Molecular Imaging and Spectroscopy with Stable Isotopes in Oncology and Neurology: Imaging metabolism and tissue structure in Multiple Sclerosis with MRI (MISSION: MIMS)

Study title:	A physiological study to assess tissue structure and metabolism in Multiple Sclerosis and normal brain using hyperpolarised carbon-13 labelled pyruvate (^{13}C-pyruvate) and sodium-23 Magnetic Resonance Imaging (^{23}Na-MRI).
Short title:	Molecular Imaging of Multiple Sclerosis with MRI (MIMS)
REC number:	15/EE/0255
Protocol version:	6.0 Dated 6th May 2022

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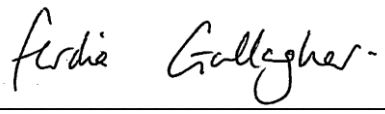
Study Sponsor:	Cambridge University Hospitals NHS Foundation Trust and University of Cambridge

1 Protocol Signatures

Chief Investigator

Name: Prof Ferdia Gallagher

Signature: 

Date: 15 Jun 2022

I have read the attached protocol entitled 'Molecular Imaging of Multiple Sclerosis with MRI (MIMS)' version 6.0 dated 6th May 2022 and agree to abide by all provisions set forth therein.

I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.

2 Study Synopsis

Study Title	A physiological study to assess tissue structure and metabolism in Multiple Sclerosis (MS) and normal brain using hyperpolarised carbon-13 labelled pyruvate (^{13}C-pyruvate) and sodium-23 Magnetic Resonance Imaging (^{23}Na-MRI).
Short Title	Molecular Imaging and Spectroscopy with Stable Isotope Oncology and Neurology: MS MISSION – MIMS
Sub-study title	Molecular Imaging and Spectroscopy with Stable Isotope Oncology and Neurology: Deuterium Metabolic Imaging MISSION – DMI
Hypotheses	1. Increased hyperpolarised carbon-13 labelled lactate can be imaged in MS lesions following intravenous injection of hyperpolarised ^{13}C-pyruvate compared to normal brain. 2. Alterations in ^{23}Na-MRI signal will correlate with the presence of MS lesions as a measure of underlying changes in tissue structure. 3. Imaging the metabolism of deuterium labelled glucose or water can provide complementary information when performed in conjunction with imaging hyperpolarised carbon-13 labelled pyruvate metabolism.
Background	<u>C13 background</u> Structural and metabolic changes occur during the inflammation that is associated with MS. These include alterations in cell size, with an associated change in tissue sodium, and elevation in tissue lactate. Using a technique termed dynamic nuclear hyperpolarisation (DNP), it is possible to increase the signal-to-noise in MRI by more than 10,000 fold. Pyruvate, the breakdown product of glucose, can be polarised using DNP and its metabolism to lactate can be demonstrated within tissue using ^{13}C-Magnetic Resonance Spectroscopic Imaging (^{13}C-MRSI). The imaging of hyperpolarised ^{13}C-lactate can help our understanding of plaque metabolism in MS and could be used to determine when patients are responding to therapy. ^{23}Na-MRI measures tissue sodium and as the concentration of sodium is an order of magnitude higher outside the cell than inside, changes in cell packing and structure can be reflected in the overall ^{23}Na-MRI signal. We will use these techniques, in conjunction with standard proton MRI (^{1}H-MRI), to study the metabolism, structure and natural history of MS plaque in patients with disease. <u>DMI background</u> As a sub-study, we are also investigating metabolic imaging with deuterium (^{2}D), which is a heavy form of hydrogen. Deuterium is naturally present in the human body wherever water is found, which makes it an ideal tracking mechanism. Similar to ^{13}Cm deuterium can be used to replace normal hydrogen in glucose with no known effect to its metabolism, while enabling glucose metabolic measurements. These metabolic measurements with ^{2}D will be used to track the change of glucose in different areas of the normal brain and ultimately leading to future working in tracking changes of types of lesions to study the effects of disease.

