen and Holmes model). Finally, the results of models using the Pennes equation do fit what limited validation data are available. However, given the limited amount of validation data we do have, it is worth examining what is known about the anatomy of the cerebral vasculature, the changes in blood flow patterns that occur as a result of localised ischaemia and how these might affect the applicability of the Pennes equation in ischaemic stroke.
Table 1.4.2 Previous studies involving heat transfer modelling in the brain.

<table>
<thead>
<tr>
<th>Modelling Paradigm</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennes or minor variation.</td>
<td>Dennis 2003 [166]</td>
</tr>
<tr>
<td></td>
<td>Dexter 1994 [156]</td>
</tr>
<tr>
<td></td>
<td>Diao [157]</td>
</tr>
<tr>
<td></td>
<td>Elwassif [154]</td>
</tr>
<tr>
<td></td>
<td>Keller [158]</td>
</tr>
<tr>
<td></td>
<td>Neimark [150] and [159]</td>
</tr>
<tr>
<td></td>
<td>Nelson [152]</td>
</tr>
<tr>
<td></td>
<td>Slotboom 2004 [163]</td>
</tr>
<tr>
<td></td>
<td>Sukstanskii [161]</td>
</tr>
<tr>
<td></td>
<td>Van Leeuwen [168]</td>
</tr>
<tr>
<td>Other Continuum Model:</td>
<td>Johansson [167] (effective conductivity model)</td>
</tr>
<tr>
<td>Discrete Vessel Modelling:</td>
<td>None</td>
</tr>
<tr>
<td>Combination:</td>
<td>Bommadavera 2002 [169]</td>
</tr>
<tr>
<td></td>
<td>Neimark [160] and [151]</td>
</tr>
<tr>
<td></td>
<td>Wang [162]</td>
</tr>
</tbody>
</table>
1.4.4 An overview of the vascular system with regard to endovascular cooling for brain ischaemia

Figure 1.4.2 An overview of arteries in the human body. [170]
The inferior vena cava and femoral vein are also labelled. Endovascular cooling devices are typically inserted via the femoral vein and the cooling surfaces rest in the inferior vena cava.
1.4.4.1 Background

The primary function of the circulatory system is to transport nutrients and oxygen to every cell in the body, and to transport waste products such as carbon dioxide, nitrogenous waste and, in the case of the brain, heat from the various extremities to the organs where these wastes can be processed or eliminated (e.g. the lungs or kidneys). The circulatory system of all mammals is a closed system, meaning that the blood remains in discrete vessels throughout its passage around the body. The majority of chemical exchange between the blood and the various tissues of the body takes place by diffusion or active transport (i.e. chemical pumps) across the walls of capillaries, which are the smallest vessels in the circulatory system (typically less than 10µm in diameter, with walls approximately 1 µm thick [171]). Because the circulatory system is contiguous, a significant change in blood temperature that occurs anywhere in the circulatory system will have an impact on the temperature of blood throughout the circulatory system.

1.4.4.2 Cooling of the blood.

The cooling catheter is inserted through the femoral vein and, under ideal circumstances, the heat exchange surfaces are positioned in the inferior vena-cava, the main vein returning blood to the heart from the lower body. The temperature of the coolant flowing through the catheter is controlled by an external thermostat, which is connected to a rectal thermometer. The thermometer controlling the cooling is not affected by the blood temperature immediately adjacent to the cooling catheter, but rather by the core-body temperature. Exact placement of the catheter will vary from one patient to the next, but the general location is in the inferior vena cava. After being cooled by the catheter, blood flowing through the femoral vein and inferior vena-cava continues into the right atrium of the heart, where it mixes with blood returning from the rest of the body. Blood is then pumped into the right ventricle, and from there it is pumped through the pulmonary artery to the lungs. The lungs are specifically adapted to maximise gas exchange between the blood in the capillaries, and the air in the alveoli. This arrangement also allows heat to be exchanged, and the blood is further cooled during its passage through the lungs. After passing through the lungs, the oxygenated (and further cooled) blood flows via the pulmonary vein to the left atrium of the heart. From here it is pumped into the left ventricle, and then around the head and body. Some of the blood flows to the trunk and legs, and this will return via the inferior vena-cava, undergoing further cooling as it passes...
over the catheter again. The remaining blood flows into the head, and this is the flow which is of most interest to stroke researchers.

1.4.4.3 The blood vessels of the head.
The arteries that supply the head are the common carotid arteries and the vertebral arteries. The common carotid arteries (left and right) branch in the neck to form internal and external carotid arteries (ICI and ECI, respectively). The external carotid arteries supply the scalp and other structures outside the skull. The internal carotid arteries branch to form the middle cerebral, anterior cerebral and posterior communicating arteries. These join the basilar artery to form the ‘Circle of Willis’. The basilar artery, in turn, is formed by the convergence of the left and right vertebral arteries.

![Figure 1.4.3, The major arteries of the brain](image)

There are also several other smaller branches from the ICI which we will not describe here. Most ischaemic strokes involve blockages distal to the Circle of Willis because the blood vessels proximal to this are quite large (and would require a large clot to occlude them) and because the Circle of Willis provides an alternative path for blood to follow if any one of the contributing blood vessels does become occluded. The diameter of the first segment of the
MCA (M1), and the blood vessels proximal to it do not change significantly in response to physiological stimuli [172]. However, all arteries distal to this are known to change in diameter in response to changes in blood pressure, CO₂ concentration and other stimuli [173]. The blood is also travelling quite fast up to this point (see Table 1.4.3).

<table>
<thead>
<tr>
<th>Artery</th>
<th>Diameter (mm)</th>
<th>Blood Flow Rate (mL/min)</th>
<th>Blood Flow Velocity (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Carotid</td>
<td>5.2±1</td>
<td>363±18</td>
<td>28.5±1</td>
</tr>
<tr>
<td>Internal Carotid</td>
<td>4.2±1</td>
<td>239±14</td>
<td>28.4±1.3</td>
</tr>
<tr>
<td>External Carotid</td>
<td>3.8±1</td>
<td>129±12</td>
<td>19.6±1.6</td>
</tr>
<tr>
<td>Vertebral</td>
<td>3.1±2</td>
<td>90±12</td>
<td>20.1±1</td>
</tr>
<tr>
<td>Middle Cerebral</td>
<td>2.5</td>
<td>170</td>
<td>63.5±4.3</td>
</tr>
</tbody>
</table>

The arteries that extend from the Circle of Willis (the right and left ACA, MCA and PCA) each supply a number of branches that directly feed the intracortical arteries (which are sometimes called penetrating arterioles), the smaller arteries that penetrate the cortex and supply the brain tissue within. An individual intracortical artery may feed directly off the MCA, for example, or the blood may have passed through as many as 5 branches of the MCA before reaching the intracortical artery [175]. Primary branches tend to have a diameter of 260-280µm and peripheral branches tend to have a diameter of between 150-180µm.

Intracortical arteries take on 6 broad forms, depending on how deep into the cortex they penetrate. The arteries that penetrate deep into the white matter tend to be large in diameter (up to 240µm), have few branches and follow a rather linear course. The arterioles that supply the cortex tend to be between 15 and 75µm in diameter, and follow an extremely convoluted course once they branch from their parent artery (see Figure 1.4.4). The terminal branches of all of these arteries are typically less than 10µm in diameter, and give way to beds of capillaries [175, 176]. Each capillary is approximately 6.5µm in diameter and may vary in length from 3 to 100µm [177].
In other parts of the body, particularly in muscles close to the skin, it is common to find metarterioles. These are small structures that connect arterioles to venules, bypassing the capillary bed. This serves an important function, allowing blood flow to the skin capillaries to be carefully controlled (by altering the diameter of the metarteriole) to control heat loss via the skin. These structures are not found in the brain, thus, blood must pass through a capillary bed before entering the venous system. However, there are anastomoses, which are similar in structure and function to the communicating arteries in the Circle of Willis [175, 178]. These small vessels connect adjacent arterioles to each other, allowing blood to flow from one arteriole into another, thus bypassing the capillary bed of the first arteriole. This may play a role in collateral blood flow after an artery or arteriole is occluded, as anastomoses downstream of the occlusion would allow blood to return to the occluded vessel from adjacent arterioles and thus perfuse capillary beds that would otherwise not have received any blood flow.
1.4.4.4 Blood flow after occlusion of a vessel

There is a considerable level of redundancy within the cerebrovascular system. In rats, it has been shown that the network of interconnected arteries can compensate for occlusion of a large vessel such as the MCA [179], or even of smaller surface arteries [180]. In these cases, blood flow through arteries and arterioles downstream of the occlusion is reversed, indicating that blood is reaching these vessels through connections, such as anastomoses, to other supplying arteries further downstream. This compensation involves dilation of the blood vessels downstream of the occlusion to minimise resistance, and over time vessels running parallel to the occluded artery may also dilate to increase blood flow to the hypoperfused region [181]. Parallel vessels that can supply an occluded artery’s territory immediately are referred to as primary collaterals, while those that must be ‘recruited’ are referred to as secondary collaterals. The presence and effectiveness of primary and secondary collateral circulation varies widely from one patient to the next, and even over time within a single patient. The recruitment of secondary collaterals is poorly understood, but is believed to take time. Furthermore, these collaterals may fail over time as ischaemic injury takes its toll on cerebral haemodynamics [181]. Effective collateral flow can help maintain perfusion levels in the penumbra, and is associated with a good clinical outcome and positive response to thrombolytic treatment [182]. However, little is known about how collateral flow may affect heat exchange. The path taken by collateral blood flow, especially through secondary collaterals, may involve an extended passage through smaller arterioles which would allow much more time for heat exchange to occur before the blood reaches the capillary bed than would be the case if the blood took the most direct route through the vasculature. The mean transit time, a parameter measured on CT or MR perfusion studies provides a rough indication of the length of time blood takes to reach a given segment of tissue from the main arteries of the brain. While this measure is not precise, or objective enough to guide modelling, it has nonetheless been shown to have a closer correlation with tissue at risk of infarction than absolute perfusion levels [183]. This may indicate that blood flow that takes too long a path to reach a region of tissue is not as effective at sustaining that tissue in terms of both supplying nutrients and removing waste, including waste heat.

Little is known about how collaterals are recruited, or why collateral blood flow appears to be more effective in some cases than in others. The implications for heat exchange are
very difficult to predict, and would most likely need to be tailored to individual patients for the purposes of modelling.

It has been demonstrated in rats that capillaries down-stream of an occluded (penetrating arteriole) vessel do dilate and that blood flow in some arterioles down-stream of an occlusion does reverse, indicating that blood is flowing from other arterioles through anastomoses downstream of the occlusion. However, there was no dilation of adjacent penetrating arterioles, and even experimentally dilating these vessels did not increase capillary flow enough to compensate for the occlusion, indicating that the level of redundancy found in larger vessels does not apply to these smaller vessels [178].

1.4.4.5 Implications for use of the Pennes bioheat transfer model

When one compares the average vessel diameters found by Duvernoy et al [175] to the calculations for equilibrium length ‘x_e’ performed by Chen and Holmes [147] several things stand out. It is likely that negligible heat exchange occurs between the heart and the initial branches of the major cerebral arteries. Indeed, even the primary branches of the major cerebral arteries, with a diameter of 260-280µm would be expected to allow little heat exchange between the blood and the surrounding tissue. However, the peripheral branches of these arteries, and the penetrating arterioles (which can feed from peripheral branches of the cerebral arteries or directly from the major arteries themselves) are within the range that Chen and Holmes predicted that most heat exchange would occur (≈175µm in diameter). Thus, it is possible that the majority of heat exchange within the human brain is taking place in penetrating arterioles and the arterial branches that feed them. These arterioles appear to be reasonably evenly distributed across a given cross-section of cortical surface, but can follow extremely sinuous courses within the cortex, making simplification of their contribution to heat exchange quite difficult. For example, some of the branches of the penetrating arteriole seen in Figure 1.4.4 loop back towards their origin and probably overlap with the branches of other penetrating arterioles. Thus, one cannot simply assume that blood temperature is a factor of depth within the cortex, which would be a simple method of accounting for heat exchange along these arterioles. Importantly, the deeper a penetrating arteriole does reach with the brain, the larger its diameter. For example, the arterioles that penetrate all the way to the white matter are approximately 250µm in diameter, which is large enough that relative heat exchange along the length of
these vessels should be quite small. Therefore the average temperature of the blood remains the same as it flows through these arteries. If this were not the case, the majority of heat exchange would be expected to occur on or near the surface of the cortex, in the peripheral branches of cerebral arteries and the shallow penetrating arterioles. This would lead to the development of a significant temperature gradient between the cortical surface and deeper brain tissues. Thus, the heat sink supplied by cerebral blood flow may be just as evenly distributed as the simplified Pennes model implies (and indeed, the fact that calculations based on this model do fit available data suggests that it is), but the heat exchange most likely occurs in the peripheral branches of cerebral arteries and the penetrating arterioles, rather than in the capillary beds they feed. This probably matters little with regard to modelling normally perfused brain tissue. However, when a penetrating arteriole is occluded, any collateral blood flow that does reach that arteriole’s territory has likely passed through other penetrating arterioles, and then anastomoses before reaching the ischaemic tissue. This may also be true of occlusions in more proximal arteries (although bigger arteries tend to have a greater level of redundancy, as mentioned in section 1.4.4.3 above). Thus, residual blood flow reaching ischaemic tissue may have already equilibrated with the temperature of the surrounding tissue, and thus would not provide the same level of cooling as the blood flowing to normally perfused tissue. This may lead to temperature gradients between healthy and ischaemic tissue in reality that would not be accounted for by the Pennes model.

### 1.4.6 Summary

The Pennes equation is a sensible choice on which to build an *in silico* model of the heat exchange during a stroke for a number of reasons, most important of which is the availability of the data required to populate it. The Pennes model may prove to be sufficient for modelling healthy brain tissue but not ischaemic brain, as outlined above. However, a model based on a more complicated algorithm is of little use if we must guess the values of the parameters that are used in the model. Thus, this thesis will examine the validity and utility of a Pennes-based model to examine heat exchange in the stroke-affected brain. As with previous studies, finding relevant *in vivo* data against which to validate the model is quite difficult due to the ethical and practical obstacles involved in collecting detailed temperature data from patients suffering from stroke. Therefore, this
validation will depend heavily on data collected via MR thermography, the other avenue of investigation examined in this thesis.
Part II:
Methods
Chapter 2.1: Magnetic resonance thermography methods

2.1.1 Scanning Protocol.

2.1.1.1 Protocol details
All MR spectroscopy examinations performed for the purposes of this thesis were single voxel spectroscopy (SVS) acquisitions using the standard Point-RESolved-Spectroscopy (PRESS) localisation sequence [184] on a clinical Siemens MRI scanner.

1.5 Tesla experiments were performed on a Siemens Magnetom Avanto scanner using the standard scanner software (VB 17). An echo time (TE) of 135ms was chosen as this produces simpler spectra (with a more even baseline and fewer resonance peaks) which are easier to interpret. The other scanning parameters were as follows: Voxel size 20mm x 20mm x 20mm. Repetition time (TR) 1500ms, vector size 1024, No. of averages: 64, Acquisition time 96s. Partial water suppression (known as “weak” water suppression within the Syngo software) was used with the default suppression bandwidth of 35Hz.

3 Tesla experiments were conducted on a Siemens Magnetom Verio using the standard scanner software (VB 17). Scanning parameters were identical to the 1.5T experiment. Automatic shimming was used in all MR experiments in this thesis, manual shimming was not performed at any stage.

2.1.1.2 Justification of protocol
The advantages and disadvantages of single voxel spectroscopy, as opposed to MRSI, were discussed in section 1.3.5.2. For the purposes of this study, it was decided that the increased accuracy of temperature estimates from single voxel spectroscopy acquisitions outweighed the extra spatial information that could be collected using MRSI.

One aim of this protocol design was to use as many standard clinical settings (i.e. scanner parameters that were pre-programmed by the manufacturer and routinely used by the
radiography staff) as possible on the MRI scanner to maximise the accessibility of the MRT paradigm for non-specialists. The standard short-TE setting for MRS on a Siemens scanner is 30ms and the standard long-TE setting is 135ms. The long-TE setting was chosen as it produces simpler spectra that are easier to interpret for a non-specialist [21] and more suitable for MR thermography [110]. See section 1.3.6.4 for further discussion on TE. This was also the rationale behind using automatic shimming, despite the fact that careful manual shimming could potentially improve the quality of the spectra acquired. Manual shimming requires a high level of MR expertise and would add to the time each patient spent in the scanner, which would be a significant drawback for patients who were neurologically compromised.

Water suppression in the scanning protocol is often avoided for the purposes of MR thermography as it may alter the shape of the water peak [21]. However, when water suppression was not used in a series of pilot scans conducted on a healthy volunteer the water peak tended to overlap the choline and creatine peaks in spectra collected in vivo, inhibiting identification of these peaks (See Figure 2.1.1). Some previous studies have solved this problem by collecting both water-suppressed and non-water-suppressed spectra and recording the frequency of the water peak from the non-suppressed spectrum, and the frequency of reference metabolite peaks from the suppressed spectrum [110].
Figure 2.1.1 An example spectrum collected from a commercial spectroscopy phantom with and without water suppression.

Top left: Choline, creatine and NAA peaks with weak water suppression.
Top right: Choline, creatine and NAA peaks acquired without water suppression.
Bottom left: Water peak acquired with weak water suppression.
Bottom right: Water peak acquired without water suppression.

Thus, the decision not to use this method was primarily a result of the type of equipment available. As can be seen in Figure 2.1.1 above, the use of water suppression did have a negative (albeit minor) impact on the shape of the water peak, as well as significantly improving the baseline around the reference peaks (cho, cr, NAA). This effect on the measurement of the reference peaks would be even more pronounced in spectra acquired in vivo, therefore it was decided to attempt the spectral acquisitions with partial (aka “weak”) water suppression. The early experiments of this thesis (particularly chapter 3.1) were designed to investigate whether the partial water-suppression setting on Siemens MRI scanners could be used in MR thermography without unduly altering temperature estimations.
2.1.2 Spectral processing

2.1.2.1 Processing details
In both 1.5T and 3T experiments spectra were processed using the standard scanner software (Siemens Syngo, Version 17).

To measure the frequency of relevant peaks, the standard automated baseline correction and Fourier transformation were performed. A Gaussian filter (which is ideal for maximising frequency resolution[185]) was applied. A filter width of 200 ms was found to be sufficient for 1.5T scans but a width of 350 ms was required for 3T scans (See Figure 2.1.2). Zero-filling was applied to 2048 data-points (original scan 1024 data points). Three macros (called ‘protocols’ on the scanner software) were created for each field strength, consisting of the above mentioned signal processing followed by zooming the screen to the regions:

- 290-310 Hz (at 1.5T) or 560-100 Hz (For 3T) to measure the water peak.
- 185-215 Hz (at 1.5T) or 355-420 Hz (For 3T) to measure the choline and creatine peaks
- 125-145 Hz (at 1.5T) or 210-280 Hz (For 3T) to measure the NAA peak.

In each case the frequency of a given peak was determined manually and was assumed to be the point of maximum amplitude for that peak. The point of maximum amplitude was relatively easy to distinguish visually, after which the frequency of this point could be determined by clicking on it with the mouse- which would generate a readout of frequency to two decimal places. Much of this method deviated from previous applications of thermography based on MRS. In particular, line-fitting software algorithms are typically used to determine the PRF of each resonance peak[112, 185] and the centre of the peak should ideally be recorded, rather than the point of maximum amplitude[112, 185]. The reasons for deviating from accepted processing methods are outlined below.
2.1.2.2 Justification of processing methods

The aim of the thermography arm of this thesis was to develop a method for temperature estimation from MRS that would be accessible to clinical and research staff conducting clinical trials of therapeutic hypothermia, who would not necessarily have access to specialised MRS software or expertise. Attempts were made to use freely-available line fitting algorithms (as mentioned previously in Chapter 1.3.6.3), and these will be described in more detail below. The lack of success in using these algorithms influenced the decision to process spectra manually rather than using specialised line-fitting software which is the standard practice in MRS thermography [99, 115, 186]. However, in hindsight the acquisition parameters that caused the failure of these algorithms was a major weakness of this study- as potentially was the resultant lack of line-fitting algorithms in the final data processing. Some research groups have developed their own in-house software for line-fitting in MR spectra [116] but without sufficient expertise in MR physics or programming this was deemed impractical for this thesis. Three options for the use of line-fitting
software were explored in this thesis, using the standard Siemens Syngo MRS software, using the freely available jMRUI software package [187] or using a commercial MRS processing software package such as LCModel[188]. Each of these options was examined and rejected in turn. Commercial MRS software packages typically cost approximately $15000. Research groups involved in clinical trials of therapeutic hypothermia, who would not typically have any expertise in MR spectroscopy, are unlikely to go to such expense purely for the purposes of MR thermography, therefore this option was rejected first. The other two line-fitting options were then tested in vitro using the same data-set examined in chapter 3.1.

The standard Siemens Syngo line-fitting algorithm was tested on the phantom data using the standard processing algorithm for single-voxel spectra with a TE of 135ms, with the filter altered to a Gaussian curve to maximise frequency resolution [103, 112, 185]. This algorithm consisted of automated baseline correction and Fourier transformation, zero-filling to 2048-data points, Gaussian filtering as mentioned above, and frequency-correction which effectively shifts the entire spectrum so that the water peak occurs at 4.7ppm, followed by Siemens-developed curve fitting to estimate the frequency, amplitude, line-width and integral of the Choline, Creatine and NAA peaks. Unfortunately, the Siemens line-fitting software only has a precision of 1Hz or 0.01ppm with regards to chemical shift, which is insufficient for temperature estimation (being equivalent to approximately 1.3°C) and this technique was not used again for this purpose, but it was used to measure the amplitude and line-width of the peaks for amplitude-weighted temperature estimations (see chapter 3.2) and estimation of spectral quality.

The jMRUI (Java-based Magnetic Resonance User Interface) package [187] was tested very early on in this study, and indeed the original plan for this thesis was to use jMRUI for all spectral processing. However, the initial in vitro experiments using jMRUI were disappointing, most likely because of differences in the way the water reference was collected (See Chapter 1.3.6.3. The phantom data analysed in chapter 3.1 was examined using a similar protocol to that used by Iain Marshall and his colleagues [110, 121, 122, 189]. Briefly, the spectra underwent automatic frequency correction that brought the water peak to a set frequency of 4.7ppm. This meant that any shift in the water peak as a result of temperature would manifest as movement of the reference metabolite peaks.
Thus, rather than plotting the chemical shift between water and water and each reference peak, it was possible to simply plot the frequency of each reference metabolite against temperature, as was done with the Siemens line-fitting algorithm. The water peak was then removed mathematically using the Hankel Lanczos Singular Value Decomposition (HLSVD) method to minimise the effects of the water peak overlapping other metabolite peaks. The frequency of each of the reference peaks was then estimated using the ‘advanced method for robust and efficient spectral fitting’ (AMARES) program within jMRUI package[110], using prior-knowledge files kindly provided by Iain Marshall (private correspondence, 2008). The use of prior knowledge is a necessary component of the line-fitting process and helps the software accurately assign the identity of each peak (by including such data as the typical frequency range of each peak, and the expected ratios of the amplitude of each peak). The resonance frequencies calculated by this method were significantly removed from the frequencies calculated using the Syngo software or manual processing, and did not correlate well with temperature (See Figure 2.1.3). This was probably a result of differences in the spectral acquisition parameters. While it is worth noting that Marshall et al used an echo-time of 145ms (where scans in this thesis used a TE of 135ms- as this was a standard MRS parameter for Siemens scanners) the more important difference was that Marshall et al used a GE scanner which acquired separate water-suppressed and non-water-suppressed spectra as described above. It is therefore likely that the processing paradigm established by Marshall et al would be more successful on spectra acquired in exactly the same manner as those used by these authors, and indeed this is possible on a Siemens MRI scanner with the newest version of Siemens Syngo available at the time of submission, but this version was not available at the time the experiments were conducted so for the purposes of this thesis, AMARES and jMRUI were judged to be unsuitable.

For the purposes of this study the point of maximum amplitude was taken as the frequency of the peak, when in fact the centre of the peak is a more accurate representation. However, attempting to estimate the centre of each peak manually would lead to an increase in inter-operator variability. The decision was made to use the point of peak amplitude to reduce this variability.
Chemical shift between Water and NAA between 30 and 41°C using manual frequency processing and AMARES line-fitting within the jMRUI package (same data-set).

The absolute value of the chemical shift is significantly different between the two processing methods, and the chemical shift as determined by manual processing is closely correlated with temperature \( (R^2=0.99) \) whereas the chemical shift determined by jMRUI is not \( (R^2=0.10) \). Siemens line-fitting produced data points, and a trend-line that very closely overlapped the manually fitted data with an \( R^2 \) of 0.90 (not shown). Unfortunately, the Siemens line-fitting software only measures chemical shift to a precision of 1Hz, which was not adequate for the purposes of this thesis.