	The ingestion of deuterium labelled glucose or water has been shown to be safe as shown in a recently published paper.
Aims of study	<u>Primary objectives</u> 1. To assess the feasibility of imaging the metabolism of hyperpolarised carbon-13 labelled pyruvate (^{13}C-pyruvate) in healthy volunteers and patients with MS. 2. To image the distribution of tissue sodium within inflammatory lesions of the brain in patients with MS. <u>Secondary Objectives</u> 1. To image the formation of hyperpolarised ^{13}C-lactate in inflammatory lesions of the brain following the injection of hyperpolarised ^{13}C-pyruvate. <u>Deuterium Metabolic Imaging (DMI) Sub-study Objectives</u> 1. To demonstrate the feasibility of imaging deuterium labelled glucose or water and its metabolites in the brain and whether this provides complementary information to the imaging of carbon-13 labelled pyruvate metabolism.
Study design	Single centre physiological study
Methodology	See study flow chart for further details (Section 3)
Study Endpoints	<u>Primary Endpoints</u> 1. Demonstration of hyperpolarised ^{13}C-lactate in inflammatory lesions of the brain following the injection of hyperpolarised ^{13}C-pyruvate. 2. Demonstration of elevated tissue sodium in inflammatory lesions of the brain. <u>Secondary Endpoint</u> 1. Demonstration of changes in metabolism of hyperpolarised ^{13}C-pyruvate over time in MS lesions. 2. Demonstration of changes in tissue sodium over time in MS lesions. <u>Deuterium Metabolic Imaging (DMI) Sub-study Endpoint</u> 1. Demonstration of deuterium labelled glucose or water metabolism in normal brains and in the brain of MS patients.
Sample Size	Up to 15 patients with MS will be successfully imaged following injection with ^{13}C-pyruvate. Patients will have up to five studies over no more than 12 months and will be followed up for one year after the last imaging test. Up to 40 healthy volunteers will be successfully imaged following injection with ^{13}C-pyruvate. An optional second imaging study up to 14 days later will be offered.

	Some of these volunteers will be asked to fast for 6 hours prior to the scan taking place. Up to 30 healthy volunteers will be recruited as part of the deuterium metabolic imaging (DMI) sub-study. Up to 15 patients with MS will be recruited as part of the deuterium metabolic imaging (DMI) sub-study.
Summary of eligibility criteria	**Inclusion criteria for MS patients (for C13 and DMI)** 1. Be aged 18 years or older. 2. Have a confirmed diagnosis of MS. 3. Be aware of and understand their diagnosis. 4. Be able to provide written informed consent according to ICH/GCP, national and local regulations. 5. Be willing and able to comply with scheduled visits, laboratory tests, imaging and other study procedures. **Inclusion criteria for healthy volunteers (for C13 and DMI)** 1. Be aged 18 years or older 2. Be able to provide written informed consent according to ICH/GCP, national and local regulations 3. Be willing and able to comply with scheduled visits, laboratory tests, imaging and other study procedures **Exclusion criteria for MS patients (for C13 and DMI)** 1. Have uncontrolled diabetes or glucose deranging conditions, or treatment that would cause such effects. 2. Have any medical condition that may increase the risk associated with study participation or in the judgement of the investigators make it unsuitable for the patient to enter the study. 3. Have a known allergy, adverse reaction or contraindication to any of the injected contrast agents used in this study including gadolinium contrast agents or pyruvate. 4. Be otherwise unsuitable for MRI e.g. having a permanent pacemaker, severe obesity or inability to lie still. 5. Be pregnant or breastfeeding **Exclusion criteria for healthy volunteers (for C13 and DMI)** 1. Have uncontrolled diabetes or glucose deranging conditions, or treatment that would cause such effects. The significance of this will be determined by the research team. 2. Have any medical condition that may increase the risk associated with study participation, or in the judgement of the investigators make it unsuitable for the patient to enter the study.

	3. Have a known allergy, adverse reaction or contraindication to any of the injected contrast agents used in this study including gadolinium contrast agents or pyruvate. 4. Be otherwise unsuitable for MRI e.g. having a permanent pacemaker, severe obesity or inability to lie still. 5. Be pregnant or breastfeeding 6. Any previous or current neurological condition deemed to be significant by the research team.
Maximum duration of enrolment of a subject	3 years

3 Flowcharts

3.1 C13 Volunteer Flowchart

Recruitment of Subjects

- Assess, recruit and successfully image up to 40 healthy control volunteers.

Volunteer Imaging

- Perform ^{1}H-MRI and ^{23}Na-MRI of the brain
- Following injection of up to 50 mL of hyperpolarised ^{13}C-pyruate intravenously, ^{13}C-MRI of the brain will be performed.
- The volunteer will spend up to two hours in the MRI scanner undergoing research imaging.
- Take up to 30 mL of peripheral blood for biochemical analysis
- Repeat imaging up to 14 days later. This is an optional part of the study.
- A subset of volunteers will be imaged following fasting for six hours prior to the imaging study. This is an optional part of the study.

3.2 DMI Volunteer Flowchart

Recruitment of Subjects

- Assess, recruit and successfully image up to 30 healthy control volunteers.