**2.1.3 Data processing**

**2.1.3.1 Chemical shift calculations**

The measured frequency, amplitude and line-width data were recorded in a spreadsheet (Microsoft Excel 2007). For each scan, four new chemical reference ‘peaks’ were created in addition to the three that had been measured from the data (i.e. \( \Delta_{\text{Cho}}, \Delta_{\text{Cr}} \) and \( \Delta_{\text{NAA}} \)). These were defined as the means of \( \Delta_{\text{Cho}} \) and \( \Delta_{\text{Cr}} \) (\( \Delta_{\text{ChoCr}} \)), \( \Delta_{\text{Cho}} \) and \( \Delta_{\text{NAA}} \) (\( \Delta_{\text{ChoNAA}} \)), \( \Delta_{\text{Cr}} \) and \( \Delta_{\text{NAA}} \) (\( \Delta_{\text{CrNAA}} \)), and the mean of all three (\( \Delta_{\text{ChoCrNAA}} \)).
The difference between $\Delta_{\text{water}}$ and each chemical shift reference ($\Delta_{\text{water-reference}}$) was then calculated using the spreadsheet and this difference was used to estimate temperature based on the calibration curves from experiment 1 (Chapter 3.1).

2.1.3.2 Amplitude-weighted temperatures.
For spectra collected in vivo, amplitude weighted temperatures were also calculated, using only the original 3 reference peaks (i.e. $\Delta_{\text{Cho}}$, $\Delta_{\text{Cr}}$ and $\Delta_{\text{NAA}}$), and the amplitude of each peak as determined by the Siemens Syngo MRS line-fitting algorithm. The line-fitting was based on the standard Siemens processing line-fitting algorithm for single voxel spectra acquired at TE=135ms with a Gaussian filter instead of the standard Hanning filter (as described in section 2.1.2.2 above). The amplitude-weighted temperatures were calculated using the following formula:

$$\frac{(T_{\text{Cho}}A_{\text{Cho}}) + (T_{\text{Cr}}A_{\text{Cr}}) + (T_{\text{NAA}}A_{\text{NAA}})}{A_{\text{Cho}} + A_{\text{Cr}} + A_{\text{NAA}}}$$

Where $T_{\text{Cho}}$ = Temperature estimated from choline peak.

$A_{\text{Cho}}$ = Amplitude of choline peak

$T_{\text{Cr}}$ = Temperature estimated from Creatine peak.

$A_{\text{Cr}}$ = Amplitude of creatine peak

$T_{\text{NAA}}$ = Temperature estimated from NAA peak.

$A_{\text{NAA}}$ = Amplitude of NAA Peak

Amplitude-squared weighted temperatures were also calculated using an almost identical formula, with the square of each amplitude ($A^2$) substituted for the relevant amplitude (A). The amplitude was estimated by line-fitting, as opposed to manually because the line-fitting software could determine the amplitude more quickly, and more accurately than would be possible with manual processing, and the precision of the software in determining amplitude was sufficient for the purposes of this thesis (unlike the precision of the software in measuring chemical shift).
2.1.4 Summary

The amplitude-weighted temperature was found to be the best overall method of data processing in healthy volunteers (see chapter 3.2), producing temperature estimations with an accuracy of approximately ±1.3°C. Therefore, from chapter 3.3 onwards, all MR thermography was performed using amplitude-weighted temperature estimation.
Chapter 2.2 Finite element modelling methods

2.2.1 Simulink Model

2.2.1.1 Software
The Simulink package within Matlab was used to create an extremely simplified model based only on the perfusion and metabolic heat generation terms of the Pennes equation (thus not accounting for conduction or radiation of heat in any way).

2.2.1.2 Model construction
Simulink allows the construction of a mathematical model using a flow-chart, where every box on the chart performs a set mathematical operation. The final model is shown in figure 2.2.1 (see figure caption for details of the mathematical operations in order). The equation formed in this way calculated the balance in heat energy between metabolic heat generation by the tissue, and heat removal by the blood while ignoring conduction of heat between adjacent regions of brain tissue, or conduction or radiation of heat from the brain into the surrounding environment. Different classes of tissue could be simulated in the Simulink model by setting the perfusion level and metabolic heat generation inputs to the appropriate values. Physical parameters such as density, specific heat capacity and baseline metabolic heat generation and perfusion were the same as those used in the Ansys model listed in Table 2.2.1.

2.2.1.3 Model execution
The Simulink model could only simulate one tissue segment at a time, and, as outlined above, does not account for interactions (such as conduction) between adjacent tissue segments. Therefore, the cerebral blood flow and cerebral metabolic rate (heat generation) for each tissue segment were input manually for each tissue segment (normal grey and white matter, oligaemic grey and white matter, penumbral grey and white matter, and infarcted grey and white matter).
The initial brain temperature was set at 37°C, and the initial blood temperature was set at 36.7°C as this blood temperature would result in a steady-state brain tissue temperature of 37°C under baseline perfusion and metabolism levels. The minimum blood temperature (saturation point) was set at 33°C and the rate of cooling was set at a rate of 1.1°C/hour (3.056x10^{-4}°C/s) for simulations that involved cooling, or 0°C/s for uncooled (i.e. ‘untreated’) simulations. The minimum temperature of 33°C was chosen as it is the target temperature for cardiac arrest patients receiving therapeutic hypothermia[63] and a number of current clinical trials for ischaemic stroke[190]. The cooling rate of 1.1°C per hour was chosen as it is the maximum rate of cooling that can be achieved with current endovascular cooling devices[75] (see Chapter 1.2).

The CBF and CMR were varied experimentally between simulations. Other physical parameters, such as the density and specific heat capacity of tissue and blood were kept constant between different simulations (and between the Simulink and Ansys models). These parameters, and the justification for the figures chosen, are outlined in section 2.2.3. The results of each simulation were read from the graph output of tissue temperature and recorded in a spreadsheet for each tissue segment under various experimental conditions outlined in Chapter 5.1. These results were compared to the results of the more complex Ansys simulations.
Figure 2.2.1 The Simulink model tested in this thesis. See opposite page for description.
Description of functions in the Simulink Model (Figure 2.2.1)

The functions shaded in red convert the cerebral blood flow rate from standard clinical units into units that can be used for subsequent calculations and, separately, for calculating blood temperature.

1: The Cerebral blood flow rate (CBF) can be input into this box in mL/100g/min (the standard units used by clinicians).

2: This is multiplied by a constant to give the CBF in kg of blood per m$^3$ of tissue per second.

3: The initial blood temperature can be input into this box, along with a rate of blood cooling (which the user must calculate externally in °C/s). The saturation box allows the user to input a minimum blood temperature, below which cooling stops. Note that this model does not simulate the process of cooling the blood itself, but rather uses the resulting arterial blood temperature as an input.

4: The tissue temperature is then subtracted from the blood temperature, the former being calculated for each iteration by the computer based on the output from the previous iteration of the simulation.

The operations shaded in blue are involved in calculating the net heat generation (NHG) of the tissue.

5: The CBF rate is multiplied by the specific heat capacity of blood (J/kg°C) and by the difference in temperature between the blood and the tissue (ΔT).

6: The resulting number is added to the metabolic heat generation of the tissue (q_m). The balance between these two inputs (heat generation by the tissue and heat removal by the blood) is the net heat generation.

Operations shaded in green calculate the effect of the net heat generation on the temperature of the tissue.

7: The net heat generation (in J/m$^3$/s) is divided by the product of the tissue density and tissue specific heat capacity to give the change in tissue temperature per second. The final operation (unshaded) is an integration loop that allow the calculated change in tissue temperature per second for each iteration to be reflected in the tissue temperature that feeds into the ΔT box of the next iteration.

8: Is an output graph allowing the user to view the tissue temperature over time.
2.2.2 Ansys Model

2.2.2.1 Software and Geometry
Ansys version 12 (Ansys, Pittsburgh USA) was used for the more complex model which included the effects of conduction between adjacent regions of tissue.

Unfortunately, the operations required for modelling heat exchange in this context (see net heat generation equations below) could, at the time of writing, only be performed in Ansys mechanical using the Ansys parametric design programming language (APDL). This necessitated using an even more simplified geometry than might have been used had the full computer aided design package within Ansys been used to build the geometry.

2.2.2.2 Geometry
The geometry of the entire simplified brain was created initially, but the unaffected hemisphere was deleted from the geometry before further input in order to expedite both coding and running the individual simulations. Structures such as the ventricles, meninges, skull and scalp were omitted for the same reason (see below for justification). The brain was modelled as a series of concentric hemispheres, consisting of an inner layer of grey matter 28mm in diameter (representing the basal ganglia) surrounded by a layer of white matter 39mm thick, which in turn was surrounded by another layer of grey matter 18mm thick (representing the cortex). These dimensions were derived from MRI measurements performed on a random healthy volunteers scanned as part of the experiment described in Chapter 3.2. Each concentric hemisphere was modelled from the same centre coordinate, so that the basal ganglia hemisphere was in the exact centre of both of the spheres representing the white matter and the cortex.

The ischaemic region was modelled as a series of concentric spheres effectively carved out of the existing brain structure. This region consisted of the infarct sphere 10mm in diameter, the penumbra sphere 15mm in diameter and the oligaemia sphere 20mm in diameter. As with the hemispheres representing the overall brain structure, the 3 spheres representing the ischaemia region were modelled from the same centre-point (although this was offset from the centre-point used for the hemispheres representing the basal ganglia, white matter and cortex). Thus, the infarct was situated exactly in the centre of the penumbra, and the infarct and penumbra were exactly in the centre of the oligaemia.
Figure 2.2.2 below illustrates the outline of the final geometry. The ischaemic region was situated so that all 3 spheres overlapped both white matter and cortex.

The scalp, skull, meninges and cerebrospinal fluid (including the ventricles) were all disregarded from the geometry of this model. The CSF could be reasonably expected to be very close to the surrounding brain tissue (in particular the choroids plexus) in temperature it was thus judged unlikely to play a significant role in heat exchange except in cases where interventions are directed specifically at the CSF temperature. The level of conduction across the meninges, skull and scalp has previously been shown to be very small [191] so the effects of this conduction were ignored, with all surfaces of the model being treated as adiabatic. The inclusion of the lateral ventricles, CSF, meninges, skull and scalp increased the time needed to run a simulation from 10-15 minutes to 6-8 hours, and increased the volume of output data created to such a point (several tens of gigabytes per simulation) that the computer available could only store the results of one simulation at any one time, and these results would have to be deleted before another simulation could be run. Given the role these structures are believed to play in heat exchange is mostly limited to providing insulation from the outside world[80, 85, 86, 192], it was decided to eliminate them from the model for the purposes of this thesis.

No attempt was made to model the brain-stem or cerebellum, as the only validation data available for comparison at the time of writing were from the cerebral hemispheres. Thus, it was decided to conduct verification and validation analysis focusing on the cerebral hemispheres, and build upon the complexity of the geometry of the model at a later date if these early experiments were successful. For the purposes of this thesis, the model was further reduced to a single cerebral hemisphere, thus the contralateral hemisphere (not affected by the simulated stroke) was not simulated. The aim of this was to further reduce the simulation times and the volume of data that needed to be stored after each simulation.

Physical parameters and baseline perfusion and metabolism levels are outlined in Table 2.2.1 and are drawn primarily from Zhu and Diao [83]. Initial brain temperature was assumed to be 37°C, and initial blood temperature was assumed to be 36.7°C as this would produce a steady-state tissue temperature of 37°C under baseline perfusion and metabolism levels, and is within the physiological range on core body temperature [171].
The use of an adiabatic (i.e. zero energy transfer) boundary condition on external surfaces is justified by the fact that conduction of heat across the skull and scalp is minimal, especially in comparison to heat exchange between the blood and brain tissue[86, 157, 166, 169].
Figure 2.2.2 A (Top) The original, more complex geometry developed for Ansys simulations B (Bottom) The more simplified geometry developed in APDL.

The Ischaemic region is modelled as a central region of infarct (I) 10mm in diameter surrounded by a region of penumbra (P) 15mm in diameter which in turn was surrounded by a region of oligaemia (O) 20mm in diameter all of which include both cortex (grey matter) and white matter. Note that the more simplified geometry omits the entire contralateral (non-affected) hemisphere of the brain as well as structures such as the meninges, skull and scalp as the thermal effect of conduction across these structures is minimal with comparison to the thermal effect of perfusion. The surfaces of the hemisphere were therefore treated as adiabatic with the only heat sink being the arterial blood. For technical reasons the more simplified version was used for Chapters 5.1 and 5.2.

2.2.2.3 Net Heat Generation Equations

In order to simulate the thermal effects of blood flow through the brain without having to model individual blood vessels, a ‘net heat generation’ (NHG) equation for each tissue segment was formulated which allowed the NHG to be calculated by the Ansys software as
a function of time and tissue temperature for every node at every time-step. The form of each NHG equation was derived from the perfusion and metabolic heat generation terms of the Pennes equation (highlighted in yellow in the complete Pennes equation below).

\[
\rho c \frac{dT_{tissue}}{dt} = -k \nabla^2 T + \omega c (T_{blood} - T_{tissue}) + qm
\]

(See Table 1.4.1 for nomenclature)

The perfusion rate (\(\omega\)) in kg/m³/s and the metabolic heat generation (Qm) were both varied between different tissue segments and on an experimental basis between the same tissue segment in different simulations. Therefore, it was necessary to program a separate NHG equation for each tissue segment (normal grey and white matter, oligaemic grey and white matter, penumbral grey and white matter and ischaemic grey and white matter) in each simulation. The specific heat capacity of blood was assumed to remain constant at 3800 J/kg/°C in all simulations (See section 2.2.3 below).

For simulations that did not involve patient cooling, the temperature of the blood was assumed to be 36.7°C throughout the simulation. For simulations involving hypothermia induction, the blood temperature was assumed to drop from an initial value of 36.7°C (average core body temperature in a healthy human) to 33.0°C (the target temperature of a number of clinical trials testing hypothermia [63, 190]) over a period of approximately 3 hours and 21 minutes, translating to a cooling rate of 0.000306°C/s, or approximately 1.1°C/hour (the same cooling rate and target temperature as tested in the Simulink model based on the temperatures involved in current clinical trials, see Chapter 1.2). It was not possible to impose a ‘saturation limit’ on blood temperature in Ansys as had been applied in the Simulink model, so rather 2 separate NHG equations were applied, one (during which blood temperature was reduced) from 0 to 12091 seconds (approximately 3 hours and 20 minutes, at which point the blood would be at 33°C), and the other (with blood temperature set to a constant 33°C) from 12092 seconds to the end of the simulation.

The baseline (normal) values of metabolic heat generation and other parameters are listed in Table 2.2.1 and are taken from Diao et al [157]. As an example, the NHG equation for normal grey matter, assuming the perfusion rate is the ‘normal’ value of 80mls/100g/min (14.7 kg/m³/s), the metabolic heat generation is 16700W/m³ and the blood temperature starts at 36.7°C and is cooled to 33°C at a rate of 1.1°C per hour (0.000306°C/s), the NHG
equations that would be coded are:

\[ NHG = (55860 \times (36.7 - 0.000306 \text{TIME}) - \text{TEMP}) + 16700 \quad \text{for\ TIME=0-12091\ seconds} \]

\[ NHG = (55860 \times (33 - \text{TEMP})) + 16700 \quad \text{for\ TiME>12091\ seconds} \]

Note that 12091 seconds (≈3 hours 20 minutes) is the time at which the blood would reach the target temperature of 33°C at this cooling rate.

TEMP in each case refers to the tissue temperature. Ansys would use the temperature at each node to complete the NHG equation for that node and then apply the resulting thermal load and calculate the results of this load on the node at each time-step.

Simultaneously, Ansys would calculate the level of conduction between adjacent nodes as a result of temperature differences between these nodes (this is the part of the Pennes equation not included in the NHG equation).

Calculating and programming the individual NHG equations could take several hours for a given experiment, but once the NHG equation for each tissue segment had been programmed into Ansys, a simulation could be run over approximately 10-15 minutes.

### 2.2.2.4 Data extraction

A path denoted ‘P’ was created in Ansys running from the centre of the basal ganglia, through the centre of the ischaemic zone through to the outer layer of the cortex (see Figure 2.2.3 below). A macro was programmed to record the temperature at every node along this path and output these temperatures to a text file that could be converted into a spreadsheet. This temperature data was used for all subsequent analysis. While this approach did omit much of the spatial data available (from all the nodes not found along path ‘P’) it had the advantage of allowing very precise temperature comparisons within the region of most interest (the ischaemic region). The best way to extract data from a broader range of nodes would be in the form of colour-coded still pictures (for showing instantaneous temperatures) or videos (for showing changes in temperature over time). An illustrative example of these colour-coded images can be found in Figure 5.2.5 in Chapter 5.2. Unfortunately, the precision of such colour-coding is significantly poorer than the precision that can be obtained with spreadsheets and graphs of the smaller volume of data obtained along path ‘P’. For the purposes of this thesis, it was decided that a smaller amount of more precise data would be more useful than a larger amount of less precise...
data. However, the future clinical applications of this modelling technique could use other data extraction techniques (such as highlighting all nodes above a certain threshold temperature) that would make use of the broader data available from such simulations.

Figure 2.2.3. The r-th ‘P’ (white arrow) along which temperature data were extracted for further analysis.

2.2.3 Model Parameters:

The starting values for various model parameters are listed in Table 2.2.1. The values of cerebral blood flow and cerebral metabolism were assumed to vary between grey matter and white matter, and also as a result of ischaemia. Furthermore, there is a significant natural variation in CBF between individual patients (and in particular with varying severity of stroke) and high level of uncertainty regarding the effects of ischaemia on CMR (see Chapter 5.2). Therefore, these parameters were varied experimentally between different simulations, which are described in detail in Chapters 5.1 and 5.2. Other physical parameters, such as density, conductivity and specific heat capacity were assumed to remain constant. There is likely to be some natural variation in these characteristics.
between individuals, and potentially within an individual. However, there are few data available regarding this natural variation, and no practical method for a clinician to measure such physical characteristics in their patients non-invasively (as could be done for CBF, for example). Therefore, variation in the density, conductivity and specific heat capacity of either the tissue or the blood were not accounted for in this model. As with the simplified geometry, it was decided to test the feasibility of the more simplified model before introducing further complexity.

There is broad agreement between different authors regarding the physical properties of brain tissue and, to a lesser extent, blood. However, this is quite possibly because authors of bioheat transfer modelling papers tend to reference other bioheat transfer modelling papers rather than actual in vivo or in vitro data. Table 2.2.2 lists a number of bioheat transfer modelling papers and the physical parameters they used. Some of the variation seen between different studies is likely due to rounding errors, as some studies have used slightly different units (e.g. calories instead of joules) and these have been converted to SI units for this table. The parameters used for this thesis, listed in Table 2.2.1 were taken from Zhu et al [83] (amongst others) and were chosen because they were the most commonly used figures across the various other modelling papers. This made comparison of the simulation results to previous models simpler, which is an important consideration with regard to model verification and validation (See Chapter 5.1).

The most obvious weakness in the physical parameters chosen is that they do not differentiate between grey matter and white matter. Due to their different compositions (in particular, the higher fat content of white matter) grey matter could reasonably be expected to show a higher specific heat capacity and a higher level of conductivity than white matter (as Collins et al assumed [193]). For the purposes of comparison, it was decided to use the most commonly accepted figures, which use the global average for brain tissue rather than individual figures for grey and white matter. However, this provides another layer of complexity that could be added to the model at a later date.

The model could also be expanded by increasing the level of anatomical detail in the anatomy, and actually modelling conduction from the brain to the external environment. However, previous modelling [161, 166] and experiments [85, 165] have indicated that this conduction does not make a meaningful contribution to heat exchange in the brain,
therefore this was not considered a major weakness of the model. Conduction between hemispheres was also ignored by this model which may prove to be a weakness, but since the conductivity of brain tissue is quite low [194] and the temperature of the contralateral hemisphere would closely match unaffected brain tissue along the boundary between the hemispheres, this is only likely to affect the results if the ischaemic region were modelled close to this boundary.

Table 2.2.1 Physical parameters used in this thesis.

<table>
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<th>Parameter</th>
<th>Grey Matter</th>
<th>White Matter</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
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<td>1050kg/m³</td>
<td>1050kg/m³</td>
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<td>Specific heat capacity</td>
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<td>Conductivity</td>
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<td>-</td>
</tr>
<tr>
<td>Baseline (100%) CBF</td>
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<td>20ml/100g/min</td>
<td>-</td>
</tr>
<tr>
<td>Baseline (100%) CMR</td>
<td>16700W/m³</td>
<td>4175W/m³</td>
<td>0</td>
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</table>
### Table 2.2.2 Physical parameters used in previous bioheat transfer modelling papers.

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<th>Study</th>
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<th>Specific Heat Capacity (J/kg/°C)</th>
<th>Density (kg/m³)</th>
<th>Notes and references for physical parameters</th>
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<td>1000</td>
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<td>3694</td>
<td>1050</td>
<td>Referenced actual experiment [195]</td>
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<td>1050</td>
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<td>3700</td>
<td>1050</td>
<td>Other modelling papers [155, 169]</td>
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<td>1050</td>
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<td></td>
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</tr>
<tr>
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<td>Johansson [167]</td>
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<td>Kotte [149]</td>
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<td>151, 159, 160]</td>
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<td>[168]</td>
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<td>Wang [162]</td>
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Part III:

Magnetic Resonance Thermography
Chapter 3.1: 
*in vitro* calibration and validation of MR thermography.

3.1.1 Background

The rationale for developing MR thermography was discussed in chapter 1.2, and the science behind thermography based on the proton resonance frequency of water was discussed in chapter 1.3. Briefly, current and future trials of therapeutic hypothermia would benefit from a non-invasive method of estimating localised brain temperature.

Magnetic Resonance Thermography (MRT) is the most promising method of achieving such temperature estimations. The most accurate and robust methods of MRT are those based on MR Spectroscopy (MRS). A major aim of this thesis is to validate a method of MRT that would be accessible to clinicians and scientists involved in hypothermia trials that is cost-effective and, ideally, fast.

The first step required to validate a new processing method for MRT is to calibrate the temperature estimations using a phantom. This allows the temperature of the object being scanned to be varied across a broader range than human volunteers could be subjected to, and allows assessment of the reliability of the technique without the random variability inherent in biological systems. This experiment therefore aimed to calibrate the temperature estimations from MR Spectroscopy using the spectral processing method described in chapter 2.1.

A further aim was to determine if the spectral peak of any one reference neurochemical allowed more accurate temperature estimations than that of any other, or whether combining reference neurochemicals by simple averaging could improve the accuracy of temperature estimations. Spectra acquired *in vivo* are never of equal quality in terms of signal-to-noise ratio and spectral dispersion as those acquired *in vitro*. Thus, validity and reliability *in vitro* do not guarantee successful implementation of the technique *in vivo*.
This is especially true in stroke patients, in whom tissue injury may alter the concentrations of the various metabolites. However, if the technique described does not allow accurate temperature estimation \textit{in vitro} (under ideal circumstances) then it will certainly not work \textit{in vivo}. Thus, it was considered prudent to validate the technique \textit{in vitro} before proceeding with human experiments.

3.1.2 Methods:

3.1.2.1 Scanning protocol

The phantom used was a commercial brain MR spectroscopy phantom (GE Healthcare Model Number 2152220). Using the scanning parameters described in chapter 2.1.1, two separate experiments were conducted, the first on a 1.5T Siemens Magnetom Avanto MRI scanner and the second on a 3T Siemens Magnetom Verio scanner. Both scanners utilised the Siemens Syngo version VB17 software platform. Manual shimming was not used for any of the MR experiments described in this thesis as it was believed this would reduce the generalisability of the protocol, particularly for neurology research groups with limited access to MR physics expertise.

For the 1.5T experiment, the phantom was heated in a water-bath to approximately 45°C. The phantom was shaken vigorously to ensure the temperature of the contents was uniform then placed in the MRI scanner and stabilised with foam pads to prevent movement. A fibre-optic temperature probe (Medrad Veris 8600) was taped to the outer skin of the phantom to monitor temperature. The manufacturer states that this probe is accurate to within ±0.2°C over the temperature range covered. The phantom was left in the scanner for approximately 15 mins to allow the contents to settle and then 12 PRESS acquisitions were conducted at 0.5-0.8°C intervals as the temperature of the phantom dropped from 40.8°C to 31.4°C.

For the 3T experiment the same basic procedure and the same temperature probe were used. However, it was not possible to heat the phantom to the same starting temperature in this experiment as a result of the equipment available. Thus, after the 15 minute settling time the phantom temperature was only 35.7°C and the experiment was terminated when the temperature of the phantom reached 31.7°C. In order to acquire more data, the scans
were conducted continuously rather than at intervals, resulting in a total of 21 PRESS acquisitions over the temperature range.