Volunteer Imaging

- Up to 10 participants will be asked to ingest an oral dose of [$^{2}H_{2}$]-water dissolved at 5% in 200 to 300 mL of potable water. The remaining participants will be asked to ingest an oral dose of [6,6'-$^{2}H_{2}$]-glucose dissolved in 200 to 300 mL of potable water giving a dose of 0.75 g/kg body weight, with a maximum of 60 g. The Chief Investigator will determine which dose the participants will be given.
- Following ingestion of the above solution, perform ^{2}H-MRI of the brain
- The volunteer will spend up to 90 minutes in the scanner undergoing research imaging.
- Some volunteers will be asked to fast for at least six hours prior to ingestion of deuterated molecule. This is **optional** and will not impact participation in the study.
- Repeat imaging up to 14 days later. This will be using the same solution as the baseline scan and is an **optional** part of the study.

3.3 C13 Patient Flowchart

Recruitment of Subjects

- Assess, recruit and successfully image up to 15 patients with MS, from the MS clinic, Addenbrooke's Hospital

Patient Imaging

- Perform ^{1}H-MRI and in some cases possibly ^{23}Na-MRI of the brain
- Perform ^{13}C-MRI scan following injection of up to 50 mL of hyperpolarised ^{13}C-pyruate intravenously.
- The patient will spend up to two hours undergoing research imaging
- Take up to 30 mL of peripheral blood for biochemical analysis.
- Each patient will be imaged once with ^{13}C-MRI.

3.4 DMI Patient Flowchart

Recruitment of Subjects

- Assess, recruit and successfully image up to 15 patients with MS, from the MS clinic, Addenbrooke's Hospital.

Volunteer Imaging

- Participants will be asked to ingest an oral dose of [6,6'-$^{2}H_{2}$]-glucose dissolved in 200 to 300 mL of potable water giving a dose of 0.75 g/kg body weight, with a maximum of 60 g.
- Following ingestion of the above solution, patients will be asked to wait at least 60 minutes prior to performing ^{2}H-MRI of the brain.
- The volunteer will spend up to 60 minutes in the scanner undergoing research imaging.
- Patients will be asked to fast for at least six hours prior to ingestion of deuterated molecule.

4 Investigators & Study Coordination

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Introduction

6 Multiple Sclerosis: An Inflammatory Disease

6.1 Introduction to Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system [1]. The disease is prevalent in young adults with a higher incidence in females than males [2]. MS leads to loss of oligodendrocytes which maintain the fatty insulating myelin sheath, and eventual breakdown of the neuronal axons. There is subsequent damage to motor, sensory, autonomic and visual function in sufferers. Patients can eventually be severely disabled, and in addition may have significant psychiatric symptoms and ultimately the disease may shorten lifespan [3] [4].

6.2 Pathogenesis and Progression of Multiple Sclerosis

The pathogenesis of the condition is likely to be activation of autoreactive T-cells in the peripheral immune system, with the cells infiltrating the blood-brain barrier, causing inflammatory damage to the neurons of the white and grey matter [5] – [10]. Whatever the initial precipitant, there is subsequent infiltration of T-cells into the brain, after the blood-brain barrier (BBB) has broken down, leading to demyelination of white matter tracts and a subsequent inflammatory reaction [11] [12]. This demyelination of the white matter results in lesions forming in the white matter tissue of the subject, visible on MRI. These lesions have a high concentration of activated T-cells present, and the level of lactate present rises with respect to normal appearing white matter, as response to the invasion and subsequent tissue demyelination [13] [14] [15].

6.3 The Clinical Course of MS

There are at least four clinical presentations of multiple sclerosis [16].

1. The first, and most common, type is relapsing-remitting MS. This form of the disease is characterised by repeated attacks of symptoms, which may or may not leave permanent disability, followed by periods of relapse [17].

2. The second type of MS is secondary-progressive. This form of MS shows an initial set of clinical events, followed by a period of remission in symptoms. On relapse, there is continuous progression of symptoms [18]. There is some evidence to suggest that this mediated by the extracellular build-up of sodium after failure of the sodium-potassium pump, leading to further cell death and extension of symptoms [19].

3. The third type of MS is known as progressive-relapsing MS, and is the least common form of the condition with only 5% of people diagnosed with this [20]. The sufferer will experience a continual increase in their symptoms, with exacerbations as they progress.

4. The final type of MS is known clinically as primary progressive, with unrelenting progression of the symptoms after the first episode [21]. This is the most aggressive form of the disease, and survival is limited to an average of 22.3 years [22]. Fortunately, only 10% of people suffering from MS are diagnosed with this form.

6.4 Treatments for MS

MS can be treated with steroids, to treat the physical manifestations of clinical relapses, and agents such as interferon-β, glatiramer, Natalizumab, fingolimod, fumarate, and alemtuzumab (recently gaining FDA and NICE approval and developed in Cambridge), are used as disease modifying therapies [5] [6]. Alemtuzumab, for example, is a monoclonal antibody which binds CD52 cell surface protein on T-cells and inhibits the cell activation which cause damage to the brain and spinal cord, and therefore slows the progression of multiple sclerosis [25].

6.5 An Overview of MRI

MRI is routinely used for the diagnosis of MS. Standard clinical MRI provides information about the distribution of water molecules and is termed proton or ^{1}H-MRI. The current method for diagnosis of MS using MRI is known as 'McDonald Criteria' [26]. MS lesions often appear as hyperintense (a bright region) on what is termed T_2-weighted imaging (which demonstrates increased water signal), or following the administration of intravenous contrast medium (which demonstrates the increased vascularity and leakiness of the lesions). One further tool available in the MRI toolkit is the use of MRS (MR spectroscopy). This technique provides a non-invasive method to distinguish individual metabolites in tissues albeit at low sensitivity [27] [28] [29]. The use of diffusion weighted MR imaging (a technique to measure how water moves through a tissue), Diffusion Kurtosis Imaging (measuring how the diffusion of water in a tissue differs from the expected normal behaviour in that tissue), and magnetization transfer imaging (assessing the pool of macromolecules in a given region of interest) have been used to assess multiple sclerosis damage and lesion development [30] [31] [32]. Such techniques may provide useful data to add in to a multi-parametric model with the other sequences used in the study, to assess lesion development and response to treatment.

6.6 Multi-Nuclear Imaging Techniques

MRI can be used to detect many other nuclei other than hydrogen. The images produced from these different nuclei (known generically as X-nuclei MRI) allow for other physical and metabolic processes to be studied in the human body, and two of these will be explored in this study.

6.7 Sodium

Sodium is at a high concentration in the body and natural abundance sodium is detectable with MRI, i.e. sodium-23 or ^{23}Na, although this is not yet applied in the clinic [26] [27] [29] [30] [37]. This technique allows the concentration of total tissue sodium to be imaged and there are a number of techniques that are being developed to distinguish intra- from extracellular sodium. This measurement can prove useful for studying conditions which may affect the sodium concentration homeostasis, for example renal failure [38]. This technique is useful in Multiple Sclerosis as the condition can lead to a large extracellular build-up of sodium [39] – [41]. Due to the properties of the MRI signal, it is possible to separate the signals from intra-cellular and extra-cellular sodium and thus better understand the changes that occur in MS.

6.8 Carbon-13

Endogenous carbon containing molecules are at a much lower concentration in the body compared to hydrogen concentration in water and fat. Furthermore, only 1.1% of natural abundance is detectable with MRI in the form of carbon-13 or ^{13}C. A novel technique for imaging carbon-13 has recently been developed and has now been translated to the clinic and is termed 'hyperpolarisation'. This technique relies upon cooling a sample of carbon-13 labelled pyruvate to 1K, pulsing microwaves through the sample, and finally

heating the sample to body temperature to be injected in to a patient. This technique produces a signal-to-noise ratio (SNR) increase of 10,000 to 100,000 times which is sufficient to allow the spatial distribution of the injected molecule to be imaged as well as the metabolites formed from it [32] [33]. The use of this technique allows the imaging of pyruvate, which is a breakdown produce of glucose, and its subsequent formation into lactate via anaerobic respiration in the cytoplasm. This provides quantitative information about the metabolic processes occurring in a living system in a non-invasive manner, for example in inflammatory conditions where lactate levels are higher than usually seen in the native healthy tissue. The technique of hyperpolarised carbon-13 has recently been translated into human imaging using a prototype device and a single human study has been performed in prostate cancer in the University of California in San Francisco [44] . Over the last four years we have been developing this technique for human use in Cambridge with substantial investment from the Wellcome Trust, Cancer Research UK and the Cambridge Cancer Centre. We will use a hyperpolariser sited next to a clinical MRI system in Addenbrooke's hospital or the Wolfson Brain Imaging Centre and this will be one of only a small number of sites in the world with this capability. This proof-of-concept proposal represents the first non-oncological application of this technique in humans and builds on the common metabolic phenotype seen in both tumours and in inflammatory lesions; we hypothesise that the elevation in tissue lactate demonstrated in the majority of cancers will also be detected in real-time in the inflammatory plaque seen in MS.