3.1.2.2 Data processing
Spectra were processed on the scanner in accordance with chapter 2.1 and the proton resonance frequency of water, choline ($\Delta_{\text{Cho}}$), the CH$_3$ resonance of creatine ($\Delta_{\text{Cr}}$) and N-acetylaspartate ($\Delta_{\text{NAA}}$) were determined manually in Hertz (all subsequent references to $\Delta_{\text{Cr}}$ refer to the CH$_3$ resonance at approximately 3.02ppm). The mathematically averaged reference peaks ($\Delta_{\text{ChoCr}}, \Delta_{\text{ChoNAA}}, \Delta_{\text{CrNAA}}$ and $\Delta_{\text{ChoCrNAA}}$) were calculated as described in chapter 2.1.3. A simple linear regression of the chemical shift between water and each reference peak against temperature was conducted and the various reference peaks were compared on the basis of goodness of fit ($R^2$ value) and the width of the 95% confidence intervals. To err on the conservative side, the maximum values of the 95% confidence intervals calculated by SPSS were quoted, i.e. those values measured at the extremes of the measured temperature range.

3.1.3 Results:

3.1.3.1 Overview
There was a strong linear correlation between temperature and the chemical shift of water (as measured against a temperature-insensitive reference) regardless of which reference chemical was used, and regardless of field strength (see Table 3.1). $R^2$ values ranged from 0.97 to 0.99 in the 1.5T experiment and 0.98 to 0.99 in the 3T experiment.

3.1.3.2 1.5T experiment
The equations relating chemical shift to temperature for each reference chemical at 1.5T are listed in Table 3.1, along with the 95% confidence intervals and $R^2$ values for temperature estimates made using each equation.

Equation 1 from Table 3.1 had a greater slope than that of equations 2 and 3 (-1.4°C/Hz compared to approximately -1.3°C/Hz, respectively). To investigate this further, the chemical shift between Cho and NAA peaks ($\Delta_{\text{Cho-NAA}}$) was regressed against temperature. The slope estimate was significantly different from 0 (k = -0.064, S.E. = 0.027, $P = 0.039$).
\[ \Delta_{\text{Cr-NAA}} \] was examined in the same way but the result was not found to be significant \((k = -0.006 \text{ S.E. } = 0.023 \text{ } P = 0.808)\).

### Table 3.1.1: Equations for calculating absolute temperature from the chemical shift of water \((\Delta_{\text{water-reference}})\) at 1.5T in vitro

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>95% CI</th>
<th>( R^2 )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation 1</td>
<td>( \Delta_{\text{Cho}} )</td>
<td>( T = -1.42(\Delta_{\text{water-cho}}) + 168.34 )</td>
<td>±0.63</td>
<td>0.97</td>
</tr>
<tr>
<td>Equation 2</td>
<td>( \Delta_{\text{Cr}} )</td>
<td>( T = -1.31(\Delta_{\text{water-Cr}}) + 171.49 )</td>
<td>±0.34</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 3</td>
<td>( \Delta_{\text{NAA}} )</td>
<td>( T = -1.32(\Delta_{\text{water-NAA}}) + 257.66 )</td>
<td>±0.40</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 4</td>
<td>( \Delta_{\text{ChoCr}} )</td>
<td>( T = -1.37(\Delta_{\text{water-ChoCr}}) + 170.75 )</td>
<td>±0.41</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 5</td>
<td>( \Delta_{\text{ChoNAA}} )</td>
<td>( T = -1.38(\Delta_{\text{water-ChoNAA}}) + 215.30 )</td>
<td>±0.47</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 6</td>
<td>( \Delta_{\text{CrNAA}} )</td>
<td>( T = -1.32(\Delta_{\text{water-CrNAA}}) + 214.90 )</td>
<td>±0.32</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 7</td>
<td>( \Delta_{\text{ChoCrNAA}} )</td>
<td>( T = -1.35(\Delta_{\text{water-ChoCrNAA}}) + 200.72 )</td>
<td>±0.37</td>
<td>0.99</td>
</tr>
</tbody>
</table>

In each equation \( T = \text{Temperature in } ^\circ\text{C} \text{ and chemical shift is measured in Hz.} \)

#### 3.1.3.3 3T experiment

The equations relating chemical shift to temperature for each reference at 3T are listed in Table 3.2, along with the 95% confidence intervals for temperature measurements made using each equation.

As in the 1.5T experiment, linear regressions of \( \Delta_{\text{Cho-NAA}} \) and \( \Delta_{\text{Cr-NAA}} \) were conducted against temperature, but neither was found to be significant. Note that the coefficient between temperature and chemical shift at 3T was approximately half that at 1.5T. This was an expected consequence of measuring chemical shift in Hertz. The coefficients in Hertz translate to values of approximately 83.54-90.29°C/ppm at 1.5T and 78.50-83.10°C/ppm at 3T.
Table 3.1.2: Equations for calculating absolute temperature from the chemical shift of water (Δwater-reference) at 3T in vitro

<table>
<thead>
<tr>
<th>Reference</th>
<th>Equation</th>
<th>95% Cl</th>
<th>R²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation 8</td>
<td>ΔCho</td>
<td>T = -0.65(Δwater-cho) + 153.44</td>
<td>± 0.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 9</td>
<td>ΔCr</td>
<td>T = -0.62(Δwater-Cr) + 158.68</td>
<td>± 0.17</td>
<td>0.98</td>
</tr>
<tr>
<td>Equation 10</td>
<td>ΔNAA</td>
<td>T = -0.62(Δwater-NAA) + 238.25</td>
<td>± 0.16</td>
<td>0.98</td>
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<tr>
<td>Equation 11</td>
<td>ΔChoCr</td>
<td>T = -0.63(Δwater-ChoCr) + 156.35</td>
<td>± 0.14</td>
<td>0.99</td>
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<tr>
<td>Equation 12</td>
<td>ΔChoNAA</td>
<td>T = -0.64(Δwater-ChoNAA) + 197.10</td>
<td>± 0.14</td>
<td>0.99</td>
</tr>
<tr>
<td>Equation 13</td>
<td>ΔCrNAA</td>
<td>T = -0.62(Δwater-CrNAA) + 198.84</td>
<td>± 0.15</td>
<td>0.98</td>
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<tr>
<td>Equation 14</td>
<td>ΔChoCrNAA</td>
<td>T = -0.63(Δwater-ChoCrNAA) + 184.29</td>
<td>± 0.14</td>
<td>0.99</td>
</tr>
</tbody>
</table>

In each equation T = Temperature in °C and chemical shift is measured in Hz.

3.1.4 Discussion:

3.1.4.1 Comparison to previous studies

The proton resonance frequency of water was closely correlated with temperature regardless of which reference PRF was used. This study has shown that it is possible to determine the temperature of a phantom with a high degree of accuracy from the difference between the proton resonance frequency of water and those of reference chemicals using commercially-available MR spectroscopy software. This was true for both 1.5T (accuracy within ±0.63°C) and 3.0T (accuracy within ±0.17°C) scanners. The accuracy of temperature estimation in this study was similar to that of previous studies using more sophisticated data-processing techniques. For example, studies at 1.5T and 3.0T scanners have previously quoted accuracies of approximately ±0.4°C and ±0.2°C, respectively [99, 111]. Likewise, the temperature coefficients found in this study (when converted to ppm) were also similar to those reported by previous studies in which values ranging from 70.13 to 135.31°C/ppm have been described [99].
3.1.4.2 Effect of magnetic field strength on temperature estimations
As expected, MRS performed at 3T allowed more accurate temperature estimations than MRS performed at 1.5T. This was due to the increased signal-to-noise ratio per unit time and greater spectral dispersion inherent at the higher field-strength. This study confirms that 3T scanners are preferable to 1.5T scanners for MR thermography studies. The choice of reference PRF also affected the accuracy of temperature estimations. In particular, combining multiple references displayed a modest benefit at 1.5T, but not at 3T.

3.1.4.3 Effect of reference chemical
With the exception of equation 1 (using choline as the reference), all equations for temperature estimation at 1.5T demonstrated identical R² and p-values. Therefore, comparison of various reference chemicals could only be based on the width of the confidence intervals for each equation. The impact of reference choice at 1.5T was significant, with 95% confidence intervals ranging from ±0.32°C (equation 6) to ±0.63°C (equation 1). On the basis of the broadest confidence intervals, the best single reference at 1.5T was ΔCr (equation 2), followed by NAA and choline. As mentioned above, combining multiple references did confer a slight advantage at 1.5T, with equation 6 (using ΔCrNAA as the reference) providing the tightest confidence intervals overall. Equation 2 (single reference) was actually better than equations 4, 5 and 7 (multiple references) but equations 6 and 7 (multiple references) were better than any of the single reference equations except equation 2. All results involving ΔCho were slightly at variance with the rest and this will be discussed further below.

At 3T, p-values for all equations were identical, and R² values ranged from 0.98 to 0.99. The width of 95% confidence intervals was similar for all equations, ranging from ±0.13°C (equation 8) to ±0.17°C (equation 9). While all of the equations using multiple references produced slightly tighter confidence intervals than equations 9 or 10 (single references), this difference was small and did not suggest an advantage of using multiple references at 3T. Indeed, the overall choice of reference was considerably less important at 3T than at 1.5T.

Taking the average of multiple chemical references will reduce the impact of random error in measurement, but will actually increase the effects of systematic error, such as the digital resolution of the MR spectra. The fact that using multiple references conferred a
benefit at 1.5T but not at 3T suggests that random errors make a greater contribution to the overall error of measurement at 1.5T than they do at 3T. Of note, there will always be a greater level of random error in vivo compared to that in vitro. Therefore, it is possible that using multiple references may still confer an advantage even at 3T when the technique is applied in vivo.

3.1.4.4 Choline

At 1.5T the chemical shift of choline produced some rather unusual results when used as a reference (either alone or in combination). Firstly, the slope of equation 1 (≈1.4°/Hz) was significantly different from those of equations 2 and 3 (≈1.3°/Hz). The slope of equation 8 (using ΔCho as a single reference at 3T) was also different from the slope of equations 9 and 10, but this difference was much less pronounced (0.65°/Hz compared to 0.62°/Hz). The difference in slope suggests that ΔCho could itself be temperature dependent. To examine this, the chemical shift between choline and NAA (ΔCho-NAA) and the chemical shift between creatine and NAA (ΔCr-NAA) were both calculated and compared to temperature. The correlation between ΔCho-NAA and temperature was found to be significant at 1.5T but not at 3T, while the correlation between ΔCr-NAA and temperature was not found to be significant at either field-strength. If ΔCho-NAA was truly dependent on temperature, one would expect this temperature-dependence to persist regardless of field-strength which was not the case. No previous study has found a correlation between ΔCho and temperature and it is therefore likely that the apparent temperature dependence of ΔCho at 1.5T was the result of random error.

3.1.4.5 Potential pitfalls in this experimental design

The most obvious weakness in this experimental design lies in the fact that the calibration thermometer was placed on the outer skin of the phantom whereas the MR spectra were collected from the fluid inside the phantom. As the phantom cooled by radiating heat to the environment, it is likely that during cooling the outer skin would be cooler than the fluid in the centre of the phantom. While the MR spectra were collected from fluid near the skin of the phantom to minimise this difference, it is possible that the temperature used to calibrate the MRT equations was lower than the actual temperature of the fluid. This would manifest as an error in the intercept of each equation. This error should become evident during in vivo testing if it exists. Unfortunately, for equipment reasons placing the temperature probe inside the liquid within the phantom was not possible, even
in a custom built phantom. A recent study [197] has also suggested that the protein and ion content of the solution from which MR spectra are acquired can affect temperature estimations performed using these spectra. The phantom used for this experiment did not contain any protein, and this could have an important effect on the temperature calibration curves, although once again the effect is likely to be more evident in the intercept of each curve than the slope [197].

3.1.4.6 Potential pitfalls with regard to applying this technique in stroke patients

The level of random error in MR spectra acquired *in vivo* will always be high compared to spectra acquired from a homogenous phantom. This is due in part to the poorer shim (i.e. a less homogenous magnetic field due to susceptibility differences within the voxel (e.g. between blood and brain tissue) which increases the effect of T2* decay on the signal[112]. The faster signal decay in turn reduces the signal-to-noise ratio. In addition, the chemical shift peaks used as references ($\Delta_{\text{Cho}}$, $\Delta_{\text{Cr}}$ and $\Delta_{\text{NAA}}$) are each attributable to a single chemical in the phantom (choline chloride, creatine hydrate and pure NAA, respectively). In the brain however, there are multiple chemicals that contribute to each peak, as described previously in section 1.3.7. This causes individual PRF peaks to demonstrate a broader line-width in spectra acquired *in vivo* which also reduces the accuracy with which the frequency of each peak can be estimated. Furthermore the concentration of each reference chemical, in particular NAA, may change as a result of pathology (including stroke). In theory, the accuracy with which the frequency of a given peak can be measured is dependent on the amplitude of the peak [198] although previous simulations [110] and experiments [116] have indicated that this accuracy is, in fact, proportional to the square of the amplitude. Thus, any reduction in the amplitude of the chemical reference peaks will have a significant effect on the accuracy of temperature estimations performed using these peaks. Amplitude- or amplitude-squared-weighted averaging of temperature estimations made using each single reference ($\Delta_{\text{Cho}}, \Delta_{\text{Cr}}$ and $\Delta_{\text{NAA}}$) has previously been shown to improve the repeatability of temperature estimations performed using other spectral processing methods [116], and the MRT technique being tested here may benefit from such averaging. This will need to be tested *in vivo*.

3.1.5 Summary

The MRT technique described in this thesis has been calibrated and validated *in vitro*. The results, in terms of both the temperature dependence of the chemical shift of water and
the accuracy of temperature estimations that can be achieved *in vitro*, are both comparable to previous studies. The choice of chemical shift reference from which the chemical shift of water is measured does have an impact on the accuracy of temperature estimations, especially at 1.5T (less so at 3T). Using the average of multiple references does confer some benefit in terms of the accuracy of temperature estimations at 1.5T, though not at 3T. The greater level of random error inherent to spectra acquired *in vivo* could favour the use of multiple references *in vivo*, even at 3T. Changes in the concentration of reference chemicals as a result of pathology could reduce the accuracy of temperature estimations performed in stroke patients, and amplitude-, or amplitude-squared-weighted averaging of temperature estimations from single references might help to compensate for this. Advanced spectral processing techniques used by previous authors for temperature estimation from MRS [111, 116, 117] can also help compensate for the increased random error in MR spectra acquired *in vivo*, which the technique described in here does not benefit from. Thus, the following experiments were designed to test the technique in healthy volunteers and so determine if it is applicable *in vivo*. 
Chapter 3.2: *in vivo* validation of MR thermography technique

3.2.1 Introduction:

MR spectra obtained *in vivo* are invariably of poorer quality than those obtained *in vitro*. This is primarily due to increased heterogeneity of the magnetic field *in vivo*, and the fact that metabolites are bound up in cellular compartments rather than being in solution as they would be *in vitro*. In addition magnetic interference from structures such as the skull and blood, patient movement (both voluntary and involuntary) and variation in the relative concentrations of the various metabolites that produce MRS signals, as well as other factors all interact to reduce the quality of spectra acquired *in vivo* (see chapter 1.3 for a more detailed description). Thus, once a new MR thermography technique has been validated *in vitro*, it is prudent to validate it *in vivo* before attempting to apply it in a clinical setting. The following experiment was therefore conducted to answer the following questions:

1: Whether the MR thermography technique validated in chapter 3.1 could be applied in healthy volunteers.

2: What level of accuracy could be achieved for temperature estimations performed *in vivo*.

3: Whether any of the reference PRFs tested in chapter 3.1 allowed more accurate estimation of temperature than any others *in vivo*.

4: Whether the use of amplitude-weighted averaging of single chemical shift references (Cho, Cr and NAA) similar to the method described by Cady et al [116] could improve the accuracy of temperature estimations performed using this technique.

It was initially hoped that calibration of temperature estimations could be performed by comparing spectra from each volunteer’s tongue to their oral temperature. Unfortunately the quality of the spectra from the tongue proved, almost universally, to be too poor to allow further processing, so calibration and validation were performed by comparing the
brain spectra to tympanic temperature, which provides a reasonable estimate of average brain temperature under normal circumstances [80].

Figure 3.2.1  An example of a spectrum acquired in the brain (A- Left) and one acquired in the tongue (B- Right). Note that the Siemens Syngo software has attempted (unsuccessfully) to fit a line representing Cho, Cr and NAA resonances in the spectrum from the tongue. NAA is not, in fact, present in the tongue but it was hoped that the Cho and Cr peaks might still allow temperature estimations against which the MRT technique could be calibrated. Unfortunately, all the spectra from the tongue were too poor to allow further processing.

3.2.2 Methods

3.2.2.1 Data collection:
All experiments involving healthy volunteers were approved by the human research ethics committees of both ACT Health (Approval ETH8.10.320) and the Australian National University (Approval 2010-553). The scanning parameters were the same as those outlined in section 2.1.1. 20 healthy volunteers (13M, 7F, 24-58 years old, mean age 33.5 years) with no known history of neurological illness were recruited to the study. Each volunteer underwent 2 MR examinations on non-consecutive days, each of which involved collection of 15 spectra, 5 in the right posterior cingulate gyrus, 5 in the left posterior cingulate gyrus and 5 in the tongue. As with the other spectral acquisitions described in this thesis, manual shimming was not used as the experimental design aimed to make the acquisition as generalizable as possible for hospitals with limited access to MR expertise. The order of scanning for the brain ROIs was alternated so that each volunteer received one exam during which the left hemisphere was scanned first and one exam where the right hemisphere was scanned first. In each case the tongue was scanned last in case a malfunction or clinical need caused the exam to be aborted early. The posterior cingulate gyrus was chosen because it is easy to identify and known to produce reliably good spectra
Each volunteer’s tympanic and oral temperatures were recorded immediately after each examination.

### 3.2.2.2 Data processing
After completion of each MR examination, spectra were processed as described in section 2.1.2. The temperature estimations from each reference were calculated using the equations derived in section 3.1.3. The amplitude and line-width of each reference peak were then estimated using the standard Siemens line-fitting algorithm, using the same Gaussian filter as was used for manual frequency processing (instead of the standard Hanning filter). Amplitude weighted and amplitude-squared weighted temperatures were then calculated from the T_{Cho}, T_{Cr} and T_{NAA} using the following formulae (as described in section 2.1.3.2):

\[
T_{A-weighted} = \frac{\left( T_{Cho}^*A_{Cho} + T_{Cre}^*A_{Cre} + T_{NAA}^*A_{NAA} \right)}{A_{Cre} + A_{Cho} + A_{NAA}} \quad \text{(Equation 15)}
\]

\[
T_{A^2-weighted} = \frac{\left( T_{Cho}^*A_{Cho}^2 + T_{Cre}^*A_{Cre}^2 + T_{NAA}^*A_{NAA}^2 \right)}{A_{Cre}^2 + A_{Cho}^2 + A_{NAA}^2} \quad \text{(Equation 16)}
\]

Where \( T_{A-weighted} \) is the amplitude-weighted temperature.

\( T_{A^2-weighted} \) is the amplitude-squared-weighted temperature.

\( T_{Cho}, T_{Cr} \) and \( T_{NAA} \) are the temperature estimates from choline, creatine and NAA and \( A_{Cho}, A_{Cr} \) and \( A_{NAA} \) are the amplitudes of the Cho, Cr and NAA peaks, respectively.

### 3.2.2.3 Statistical analysis
While tympanic temperature provides a reasonable estimate of average brain temperature, it cannot be considered the ‘gold standard’ of measurement for the temperature for the cingulate gyrus (the gold standard would require surgically implanting a thermometer into the cingulate gyrus of each volunteer, which was neither safe nor practical). Therefore, the most appropriate tool to compare the two temperature measurements (tympanic and MRS-derived) was a Bland-Altman plot[199, 200]. A Bland-Altman plot is not suitable for comparing repeated measurements from the same patient as it assumes that the individual data-points are not correlated with each other [199]. Bland and Altman did publish a method for examining multiple pairs of measurements from a single patient [200], but it requires that each measurement using one method (e.g. MRT) is paired with a measurement using the other method. That is, it would require the tympanic temperature to be measured after each individual spectrum was collected rather than simply measuring
the tympanic temperature once at the end of the exam. Therefore, the data were divided up into four subsets on which a Bland-Altman analysis could be carried out. The data were first divided by ROI (left or right hemisphere), and then exams were divided into series 1 (exams in which the left hemisphere was scanned first, not necessarily the first exam of every volunteer) and series 2 (exams in which the right hemisphere was scanned first). The four data subsets (left hemisphere series 1, right hemisphere series 1, left hemisphere series 2 and right hemisphere series 2) were then subjected individually to statistical analysis. Each data subset still contained 5 scans, which could not be analysed separately using a Bland-Altman plot. Therefore, for each MRT processing method, the average temperature estimation over the 5 spectra was calculated, and this was plotted against the tympanic temperature. The standard deviation of each of the resulting Bland-Altman plots demonstrated the limits of agreement between using the average of 5 MRT-derived temperature measurements, and the tympanic temperature. The average difference (between the MRT-derived temperature and the tympanic temperature) from each Bland-Altman plot also provided an estimate of any bias inherent in the MRT measurements (e.g. as a result of temperature differences between the fluid inside the phantom and the outer surface of the phantom).

The average of 5 MRT-measurements would be more accurate than any single MRT-measurement, and this needed to be accounted for as clinical applications of this technique would likely involve only a single MRT-measurement from each region of interest. Therefore, the additional variance of individual spectra was calculated using mixed-models analysis in SPSS. The residual variance, after accounting for differences in temperature between individuals and changes in each individual’s temperature between scans, was added to the variance from the Bland-Altman plot to find the total variance (note variances sum together, standard deviations do not). The total variance was then used to calculate the total standard deviation for individual MRT measurements, which provided an estimate of the confidence intervals for the actual temperature from a given MRT measurement (95% confidence interval is ±2 standard deviations).
3.2.3 Results

3.2.3.1 Example Bland-Altman analysis of a single data subset

The Bland-Altman plot for each MRT-processing method is shown in Figure 3.2.2. The plots presented are those from the data subset which produced the poorest results (i.e., the broadest estimated confidence intervals for temperature estimation). Each MRT-processing method demonstrated a bias in temperature estimation. Equations 2 and 3 (using Cr and NAA, respectively as the chemical shift reference) both tended to underestimate temperature by approximately 1.2°C. Equation 1, using choline as the reference, tended to overestimate but this was the only equation to do so. The bias of each equation and the standard deviation of each BA plot can be found in Table 3.2.2.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Reference</th>
<th>Bias</th>
<th>Standard Deviation</th>
<th>Variance (SD^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cho</td>
<td>0.23</td>
<td>1.06</td>
<td>1.117</td>
</tr>
<tr>
<td>2</td>
<td>Cr</td>
<td>-1.16</td>
<td>1.23</td>
<td>1.508</td>
</tr>
<tr>
<td>3</td>
<td>NAA</td>
<td>-1.22</td>
<td>0.79</td>
<td>0.616</td>
</tr>
<tr>
<td>4</td>
<td>ChoCr</td>
<td>-0.52</td>
<td>0.55</td>
<td>0.299</td>
</tr>
<tr>
<td>5</td>
<td>ChoNAA</td>
<td>-0.33</td>
<td>0.60</td>
<td>0.358</td>
</tr>
<tr>
<td>6</td>
<td>CrNAA</td>
<td>-1.19</td>
<td>0.76</td>
<td>0.582</td>
</tr>
<tr>
<td>7</td>
<td>ChoCrNAA</td>
<td>-0.72</td>
<td>0.54</td>
<td>0.293</td>
</tr>
<tr>
<td></td>
<td>Amp-Weighted</td>
<td>-0.85</td>
<td>0.54</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Amp^2-Weighted</td>
<td>-0.97</td>
<td>0.58</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Figure 3.2.2 Bland Altman plots for the various MRT-processing methods tested in this experiment. Each BA plot shows the difference between the MRT-derived temperature estimations and the tympanic temperature (vertical axis) and the mean of the MRT-derived and tympanic temperatures (horizontal axis). The horizontal lines within each BA plot show the average difference (the bias) and the 95% confidence intervals for the limits of agreement between MRT-derived temperature estimations and tympanic temperature. Note that the amplitude-weighted and amplitude-squared-weighted temperatures were derived from equations which had been corrected for the bias found in BA-plots A, B and C, hence the bias for amplitude and amplitude-squared-weighted temperature estimations are both very close to zero.


3.2.3.3 Total variance analysis of a single subset of data

The results outlined in this section relate to the same data subset analysed in section 3.2.3.2. Table 3.2.2 shows the residual variance for temperatures derived from each MRT-processing method, along with the variance derived from the BA plots which were added.
together to form the total variance. The standard deviation calculated from the total variance of each equation provides an estimate of the 95% confidence intervals for the temperature estimations made using that equation.

### Table 3.2.2 Variance components and total variance for each of the temperature estimation equations within this data subset.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Reference</th>
<th>BA variance</th>
<th>Residual Variance</th>
<th>Total Variance</th>
<th>Total SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cho</td>
<td>1.117</td>
<td>0.138</td>
<td>1.255</td>
<td>1.121</td>
</tr>
<tr>
<td>2</td>
<td>Cr</td>
<td>1.508</td>
<td>0.000</td>
<td>1.508</td>
<td>1.228</td>
</tr>
<tr>
<td>3</td>
<td>NAA</td>
<td>0.616</td>
<td>0.091</td>
<td>0.717</td>
<td>0.841</td>
</tr>
<tr>
<td>4</td>
<td>ChoCr</td>
<td>0.299</td>
<td>0.055</td>
<td>0.354</td>
<td>0.592</td>
</tr>
<tr>
<td>5</td>
<td>ChoNAA</td>
<td>0.358</td>
<td>0.040</td>
<td>0.398</td>
<td>0.631</td>
</tr>
<tr>
<td>6</td>
<td>CrNAA</td>
<td>0.582</td>
<td>0.072</td>
<td>0.654</td>
<td>0.809</td>
</tr>
<tr>
<td>7</td>
<td>ChoCrNAA</td>
<td>0.293</td>
<td>0.040</td>
<td>0.581</td>
<td>0.762</td>
</tr>
<tr>
<td>Amp-weighted</td>
<td></td>
<td>0.286</td>
<td>0.059</td>
<td>0.766</td>
<td>0.586</td>
</tr>
<tr>
<td>Amp(^2)-weighted</td>
<td></td>
<td>0.341</td>
<td>0.078</td>
<td>0.805</td>
<td>0.648</td>
</tr>
</tbody>
</table>

### 3.2.3.4 Comparison of results between data subsets

There was a considerable degree of variation in the bias (as determined from the Bland-Altman plots) and the variance found for each equation across the 4 data sub-sets. Table 3.2.3 lists these values for each subset (note that the subset described in detail above was subset 1-R, i.e. the right hemisphere ROI of set 1). The equation that demonstrated the most consistent total variation was equation 4 (using the average of choline and creatine as the reference) followed by the amplitude-weighted average temperature. Notably, each data subset produced a different result regarding the equation with the lowest total variance (the lowest variance within each subset has been highlighted in Table 3.2.3).
<table>
<thead>
<tr>
<th></th>
<th>Series 1 (Left hemisphere)</th>
<th>Series 1 (Right hemisphere)</th>
<th>Series 2 (Left hemisphere)</th>
<th>Series 2 (Right hemisphere)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho Residual</td>
<td>0.0993</td>
<td>0.1378</td>
<td>0.1169</td>
<td>0.1820</td>
</tr>
<tr>
<td>Cho BA variance</td>
<td>0.2446</td>
<td>1.1990</td>
<td>0.4079</td>
<td>0.2008</td>
</tr>
<tr>
<td>Cho BA bias</td>
<td>0.89</td>
<td>0.11</td>
<td>0.83</td>
<td>0.93</td>
</tr>
<tr>
<td>Cho total SD</td>
<td><strong>0.5864</strong></td>
<td><strong>1.1562</strong></td>
<td><strong>0.7244</strong></td>
<td><strong>0.6187</strong></td>
</tr>
<tr>
<td>Cho A</td>
<td>25.8 (3.4)</td>
<td>26.5 (4.0)</td>
<td>26.4 (4.5)</td>
<td>26.4 (3.6)</td>
</tr>
<tr>
<td>Cr Residual</td>
<td>0.0897</td>
<td>0.1089</td>
<td>0.2008</td>
<td>0.0995</td>
</tr>
<tr>
<td>Cr BA variance</td>
<td>0.2468</td>
<td>1.5601</td>
<td>0.2617</td>
<td>0.1891</td>
</tr>
<tr>
<td>Cr BA bias</td>
<td>-1.55</td>
<td>-1.21</td>
<td>-1.53</td>
<td>-1.56</td>
</tr>
<tr>
<td>Cr total SD</td>
<td><strong>0.5801</strong></td>
<td><strong>1.2490</strong></td>
<td><strong>0.6088</strong></td>
<td><strong>0.5372</strong></td>
</tr>
<tr>
<td>Cr A</td>
<td>26.7 (2.3)</td>
<td>26.6 (2.8)</td>
<td>27.3 (3.3)</td>
<td>27.1 (2.7)</td>
</tr>
<tr>
<td>NAA Residual</td>
<td>0.0935</td>
<td>0.0909</td>
<td>0.0719</td>
<td>0.1127</td>
</tr>
<tr>
<td>NAA BA variance</td>
<td>0.2749</td>
<td>0.6140</td>
<td>0.4931</td>
<td>0.2971</td>
</tr>
<tr>
<td>NAA BA bias</td>
<td>-0.97</td>
<td>-1.22</td>
<td>-1.00</td>
<td>-1.06</td>
</tr>
<tr>
<td>NAA total SD</td>
<td><strong>0.6069</strong></td>
<td><strong>0.8396</strong></td>
<td><strong>0.7517</strong></td>
<td><strong>0.6402</strong></td>
</tr>
<tr>
<td>NAA A</td>
<td>47.8 (5.9)</td>
<td>51.7 (5.4)</td>
<td>48.8 (7.0)</td>
<td>52.0 (6.0)</td>
</tr>
<tr>
<td>ChoCr Residual</td>
<td>0.0646</td>
<td>0.0546</td>
<td>0.0784</td>
<td>0.0969</td>
</tr>
<tr>
<td>ChoCr BA variance</td>
<td>0.2376</td>
<td>0.2909</td>
<td>0.2742</td>
<td>0.2052</td>
</tr>
<tr>
<td>ChoCr BA bias</td>
<td>-0.34</td>
<td>-0.53</td>
<td>-0.40</td>
<td>-0.37</td>
</tr>
<tr>
<td>ChoCr total SD</td>
<td><strong>0.5498</strong></td>
<td><strong>0.5878</strong></td>
<td><strong>0.5938</strong></td>
<td><strong>0.5496</strong></td>
</tr>
<tr>
<td>ChoCr A</td>
<td>26.7 (2.3)</td>
<td>26.6 (2.8)</td>
<td>27.3 (3.3)</td>
<td>27.1 (2.7)</td>
</tr>
<tr>
<td>ChoNAA Residual</td>
<td>0.0689</td>
<td>0.0401</td>
<td>0.0094</td>
<td>0.0396</td>
</tr>
<tr>
<td>ChoNAA BA variance</td>
<td>0.2545</td>
<td>0.3950</td>
<td>0.3969</td>
<td>0.2509</td>
</tr>
<tr>
<td>ChoNAA BA bias</td>
<td>-0.08</td>
<td>-0.40</td>
<td>-0.18</td>
<td>-0.15</td>
</tr>
<tr>
<td>ChoNAA total SD</td>
<td><strong>0.5687</strong></td>
<td><strong>0.6597</strong></td>
<td><strong>0.6374</strong></td>
<td><strong>0.5390</strong></td>
</tr>
<tr>
<td>CrNAA Residual</td>
<td>0.0366</td>
<td>0.7219</td>
<td>0.0008</td>
<td>0.0000</td>
</tr>
<tr>
<td>CrNAA BA variance</td>
<td>0.2500</td>
<td>0.5861</td>
<td>0.3520</td>
<td>0.2481</td>
</tr>
<tr>
<td>CrNAA BA bias</td>
<td>-1.24</td>
<td>-1.19</td>
<td>-1.29</td>
<td>-1.33</td>
</tr>
<tr>
<td>CrNAA total SD</td>
<td><strong>0.5354</strong></td>
<td><strong>0.8114</strong></td>
<td><strong>0.5940</strong></td>
<td><strong>0.4981</strong></td>
</tr>
<tr>
<td>ChoCrNAA Residual</td>
<td>0.0938</td>
<td>0.0402</td>
<td>0.0078</td>
<td>0.0021</td>
</tr>
<tr>
<td>ChoCrNAA BA variance</td>
<td>0.2446</td>
<td>0.3097</td>
<td>0.3311</td>
<td>0.2309</td>
</tr>
<tr>
<td>ChoCrNAA BA bias</td>
<td>-0.56</td>
<td>-0.71</td>
<td>-0.64</td>
<td>-0.63</td>
</tr>
<tr>
<td>ChoCrNAA total SD</td>
<td><strong>0.5817</strong></td>
<td><strong>0.5915</strong></td>
<td><strong>0.6236</strong></td>
<td><strong>0.4827</strong></td>
</tr>
<tr>
<td>Amp- weighted T Residual</td>
<td>0.0881</td>
<td>0.0585</td>
<td>0.0434</td>
<td>0.0788</td>
</tr>
<tr>
<td>Amp- weighted BA variance</td>
<td>0.2487</td>
<td>0.2857</td>
<td>0.3956</td>
<td>0.2642</td>
</tr>
<tr>
<td>Amp-weighted T BA bias</td>
<td>-0.64</td>
<td>-0.85</td>
<td>-0.71</td>
<td>-0.68</td>
</tr>
<tr>
<td>Amp-weighted T total SD</td>
<td><strong>0.5804</strong></td>
<td><strong>0.5862</strong></td>
<td><strong>0.6625</strong></td>
<td><strong>0.5857</strong></td>
</tr>
<tr>
<td>Amp(^2)- weighted T Residual</td>
<td>0.0337</td>
<td>0.0782</td>
<td>0.0055</td>
<td>0.0740</td>
</tr>
<tr>
<td>Amp(^2)- weighted BA variance</td>
<td>0.2652</td>
<td>0.3413</td>
<td>0.4385</td>
<td>0.2987</td>
</tr>
<tr>
<td>Amp(^2)- weighted BA bias</td>
<td>-0.74</td>
<td>-0.97</td>
<td>-0.81</td>
<td>-0.80</td>
</tr>
<tr>
<td>Amp(^2)- weighted T total SD</td>
<td><strong>0.5467</strong></td>
<td><strong>0.6477</strong></td>
<td><strong>0.6643</strong></td>
<td><strong>0.6105</strong></td>
</tr>
</tbody>
</table>
3.2.4 Discussion

3.2.4.1 Summary of results
The results of the statistical analysis described in sections 3.2.3.2 and 3.2.3.3 were described in detail for the sake of clarity and should not be considered more important or relevant the results from the other data subsets. The results from each data subset indicated a different equation would yield the most accurate temperature estimations. Furthermore, the individual components of each total variance (residual variance and Bland-Altman variance), as well as the apparent bias of each equation varied across the data subsets. This made meaningful analysis difficult, as the only trend that was found to be consistent across all 4 data subsets regarded the direction of the bias from the Bland-Altman plots. Namely, equation 1 tended to consistently overestimate temperature, and all other equations tended to underestimate temperature, regardless of the data subset. Overall, equation 4 yielded the most consistent total variance across the 4 data subsets (as measured by the standard deviation of the total variance across the subsets), followed closely by the amplitude-weighted temperature. However, it is noteworthy that the total variance of the amplitude-weighted temperature was remarkably consistent across 3 of the data subsets, with 1 subset (2-L) being a possible outlier.

3.2.4.2 Correction of intercepts for each equation from section 3.1
The most logical way to correct for the bias of equations 1-7 was to use the average of the biases from each of the 4 data subsets. Table 3.2.4 lists the corrected equations that will be used for the remainder of this thesis. Rather than correcting the apparent bias of the amplitude- and amplitude-squared-weighted averages, these equations were left unchanged but from this point will be calculated from corrected temperatures. The confidence intervals listed are the broadest intervals found across the 4 data subsets in order to avoid overestimating the accuracy of temperature estimations performed using each technique.

The fact that equations 2-7 tended to underestimate temperature, while equation 1 tended to overestimate temperature across all 4 data subsets supports the hypothesis that each equation was subject to a bias as a result of the design of the phantom calibration experiment (see section 3.1.4.5). If this was purely because of a temperature gradient between the fluid where the MRS spectra were acquired and the skin of the phantom
where the calibration temperatures were measured, one would expect all the equations to underestimate temperature. However, the choline reference produced some anomalous results *in vitro* (see section 3.1.4.4), so this possibility cannot be ruled out. It is likely that the systematic bias in each equation resulted from a combination of the temperature gradient between the fluid in the phantom and the calibration thermometer as well as differences in the protein content between the phantom and brain tissue [197].

<table>
<thead>
<tr>
<th>Chemical Shift Reference</th>
<th>Equation</th>
<th>95% Limits of agreement of average of 5 measurements</th>
<th>95% Limits of agreement of individual measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation 17 <em>Δ</em> Cho</td>
<td>$T = -1.414Δ_{\text{water-cho}} + 167.65$</td>
<td>±2.1144</td>
<td>±2.2274</td>
</tr>
<tr>
<td>Equation 18 <em>Δ</em> Cr</td>
<td>$T = -1.309 Δ_{\text{water-cr}} + 172.95$</td>
<td>±2.4563</td>
<td>±2.5429</td>
</tr>
<tr>
<td>Equation 19 <em>Δ</em> NAA</td>
<td>$T = -1.315 Δ_{\text{water-NAA}} + 258.72$</td>
<td>±1.5698</td>
<td>±1.6824</td>
</tr>
<tr>
<td>Equation 20 <em>Δ</em> ChoCr</td>
<td>$T = -1.367 Δ_{\text{water-ChoCr}} + 171.16$</td>
<td>±1.0948</td>
<td>±1.2508</td>
</tr>
<tr>
<td>Equation 21 <em>Δ</em> ChoNAA</td>
<td>$T = -1.368 Δ_{\text{water-ChoNAA}} + 215.50$</td>
<td>±1.1958</td>
<td>±1.3036</td>
</tr>
<tr>
<td>Equation 22 <em>Δ</em> CrNAA</td>
<td>$T = -1.315 Δ_{\text{water-CrNAA}} + 216.16$</td>
<td>±1.52596</td>
<td>±1.6200</td>
</tr>
<tr>
<td>Equation 23 <em>Δ</em> ChoCrNAA</td>
<td>$T = -1.351 Δ_{\text{water-ChoCrNAA}} + 201.36$</td>
<td>±1.0817</td>
<td>±1.5602</td>
</tr>
</tbody>
</table>

### 3.2.4.3 Best MRT-method for ongoing use

Because each subset produced a different answer to this question, it is difficult to answer. Logically the equation with the most consistent results across the 4 data subsets is probably the best. By one measure (SD of the 4 different values for total variance) this would be equation 20 (corrected version of equation 4). However, given the difficulty in identifying choline and creatine peaks in poor spectra, and the remarkable consistency of amplitude-weighed temperature across 3 out of 4 subsets, it seems unwise to disregard the information that can be gleaned from the NAA peak. In addition, the advantages of amplitude-weighted temperature estimations will likely become more apparent in poor spectra than those examined in this study, as the amplitude-weighting can help compensate for reduction in the amplitude (or increase in linewidth) of any individual peak.
as a result of spectral quality or pathology. Hence, the amplitude-weighted temperature estimation method will be the method used for the remainder of this thesis.

3.2.4.4 Other questions/issues
While the amplitude-weighted temperature estimation method should help compensate for changes in the concentration of various metabolites as a result of regional variation or pathology, a reduction in the amplitude of any of the chemical shift reference peaks could reduce the accuracy of temperature estimation. Thus, it cannot be assumed that the accuracy of temperature estimations achieved in this experiment can also be achieved in ischaemic tissue or, indeed, in regions of the brain where spectral quality tends to be poor or the concentration of metabolites tends to be lower, especially if the amplitude of all three chemical shift reference peaks were reduced.

3.2.4.5 Summary
The variation found between the 4 data subsets suggests that there is a great deal of noise in the temperature estimations performed in this experiment which could not be attributed to variation between individuals, or variation between regions of interest or over time within an individual. Since the spectra themselves tended to be of high quality, it must be concluded that this noise is a result of the temperature estimation method. The overall suitability of the MRT-processing method under investigation will be discussed in Chapter 6 in light of all the experimental data collected for this thesis, but the results of this experiment are not particularly encouraging. The amplitude-weighted average temperature does appear to be the most promising of the temperature estimation equations being investigated and will therefore be further explored in the next chapter. The accuracy of temperature estimations performed using this method appears to be approximately ±1.3°C.

A logical approach to detecting differences in temperature as a result of ischaemia is to compare the infarct or penumbra temperature to another region of the patient’s brain, e.g. the corresponding region of the contralateral hemisphere. There is little data available on temperature variation within the healthy brain and between corresponding contralateral regions. It is generally assumed that the temperature of the brain is fairly uniform, and that any temporary variations are random (i.e., that there are no systematic differences between hemispheres or between different regions within a hemisphere). However, there
is little data available to support this assumption in intact tissue. Furthermore, there may be regional variations in the quality of spectra that can be acquired, or the concentration of certain metabolites such as neurotransmitters which may affect temperature estimations from MR spectra. The effects of these variations would be dependent on the method of processing the MR spectra. Hence it is worth collecting more data. The next experiment (Chapter 3.3) was therefore designed to examine multiple regions of interest within the brain of a single volunteer using the MRT processing method chosen here (amplitude-weighted averaging) to determine what level of temperature variation can be considered ‘normal’, and what can be considered pathological.
Chapter 3.3 Regional and temporal variation in healthy brain temperature.

3.3.1 Background
Previous studies that examined brain temperature in stroke have typically compared the temperature of stroke-affected brain tissue (infarct and peri-infarct or penumbra) to unaffected tissue. This is a very sensible approach, as the ‘normal’ temperature of healthy brain can vary between different patients and from one day to the next for a given patient, just as normal body temperature can. However, the data available regarding normal variation in temperature across different regions of the brain are quite limited. It was therefore thought prudent to collect some data on the normal level of temperature variation between different regions of an individual’s brain to provide some context for the temperature variations that might be found in stroke patients. In addition, since one of the following experiments will examine changes in temperature over time in stroke patients, some data regarding normal variation in brain temperature over time would also be helpful. Thus, the following experiment was designed to collect such data.

3.3.2 Methods
3.3.2.1 Data collection
All experiments involving healthy volunteers were approved by the human research ethics committees of both ACT Health (Approval ETH8.10.320) and the Australian National University (Approval 2010-553). Each volunteer underwent 1 exam, involving scans of 5 different ROIs in each hemisphere (10 acquisitions in total).

3.3.2.2 Data processing
Spectra were processed in the manner described in Chapter 2.1 and the amplitude-weighted temperature for each spectrum was calculated as described in section 2.1.3.2.
The data-set was examined using a linear mixed-model analysis in SPSS to determine whether there were any systematic differences in temperature between different ROIs within a hemisphere, or between hemispheres. The spectra from individual ROIs were also compared in terms of the line-width and amplitude of the various reference peaks to determine whether spectra of sufficient quality could be consistently acquired from each ROI.

3.3.3 Results

3.3.3.1 Quality of Spectra
Table 3.2.1 lists metrics of the linewidth (full width at half-max height) of NAA across all spectra examined, and individually for the separate regions of interest. There was considerable heterogeneity in the spectral quality across the different regions of interest, as evidenced by the variation in the linewidth of NAA.

<table>
<thead>
<tr>
<th>Region</th>
<th>Whole Brain</th>
<th>BG</th>
<th>CBL</th>
<th>OL</th>
<th>FL</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.15</td>
<td>5.80</td>
<td>5.71</td>
<td>4.56</td>
<td>5.51</td>
<td>4.21</td>
</tr>
<tr>
<td>SD</td>
<td>1.29</td>
<td>1.23</td>
<td>1.46</td>
<td>0.83</td>
<td>1.35</td>
<td>0.64</td>
</tr>
<tr>
<td>Maximum</td>
<td>10.80</td>
<td>8.60</td>
<td>10.80</td>
<td>6.60</td>
<td>7.80</td>
<td>5.50</td>
</tr>
<tr>
<td>Minimum</td>
<td>3.30</td>
<td>4.20</td>
<td>4.50</td>
<td>3.50</td>
<td>3.90</td>
<td>3.30</td>
</tr>
<tr>
<td>Range</td>
<td>7.50</td>
<td>4.40</td>
<td>6.30</td>
<td>3.10</td>
<td>3.90</td>
<td>2.20</td>
</tr>
</tbody>
</table>

3.3.3.2 Average brain temperature.
The average brain temperature across all ROIs and all volunteers was 36.33°C with a standard deviation of 0.54. The average brain temperature and standard deviation for each volunteer can be found in Table 3.3.2
Table 3.3.2 Mean and standard deviation of brain temperature for each volunteer (°C)

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Mean Temp</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.87</td>
<td>0.37</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>36.35</td>
<td>0.44</td>
<td>1.22</td>
</tr>
<tr>
<td>3</td>
<td>36.11</td>
<td>0.71</td>
<td>2.39</td>
</tr>
<tr>
<td>4</td>
<td>36.50</td>
<td>0.42</td>
<td>1.32</td>
</tr>
<tr>
<td>5</td>
<td>36.30</td>
<td>0.57</td>
<td>1.87</td>
</tr>
<tr>
<td>6</td>
<td>36.68</td>
<td>0.23</td>
<td>0.65</td>
</tr>
<tr>
<td>7</td>
<td>36.24</td>
<td>0.41</td>
<td>1.22</td>
</tr>
<tr>
<td>8</td>
<td>36.13</td>
<td>0.38</td>
<td>1.30</td>
</tr>
<tr>
<td>9</td>
<td>36.27</td>
<td>0.68</td>
<td>2.09</td>
</tr>
<tr>
<td>10</td>
<td>35.90</td>
<td>0.45</td>
<td>1.45</td>
</tr>
</tbody>
</table>

3.3.3.3 Mixed model analysis of brain temperature

Neither region of interest nor hemisphere was found to be significant overall in terms of their effect on temperature. However, a post-hoc analysis found that the basal ganglia were cooler than the rest of the brain on average and this effect was statistically significant (p<0.041). The average temperature of each ROI is listed in Table 3.3.3. Table 3.3.4 lists the differences between ROIs relative to the parietal lobe and the p-values of these differences. Note that the parietal lobe was simply chosen as the reference point at random by the statistics software.

Table 3.3.3 Average absolute temperature of each region of interest within the brains of 10 healthy volunteers (°C)

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Average Temperature</th>
<th>Standard Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>36.45</td>
<td>0.47</td>
<td>35.50 – 37.40</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>35.38</td>
<td>0.46</td>
<td>34.46 – 36.31</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>36.42</td>
<td>0.49</td>
<td>35.45 – 37.39</td>
</tr>
<tr>
<td>Frontal</td>
<td>36.09</td>
<td>0.46</td>
<td>35.16 – 37.01</td>
</tr>
<tr>
<td>Occipital</td>
<td>36.43</td>
<td>0.46</td>
<td>35.50 – 37.35</td>
</tr>
</tbody>
</table>
Table 3.3.4 Average relative temperature differences between different regions of interest, as measured against the parietal ROI (°C)

<table>
<thead>
<tr>
<th>Region Of Interest</th>
<th>Temperature difference (relative to parietal ROI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>-1.81</td>
<td>0.041</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-0.17</td>
<td>0.846</td>
</tr>
<tr>
<td>Frontal</td>
<td>-0.20</td>
<td>0.819</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.08</td>
<td>0.924</td>
</tr>
</tbody>
</table>

3.3.3.4 Analysis of reference PRF amplitude

Region of interest was found to have a statistically significant effect on the amplitude of the choline, creatine and NAA resonance peaks (p<0.001). Table 3.3.5 lists the average amplitude of each peak in the different regions of interest. Hemisphere was not found to be statistically significant with regard to any of the peak amplitudes.

Table 3.3.5 Amplitude of the choline, creatine and NAA peaks in each region of interest.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Cho Amplitude (SE)</th>
<th>Cr Amplitude (SE)</th>
<th>NAA Amplitude (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>27.82 (1.18)</td>
<td>26.53 (1.00)</td>
<td>54.65 (1.93)</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>23.70 (1.16)</td>
<td>22.32 (0.98)</td>
<td>40.66 (1.89)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>36.23 (1.21)</td>
<td>36.90 (1.03)</td>
<td>45.17 (1.97)</td>
</tr>
<tr>
<td>Frontal</td>
<td>30.72 (1.16)</td>
<td>27.36 (0.98)</td>
<td>47.04 (1.89)</td>
</tr>
<tr>
<td>Occipital</td>
<td>22.72 (1.16)</td>
<td>25.51 (0.98)</td>
<td>49.32 (1.89)</td>
</tr>
</tbody>
</table>

A post-hoc analysis found that not all of the differences in amplitude between different ROIs were statistically significant. Table 3.3.6 below lists the differences between ROIs relative to the parietal lobe and the p-values of these differences for each reference PRF (once again these differences are expressed relative to the parietal lobe). Careful manual shimming may have improved the homogeneity of the linewidth and amplitude of each peak across the different regions of interest, but at the expense of the generalisability of the MR thermography technique for research groups without significant MR expertise.
Table 3.3.6 Differences in the amplitude of each peak between regions of interest. All differences are expressed relative to the Parietal lobe.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Cho amplitude difference</th>
<th>p value (&lt;)</th>
<th>Cr amplitude difference</th>
<th>p value</th>
<th>NAA amplitude difference</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parietal</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td>-4.93</td>
<td>0.010</td>
<td>-5.89</td>
<td>0.002</td>
<td>-16.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>8.09</td>
<td>0.001</td>
<td>9.71</td>
<td>0.001</td>
<td>-10.31</td>
<td>0.001</td>
</tr>
<tr>
<td>Frontal</td>
<td>2.84</td>
<td>0.134</td>
<td>0.30</td>
<td>0.873</td>
<td>-8.14</td>
<td>0.007</td>
</tr>
<tr>
<td>Occipital</td>
<td>-6.00</td>
<td>0.002</td>
<td>-1.14</td>
<td>0.543</td>
<td>-6.43</td>
<td>0.033</td>
</tr>
</tbody>
</table>

3.3.4 Discussion

3.3.4.1 Overview of results

As noted in Section 3.3.3.1 above, there was considerable heterogeneity in the spectral quality across the various ROIs examined in this experiment. The parietal and occipital lobes produced the highest quality spectra, while the cerebellum produced the least consistent results (as evidenced by the range and standard deviation of the linewidth of the NAA peak in Table 3.3.1).