6.9 Deuterium (^{2}H)

Deuterium (^{2}H), also known as heavy hydrogen is an isotope of hydrogen which contains one additional neutron within its nucleus compared to ordinary hydrogen (^{1}H), giving it double the mass of ^{1}H. Deuterium is found in low abundance in the body with 0.0115% of the natural abundance signal of water. ^{2}H-labelled substrates can be detected using magnetic resonance spectroscopy and the SNR for detection is increased significantly after the administration of ^{2}H-labelled molecules. This can be either in the form of heavy water ^2H_2O or ^{2}H-labelled substrates. The latter include ^{2}H-labelled glucose and this has been termed deuterium metabolic imaging (DMI). Reliable differentiation between different metabolic states can be achieved when signals from ^{2}H-labelled glucose and its metabolites are detected in real time. Two recent studies have utilised DMI in vivo to study changes in brain metabolism and only one of which had human volunteers. The studies confirm the ability of DMI to reliably measure spatiotemporal changes in cerebral glucose consumption rates (CMRgluc) and Tricarboxyclic acid cycle flux (VTCA) though detection of ^{2}H-labelled glucose, glutamate/glutamine (Glx) and lactate in the brains of rats and humans both healthy volunteers and with brain tumours.

Both ^{13}C-MRS and DMI both assess the dynamics of isotope labelling on intermediate metabolites after injection of isotope-labelled glucose or its breakdown products: carbon-13 MRS has better spectral resolution compared to ^{2}H detection but DMI has a narrower chemical shift range for the metabolic molecules of interest leading to less chemical shift displacement error. ^{2}H also has more than a 5-fold shorter T1 relaxation time than for ^{13}C and has less contamination of signal from endogenous molecules, allowing for better spatio-temporal resolution. This sub-study is aims to demonstrate the feasibility of undertaking both hyperpolarised ^{13}C-MRS following pyruvate injection and DMI in the same volunteers for the first time to assess how they may provide complement information.

Study Design

7 Hypothesis of the study

The study will assess novel imaging techniques to study tissue structure and metabolism within acute inflammatory plaques secondary to Multiple Sclerosis. In particular, it will assess the distribution of tissue sodium and the physiological metabolism of a glucose breakdown product, pyruvate.

8 Objectives of the Study

8.1 Primary Objectives

1. To assess the feasibility of imaging the metabolism of hyperpolarised carbon-13 labelled pyruvate (^{13}C-pyruvate) in healthy volunteers and patients with MS.
2. To image the distribution of tissue sodium within inflammatory lesions of the brain in patients with MS.

8.2 Secondary Objective

1. To image the formation of hyperpolarised ^{13}C-lactate in inflammatory lesions of the brain following the injection of hyperpolarised ^{13}C-pyruvate.

8.3 Deuterium Metabolic Imaging (DMI) sub-study Objective

1. To demonstrate the feasibility of imaging deuterium labelled glucose or water and its metabolites in the brain and whether this provides complementary information to the imaging of carbon-13 labelled pyruvate metabolism.

9 Summary of the Study

9.1 Healthy Volunteers

Up to 40 healthy volunteers will be successfully injected with hyperpolarised ^{13}C-pyruvate and up to 30 will undergo deuterium metabolic imaging (up to 10 participants will be asked to ingest deuterated water and the remaining will be asked to ingest deuterated glucose, as determined by the Chief Investigator) to study tissue structure and metabolism in normal brain. Where possible, these will be performed in the same volunteers. Volunteers will be excluded if they have any previous or current neurological condition, any significant medical condition as determined by the investigators, or meet any of the exclusion criteria as listed below. Volunteers will be recruited by advertising locally with posters on the biomedical campus, through e-mails and utilising the NIHR BioResource.

Healthy volunteers will be imaged on at least one occasion using the same imaging protocol as patients with MS including a combination of ^{1}H-MRI (and may include gadolinium-enhanced imaging), ^{23}Na-MRI,^{13}C-MRI (following an injection of hyperpolarised ^{13}C-pyruvate) and ^{2}H-MRI (following oral uptake of either ^{2}H-labelled water or ^{2}H-labelled glucose in potable water). An optional second scan up to 14 days later will be offered and a subset of volunteers will be asked to fast for six hours prior to the scan taking place.

Any imaging or results of healthy volunteers obtained in this study are not intended to form part of the participants medical records or designed to diagnose any illnesses they may have. However, there is a chance of less than 1:100 that a significant abnormality becomes apparent in this research. In such circumstances, the research team will either contact the participant or their GP (if permission granted in consent form) to discuss the results and whether any further tests are required. It will be mandatory that the participants agrees to be contacted if any incidental findings are discovered and they will not be allowed to join the study if they decline this. It will be **optional** that we contact their GP regarding any possible incidental findings and this will not affect their participation in the study.