The average brain temperature across all volunteers (36.33°C) was reasonably close to the widely accepted average value of 37°C. The range of temperatures found within each volunteer (maximum of 2.4°C) was within the estimated margin of error for these temperature estimations found in chapter 3.2 (±1.3°C). Region of interest and hemisphere were not found to have any statistically significant effect on temperature with the exception of the basal ganglia, which tended to be cooler than the rest of the brain.

The effect of region of interest, but not hemisphere, was found to be statistically significant for the amplitudes of all three reference peaks. This made sense as the concentrations of the chemicals in question are known to vary between grey matter and white matter [21], and between different regions of the brain [104]. Table 3.3.6 ranks each region of interest with the regard to the amplitude of each reference peak. It is noteworthy that the cerebellum demonstrated the second lowest average amplitude for NAA, but the highest amplitude for choline and creatine.
Table 3.3.6 Ranking of regions of interest according to the amplitude of each reference peak.

<table>
<thead>
<tr>
<th>Region of interest (highest amplitude to lowest)</th>
<th>Peak 1 (highest)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 (lowest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho</td>
<td>Cerebellum</td>
<td>Frontal</td>
<td>Parietal</td>
<td>Basal Ganglia</td>
<td>Occipital</td>
</tr>
<tr>
<td>Cr</td>
<td>Cerebellum</td>
<td>Frontal</td>
<td>Parietal</td>
<td>Occipital</td>
<td>Basal Ganglia</td>
</tr>
<tr>
<td>NAA</td>
<td>Parietal</td>
<td>Occipital</td>
<td>Frontal</td>
<td>Cerebellum</td>
<td>Basal Ganglia</td>
</tr>
</tbody>
</table>

Not all differences between the regions of interest were statistically significant. As noted in Table 3.3.5 above, the differences between the parietal lobe and all other ROIs were significant for NAA, but for Cho the frontal lobe was not found to be significantly different and for Cr this was true of both the frontal lobe and the occipital lobe.

### 3.3.4.2 Temperature variation within the brain

The range of temperatures found within any one patient did not exceed 2.4°C. Given that the accuracy of the temperature estimated is approximately ±1.3°C, this range of temperatures may be explained by the limits of precision of the temperature estimations themselves. Previous studies using MR thermography have also failed to find any consistent temperature gradients within healthy human brains [105, 115]. Childs et al measured 4 different sites within the brains of 8 volunteers using single-voxel spectroscopy. There was no consistent pattern to the temperature differences between regions within each patient except for the fact that temperature variations tended to be small (no greater than 1.6°C within any single patient) [111]. Childs also examined healthy brains using MRSI, as did Marshall et al [110] and while both authors reported greater variation in temperature estimations between voxels than those found using single-voxel MRS, neither found any consistent variation in regional brain temperature. Corbett et al did find that the thalamus tended to be slightly warmer than the frontal lobe (37.7±0.6 vs 37.2±0.6 respectively) in 10 patients examined using single-voxel-spectroscopy and found that this difference was statistically significant, albeit small [105]. Ishigaki et al examined differences between right and left hemispheres and found this difference was less than 0.24°C in healthy volunteers (although could be greater in patients suffering severe carotid stenosis) [131]. Therefore, despite the small sample size of this study (and indeed all previous studies) it appears likely that any systematic temperature variations that do exist within healthy human brains are small enough that they cannot be detected with current MR thermography techniques. This may change as the accuracy of MR thermography improves.

128
3.3.4.3 Variation in reference peak amplitude

This study did find significant systematic variations in the amplitudes of the various reference peaks. This supports the decision to use the amplitude-weighted average temperature rather than relying on any single reference. This method was originally proposed to ameliorate the effects of pathology on the amplitude of these reference peaks, especially that of NAA. However, the results of this study indicate that the amplitude of each peak, and thus the accuracy of temperature estimations made from these peaks can vary considerably between different regions even within healthy brain tissue. Notably, there was no significant difference found between equivalent ROIs in different hemispheres of the brain, and the effects of location were different for the different peaks. This indicates that the variation seen in the peak amplitude was not solely the result of variations in the magnetic interference from the skull or CSF degrading the signal-to-noise ratio in some ROIs more than others (although this may have been the case in the basal ganglia), otherwise all three reference peaks should have been affected the same way in each ROI. The results presented here indicate that there are variations in the concentration of choline, creatine and NAA between different regions of the human brain.

As noted above, not all the regional variation in amplitude for the various peaks was found to be significant. The amplitude of the NAA peak varied significantly regardless of ROI. However, the choline and creatine peaks were less likely to show significant variation between the different ROIs within the cortex (frontal, parietal and occipital). It was also noted above that there was no significant variation in peak amplitudes between equivalent ROIs in contralateral hemispheres. Together, these results indicate that the variation in the peak amplitudes (which indicate variation in the concentration of the relevant metabolites) may be related directly to the function of the tissue. A larger sample size would need to be examined to further explore this possibility.

3.3.4.4 Implications for ongoing MR Thermography experiments

The results of this experiment further support the decision to use the amplitude-weighted average temperature rather than relying on any single reference peak. The use of amplitude-weighting was originally intended to ameliorate the effects of changes in peak amplitude due to pathology [116]. However, this experiment suggests that regional variation in healthy tissue may be just as significant a source of error in MR thermography measurements. The lack of significant variation in peak amplitude between equivalent...
ROIs in contralateral hemispheres suggests that comparing the temperatures of such matched contralateral ROIs is a more valid method than comparing randomly located ROIs based purely on their pathological classification. However, a number of previous studies [110, 121, 122, 201] have used MR spectroscopic imaging and compared the average temperatures of tissues based purely on pathological classification (i.e. infarct, penumbra, possible penumbra, healthy tissue) using a single chemical shift reference (NAA). The results presented here suggest that if some allowance was made for the variation in NAA peak amplitude between different voxels (for example by using the amplitude-weighted average temperature of each voxel) then these studies may have achieved more accurate results. Careful manual shimming of individual voxels may also reduce the variation in spectral quality between different voxels and allow more valid comparisons between voxels.

3.3.4.5 Summary.
This study found no consistent variation in temperature between different regions of interest in the brains of healthy volunteers, indicating that any systematic variation in temperature is within the margin of error for temperature estimations performed using the technique being used for this thesis. The variation in linewidth of the NAA peak across various ROIs suggests that manual shimming may improve the quality of temperature estimations if the time and expertise to conduct this procedure are available. There was significant variation in the amplitudes of the various reference chemical shift peaks between different ROIs, although not between matched contralateral ROIs. This may partly have been a result of the variation in spectral quality between ROIs, indicated by the variation in the linewidth of the NAA peak. Thus, the variation in peak amplitude and temperature estimations may be partly explained by variation in spectral quality rather than any underlying variation in metabolite concentrations. Overall, these results indicate that even in healthy tissue, the use of amplitude-weighting is likely to demonstrate a benefit in terms of the accuracy of temperature estimations based on MR spectroscopy. Furthermore, this study indicates that where a single chemical shift reference has been used, temperature estimations would be most valid if they were confined to contralateral matched regions of interest.
Part IV:

Natural History of Regional Brain Temperature in Stroke Patients
Chapter 4.1 Application of MR Thermography to Ischaemic Stroke Patients

4.1.1 Background

The reasons for interest in the effects of stroke on brain temperature were outlined in chapter 1.2, and the science behind MR thermography based on the proton resonance frequency of water have been discussed previously in detail (chapters 1.2 and 1.3, respectively). The purpose of this experiment was to apply the magnetic resonance thermography (MRT) technique developed in chapters 3.1-3.3 to patients suffering from acute and subacute ischaemic stroke. There is still very little data available regarding the variations in temperature that occur in the human brain after a stroke, either between different regions of the brain, or over time (See chapter 1.2). It is important to attempt to collect such data in order to have a basis of comparison for data collected from patients subjected to therapeutic hypothermia. It is also likely to be more difficult to obtain useful proton resonance spectra from injured brain tissue than it is from healthy brain tissue. It is therefore worthwhile attempting to use the MRT technique described in this thesis to ensure that the data necessary for temperature estimation can, in fact, be collected from ischaemic tissue (See Chapter 1.2).

The accuracy of the MRT technique being used was tested in Chapter 3.2 and estimated to be approximately ±1.3°C. There is a strong possibility that the temperature changes that occur in the brain after a stroke are too small to be detected with this technique. However, this is not known for certain and is hence worth testing experimentally.

Therefore, this pilot study was designed to answer the following questions:

1: Is it possible to obtain the spectral data necessary for the MR thermography paradigm developed in this thesis from ischaemic stroke patients?

2: Do temperature variations large enough to be detected by this MRT technique occur
after an ischaemic stroke?

3: Does regional brain temperature correlate with clinical outcome?

**4.1.2 Methods**

**4.1.2.1 Patient recruitment**

The project was approved by the Australian Capital Territory health human research ethics committee and the Australian National University human research ethics committee (Approval references ETH11.09.1014 and 2010-392 respectively). Informed consent was obtained from each patient or from next of kin if the patient was unable to give informed consent as a result of their brain injury.

All patients were recruited within 24 hours of suffering an ischaemic stroke in the territory perfused by the middle cerebral artery (MCA). Recruitment was limited to MCA strokes because this territory consists largely of tissue from which good MR spectra can generally be obtained. Furthermore, MCA strokes are the single most common class of ischaemic stroke [18]. Patients were excluded if they were under 18 years of age, if they had any known contraindications to MRI, or if they could not receive their first MRI within 24 hours of stroke onset (or time last known well for patients with an unknown stroke onset).

**4.1.2.2 Imaging**

The study design called for each patient to undergo a total of 5 MR examinations, with the first of these being conducted as soon as practical after stroke onset, subsequent examinations were conducted every 24 hours after the initial examination. All examinations were conducted on the 1.5T Siemens Magnatom Avanto MRI scanner at the Canberra Hospital.

The protocol for the first MR examination involved Diffusion-weighted imaging (DWI), which shows acute ischaemia very effectively [22] in 3 planes. These triplanar images were then used to guide the location of 3 single-voxel MR spectroscopy (MRS) acquisitions identical to those carried out on healthy volunteers in Chapters 3.2 and 3.3. The first MRS voxel was located within the infarct, recognisable as a region hyper-intensity on the DWI. The second voxel was positioned in tissue as close to the infarct as possible (peri-infarct), while avoiding structures such as the skull or ventricles that would have degraded the
quality of the spectrum. The final spectrum was positioned in the contralateral hemisphere, as close as practical to the ‘mirror image’ of the infarct voxel.

Perfusion-weighted imaging (requiring an injection of gadolinium-based contrast agent) was performed after the acquisition of the final MR spectrum to provide a cerebral blood flow value for the purposes of future finite element modelling (see chapters 5.1 and 5.2). If patients were unable to receive the gadolinium contrast agent for any reason (most commonly because of poor kidney function [202]) they were still enrolled in the study and the PWI scan was simply omitted from the scanning protocol. Consideration was given to conducting the PWI before the MRS acquisitions, and using the PWI/DWI mismatch to identify penumbra for voxel placement. Unfortunately, the MRI scanner being used did not have this capability, as the PWI scans had to be post-processed off-site. Therefore, the PWI was conducted after the MRS scans in order to reduce the risk of the gadolinium in the patient’s blood affecting the MR spectra [203].

The second and subsequent MR examinations all began with a 3 dimensional fluid attenuated inversion recovery (FLAIR) scan. This is a form of T2-weighted imaging (See Appendix 1) which shows sub-acute ischaemia very effectively and produces clearer anatomical images than DWI. The 3 dimensional FLAIR data were then post-processed into 3 plane images, similar to the 3 plane diffusion-weighted images used in the first scan. These triplanar images were used to position 3 MRS voxels. The MRS voxels were manually positioned as close as possible to the location of the 3 voxels acquired in the first MR examination. PWI and DWI were not conducted during these examinations.

4.1.2.3 Other data collection
The severity of each patient’s stroke symptoms was assessed using the national institute of health stroke scale (NIHSS) [204, 205]. Relevant information such as time of symptom onset, age, medications, risk factors and previous ischaemic events was obtained from the patient’s medical notes and from bedside interviews conducted with the patients or their relatives. The patient’s tympanic temperature was recorded at the end of each MR examination. Patient outcome was recorded by means of the modified Rankin score, which was assessed via telephone interview 90 days after the patient’s stroke. Both the NIHSS and the
modified Rankin score are very common assessment tools in stroke studies [204, 206, 207] and using these tools made comparison to previous studies easier. Furthermore, while this study was never designed to recruit enough patients to derive meaningful conclusions about the effects of regional brain temperature on outcome, by recording the NIHSS and modified Rankin score the door was left open for these data to be included in a subsequent larger data set or meta-analysis.

4.1.2.4 Data processing
MRS data were processed in the manner described in chapter 2.1, and the amplitude-weighted temperature was calculated for each voxel (see chapters 3.2 and 3.3). These temperatures were recorded in a spreadsheet for further analysis. The raw data from each PWI scan was burned onto a DVD and sent to Dr Andrew Bivard at John Hunter Hospital in Newcastle, NSW, for processing on the MIStar software package (Apollo Medical Imaging, Melbourne, Vic). Unfortunately, absolute CBF values are difficult to determine accurately using the PWI scans available at Canberra Hospital. Therefore, MIStar was used to map the relative CBF (CBF values relative to the average value across the entire patient’s brain) and the relative CBF value corresponding to each MRS voxel was recorded.

No statistical analysis was carried out as the sample size obtained was too small for such analysis to yield meaningful results.

4.1.3 Results

4.1.3.1 Recruitment
Despite the data collection period being extended several times, the total number of patients successfully recruited was only 3 (2 males, 1 female). A number of factors impeded recruitment, but the following were likely the most significant.  
1: Research MRI scans generally had to be scheduled during normal operating hours of the MRI scanner (8am-4pm Monday-Friday). Arrangements could be made to scan on the weekend but only with several days notice. This meant that patients arriving on Friday afternoon or at any time over the weekend were unlikely to be scanned within 24 hours, particularly since Monday mornings were often filled with patients for whom clinicians had requested MRI scans over the course of the weekend.
2: Since the research scans were being conducted during standard scanner hours if a
patient needed an urgent MRI for clinical reasons at any time once a research subject had been recruited, the research scan was much more likely to be cancelled or rescheduled than a scan that was required for medical reasons.

3: Industrial issues at the The Canberra Hospital imaging department lead to the cancelling of all research MRIs for several months.

4: There was a temporary drop-off in the number of patients admitted to TCH specifically suffering MCA-territory strokes.

In addition to the problems recruiting patients, none of the three patients successfully recruited received the full complement of 5 MRI examinations. One required surgery after the third scan that left him unable to undergo an MRI safely. Another was recruited 4 days before the Christmas shutdown of the MRI unit, so only received the first 4 examinations. The family of the last patient withdrew their consent after she had received her third examination because they believed she was finding the scans too traumatic (the patient herself was unable to communicate effectively one way or the other). Patient 1 was unable to undergo perfusion-weighted imaging due to poor kidney function which is a contraindication for a gadolinium injection [202].

4.1.3.2 MR Spectra
The spectra acquired from infarcted tissue tended to be of a poorer quality than those acquired from healthy tissue (see Figure 4.1.1 below) but were mostly adequate for thermography processing. One of the spectra acquired from the infarct region in patient 2 was so poor that the creatine and choline peaks could not be identified accurately. This spectrum was processed using the equation for determining temperature from NAA alone (see chapter 3.2) which was estimated to be accurate to within ±1.7°C (compared to 1.3°C for the amplitude-weighted temperature). All of the spectra acquired from the second and third examinations of patient 3 were too poor to process and thus no temperature data exists for these examinations, for either ischaemic or healthy tissue.

4.1.3.3 Spatial and temporal variation in temperature
To highlight spatial variation in temperature in the immediate aftermath of a stroke, Figure 4.1.2 shows the temperature of each voxel relative to the patient’s tympanic temperature during their first MRI examination.
Figure 4.1.3 below shows the various temperature estimations from each patient and the changes in temperature over time for the different regions examined (infarct, peri-infarct and contralateral). Tympanic temperatures are also included for comparison. Absolute temperature estimations are less reliable than relative differences in temperature between different regions of interest within the same examination. However, this does not apply to temperature differences between different MRI examinations (i.e. on different days). Therefore, it was considered worthwhile presenting the absolute temperature estimations in this figure in order to demonstrate the change in temperature over time for each region of interest.
Figure 4.1.1 Example spectra from a healthy voxel (top), a peri-infarct voxel (middle) and an infarcted voxel (bottom). Note the elevated noise level at the far left (<220Hz) of the infarct spectrum.
Figure 4.1.2 Tissue temperature relative to contralateral temperature within each patient during their earliest MR examination.
Figure 4.1.3 Absolute temperature estimations of the 3 brain regions examined in each patient plotted against time since stroke onset for [A] (top) Patient 1, [B] (centre) Patient 2 and [C] (bottom) Patient 3. Note that temperature data could not be successfully extracted from the second and third MR examinations of Patient 3.
4.4.3.4 Temperature and cerebral blood flow

Figure 4.1.4 below shows the relative CBF data available from Patient 2 (A) and Patient 3 (B) plotted alongside the relative temperature data from each patient. In this case relative CBF is recorded as a percentage of the average CBF across the entire patient’s brain (including the ischaemic zone) different software packages may use different algorithms. Predictably, the highest rCBF level in each patient was found in the contralateral region, and the lowest rCBF was found in the infarcted region, although Patient 3 appeared to have largely recanalised by the time the perfusion scan was completed. None of the regions of interest on which temperature estimations were performed were found to have 100% rCBF.
Figure 4.1.4 Regional brain tissue temperature relative to contralateral temperature (left-hand vertical axis) and relative CBF (right-hand vertical axis) for [A] (top) Patient 2 and [B] (bottom) Patient 3. No CBF data are available for Patient 1. Note that relative CBF (rCBF) in this case is the local CBF relative to the average CBF value over the entire brain (including the ischaemic zone). It is interesting to note that even the contralateral tissue did not register 100% rCBF in either patient.
4.1.4 Discussion

4.1.4.1 Summary of results
Temperature data could generally be extracted from infarcted tissue provided other factors such as patient movement did not interfere. When patient movement was a factor, it affected healthy tissue as well as ischaemic tissue. Most, but not all of the temperature variation seen between different tissue segments during any one examination was within the limits of precision of the temperature estimation technique. Furthermore, all of the temperature variation within each examination was within the maximum variation found between different regions in healthy volunteer’s brains (2.4°C, see Table 3.3.1) except the first examination of Patient 1. In this particular examination, the difference in temperature between the infarct and peri-infarct regions was 3.6°C.

The sample size was, of course, too small to draw meaningful conclusions about temperature but there were some apparent trends. Peri-infarct was universally warmer than any other brain tissue or tympanic temperature during the first 24 hours. Infarct was cooler than contralateral in 2/3 cases, and cooler than tympanic temperature in all 3 cases. Patient 3 had the smallest variation in temperature between different tissues but this may have been because she had recanalised by the time the MR examination was performed.

Changes in temperature over time were quite different between the two patients for whom data were obtained but there were some similarities. The infarct got warmer at first and then began cooling sometime after approximately 70 hours post stroke. The peri-infarct region got cooler between 24 and 48 hours post stroke and then remained relatively steady. The contralateral tissue remained relatively steady in Patient 2, although it did drop slightly and then increase by the third examination. The contralateral temperature in Patient 2 increased noticeably between the first and third examinations (approximately 16 hours and 68 hours post symptom onset, respectively). It should be noted that recanalisation would have occurred at different times in different patients and the time-course of recanalisation would probably have an effect on the time-course of temperature changes in the brain. If recanalisation had occurred by the time the initial MRI examination was conducted (as it had in patient 3) this could be detected by the PWI scan. Otherwise, recanalisation could have occurred at any time after the initial MRI examination and it could not be known which temperature readings had been affected by this.
Relative cerebral blood flow (rCBF) was found to be lowest in the infarct and highest in the contralateral region in both patients who underwent perfusion scanning.

4.1.4.2 Comparison to previous studies
As mentioned previously, there is a scarcity of data regarding changes in brain temperature after a stroke and virtually all the data available have been collected by MR thermography (see chapter 1.3). Karaszewski et al [121] applied their spectroscopic imaging technique to a total of 40 ischaemic stroke patients and managed to find a number of statistically significant trends [121]. The authors in this case found that ‘possibly abnormal’ tissue (where the MR voxel was partially, but not fully made up of tissue that appeared abnormal on DWI) tended to be warmer than ‘definitely abnormal’ (infarcted) tissue. They also found that voxels bordering those containing abnormal tissue tended to be warmer than definitely abnormal tissue (but cooler than ‘possibly abnormal’ tissue), and all three of these tissue categories tended to be warmer than distal normally perfused brain tissue. The average differences reported by the authors were all less than 0.5°C but the differences recorded within individual patients were as large as 2.9°C [121]. In a subsequent study of 40 patients[124] Karaszewski et al found once again that “possible penumbra” was warmer than either infarct or contralateral tissue. Thus, the general trend found in this study of peri-infarct tissue being warmer than infarcted or healthy tissue does seem to fit with the results of Karaszewski et al, although the reliability of Karaszewski’s temperature estimations is likely to be just as uncertain as those reported on here (see chapter 1.3 and chapters 3.1 and 3.2 for discussion). The elevated temperature in the penumbra fits with the hypothesis that the penumbra continues to generate heat through metabolism, while the reduction in perfusion to this region reduces the amount of heat that is removed by the blood (see Part V for further discussion).

4.1.4.3 Cerebral Blood Flow
It was curious that none of the relative cerebral blood flow (rCBF) measurements were above 70%. Given that the baseline for these relative CBF calculations was the average CBF across the entire patient’s brain (including the ischaemic region), in theory normally perfused brain tissue should have had a relative CBF close to, or even over 100%. The fact that the contralateral region of interest in Patients 2 and 3 was well below this value may indicate that an ischaemic stroke can affect cerebral blood flow well beyond the territory of the occluded blood vessel. The reduction in activity in an undamaged region of the brain
which shares neural connections to a damaged region of brain tissue is known as diaschisis and has been recorded in the past, particularly in the cerebellum [18]. The possibility that diaschisis also occurs within the cerebral cortex, in turn, might have implications for the successful implementation of therapeutic hypothermia. However, as with the rest of this study, the sample size was too small to draw any meaningful conclusions.

4.1.4.4 Possible explanations for temperature variation
Brain temperature is believed to be primarily influenced by the balance between waste heat production as a result of cerebral metabolism and the removal of heat by the blood (see chapter 1.4). Thus, it is unsurprising that temperature was elevated in the peri-infarct region, as this region is likely to experience reduced blood flow (thus reduced heat removal) while still generating a significant amount of heat. Conversely, the infarcted tissue might have produced less metabolic heat as a result of neuronal death within this region. The interactions between cerebral blood flow, cerebral metabolism and temperature will be explored further in chapters 5.1 and 5.2.

The fact that the temperature in the peri-infarct region appeared to decrease over time after the first 24 hours might have been a result of the restoration of normal perfusion levels to this tissue. The infarct appeared to increase in temperature over time after the first 24 hours. This could have been a result of a temporary influx of metabolically active lymphocytes and microglia into the infarcted region. The proliferation of microglia is believed to peak 48-72 hours after stroke onset [208], which would fit with the timing of the temperature increases detected in this study.

4.1.4.5 Feasibility of MR thermography in stroke patients
What little data are available suggest that there may be temperature changes large enough to detect using this MRT technique in some patients. This is significant as temperature variation of such a magnitude could affect the success of hypothermia therapy. The reliability of these temperature estimations is uncertain (see chapter 3.2) but the results of this pilot study indicate that ischaemic injury is not itself a barrier to the application of MR thermography.
4.1.5 Conclusions

The utility of temperature estimations based on the MR thermography technique described in this thesis is limited by the amount of noise present in the temperature data (see section 3.2.4). Encouragingly, MR spectra sufficient for thermography processing were obtained from each of the patients examined (although there were some unsuccessful examinations) and the results of this pilot study do broadly fit with what is known about changes in brain temperature after stroke. This suggests that ischaemic brain injury is not, in itself, a barrier to successful MR thermography. Therefore, if the thermography technique can be improved in healthy volunteers, as it could be by changes as simple as using higher field-strength MRI scanners (see section 3.2.4), then these improvements are likely to apply to temperature estimation in stroke patients. The interaction between cerebral blood flow, metabolism and temperature after a stroke are worth investigating further, as this interaction is likely to have a significant bearing on the success or otherwise of therapeutic cooling of the penumbra (see chapters 1.2 and 1.4). In the absence of accurate temperature measurements from the stroke-affected brain, this investigation could be performed using computer modelling. This is the focus of the following experimental chapters.
Part V:

Finite Element Modelling
Chapter 5.1 Finite element modelling of heat exchange in the stroke-affected brain.

5.1.1 Background

Little is known about temperature changes in the brain as a result of ischaemic stroke. What data are available, as outlined in Chapter 1.4, are derived from MR thermography experiments which typically report the average temperature of infarcted tissue, penumbra and healthy tissue, or even just average differences in temperature between these regions. Finite element modelling has the potential to add more spatial and temporal detail to these temperature maps. In particular, it may shed light on temperature inhomogeneity within each tissue region (such as across the penumbra) and expose greater variations in temperature that are concealed by the measurement of average temperatures. The aims of this experiment were:

1: To verify and validate a simple finite element model based on the Pennes bioheat equation.
2: To examine the temperature variation associated with typical changes in blood flow during an ischaemic stroke.

As noted in previous chapters, verification involves comparing the results of the model to one’s conceptual model, detecting any programming errors and testing the sensitivity of the modelling results to software settings such as time-step or mesh size (see chapter 1.4). This step does not involve comparing the modelling results to real world data (this is performed during validation). The verification step of this study made use of the Simulink model outlined in chapter 2.2.1. The results of the Simulink model were used as a ‘ball-park figure’ against which to compare the results from the Ansys simulations in order to detect any major programming errors. In addition, if the results from the Ansys and Simulink models were similar enough, an argument could be made for abandoning Ansys
and simply using the Simulink model (which is much easier to use, cheaper, and faster to program).

5.1.2 Methods

5.1.2.1 Simulink model
The Simulink model described in Chapter 2.2.1 was run for each individual tissue segment using the cerebral blood flow and metabolism rates outlined below (Table 5.1.1). The equilibrium temperature reached in each Simulink simulation was recorded and compared to the temperature profile of the equivalent tissue from Ansys simulations.

5.1.2.2 Model geometry
The geometry outlined in chapter 2.2.2 was used for all FEM studies in this thesis. The initial maximum mesh size was set at 5mm. Results were examined by extracting the temperature along path ‘P’ which passed from the centre of the brain, through the basal ganglia, and through the centre of the ischaemic region. This allowed examination of temperature in each of the separate tissue compartments (healthy grey- and white-matter, oligaemic grey- and white-matter, penumbral grey-and white-matter and infarcted grey- and white-matter).

5.1.2.3 Model parameters
Physical parameters such as tissue density, conduction and specific heat capacity were assumed to be constant throughout the brain and can be found in section 2.2.3. Initial brain temperature was set to 37°C and initial blood temperature was set to 36.7°C. The surfaces of the brain hemisphere were treated as adiabatic. These figures and assumptions are justified in Chapter 2.2.

The CBF and CMR were assumed to vary between grey matter and white matter as a result of the greater energy requirements of grey matter, and were also varied experimentally to simulate an ischaemic stroke. The baseline values were adapted from [209, 210] and are outlined in Table 2.2.1, and again in Tables 5.1.1 and 5.1.2, along with the values used to simulate ischaemia.
Table 5.1.1 Cerebral blood flow values simulated in this experiment. The stroke-affected region was located such that the infarct, penumbra and oligaemia all encompassed both white-matter and grey matter. Note that grey matter and white matter are different structural tissue types, whereas the terms “Oligaemia”, “Penumbra” and “Infarct” are essentially functional descriptions related to the magnitude of changes in the CBF as a result of a vessel occlusion.

<table>
<thead>
<tr>
<th>Blood flow classification</th>
<th>Absolute value in Grey Matter</th>
<th>Absolute value in White Matter</th>
<th>Relative value (to baseline levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80mL/100g/min</td>
<td>20mL/100g/min</td>
<td>100%</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>64 mL/100g/min</td>
<td>16 mL/100g/min</td>
<td>80%</td>
</tr>
<tr>
<td>Penumbra</td>
<td>32 mL/100g/min</td>
<td>8 mL/100g/min</td>
<td>40%</td>
</tr>
<tr>
<td>Infarct</td>
<td>16 mL/100g/min</td>
<td>4 mL/100g/min</td>
<td>20%</td>
</tr>
</tbody>
</table>

While CMR and CBF are closely coupled under normal circumstances, an ischaemic stroke disrupts the homeostatic mechanisms that normally preserve this coupling. Unfortunately, the available data concerning changes in cerebral metabolic (CMR) rate during a stroke are extremely limited. Therefore, rather than simulating a single ‘typical’ scenario with regard to the CMR, 3 possible representative scenarios were simulated as outlined in Table 5.1.2 below.

Table 5.1.2 CMR scenarios simulated in this study. All CMR values are shown relative to the baseline values of 16700W/m³ for grey matter and 4175W/m³ for white matter.

<table>
<thead>
<tr>
<th>Blood flow classification</th>
<th>Scenario A (Relative CMR)</th>
<th>Scenario B (Relative CMR)</th>
<th>Scenario C (Relative CMR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100%</td>
<td>60%</td>
<td>100%</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>100%</td>
<td>60%</td>
<td>100%</td>
</tr>
<tr>
<td>Penumbra</td>
<td>70%</td>
<td>40%</td>
<td>150%</td>
</tr>
<tr>
<td>Infarct</td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Scenario A represented the most likely changes in CMR, with metabolism dropping to 70% of baseline in the penumbra (based on data from [211]) and dropping to zero in the infarct due to the death of the brain tissue in this region (see Chapter 1.1.2). Scenario B represented a situation where the patient was experiencing reduced cerebral metabolism throughout their brain as a result of medication or the side effects of stroke. The CMR in the penumbra in this scenario was modelled as two thirds of the value of CMR in the healthy tissue, which was a similar ratio to that modelled in scenario A. The final scenario represented a situation where, as a result of excitotoxicity, the penumbra actually experienced an increase in metabolism during ischaemia (See Chapter 1.1.2). In this scenario, metabolism in the infarct was reduced but did not cease altogether, which is an important possibility to consider. All three scenarios were modelled independently and the
temperature profile from each scenario was compared to the other scenarios and to available data from the literature.

5.1.2.4 Verification
The process of verification involves testing the sensitivity of the model to software parameters that are not being varied experimentally. Only scenario 1A was used for this process, and the results were analysed by comparing the temperature profiles of the various simulations at the 1-hour time-point, as all simulations had reached equilibrium by this time-point making such comparison easier. Ansys allows a maximum and minimum time-step to be set by the programmer, after which the software will alter the time-step as necessary, negating the need for the programmer to test different time-steps in this case. The maximum mesh size (the size of the segments the software divides the model up into) was set to 5mm and the software was allowed to create smaller segments where necessary. A coarse mesh simulation (maximum mesh size 6mm) and a fine-mesh simulation (maximum size 4mm) were also performed and the temperature profiles were analysed for differences. A separate, purely theoretical, simulation was also conducted with the conductivity of the tissue reduced to near 0 (0.001W/M/K) to investigate the overall contribution of conduction to the final temperature profile of the ischaemic brain tissue.

5.1.2.5 Validation
Validation was performed using data from a series of experiments performed on monkeys [201] using thermography based on MR spectroscopic imaging (MRSI), similar to the technique used by Karaszewski et al in humans [121]. The Sun study was chosen for this purpose because it presents data from very early after the stroke, and contains reasonably precise time-points for the data, both of which are typically lacking for data collected from human patients. However, the MRSI technique chosen by Sun and Karaszewski has an important flaw. As noted in section 1.3.5.2, MRSI cannot provide accurate measurements of individual voxels. Instead, the authors derive the average temperature of a variety of tissue segments (classified according to apparent injury) and then present the average differences in temperature between these tissue segments (since the authors were more confident of measuring relative temperature differences than they were of measuring absolute temperature). Therefore, to derive measurements from the simulations that were directly comparable to the validation data, the average temperature of each tissue
segment within the model was calculated and these were used as the primary basis for validation, although the maximum temperature differences between nodes were also examined. Sun et al divided their tissue segments into infarct core, penumbra, and oligaemia using perfusion/diffusion mapping and used the contralateral hemisphere as the ‘normal tissue’ reference. They did not distinguish between grey matter and white matter but presented the average temperature of each tissue segment (i.e. normal tissue, penumbra and infarct).

5.1.3 Results

5.1.3.1 Simulink simulation results

The results of the Simulink simulations can be found in Table 5.1.3 (scenario A), 5.1.4 (scenario B) and Table 5.1.5 (scenario C) below.

<p>| Table 5.1.3 Equilibrium temperatures of the various tissue regions from Simulink simulation of Scenario A. All temperatures in °C. |</p>
<table>
<thead>
<tr>
<th>Blood flow classification</th>
<th>Grey Matter (°C)</th>
<th>White Matter (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>37.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>37.1</td>
<td>37.1</td>
</tr>
<tr>
<td>Penumbra</td>
<td>37.2</td>
<td>37.2</td>
</tr>
<tr>
<td>Infarct</td>
<td>36.7</td>
<td>36.7</td>
</tr>
</tbody>
</table>

<p>| Table 5.1.4 Equilibrium temperatures of the various tissue regions from Simulink simulation of Scenario B. All temperatures in °C. |</p>
<table>
<thead>
<tr>
<th>Blood flow classification</th>
<th>Grey Matter (°C)</th>
<th>White Matter (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>36.88</td>
<td>36.88</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>36.92</td>
<td>36.92</td>
</tr>
<tr>
<td>Penumbra</td>
<td>37.00</td>
<td>37.00</td>
</tr>
<tr>
<td>Infarct</td>
<td>36.70</td>
<td>36.70</td>
</tr>
</tbody>
</table>

<p>| Table 5.1.5 Equilibrium temperatures of the various tissue regions from Simulink simulation of Scenario C. All temperatures in °C. Blood temperature was 36.70°C. |</p>
<table>
<thead>
<tr>
<th>Blood flow classification</th>
<th>Grey Matter (°C)</th>
<th>White Matter (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>37.00</td>
<td>37.00</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>37.07</td>
<td>37.07</td>
</tr>
<tr>
<td>Penumbra</td>
<td>37.82</td>
<td>37.82</td>
</tr>
<tr>
<td>Infarct</td>
<td>37.45</td>
<td>37.45</td>
</tr>
</tbody>
</table>
5.1.3.2 Ansys simulation results

Figure 5.1.1 shows the results of modelling scenario A with various mesh densities.

Figure 5.1.2 shows the results of modelling scenario A when the conductivity of brain tissue was assumed to be near 0.

Figure 5.1.3 shows the results of modelling the three different CMR scenarios.

Figure 5.1.1 Temperature profile along path ‘P’ 1 hour after stroke onset for scenario A when simulated using a mesh size of 4mm (Fine Mesh), 5mm (Normal Mesh) and 6mm (Coarse Mesh). There was no discernible difference between the results so the ‘normal mesh’ was used for all remaining simulations.
Figure 5.1.2 Temperature profile along path ‘P’ 1 hour after stroke onset for scenario A when simulated using the accepted figure for the conductivity of tissue (‘normal k’) and a conductivity of near zero (‘low k’). This simulation was used for verification only, to examine the role of conduction between adjacent brain tissue in this model and to test the sensitivity of the model to variation in tissue conductivity.

Figure 5.1.3 Temperature profiles along the path ‘P’ 1 hour after stroke onset for the 3 scenarios being simulated in this study.

Table 5.1.6 below lists the average temperature of each tissue segment across the 3 scenarios simulated in this study. These figures were derived by taking the temperature
from every node within each tissue segment and calculating the mean of these temperatures.

Table 5.1.6 Average temperature of each tissue segment across the 3 scenarios simulated in this study.

<table>
<thead>
<tr>
<th>Tissue segment</th>
<th>Scenario A (°C)</th>
<th>Scenario B (°C)</th>
<th>Scenario C (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy tissue</td>
<td>37.01</td>
<td>36.88</td>
<td>37.01</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>37.03</td>
<td>36.90</td>
<td>37.05</td>
</tr>
<tr>
<td>Penumbra</td>
<td>37.05</td>
<td>36.91</td>
<td>37.14</td>
</tr>
<tr>
<td>Infarct</td>
<td>37.00</td>
<td>36.95</td>
<td>37.25</td>
</tr>
</tbody>
</table>

Table 5.1.7 Average temperature of each tissue segment across the 3 scenarios simulated in this study relative to healthy tissue.

<table>
<thead>
<tr>
<th>Tissue segment</th>
<th>Scenario A (°C)</th>
<th>Scenario B (°C)</th>
<th>Scenario C (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy tissue</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Penumbra</td>
<td>0.04</td>
<td>0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>Infarct</td>
<td>-0.01</td>
<td>0.07</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 5.1.8 Range of temperatures (maximum, minimum and range) found across all tissues in each simulation.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Ansys simulations</th>
<th>Simulink simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max Temp (°C)</td>
<td>Min Temp (°C)</td>
</tr>
<tr>
<td>A</td>
<td>37.09</td>
<td>36.95</td>
</tr>
<tr>
<td>B</td>
<td>36.93</td>
<td>36.85</td>
</tr>
<tr>
<td>C</td>
<td>37.29</td>
<td>37.00</td>
</tr>
<tr>
<td>A(low k)</td>
<td>37.30</td>
<td>36.70</td>
</tr>
</tbody>
</table>
Table 5.1.9 Experimental results from monkey stroke model ([201] for comparison. 95% confidence intervals are listed wherever available from the original paper.

<table>
<thead>
<tr>
<th>Tissue region</th>
<th>Infarct (°C)</th>
<th>Penumbra (°C)</th>
<th>Oligaemia (°C)</th>
<th>Contralateral (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp relative to baseline</td>
<td>1.08 (±0.19)</td>
<td>1.72 (±0.08)</td>
<td>0.62 (±0.14)</td>
<td>0.23</td>
</tr>
<tr>
<td>Temp relative to contralateral hemisphere</td>
<td>0.80</td>
<td>1.44</td>
<td>0.34</td>
<td>0</td>
</tr>
</tbody>
</table>

5.1.4 Discussion

5.1.4.1 Summary of results

The results of the Simulink simulations match the average temperatures of the various tissue segments found in Table 5.1.7 relatively closely. In each case the healthy tissue was the same temperature in both simulations or very slightly (0.1°C) warmer in the Ansys simulation compared to the Simulink simulation. Furthermore, the tissue experiencing reduced blood flow (oligaemia, penumbra and infarct) were all cooler in the Ansys simulations than in the Simulink simulations. This can be explained by the fact that the Ansys simulations account for the conduction of heat from warmer oligaemic and ischaemic tissue to cooler healthy tissue.

The average temperature of each tissue segment concealed considerable temperature gradients within each segment, suggesting that the more complex Ansys simulations are likely to provide clinically important detail that Simulink simulations would not. The results of simulating scenario A in Ansys were not affected by altering the mesh size, but were affected significantly by altering the assumed conductivity of the tissue.

Ansys modelling of scenarios A and B produced temperature profiles that were similar in shape but offset by approximately 0.1°C due to the lower overall brain temperature that would result from the lower baseline CMR (scenario B). In each of these simulations, the temperature was increased in the oligaemia and penumbra but reduced in the infarct core. The maximum temperature difference found within these results was 0.14°C for scenario A and 0.08°C for scenario B. The results of simulating scenario C were markedly different, most notably because the temperature peak in this simulation was in the infarct. The difference in temperature between the warmest and coolest tissues in scenario C was 0.29°C, more than double the largest temperature gradient found in scenario A.
None of the Ansys simulations produced temperature gradients of the magnitude found in the experimental data from [201] (See Table 5.1.9). The differences between the average temperatures of the various tissue classifications were very small in the simulation results compared to the in vivo results from [201]. The difference between the maximum and minimum temperatures found within each simulation were considerably greater in magnitude than the differences between average tissue temperatures, but were still smaller than those found in the experimental data.

5.1.4.2 Verification

The differences between the results of the Simulink simulations and the average tissue temperatures from the equivalent Ansys simulations were minor and could all be explained by the fact that conduction of heat from warmer tissue to adjacent regions of cooler tissue (e.g. from the penumbra into the infarct or from the tissue experiencing oligaemia to the surrounding healthy tissue) which was accounted for by the Ansys model but not the Simulink model. This suggests that there were no major coding errors in the Ansys model despite the increased level of complexity in the code. Altering the mesh size did not have any discernible effects on the results. This indicates that the mesh size chosen was appropriate for the task. Indeed, it would have been acceptable to use the coarser mesh (6mm) but the decision was made to use the mesh size of 5mm, which was verified in this study, as this size already provided relatively rapid simulations (approximately 10-15 minutes per simulation). The lack of effect for the mesh size was unsurprising as Ansys has the ability to use a finer mesh wherever necessary (for example, in smaller structures or regions with steep temperature gradients). Thus, mesh size verifications are more important in other forms of modelling such as complex fluid dynamics than in finite element modelling, but it was decided that it was worth running the test just in case, as this specific type of bioheat transfer modelling has not been thoroughly tested in Ansys.

Altering the assumed conductivity of tissue did have a significant effect on the results (see Figure 5.1.2) and produced results closer (but not identical) to the equivalent Simulink simulation. This suggests that errors in the assumed physical properties of the tissue (such as density and specific heat capacity as well as conductance) can have a significant effect on results of simulations. This is important as the literature values of these physical
properties are average values from a collection of studies, and different studies did find differing values for many of these physical properties [196].

In the Simulink simulation, the warmest tissue was the penumbra, but in the low conductivity Ansys simulation the temperature peaked in the infarcted tissue. The scale of the temperature gradient was also slightly larger in the low-conductivity Ansys simulation compared to the (no conductivity) Simulink simulation (0.60°C and 0.52°C, respectively). This indicates that even an extremely low level of conduction produces results that are different to those obtained when conduction is completely absent. This reinforces the notion that the simple Simulink model is probably too simplified to provide realistic simulations.

5.1.4.3 Validation

The results of the simulations in healthy brain tissue (i.e., tissue receiving normal blood flow) were quite realistic in terms of the equilibrium temperature difference between the blood and the brain tissue. However, the results were less successful in ischaemic tissue. None of the simulations in Ansys or Simulink produced results that closely matched the experimental data (See Tables 5.1.3-5.1.9 above). Some of the differences may be explained by the choice of parameters used in this study, but some of the differences, in particular the scale of the temperature differences between the ischaemic tissue and healthy tissue were much greater in the experimental data than any of the simulation results. The key differences, and the factors that may have caused them, will be dealt with one by one, but first it is worth examining the results of the simulations in detail.

In scenario A, the peak temperature (37.09°C) was found to be in the penumbra, and the lowest temperature (36.95°C) was found in the infarct. The average temperature of each tissue segment followed a similar pattern, albeit with much smaller temperature variation (See Table 5.1.7). The Penumbra had the highest average temperature, followed by the oligaemia, then normal tissue, and the infarct had the coolest average temperature. Scenario B produced less straightforward results. Once again, the peak temperature (36.93°C) was found in the penumbra (although the adjacent oligaemia was just as warm) and the coolest temperature (36.85°C) was found in the infarct. However, the average temperature of the infarct was actually warmer than the average temperature of any other tissue segment, followed by the penumbra, oligaemia and normal tissue respectively. The
maximum temperature and all of the temperature differentials were lower in scenario B compared to scenario A, which is unsurprising given the lower baseline brain temperature. The results of scenario C were significantly affected by the presence of residual metabolism in the infarct. Thus, the peak temperature occurred in the infarct, and the coolest temperature occurred in normal tissue. The average temperature of each segment followed the same pattern as scenario B (albeit with greater temperature differentials), with the infarct being the warmest on average, followed by the penumbra, oligaemia and normal tissue respectively. Interestingly, in scenario C the ischaemic and oligaemic grey matter tended to be warmer than the equivalent white matter, whereas for scenarios A and B the reverse was true. This is likely because the 50% increase in metabolism simulated in the penumbra for scenario C was much greater in absolute terms in the grey matter compared to the white matter, and this tended to dominate the results of the simulation.

In contrast, in the experimental data these simulations were being compared to, the penumbra was found to have the warmest average tissue temperature, followed by the infarct, then the oligaemia and the healthy tissue. Furthermore, the difference in temperature between the penumbra and healthy tissue was 1.44°C, whereas none of the simulations that assumed normal physical properties generated temperature differences greater than 0.29°C. The differences in average tissue temperature for each of the simulations were no greater than 0.24°C, and most were less than 0.1°C (See Table 5.1.7).

As mentioned above, some of these differences can be attributed to the choice of parameters used in the simulations. For example, the fact that the infarct was cooler than normal tissue in scenario A (which was the ‘most likely’ scenario) was probably due to the assumption that metabolism in the infarct instantly dropped to zero. It is quite conceivable that there would be some residual metabolic heat generation in the infarct core, especially during the first hour of ischaemia (which was when this temperature data was collected in the monkeys). Scenario C did assume that cerebral metabolism in the infarct merely dropped to 50% of the baseline value, rather than stopping entirely. In this scenario, the infarct was warmer than the penumbra (which also fails to match the in vivo experimental data). However, this could simply be because 50% of baseline metabolism was unrealistically high. Therefore, there almost certainly exists a level of metabolic heat
output somewhere between zero and 50% that would cause the infarct to be warmer than the normal tissue, but not as warm as the penumbra (which is what was found in the monkey experiment). The geometry of the ischaemic region could also contribute to this. A smaller infarct, effectively experiencing a greater surface area to volume ratio (referring to the surface area exposed to the penumbra) would have an average temperature closer to that of the penumbra because heat energy would be conducted from the penumbra throughout the infarct more efficiently. Thus, there should be an alternative geometry where the infarct would equilibrate at a temperature warmer than that of the healthy tissue but cooler than that of the penumbra.

The method used to measure temperature in the monkey experiment may also have affected the results. The authors used a magnetic resonance spectroscopic imaging (MRSI) method (See Chapter 1.3 for a more explanation of this technique and the associated advantages and disadvantages) that effectively produced a 6x6 grid of voxels across the volume of interest and a temperature estimate for each voxel. However, the temperature estimates for individual voxels produced by this technique are not accurate enough to be used alone. This is why the authors reported the average temperature of each tissue classification. Unfortunately, some of the voxels produce spectra that were so poor that temperature estimation cannot be performed at all, especially in ischaemic tissue. Thus, the number of voxels that are averaged together for each tissue segment can vary widely, with a corresponding variation in the confidence that should be attached to each average temperature. Furthermore, if only 1 or 2 voxels were available in the infarct of a given monkey, it is possible that these voxels did not accurately represent the average temperature of the infarct (or any other tissue segment). The authors did not report the number of voxels they were forced to reject, nor the number of voxels available within each tissue segment. It is noteworthy that the temperatures reported for the infarct and oligaemia had larger confidence intervals than the temperatures reported for the penumbra (0.19°C and 0.14°C compared to 0.08°C, respectively). This suggests that there were fewer voxels available in the infarct and oligaemia than there were in the penumbra. It is also possible that the many of the spectra from the ischaemic tissue were of slightly poorer quality without being poor enough for the authors to reject. This would cause less accurate temperature estimations (and hence greater confidence intervals) but would be expected to affect the penumbra more than the oligaemia. It is more likely therefore, that
this source of error was simply unaccounted for by the authors and the number of voxels available in each tissue segment was the primary cause of the increased confidence intervals in the infarct and oligaemia. The confidence intervals reported by Sun et al refer to the variation in the temperature estimated across the individual monkeys in the study, and it is not known how these confidence intervals relate to the absolute temperature, or even the relative temperature of each tissue segment. This was always going to be an issue with using MR thermography data for validation, but unfortunately it was the best data-set available. The MR thermography experiments conducted for the purpose of this thesis indicated that the accuracy of temperature estimation from a single voxel was no better than \( \pm1.25^\circ C \), and when using NAA as the sole chemical reference (as Sun et al did) the accuracy was \( \pm1.68^\circ C \). Estimates based on single voxels from an MRSI scan are likely to be even worse (See Chapter 1.3 for an explanation of the problems associated with MRSI).