9.2 Patient Selection

Up to 15 patients with multiple sclerosis will be recruited, consented and successfully injected with hyperpolarised ^{13}C-pyruvate and up to 15 patients with multiple sclerosis will be recruited, consented and will undergo deuterium metabolic imaging from the multiple sclerosis clinics run at Addenbrooke's hospital.

Patients will be imaged on one occasion using the same imaging protocol as the healthy volunteers. Depending on the substudy, they will either undergo ^{13}C-MRI (following an injection of hyperpolarised ^{13}C-pyruvate) or ^{2}H-MRI (following oral uptake of ^{2}H-labelled glucose in potable water). Where possible, patients will undergo these research scans at the same time as their clinically required ^{1}H-MRI (proton) scan.

9.3 Inclusion Criteria

Inclusion criteria for MS patients (for C13 and DMI)

1. Be aged 18 years or older.
2. Have a confirmed diagnosis of MS.
3. Be aware of and understand their diagnosis.
4. Be able to provide written informed consent according to ICH/GCP, national and local regulations.
5. Be willing and able to comply with scheduled visits, laboratory tests, imaging and other study procedures.

Inclusion criteria for healthy volunteers (C13 and DMI)

1. Be aged 18 years or older
2. Be able to provide written informed consent according to ICH/GCP, national and local regulations
3. Be willing and able to comply with scheduled visits, laboratory tests, imaging and other study procedures

9.4 Exclusion Criteria

Exclusion criteria for MS patients (for C13 and DMI)

1. Have uncontrolled diabetes or glucose deranging conditions, or treatment that would cause such effects. The significance of this will be determined by the research team.
2. Have any medical condition that may increase the risk associated with study participation or in the judgement of the investigators make it unsuitable for the patient to enter the study.
3. Have a known allergy, adverse reaction or contraindication to any of the injected contrast agents used in this study including gadolinium contrast agents or pyruvate.

4. Be otherwise unsuitable for MRI e.g. having a permanent pacemaker, severe obesity or inability to lie still.
5. Be pregnant or breastfeeding

Exclusion criteria for healthy volunteers (for C13 and DMI)

1. Have uncontrolled diabetes or glucose deranging conditions, or treatment that would cause such effects.
2. Have any medical condition that may increase the risk associated with study participation or in the judgement of the investigators make it unsuitable for the patient to enter the study.
3. Have a known allergy, adverse reaction or contraindication to any of the injected contrast agents used in this study including gadolinium contrast agents or pyruvate.
4. Be otherwise unsuitable for MRI e.g. having a permanent pacemaker, severe obesity or inability to lie still.
5. Be pregnant or breastfeeding
6. Any previous or current neurological condition

9.5 Main Study Protocol (for C13 in MS patients or healthy volunteers)

Prior to MRI imaging each participant in the C13 studies will undergo screening which will involve review of medical records and also discussions between a clinical member of the research team and the participant. The following procedures and assessments will take place:

- Informed consent
- Medical history
- Gender, height, weight (kg), age and date of birth
- Pregnancy test for women of childbearing potential (WOCBP)

After screening, participants will be imaged once for ^{13}C-MRI following an injection of hyperpolarised ^{13}C-pyruvate which will be given via a peripheral cannula. The ^{13}C-MRI images will be co-registered with clinical proton MRI (^{1}H-MRI). Where possible, this will be performed at the same time as the research imaging.

Healthy volunteer participants will be asked if they wish to have their personal information stored on a secure database. This data could be used for recruitment for further studies undertaken.

As part of the main study protocol, healthy volunteers (not diagnosed with MS) will be recruited to compare the imaging techniques with MS patient images, as mentioned in Section 9.1.

9.6 Deuterium Metabolic Imaging (DMI) Sub-study Protocol

In this sub-study, healthy volunteers and MS patients will also be consented to participate in imaging using deuterium labelled molecules. Screening will be required to make sure the participants consider themselves healthy and that it is safe to scan them in an MRI scanner. Up to 30 healthy volunteers will be given either ^{2}H-labelled glucose or ^{2}H-labelled water dissolved in potable water for ingestion. Up to 15 MS patients will be given ^{2}H-labelled glucose dissolved in potable water for ingestion. A small blood sample (up to 50 mL) over the course of the study visit may be taken at the scanning facility. This would be taken from a cannula that is placed in the arm or hand of the participant and we will try to use the same cannula for all time points. This is optional and will not affect participation in the study if the participant decline blood sampling. Blood samples will be analysed for various chemicals within the blood and will be compared to samples taken prior to and after the deuterated drink. Following ingestion, participants will undergo ^{2}H-MRI within

two hours. Images of glucose metabolism will be acquired dynamically over time (up to 90 minutes). The results will be used to map the cerebral glucose consumption rate (CMRgluc) and tricarboxylic acid cycle flux (VTCA) within different parts of the brain. The images obtained will be compared to the images obtained from the carbon-13 MRI images from the same corresponding participant (where possible). ^{1}H-MRI, ^{23}Na-MRI and carbon-13 MRI following injection of hyperpolarised pyruvate will be performed in the same participant where possible.