The most glaring difference between the simulation results and the experimental data was the magnitude of the temperature differences. Whereas temperature differences of 1\(^\circ C\) or more between tissue segments were found to be the norm in the experimental data, the Ansys simulations resulted in maximum temperature differences of the order of 0.1\(^\circ C\), and even smaller differences when the average temperature of each tissue segment (which more closely matched the method of data collection from the monkey experiments) was considered. While one could, in theory, find a combination of CBF and CMR that recreated the temperature elevations found in the monkey experiments, the simulation parameters would have to be well outside realistic limits. The 50% increase in metabolism simulated in the penumbra for scenario C was purely hypothetical, as what data are available suggest that metabolism in the penumbra decreases as a result of nutrient deficiencies [8, 212]. With all the problems and pitfalls associated with MR thermography, especially when using MRSI, it is possible that the experimental data that this study has relied on was simply wrong. However, it is just as likely (if not more so) that the fault lies in the model.

If the physical characteristics of the tissue (such as density, specific heat capacity or conductivity) assumed in this study were incorrect, this would invalidate the model. This scenario is not unlikely. When it comes to the physical properties of the tissue, engineers publishing models of the brain tend to reference previous modelling papers, rather than the experiments that actually measured the physical properties directly. The most recent
paper found in the literature search for this thesis that actually referenced such an experiment was published in 1988 [196] and the papers the authors referenced were published no later than 1982. Many of these original sources are no longer readily available to authors. Werner and Buse list physical characteristics of ‘cortex’ but did not differentiate between grey matter and white matter. Subsequent engineering models, including the one described in this study, have therefore assumed that grey matter and white matter have identical physical properties. However, white matter has a significantly higher lipid content compared to grey matter [213] and the fat content of tissue has been shown to have an effect on such physical properties as specific heat capacity [214]. Furthermore, it is likely that there are small differences in some of the physical properties between individuals, and even more likely that such differences exist between monkeys and humans. Therefore, experiments to update, or at least confirm the validity of the physical parameters being used by biomedical engineers would be extremely helpful, especially if the authors of such experiments were to measure tissue from more than one individual and publish confidence intervals for each parameter. That being said, previous studies have successfully modelled heat exchange in the human brain using the same physical parameters as this study [155, 156, 158, 167], which would suggest that the values used were at least plausible.

One of the most vulnerable assumptions made in this model is that blood reaches the small arterioles at core body temperature. One of the key reasons that this assumption may hold is that the high velocity of the blood and small surface area to volume ratio of the larger arterioles minimise the level of heat exchange between the blood and the surrounding tissue before the blood reaches the small arterioles. The realistic results obtained in healthy brain tissue suggest that this is a reasonable assumption under normal circumstances. However, the changes in the cerebral blood flow during a stroke almost certainly extend beyond the mere reduction in the overall rate of blood flow, which may render this assumption implausible. Even a partial occlusion of a larger or medium-sized artery would reduce the velocity of the remaining blood flow through that artery. Furthermore, if the blood flowing to ischaemic tissue were reaching that tissue via downstream anastomoses (See Chapter 1.4.4) it may first have to travel through the small arteries and arterioles of surrounding brain tissue. This would mean that the blood would
have already warmed to the temperature of the surrounding brain tissue, which would significantly reduce the efficacy of the heat sink provided by the blood.

**5.1.4.4 Conclusions and follow-up**

While it is possible that the model is in fact valid, and that the MR thermography data that the model results were compared to was faulty, the most obvious conclusion of this study was that the model described does not appear adequate for simulating cerebral ischaemia in its current form. One would expect errors in the physical parameters such as tissue density and specific heat capacity to affect simulations of healthy tissue as well as ischaemia and there is no evidence of this in the current model or previous studies applying the Pennes equation to brain tissue. This being said, an updated set of physical characteristics for the various tissue types, including confidence intervals, would be a thoroughly worthwhile publication as it would allow engineers conducting such simulations in the future to have more confidence in the parameters they were using.

While the simplifying assumptions made for the purposes of this model appear to be fair in normally perfused tissue, this does not appear to be the case in ischaemic tissue. This in itself is actually a useful finding of the current study, as it suggests that there are factors affecting heat exchange in ischaemic tissue that have not hitherto been accounted for. One possible explanation is that the path the blood takes to the ischaemic tissue allows more heat exchange between the blood and surrounding brain tissue before the blood reaches the small arterioles and capillaries of the ischaemic tissue, as outlined above. The only way to improve the effectiveness of the model is to incorporate more data into it. Unfortunately, the clinical data that could potentially be incorporated are extremely limited.

There are a number of perfusion parameters that can be measured by current medical imaging techniques, particularly by contrast-enhanced CT scanning. Perhaps the most promising of these for the current study is the delay time, which provides an indication of how long the blood takes to travel from a large artery to the tissue in question. This delay time has been shown to correlate well with the penumbra [16] and may in time provide a readily measurable parameter that could be incorporated into the finite element model as a proxy for the heat exchange experienced by the blood before it reaches a given segment of tissue. However, the delay time and other similar perfusion parameters are currently
subject to a considerable degree of variation between software packages and even between operators on the same software package, as they do require a certain amount of manual processing. Until this issue is resolved, perfusion parameters measured by medical imaging are unlikely to solve the problems with finite element modelling discovered in this study. This said, medical imaging and computer modelling techniques are constantly improving, and these will provide avenues to improve the effectiveness of the current finite element model. In the meantime, while no firm conclusions can be drawn from the results of simulations using the model described here, certain trends (such as the direction of temperature gradients) were successfully simulated using the current model, and it may be useful to examine the implications of these trends for therapeutic hypothermia in stroke. This will be the focus of the following chapter.
Chapter 5.2 Finite element modelling of stroke-affected brain tissue during therapeutic hypothermia.

5.2.1 Introduction
One of the key reasons we are interested in brain temperature is the potential for hypothermia to be used as a therapy in stroke. The ultimate aim of acute ischaemic stroke treatments, including therapeutic hypothermia, is to prevent the penumbra from progressing to infarction[11]. As mentioned in chapter 1.2.2.6, current trials of therapeutic hypothermia typically involve whole-body hypothermia and assume that cooled blood will reduce the temperature of the brain tissue[77, 190, 215]. This is a reasonable assumption in cardiac arrest patients, where hypothermia is typically induced after normal blood flow has been re-established for the entire brain[63]. Conversely, in stroke patients blood flow to the ischaemic tissue is typically still impaired when the patient presents at hospital, and may remain so throughout the cooling period[77]. Hence, it is not known whether current cooling techniques actually deliver a temperature reduction to the region where it is needed most, namely, the penumbra. Therefore, this study was designed to use finite element modelling to investigate the magnitude and time-course of temperature changes in ischaemic brain tissue during induction of therapeutic hypothermia.

5.2.2 Methods

5.2.2.1 Model geometry
The geometry from chapter 5.1 was used once again for this study, with the same mesh and time-steps.
5.2.2.2 Model parameters

Physical parameters such as density, specific heat capacity etc. were identical to those used in chapter 5.1. The temperature of all of the brain tissue was assumed to be 37°C at the start of the simulation. As mentioned above, the clinical use of therapeutic hypothermia involves the induction of systemic hypothermia, thus reducing the temperature of the arterial blood reaching the brain. Arterial blood temperature is therefore the key input to the model that was varied to simulate the induction of hypothermia in these simulations.

The temperature of the blood was assumed to begin at 36.7°C (see Chapter 2.1 for justification of initial conditions) and drop by 1.1°C per hour to a target temperature of 33°C. This represents the most rapid cooling that can be achieved with current endovascular cooling devices, and the target core body temperature used in the treatment of cardiac arrest victims[63, 216] and certain clinical trials for ischaemic stroke[190]. The simulations were restricted to the first 5.5 hours of cooling, as this would allow enough time for the blood to reach target temperature and the brain tissue to reach a steady state matching the target blood temperature. All parameters except temperature were assumed to remain constant throughout the simulated timeframe.

5.2.2.3 CMR and CBF variations

The same combinations of cerebral metabolic rate that were investigated in chapter 5.1 were investigated in this study. The level of residual cerebral blood flow (CBF) in ischaemic tissue can vary considerably between different patients, and even over time within an individual patient. Variations in CBF may have a significant effect on the amount of heat that can be removed from tissue by the blood. Therefore, this study investigated not only the typical CBF levels that were simulated in chapter 5.1, but also two other possible combinations, all of which are listed in Table 5.2.1.
Table 5.2.1 Sets of cerebral blood flow (CBF) investigated in this study. All values are percentages of the standard values.

<table>
<thead>
<tr>
<th>Tissue classification</th>
<th>CBF set 1</th>
<th>CBF set 2</th>
<th>CBF set 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (unaffected tissue)</td>
<td>100%</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>Oligaemia</td>
<td>80%</td>
<td>55%</td>
<td>80%</td>
</tr>
<tr>
<td>Penumbra</td>
<td>40%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>Infarct</td>
<td>20%</td>
<td>10%</td>
<td>10%</td>
</tr>
</tbody>
</table>

CBF set 1, as described in chapter 5.1, represents the ‘text-book’ values for each tissue classification. Set 2 represents a lower baseline level of CBF in healthy tissues, and correspondingly lower values in the ischaemic tissue. This could occur as a result of simple variation in the baseline CBF between individuals, or could be a direct result of the medications that are administered to patients during hypothermia, or indeed the hypothermia itself. Set 3 represents a normal baseline CBF in the healthy tissue and only mildly reduced in the tissue experiencing benign oligaemia, but a more pronounced reduction in CBF in the penumbra and infarct. This could occur as result variations in the size and location of the clot causing the stroke, or the level of collateral circulation in the ischaemic tissue.

Each CBF set was simulated with three CMR sets, resulting in a total of 9 scenarios being simulated. These scenarios were designated 1A (CBF set 1 with CMR set A), 2B (CBF set 2 with CMR set B) and so on. Table 5.2.2 lists all nine scenarios.

Table 5.2.2 Scenarios investigated in this study.

<table>
<thead>
<tr>
<th>CBF set</th>
<th>CMR set A</th>
<th>CMR set B</th>
<th>CMR set C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scenario 1A</td>
<td>Scenario 1B</td>
<td>Scenario 1C</td>
</tr>
<tr>
<td>2</td>
<td>Scenario 2A</td>
<td>Scenario 2B</td>
<td>Scenario 2C</td>
</tr>
<tr>
<td>3</td>
<td>Scenario 3A</td>
<td>Scenario 3B</td>
<td>Scenario 3C</td>
</tr>
</tbody>
</table>

5.2.2.4 Data analysis

The temperature along the path ‘P’ (see Figure 2.2.3 was extracted at hourly intervals for each simulation in the same manner as it was in chapter 5.1. The temperature was plotted against distance for each time-point to allow analysis of the temperature within each region of interest at each time-point. The temperature for individual ROIs was also plotted against time, allowing analysis of the time-course of temperature changes for each ROI.
These plots allowed a comparison of the effects of variations in cerebral blood flow and cerebral metabolic rate on the rate and depth of cooling achieved in each tissue segment, though particular attention was paid to the penumbra as the temperature of this segment would be the most important in a clinical setting.

5.2.3 Results

5.2.3.1 Spatial variation in temperature
Spatial variations in temperature are illustrated here at the 5-hour time-point to demonstrate the relative contributions of CMR and CBF to the final temperature equilibrium. Figure 5.2.1 shows the temperature along path ‘P’ for each of the simulations grouped by CMR set, to demonstrate differences between CBF sets. Figure 5.2.2 shows the same data grouped by CBF set, allowing comparison of the effects of varying CMR with constant CBF values.
FIGURE 5.2.1: Comparison of varying levels of CBF on regional temperature at 5 hour time-point.

A (Top): With CMR set A
B (Centre): CMR set B
C (Bottom): CMR set C

Vertical axis: Temperature (°C) Horizontal Axis: Distance along path ’P’ (mm)

Figure 5.2.2: Effects of varying levels of CMR on regional temperature at 5 hour time-point.

A (Top): With CBF set 1
B (Centre): CBF set 2
C (Bottom): CBF set 3

Vertical axis: Temperature (°C) Horizontal Axis: Distance along path ‘P’ (mm)

5.2.3.2 Time-course of temperature changes

Figure 5.2.3 shows the temperature profile along path ‘P’ at various time-points from the simulation of scenario 2C. Other scenarios followed a similar pattern, with the relative temperature differences between various tissue segments being established by the 1-hour time-point and remaining constant as the absolute temperature of the brain fell in line with blood temperature. The temperature profile did not change between 4-hour and 5-hour time-points, therefore the 5-hour data are not shown in this figure.

![Figure 5.2.3 Temperature versus distance along path ‘P’ from the basal ganglia through the ischaemic region for simulation 2C at various time-points.](image)
Figure 5.2.4 shows the temperature of a selected segment of penumbral grey matter adjacent to the infarct. This tissue would be at highest risk of infarction as the infarct core expanded over time. As this figure illustrates, the rate of cooling (the slope of the temperature/time line) did not vary between different combinations of CBF and CMR, and was in line with the rate of blood cooling.

Figure 5.2.5 shows the temperature changes in the ischaemic region over time represented on a colour-coded graphic.
5.2.5 Colour-coded graphical representation of the temperature changes within the ischaemic brain tissue.

This image is presented as if the viewer were looking down from above the patient’s head (i.e. axial section), with relevant layers of tissue removed to allow viewing into the centre of the ischaemic zone. A: Approximately 2 minutes after cooling is initiated. B: Approximately 10 minutes. C: Approximately 30 minutes. D: Approximately 1 hour.

Note that between image B and image D the colours do not change significantly, but the temperature scale that corresponds to those colours does change. These graphics are useful for pointing out gross trends or areas of maximum and minimum temperature but do not convey detailed data as effectively as the graphs in Figures 5.2.1-5.2.4.

5.2.4 Discussion

5.2.4.1 Summary of results within the conceptual model

The results of these simulations demonstrated that (within the simulations at least) the relative temperature differences between different tissue segments, as well as the absolute temperature reduction achieved in each tissue segment were dependent on the combination of CBF and CMR. Conversely, the rate of cooling was independent of these factors and was driven instead by the rate of blood cooling. Predictably, the higher the rate of CBF and the lower the rate of CMR in a given segment of tissue, the greater the depth of
cooling that was achieved in that tissue. It is noteworthy that the temperature of the penumbral tissue was reduced to below 34°C (which is considered a therapeutic level of hypothermia) in all simulations, regardless of CBF or CMR.

The effects of CBF and CMR were interdependent. In the scenarios where CMR was universally elevated (Figure 5.2.3), the temperature of the ischaemic tissue varied by as much as 0.5°C within each tissue segment as a result of variations in CBF. Conversely, when CMR was universally reduced (Figure 5.2.2) the temperature of each tissue segment did not vary by more than 0.1°C between simulations of different CBF levels. Similarly, the temperature difference between healthy and ischaemic tissue did not exceed 0.2°C when CMR was universally reduced.

The level of CBF in adjacent healthy tissue (including tissue experiencing oligaemia) did affect the final temperature of the penumbra, but the magnitude of this temperature difference was no more than 0.1°C in the infarct, and even less in the penumbra (compare the results from CBF set 2 to those from CBF set 3 in Figures 5.2.1-5.2.3). The level of CBF in the infarct, on the other hand, did contribute to significant temperature heterogeneity across the penumbra, particularly when the CMR in the infarct was not zero. This suggests that the precise anatomy of the ischaemic region (the relative size and position of the infarct and penumbra, for example) could have a significant effect on the level of cooling achieved in the penumbra, especially if the infarct were still metabolically active.

5.2.4.2 Interpretation of results with respect to potential failings of the conceptual model

It is highly likely that the results of these simulations underestimate the temperature differences between different tissue segments, just as the simulations of uncooled stroke were found to (See Chapter 5.1). These simulations indicate that any level of CBF that is sufficient to prevent immediate cell death (i.e. infarct core) will also be sufficient to cool the tissue to a therapeutic level. This, unfortunately, is unlikely to translate to clinical practice due to the discrepancy between the simulations and *in vivo* data mentioned above. It is, nonetheless, interesting to note that the absolute level of CBF in the penumbra is not itself a factor that would prevent effective cooling.

The finding that reduced CMR not only reduced overall brain temperature but also minimised temperature differences may translate to clinical findings, particularly since the
model does appear to accurately simulate the direction of temperature gradients found in
the brain, if not their magnitude. Drugs that reduce CMR, such as barbiturates have
previously been shown to reduce brain temperature [44] and it is possible that they would
help minimise temperature differences across the stroke-affected brain. The investigation
of this possibility would be a worthwhile project for the future.

In these simulations the rate of temperature change in the blood was found to be the
primary factor affecting the rate of temperature change in the brain. What limited
experimental data are available suggest that this is the case in non-ischaemic brain [90] but
it is not yet known whether this applies to ischaemic tissue. From the results in Chapter 5.1
it appears that there are factors besides the CBF, CMR and arterial blood temperature
affecting the equilibrium temperature of ischaemic brain tissue. Whatever these factors
are, it is possible that they will affect both the rate of cooling, and the magnitude of
temperature change that can be achieved in ischaemic tissue.

5.2.5 Conclusions

Models such as the one developed in this thesis can provide useful insight into the
processes they simulate even if the simulations themselves do not completely match
reality. This model obviously requires further development before it can be used to guide
treatment for individual patients, but the trends identified in this chapter still have clinical
significance. The absolute rate of CBF found in the penumbra is not in itself a factor that
will prevent effective cooling of the penumbra. Further research is still required to identify
what factors will limit cooling of the penumbra, and these are likely to include the details of
the path the blood takes through the arterioles and microvasculature in the ischaemic
tissue, as described in Chapter 5.1.4. This research can be guided in part by the finding that
absolute CBF rate is unlikely to be important. The utility of reducing CMR by
pharmacological means is also worth investigating. In particular, the possibility that a
reduction in overall CMR could help minimise the temperature difference between
ischaemic and healthy tissue deserves further research. The geometry of the ischaemic
region, in particular the relative size and CBF/CMR rates of the infarct, penumbra and
oligaemia may have an effect on the temperature profile of the ischaemic region.
Therefore, due to the complexity of the geometry of any real brain (and the ischaemic
region inside it), some form of automation for the creation of a geometric model from
patient scans (in particular from CT-perfusion scans[20]) would be extremely helpful if the model is ever developed to the point where it can be applied to modelling individual patients and guiding their treatment. The software required for this automation could be developed while the heat transfer model itself is improved.
Part VI:

Discussion and Conclusions
Chapter 6
Thesis Discussion and Conclusions

6.1 Objectives of this thesis
The experiments in this thesis were originally designed to augment a Phase III clinical trial testing mild hypothermia for the treatment of acute ischaemic stroke (the Cerebral Hypothermia in Ischaemic Lesion trial, ‘CHIL’)\[190\]. Since then, patient recruitment for the CHIL trial has been suspended indefinitely (personal communication, Prof Chris Levi October 2014) but therapeutic hypothermia remains an important potential avenue of clinical research. The central aim of this thesis was to develop methods to measure whether, and to what degree, it is possible to cool ischaemic brain tissue. Two such methods were tested, magnetic resonance thermography (MRT) and finite element modelling (FEM). These methods were chosen for their practical applicability in a clinical trial of hypothermia where specialist expertise in areas such as MR physics is often not available. The suitability of both tools will be discussed separately.

6.2 Overall suitability of the magnetic resonance thermography technique described in this thesis
In its current form, the MRT method described in this thesis is not yet ready for clinical implementation. A number of problems described in Chapters 3 and 4 would affect the confidence with which this MRT technique could be applied. The absolute accuracy of temperature estimations (determined to be ±1.3°C in healthy tissue) is one such problem, as most of the temperature variation observed within both healthy volunteers and stroke patients was less than 2.5°C, and therefore the possibility that the temperature variation was zero cannot be ruled out in most cases. However, the estimations should be accurate enough to allow researchers to detect the 3-4°C temperature decrease targeted in clinical trials such as CHIL. The greater concern is the variation demonstrated in the apparent accuracy of temperature estimations between different data subsets (see Table 3.2.3). This raises the possibility that if another series of spectra was acquired and analysed the
apparent accuracy of the temperature estimations would be even poorer. The confidence that can be placed in any particular temperature estimation is therefore quite limited. Furthermore it is expected that in ischaemic tissue, particularly tissue that has already infarcted, the accuracy of temperature estimations based on MR spectroscopy will be poorer regardless of the individual method used to acquire and process the spectra, but the magnitude of this effect cannot be quantified with any confidence, particularly since it is likely to vary from patient to patient. The experiments in this thesis indicate that it is possible to conduct temperature estimations in infarcted tissue, but did not indicate what level of confidence should be attached to these temperature estimations. On a more promising note, the temperature of the infarct is not typically as important to clinicians as the temperature of the penumbra, and the spectra from the penumbra did tend to be of similar quality to those from healthy tissue in the small cohort of patients examined in this thesis, suggesting that the accuracy of temperature estimations should also be similar.

It is possible that using line-fitting algorithms to determine the proton resonance frequency of the water and reference metabolites would improve the accuracy of temperature estimations based on these resonance frequencies. Indeed, line-fitting is considered mandatory by many authors with regards to MR thermography [105, 110, 115, 217]. The standard Siemens Syngo line-fitting algorithm was found to be insufficient for the purposes of MR thermography during the course of the early phantom experiments described in Chapter 3.1. One of the goals of this thesis was to test a method of MR thermography that would be widely and easily accessible to clinicians conducting trials of therapeutic hypothermia. The need to export data to a separate work-station before processing, not to mention the expense of purchasing specialised software may act as a barrier to the widespread utilisation of any thermography technique dependent on software packages such as LCModel. Therefore the decision was made to avoid using specialised MR spectroscopy software that could have allowed automated interpretation of the spectra. The resulting temperature estimations were not found to be adequate for their intended use, and other methods of processing spectra for MR thermography, that have only recently become available, may provide a better alternative. For example, the latest version of jMRUI [187] is available with a ‘thermography plug-in’ [218]. This is promising as jMRUI is available free to academic users. This temperature plug-in requires further validation but is worth investigating as a possible alternative to the MRT method described
In this thesis, improvements could also be made to the process of acquiring the spectra. The lack of an unsuppressed water reference has already been described as a major weakness of this study. This flaw has already been corrected as far as future acquisitions go by the updates to the Siemens Syngo software, which now collect a separate (unsuppressed) water reference by default. Careful manual shimming may also improve the quality of the spectra acquired in vivo, and allow greater consistency in the spectral quality across different regions of the brain. Improved methods of automatic shimming have also become more widely available since these experiments were conducted. Techniques such as “FASTMAP” and “PACMAP” [219] allow highly accurate shimming while preserving the generalisability of the protocol that was one of the intended features of the MRT paradigm tested in this thesis.

The possibility cannot be ruled out that MR thermography will never be accurate enough to provide worthwhile clinical data on individual patients. Many of the problems encountered with MR thermography in this thesis are not unique to the processing method described here. For example the accuracy of the temperature estimations, both in vitro and in vivo, was comparable to those found in previous studies (as mentioned in Chapters 3.1 and 3.2). The variation in the apparent accuracy of the temperature estimations between different data subsets in Chapter 3.2 is still a concern, but no other MR thermography technique has been subjected to this form of cross-data-set analysis (or at least, the results of such analysis have not been published). It may therefore be the case that other methods of MR thermography would display similarly problematic results were they subjected to such an analysis. The inability to quantify the effects of tissue injury on the accuracy of temperature estimations from ischaemic tissue is a factor regardless of the method of MR thermography processing used.

It is possible that simple measures such as increasing the field strength of the MRI scanner, or using a different spectral processing method such as the jMRUI plug-in or other specialised MR spectroscopy software will allow accurate and reliable temperature estimations in stroke patients. In order to have full confidence in these temperature estimations validation experiments in animals would arguably be required. The possible structure of these experiments will be discussed below. It is also worth noting that, since
Siemens have changed the way the water signal is acquired for MRS scans in their latest version of the Syngo scanner software, the processing method described in this thesis is no longer readily applicable. Even old Siemens scanners will typically have received a software upgrade and now collect a separate, unsuppressed water signal against which other metabolite PRFs could be compared. This, combined with the problems outlined above, means that the MR thermography method described will probably never be widely used, but does not detract from the validity of the experiments described in this thesis. As mentioned above, there are many difficulties and uncertainties involved in MR thermography and these may never be overcome. Nevertheless this remains an important avenue of research as there are still very little data available regarding temperature changes in the brain after a stroke. Furthermore, the other avenue of investigating these changes discussed in this thesis, computer modelling of heat exchange, also requires more in vivo data before it can be developed further, and some of this data will probably have to come from MR thermography or much more invasive neurosurgical techniques.