Participants will be asked to fast for six hours prior to the glucose drink, which will involve not eating anything and only have small amounts of water over this time.

9.7 Patient Withdrawal From The Study

If, during, the course of the study, any patient develops a condition which would prevent them from receiving an injection of pyruvate or gadolinium, or fails to show an enhancing lesion after three monthly MRI scans, then they will be withdrawn from the study.

9.8 Primary Outcome Measure

Establishing the physical presence and metabolism of pyruvate in the brain, in both healthy tissue and multiple sclerosis white matter lesions.

Quantifying the absolute concentration of extra-cellular sodium in MS white matter plaques, and healthy brain.

9.9 Secondary Outcome Measure

Quantifying the sensitivity and spatial resolution of carbon-13 imaging of pyruvate and lactate in the human brain.

9.10 Follow-Up

Each patient from both the main study and stroke sub-study will be followed up for up to one year, to assess clinical symptom progression as part of routine care.

9.11 Data Handling

Data will be securely stored using password-protected storage media, in addition to password-protected servers. Patient lists will be stored securely in a locked facility in the Department of Radiology. This will all be carried out as per trust policy.

Image data acquired at the Wolfson Brain Imaging Centre of the Department of Neuroscience of the University of Cambridge will be stored non-anonymised and encrypted for 10 years after the time of scanning and may be transferred to the NHS for clinical purposes, but access is heavily restricted and the data is stored on encrypted disks.

9.12 Confidentiality

All images will be anonymised and kept on the secure hospital network or non-anonymised and encrypted and stored on the secure University of Cambridge network. Any analysis will only be performed on anonymised images. Each patient will be assigned a non-patient identifiable number, and data analysed with knowledge of that number. The personal information of voluntary participants will be stored on a

secure database. Blood samples will also be anonymised with a unique participant code and will be disposed of appropriately after analysis and results recorded using a non-patient identifiable number.

9.13 Statistics

Sodium and carbon MRI will then be compared with the clinical measures of inflammatory activity and tissue destruction and conventional proton MRI.

Up to 15 patients and up to 40 healthy controls will be successfully injected with hyperpolarised pyruvate and will be analysed with subsequent spectroscopic imaging to determine whether there is a significant association between the presence of a multiple sclerosis lesion and increased hyperpolarised lactate concentration within the lesion, in comparison to normal brain tissue. The power to detect a true association depends on the correlation between the covariates (the higher the correlation, the lower the power) and the correlation between each covariate and the amount of hyperpolarised lactate (the higher the correlation, the higher the power). Given the relative paucity of human data from hyperpolarised carbon-13 imaging, a formal power calculation can only be approximated; for example, patients with MS have been shown to have elevated blood lactate and it is assumed that the cerebral concentration of lactate in active lesions is higher. If the difference between control and MS patients is ~ 2 mM with a standard deviation of ~ 1 mM then a study group of 15 patients should have a statistical power of at least 90% to detect a difference between the two groups with a p-value of 0.05. If there are multiple lesions in each patient and the differential between lactate within an MS lesion and normal brain is even higher, the significance and power of the study may be greater.

In the deuterium metabolic imaging (DMI) sub-study, images obtained will be mapped onto the corresponding proton/multi-nuclear MRI images of the volunteers.

9.14 Study Management

The study will be conducted and coordinated in the Department of Radiology by a research team, including a research nurse, research assistant, and study coordinator. MR support will be provided by the medical physics department, clinical radiology support by the radiology department, and clinical neurology support by the neurology clinic.

9.15 Ethics

Ethical permission is being sought from the East of England Cambridge South Research Ethics Committee.

9.16 Summary

In summary, due to the inflammatory reaction of the cells in the region of demyelination in MS, and the use of pyruvate/lactate imaging, it is thought to be possible to image the multiple sclerosis lesions, and their large pools of lactate, in the human brain. This technique may provide early information into the development of multiple sclerosis lesions, from a biochemical analysis of the kinetics of the metabolism in the plaque itself. Further study with known techniques, such as spectroscopy, will provide verification of the carbon imaging, and other proton sequences will be used to correlate with the data.