6.3 Overall utility of the finite element model developed in this thesis

The model in its current form appears to be adequate in normally perfused tissue but not in ischaemic brain tissue. This in itself is an important finding as it suggests that heat exchange in ischaemic brain tissue is altered beyond the level that could be explained by the absolute changes in the volume of blood flow. This is obviously quite relevant in the context of therapeutic hypothermia, but may also have implications for the delivery of other stroke therapies. The mechanism of the changes in heat exchange is not currently known, but is likely to involve changes in the path blood takes to reach ischaemic tissue on a scale beyond the resolution of current medical imaging techniques. While chemical exchange between the blood and the tissue is separate to heat exchange, anything that affects heat exchange also has the potential to affect chemical exchange. Therefore, further investigation of heat exchange in ischaemic brain tissue may yield important information relevant to the delivery of chemical therapies such as neuroprotective drugs.

The model developed in this thesis is a starting point for further in silico investigation of heat exchange in the stroke-affected brain. As mentioned in Chapter 5.1, there are
several bioheat-exchange equations that are more complex, and hence more thorough, than the Pennes equation. The problem with applying these equations in the human brain is collecting enough *in vivo* data on which to apply the equations. Given the problems found with using the Pennes equation in this thesis, it seems likely that improvement in the finite element modelling of heat exchange will be heavily dependent on finding ways to collect such *in vivo* data. Such methods could involve improved MR thermography techniques to provide more temperature data, and improved perfusion imaging techniques to shed more light on the minute details blood flow in ischaemic tissue. Other avenues of *in silico* investigation might still prove useful. The most likely source of error in the model described in this thesis is the assumption that blood temperature in the small arteries of the ischaemic tissue is the same as the blood temperature in the major arteries. Computer simulations of blood flow itself through a cerebral blood vessel network, including simulating the changes that might occur when one artery is occluded could provide some clues as to the changes in blood flow that affect heat exchange. Once again, acquiring the *in vivo* data against which to validate such a model would be challenging, but the structural data on which to base the model are available currently. The construction of this model could in itself provide the basis for a separate PhD thesis, but a proposed basis of such a model will be described below.

6.4 The next step

With regard to MR thermography, it would be worthwhile identifying a single thermography paradigm that could be applied in a clinical setting by researchers conducting clinical trials. Such trials are typically multi-centre (i.e. involve staff and patients from multiple hospitals in multiple cities) and ideally the temperature data collected across the various centres should be comparable. This could potentially involve one coordinating site investing in expensive MR spectroscopy software and MR physicist support, allowing other centres to collect spectral data from patients and send this spectral data to the coordinating site for processing into temperature estimations. This would allow widespread use of MR thermography in clinical trials of hypothermia without placing an undue cost burden on individual hospitals. However, before this system is put into place a single method of MRT should be chosen and fully validated. In order to achieve these goals, the following experiment would be suitable, though logistically challenging.
1: An animal model such as a pig or sheep could be chosen and the experiment should be conducted in a standard clinical MRI scanner (preferably a 3 Tesla system). The steps below are described for a single animal but the test would need to be repeated on multiple animals to ensure the validity of the results across multiple datasets.

2: Surgical arrangements should be made to allow the implanting of temperature probes in at least one and preferably two locations within the animal’s brain.

3: A mechanism should be put in place to allow the experimenters to control the animal’s temperature.

4: With the animal at a steady temperature, repeated spectra could be acquired using a number of spectroscopy sequences, including single-voxel and multi-voxel techniques.

5: This process could then be repeated as the animal’s temperature was raised through a series of temperature steps, 0.5 to 1 degree apart. At each temperature step multiple spectra would need to be collected using each individual spectroscopy sequence to establish the repeatability of each sequence.

6: MR thermography processing could then be conducted on each spectrum using a variety of processing methods such as manual processing (as was used in this thesis), the jMRUI temperature plug-in and other professional spectroscopy software such as LC-Model.

7: At the final temperature step, a stroke could be induced in the animal, ideally in vascular territory that included one of the temperature probes.

8: The results of each combination of acquisition sequence and spectral processing could then be compared on the basis of repeatability (i.e. how closely repeated acquisitions at a steady temperature match each other) and calibrated against the temperature of the brain as measured by the implanted temperature probes.

The proposed experiment would be logistically very difficult and potentially quite expensive, particularly since each animal would need to remain in the MRI scanner for many hours (the cost of scanning a phantom for 2 hours at John Hunter Hospital was $880). Most clinical MRI scanners, even at research intensive hospitals, are not available for animal experiments. If this experiment cannot be conducted the experiments outlined in Chapters 3.1-3.3 could be repeated with other methods of spectrum acquisition and processing. This would not validate the MR thermography as thoroughly as the experiment outlined above, but could be conducted at almost any hospital equipped with an MRI
scanner, which is why this experimental paradigm was chosen for this thesis. In medicine, studies must often be pragmatic rather than perfect.

A logical next step regarding in silico investigation of heat exchange in ischaemic brain would be to develop a model of blood flow through the small arterioles and capillaries in brain tissue. This would arguably be best be conducted using a modelling technique called complex fluid dynamics (CFD). This technique allows the investigator to model blood flow within individual small vessels and model the effects of changes in pressure and other variables that might occur during as the result of a stroke. It is not currently possible to collect data on blood flow at the scale that would be useful to such a model using medical imaging. However, using the anatomical data detailed in Section 1.4.4, it would be possible to build up a model of a representative section of brain tissue a few cubic centimetres in volume. Such a representative sample should include several penetrating arterioles and their associated capillary beds, anastomoses and venous drainage. The parameters of the blood feeding into these arterioles could be based on the data from Table 1.4.3. Simulations could then be run to investigate the effects of occluding one arteriole under different circumstances (e.g. changing the number, location and length of anastomoses, changing the pressure in the capillary bed as a result of oedema in the surrounding tissue). The model could then be expanded to involve multiple branches of a cerebral artery feeding different penetrating arterioles, with various anastomoses between arteries and arterioles. The time it would take to code this model and run each simulation would be considerably longer than the finite element model described in this thesis. Indeed, the construction of the model and running the short list of simulations outlined above could arguably constitute an entire PhD thesis. As with the model described in this thesis, validation would be extremely difficult as a result of the difficulties in obtaining in vivo data, nevertheless the insights that could be gained from the proposed experiments would potentially be invaluable.

6.5 Concluding remarks
Ischaemic stroke remains one of the most significant causes of morbidity and mortality in both developed and developing nations around the world. Research dedicated to improving our knowledge of stroke, and improving treatment for stroke victims is ongoing and stroke management improves every year. However, most of these improvements are
in the area of improved recanalisation strategies [11, 26] or better selection of patients who likely to benefit from dangerous recanalisation therapies [16, 220]. The acute treatments currently available to stroke patients all have extremely limited time-windows within which they must be administered. In addition to being potentially neuroprotective in its own right, hypothermia has the potential to extend the time-window within which other treatments may be effective. Unfortunately, current evidence suggests that hypothermia itself may have a limited effective time-window and the logistics of inducing moderate hypothermia have proved difficult, severely limiting patient recruitment into clinical trials thus far. The CHIL trial failed, in part, because of the requirement that patients be admitted to intensive care units in order to receive hypothermia treatment. Intensive care beds were rarely, if ever, available when suitable patients arrived at the hospital (personal communication with Prof Christopher Levi, October 2014). At least one clinical trial, called Eurohyp I [215] is currently underway testing a milder level of hypothermia, which does not necessarily require admission to intensive care and this trial may have a greater chance of success. MR thermography could provide valuable data from patients enrolled in the Eurohyp I trial, although not in the form tested in this thesis. In the absence of an MR thermography paradigm that is readily usable by clinical or research staff at the various hospitals involved in the Eurohyp trial, it could be argued that one of the several MR research centres involved in MR thermography could be contracted to provide MR thermography support for all the centres involved in the Eurohyp trial. This is potentially feasible because individual MR spectra are typically small enough to be sent by email after being de-identified. Such an undertaking would require a high level or coordination between the MR thermography team and the designers of the clinical trial to ensure the correct MR sequences are acquired, particularly as different hospitals are likely to have different types of MRI scanner. However, overall this is arguably achievable and, as with previous MR thermography studies [110, 121, 122], has the potential to contribute statistical data regarding average temperature changes during hypothermia treatment even if the temperature estimations from individual patients are not fully validated.

**In silico** modelling of cerebral ischaemia is a growing area of research, with some models being developed to investigate the role biochemical effects of temperature in cerebral ischaemia [221, 222]. In time, such modelling could be constructed using data from an individual patient, to help guide that patient’s treatment. However, at present, the
collection of such data from individual patients is not feasible in the acute stroke setting. As medical imaging and biochemical techniques improve, this will hopefully change. In the meantime, *in silico* modelling has the potential to make an important contribution to the body of knowledge regarding stroke. While much of this in silico research, like the finite element model tested in this thesis, will produce more questions than answers, it will provide an economical, ethical, and exciting avenue of research in the future.
References


Appendix 1: A Brief Introduction to Nuclear Magnetic Resonance

The following is a very brief introduction to the physics and signal acquisition behind MR spectroscopy to allow a reader unfamiliar with MRI and MRS to understand some of the terminology used in Part III of this thesis. It is not a comprehensive overview of MRS techniques.

A1 Nuclear Magnetic Resonance

A1.1 Atomic particles
To understand the phenomenon of nuclear magnetic resonance it is first necessary to understand the basics of atomic structure. Each atom is composed of a nucleus surrounded by electrons. Electrons are negatively charged and move constantly (and at very high speed) in a series of probability clouds around the nucleus (these clouds are often referred to, somewhat inaccurately, as orbits). Electrons do not play a major role in MRI or MRS, but the overlap of electron probability clouds is involved in chemical bonding [1] and the motion of electrons does form a very small magnetic field, as the motion of all charged particles does, which can shield the nucleus from an external magnetic field to a very small degree [2].

The nucleus, in turn, is made up of protons, which are positively charged, and neutrons, which bear no charge. The number of protons in a nucleus determines the chemical identity of the atom. The simplest nucleus is that of hydrogen, which (for the most common isotope, \(^1\)H) consists of a single proton [1].

A1.1.2 Spin
Protons move in a series of motions determined by the laws of quantum mechanics [3]. One of these motions involves the proton spinning around its own axis. The total angular momentum for all the protons in a given nucleus is referred to as the ‘nuclear spin’ and this nuclear spin generates a magnetic moment around similar to that of a bar magnet, which is
orientated along the axis of spin (See Figure A1 below). For a hydrogen nucleus, which is the focus of MRI and most clinical MRS [3], there is only 1 proton so the total magnetic moment of the nucleus is equal to the magnetic moment of that proton. The magnetic field generated in this fashion allows the nucleus to interact with external magnetic fields and such interactions form the basis of MRI and MRS. It should be noted that not all nuclei possess nuclear spin. Nuclei with an even number of protons and an even number of neutrons such as carbon 12 (\textsuperscript{12}C) do not possess spin and hence are invisible in terms of MRI [3]. Other isotopes such as carbon 13 (\textsuperscript{13}C) and phosphorous (\textsuperscript{31}P) are visible to MRS, and are examined using MRS in vivo but this discussion will focus on hydrogen.

![Figure A1: The angular momentum of a hydrogen nucleus and the resulting magnetic moment (M).](image)

**A1.3 Interactions with a magnetic field**

Under normal circumstances, the magnetic moments of the nuclei in a given volume are aligned randomly, and the vector sum of the spins is zero. When a strong magnetic field is applied each nucleus begins to wobble or precess at a slight tilt to the axis of the magnetic field as a result of the interactions between the nucleus and the magnetic field. The motion of this precession is similar to that of a gyroscope wobbling about its axis and the
frequency at which the nucleus precesses is determined in part by a property called the gyromagnetic ratio, denoted \( \gamma \). Each MR-visible nucleus possesses a characteristic gyromagnetic ratio, and the precession frequency (\( \omega_0 \), also known as the Larmor frequency) of the nucleus in a magnetic field strength of \( B_0 \) Tesla is given by the famous Larmor equation.

\[
\omega_0 = \frac{\gamma B_0}{2\pi}
\]

The gyromagnetic ratio is measured in radians/T/s and for hydrogen is \( 26.752 \times 10^7 \) rad/T/s, which translates to 42.557 MHz/T [3].

By convention, the direction of the magnetic field applied to the volume of interest is designated as \( z \). While the magnetic field is applied, slightly more than half of the nuclei will align parallel to the field (in the direction \( +z \), often referred to as ‘spin up’) and the rest will align anti-parallel (in the direction \( -z \), or ‘spin down’). The imbalance in numbers is due to the fact that the spin down configuration is a higher energy state than the spin up configuration. The difference in energy between these two states is directly proportional to the magnetic field strength and is the reason for increased signal and resolution as field strength increases. At this point, each individual magnetic moment has a \( z \) component, which is quantized, and ever-shifting \( x \) and \( y \) components as a result of precession. At the macroscopic level, because there are more nuclei in the spin up configuration than the spin down configuration, there is a net magnetization vector \( M_0 \) in the direction of the \( z \) axis. Because the \( x \) and \( y \) components of the various nuclei are still random they cancel out, meaning at this point \( M_0 \) has zero \( x \) and \( y \) components.
Figure A2: The precession of a magnetic moment of a nucleus aligned parallel (spin up) and antiparallel (spin down) to the magnetic field \((B_0)\). Adapted from [4].

Figure A3 shows the macroscopic view and the resulting magnetic vector; \(M_0\). This the lowest energy state of the nuclei and the equilibrium to which the system will return following perturbation. Perturbing this system in order to manipulate \(M_0\) is the basis generating a signal that can be detected by an MRI scanner.
A1.4 Excitation

The nuclei under the conditions outlined above possess a characteristic resonance frequency at which they will absorb electromagnetic radiation and ‘flip’ from the spin up configuration to the spin down configuration, or emit said energy in order to flip from the spin down configuration to spin up. The resonance frequency is dependent on the magnetic field strength and the identity of the nucleus and the chemical environment the nucleus is in i.e. what other atoms the nucleus is bound to. Thus, at a given magnetic field strength all the hydrogen nuclei within water will resonate at the same frequency (in a perfectly homogenous magnetic field) while those in creatine molecules, for example, will resonate at a separate frequency. For hydrogen nuclei at all relevant field strengths the resonance frequency falls in the radio frequency (RF) range.

When a pulse of RF that is perpendicular to the base magnetic field (e.g. along the x axis) is applied to the sample, the number of nuclei in the spin up and spin down configurations begins to change. If the number of nuclei in the spin down configuration becomes equal to
the number in the spin up configuration, $M_0$ is effectively reduced to zero, and if the number of spin down nuclei exceeds the number of spin up nuclei, $M_0$ can become negative. At the same time, the $x$ and $y$ components of the various magnetic moments in the sample begin to converge (at which point they are said to be coherent). This results in the generation of a net magnetization vector along the $x$ axis. Because the nuclei are still precessing around the $z$ axis, this net magnetization vector actually rotates in the $xy$ plane at the Larmor frequency ($\omega_0$). For the sake of illustration, imagine an observer standing on a platform that rotates around the $z$ axis at $\omega_0$, from the perspective of the observer the $x$ and $y$ components of $M_0$ will appear to be stationary even though they are, in fact, moving. The macroscopic effect of these combined phenomena is that $M_0$ appears to rotate from the $z$ axis into the $x$ axis. If the RF pulse is calibrated to reduce the $z$ component of $M_0$ to zero (i.e. equal numbers of spin up and spin down nuclei) $M_0$ will appear to have rotated 90°. This is referred to as a ‘90° pulse’. The RF pulse can be carefully calibrated to produce a specific change in $M_0$, and pulses are typically designated in terms of the apparent angle of rotation of $M_0$ (e.g. 45°, 90° or 180°). However, it should be noted that the generation of coherence along the $x$ axis (which manifests as the increase in $M_0$ along the $x$ axis) and the change in the ratio of nuclei in the spin up and spin down configurations (which manifests as the reduction of $M_0$ along the $z$ axis) are separate, albeit intertwined, processes. Once the RF pulse has finished (i.e. when the RF radiation is no longer being applied to the sample) both components of $M_0$ will return to their original values. This is referred to as relaxation.

**A 1.5 Relaxation**

After the RF pulse has ceased, some of the nuclei in the spin down configuration will emit electromagnetic energy at their resonance frequency and return to the spin up configuration. This occurs until the original ratio of spin up to spin down nuclei has recovered, at which point the $z$ component of $M_0$ will return to its equilibrium level. This is known as spin-lattice relaxation or T1 relaxation. Simultaneously, the $x$ (and $y$) components of the various magnetic moments begin to lose coherence, resulting in the net magnetization along the $x$ axis returning to zero. This is called T2 relaxation or spin-spin relaxation and it is a result of interactions between the oscillating magnetic moments of adjacent nuclei. T2* relaxation is the combination of T2 relaxation with other factors that cause the magnetic moments to lose coherence, such as minor heterogeneity of the base
magnetic field.
Both T1 and T2 relaxation occur in an exponential manner. The time taken for the z component of $M_0$ to return to 63% of its original value is referred to as the T1 time. The time taken for the x component of the net magnetization vector to decay to 37% of its original value is referred to as the T2 time. Both types of relaxation affect the signal that is detected by the MRI scanner.

A 1.6 The MR signal

While the changes in the x and y components of $M_0$ are described above from a rotating plane of reference, it is important to remember that these components are actually constantly changing as $M_0$ precesses around the z axis. If a conductive coil is placed alongside the sample, rotating magnetization vector will generate an electric current in that coil in accordance with Faraday’s law. The current induced in the coil will oscillate with a frequency of $\omega_0$, and the amplitude of the current will decay exponentially over time (see Figure A4 below). This signal is called a Free Induction Decay (FID) and forms the raw data that can be processed into an image or a spectrum by the computer attached to the MRI scanner. Figure A4 shows the FID that would be produced by a single net magnetization vector (e.g. by a sample containing only one chemical, such as water). In reality, each FID collected in vivo will contain a number of overlapping components, each with a slightly different frequency of oscillation and a different rate of decay (See Figure A5). The MRI scanner uses a process called Fourier Transformation to separate out the various components of the FID into either an image (MRI) or a spectrum (MRS).
Figure A4: Rotation of M0 in the transverse (x/y) plane and the resulting signal detected by a receiving coil. The y component of the signal cannot be detected (assuming the receiver is orientated along the x axis) but can be extrapolated. The x component is referred to as the ‘real’ part of the spectrum while the y component is referred to as the ‘imaginary’ part. This disconcerting notation arises from the fact that the y component of the signal must be calculated using complex numbers. Image from [4], reproduced with permission.
A1.7 The acquisition sequence

Given the level of electromagnetic noise that will also be detected by the MR receiving coil, it is not possible to generate a useful spectrum from a single FID. In practice, each MRS acquisition involves generating multiple FIDs and averaging the results [3]. The number of FIDs collected before further processing is referred to as the ‘number of averages’. The generation of each FID requires a separate RF pulse (or more often a series of RF pulses depending on the type of sequence). The time between the application of successive excitation pulses is called the repetition time or TR. The time between the end of the excitation pulse and the first measurement of the resulting signal is called the echo time or TE. Signals from metabolites with fast T2 relaxation times, such as glutamate, can only be measured with a short TE (e.g. 30ms)[3]. Signals from metabolites such as creatine, which possess slower T2 relaxation times, can be measured after a longer TE (e.g. 135ms). The choice of TE will vary depending on the metabolites that are under examination [5].
A1.8 Signal processing

The first step in processing the FID signal is to convert each analogue FID into digital form. This process is carried out by a device called an analogue to digital converter, which measures the instantaneous value of the analogue signal at discrete intervals over a set time. The number of data points measured for a given signal will determine the digital resolution of the acquisition. There is a practical limit on the number of data points that can be measured before the signal decays to zero as a result of T2 relaxation. Any measurements of the FID that are made after this will detect noise but not signal, hence reducing the signal to noise ratio (SNR). The simplest way to solve this is to append a number of data points that are designated zero to the end of the FID (after the point where the signal has actually decayed to zero). This process is called zero-filling and it simulates continuing to collect data without collecting the associated noise (see figure A6 below).

Figure A6: Zero filling of a FID (left) and the effect on the resulting spectrum (right). Note that because the data points added onto the FID do not contain any signal, they do not add any new information to the spectrum, they simply improve resolution. From [4] reproduced with permission.
The other post-processing technique that is often performed at this point is called apodization or filtering. This involves multiplying each data point by a filter function which decays at a similar rate to the rate of decay of the signal itself. The result is that data points from the beginning of the FID, where the signal amplitude is highest, are given more weight in the final spectrum than the points toward the end of the FID when the signal amplitude is lowest. The shape of the filtering function can be chosen to enhance SNR (exponential or Lorentzian function), or spectral resolution (Gaussian function) but generally not both.

Fourier Transformation converts the FID, which appears as changes in current over time, into a spectrum. A spectrum displays the various signal components separated according to their frequency and appears as a graph with amplitude along the vertical (y) axis and frequency along the horizontal (x) axis (see bottom row of Figure A5). As mentioned above, the precise frequency of each signal will be determined by the identity of the nucleus (e.g. $^1$H or $^{13}$C), the strength of the magnetic field and the chemical identity of the nuclei generating the signal. The effects of chemical identity are quite small compared to the other two factors. In general, the resonance frequencies of a given nucleus in different chemical species will vary by a matter of a few hundred Hz at 1T. The resonance frequencies of different nuclei at 1T will vary by tens of millions of Hz. This has given rise to the concept of the ‘chemical shift’ i.e. the shift in resonance frequency of a given nucleus as a result of chemical identity. The chemical shift is expressed as the difference in resonance frequency from a reference chemical, historically tetra-methyl silane for $^1$H, typically as a proportion of the resonance frequency for the reference chemical. Since the reference frequency is usually in the order of MHz, whereas the chemical shift is in the order of Hz, this proportion is expressed as parts-per-million ppm. The advantage of using this scale is that it automatically corrects for magnetic field strength (since the reference frequency is dependent on field strength). Thus, chemical shift measurements made in ppm will be the same at 1T, or 3T or even 7T, greatly simplifying the description and interpretation of MR spectra.

A1.9 Other resources
This appendix has been included to allow a reader unfamiliar with MRI and MRS to make sense of some of the terminology used in Section 3 of this thesis. For an excellent text-
book regarding all aspects of MRS see “in vivo NMR Spectroscopy Principles and Techniques” by Robin de Graaf [3]. For an excellent introduction to the clinical uses of MR spectroscopy see [5] and [6]. For a more thorough explanation of the various post-processing techniques available for the clinical MR spectroscopist see [4].

References
3. de Graaf, R.A., in vivo NMR Spectroscopy Principles and Techniques. 2nd ed. 2007, Chichester: John Wiley and Sons Ltd.
Appendix 2: MRI Safety Screening Questionnaire.

MRI SAFETY QUESTIONNAIRE

WARNING: Due to the MRI system having a very strong magnetic field that is always on, it may be inappropriate/hazardous for some individuals to have a scan. Therefore all questions must be answered accurately to determine your eligibility. Incorrect information could result in serious injury. If you do not fully understand any of the questions please ask for help.

Have you ever had an operation of any kind, at any time in your life?  Yes  No

List all operations, approximate dates, and mark the operation areas on the figure opposite.

Have you had an MRI examination before?  Yes  No If yes, when and where?

Do you have any of the following? (Please circle Yes or No for all questions)

Yes  No
Cardiac pacemaker/ Defibrillator/ Pacing wires
Artificial heart valve
Anaesthetic clip
Ear implant (e.g. Staples, Cochlear) Bionic ear
Vascular implant/ Stent/ Coll/ Filter/ Aortic graft
Neurostimulator or Drug infusion pump
Electronic or Magnetically activated implant or device
Brain shunt
Any other type of prosthesis/ implant (eyes, penis etc.)

If you answer "Yes" to any of these questions, please call 4040 0805 prior to your booking.

Yes  No
Joint replacement or artificial limb
Metal rods, plates or screws in/ on bones
Metal Shrapnel/ Bullet injuries
Hearing aid
Have you ever been a metal worker (e.g. welding, grinding, fabrication)?
Have you ever had metal in your eye following an injury or operation? If yes, has it been removed?  Yes  No
Is there any possibility of metal in your body through injury or surgery, other than that stated on this sheet?
Do you have a history of kidney (renal) disease?
Have you ever had an allergic reaction to contrast media?
Do you suffer from claustrophobia?
Is there any possibility you could be pregnant?
Have you filled out and understood all questions on this form?

I hereby confirm that I have read, understood and correctly answered the above questions and agree to have an MRI and procedures necessary to complete the examination.

Signature of person completing form: __________________________ Date ______/____/____

Form completed by:  [Patient]  [Relative]  [Doctor]  [Other (please specify)]

Print name and contact number/page number: __________________________

WARNING: Before entering the scan room all metallic objects must be removed including watches, jewellery, hearing aids, wets, credit cards, keys, pens/pencils, scissors, mobile phones, hair pins, clips, piercings, clothing with metal e.g. bras, jeans, zips, stud. Locked available.

MRI OFFICE USE ONLY

Form information reviewed by: __________________________ Signature
Second Time Out identification by: __________________________ Signature

Designation

Designation