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11 Document History

Amendment Number	Protocol Version Number	Date Issued	Author(s) of Changes	Details of Changes Made
0.0	1.2	19/08/2015	Mr James Grist, Mrs Rose Eichenberger, Mrs Marie-Christine Laurent, Dr Ferdia Gallagher	New study documents to REC, changes as required by REC initial review
1.0	2.1	14/11/2016	Mr James Grist, Mrs Marie-Christine Laurent	1. Protocol: Increase of healthy volunteer number from 5 – 15 2. Protocol: Inclusion of stroke and blood sub-study 3. Protocol & Healthy Volunteer Information Sheet & Consent form: Addition of optional volunteer database 4. Protocol, Healthy Volunteer & Patient Information Sheets: Inclusion of a screening visit 5. Healthy Volunteer Information Sheet: Inclusion of reasonable travel and parking costs can be reimbursed 6. Patient Consent Form: Inclusion of allowing research team to view medical records and images for one year after study completion 7. Patient Information Sheet: Inclusion of optional repeat scan
2.0	3.0	03/07/2018	Ms Amy Frary, Dr Ferdia Gallagher, Dr Stephan Ursprung, Mr James Grist, Mr Matthew Locke	1. Protocol: Increase of healthy volunteer number from 15 to 40 2. Protocol: The addition of recruiting healthy volunteers from the NIHR BioResource 3. Protocol & Healthy Volunteer Information Sheet: The addition of an optional second scan 3. Protocol & Healthy Volunteer Information Sheet: The addition of optionally fasting prior to a scan 4. Protocol & Healthy Volunteer Information Sheet: Update list of investigators, study coordination & study contacts 5. Removal of ‘untreated’ from Multiple Sclerosis inclusion criteria 6. Protocol: Insertion of document history 7. Protocol, Information Sheet (Patient and HV) and Consent Forms (Patient and HV): inclusion of Wolfson Brain Imaging Centre as a scanning location

Amendment Number	Protocol Version Number	Date Issued	Author(s) of Changes	Details of Changes Made
				8. Protocol, Information Sheet (Patient and HV) and Consent Forms (Patient and HV): updating data protection and confidentiality wording to current GDPR 2018 9. All documentation: General spelling (UK vs US English), consistent wording and formatting to match other MISSION studies
3.0	4.0	29/08/2019	Dr Ferdia Gallagher, Dr Joshua Kaggie, Mr Matthew Locke	1. Protocol, inclusion of Deuterium Metabolic Imaging (DMI) sub-study. 2. Creation of new PIS and Consent form for DMI sub-study.
	4.1	14/01/2020	Mr Matthew Locke	1. Incorrect labelling of deuterated water on Page 8. Changed from "*Up to 10 participants will be asked to ingest an oral dose of **[6,6'-$^2H_2$]**-water dissolved at 5% in 200 to 300 mL of potable water. The remaining participants will be asked to ingest an oral dose of [6,6'-$^2H_2$]-glucose dissolved in 200 to 300 mL of potable water giving a dose of 0.75 g/kg body weight, with a maximum of 60 g. The Chief Investigator will determine which dose the participants will be given.*" to "*Up to 10 participants will be asked to ingest an oral dose of **[$^2H_2$]**-water dissolved at 5% in 200 to 300 mL of potable water. The remaining participants will be asked to ingest an oral dose of [6,6'-$^2H_2$]-glucose dissolved in 200 to 300 mL of potable water giving a dose of 0.75 g/kg body weight, with a maximum of 60 g. The Chief Investigator will determine which dose the participants will be given.*"
4.0	5.0	09/06/2020	Prof. Ferdia Gallagher, Dr Josh Kaggie, Mr Matthew Locke	1. The inclusion of blood samples (up to 50 mL) to be taken from healthy volunteers under the DMI substudy on the day of imaging. These will be taken in the time frame of immediately prior to imaging up to two hours post oral consumption. Where possible a single cannula will be used to take these blood samples to avoid multiple venepunctures. 2. The inclusion of an **optional** fasting for six hours prior to imaging in the DMI substudy. This will be to compare between fasted and non-fast images. 3. The removal of full screening in the Clinical Research Facility for healthy volunteers on the DMI substudy. This is not considered a requirement due to the reduced risks of oral consumption as opposed to intravenous injection in the C13

Amendment Number	Protocol Version Number	Date Issued	Author(s) of Changes	Details of Changes Made
				substudy. Full screening will remain in place for the C13 healthy volunteer and patient studies. 4. Updated information about reporting incidental findings on healthy volunteer studies. This is for both C13 and DMI healthy volunteer substudies. These are updates from local guidance to be made on all healthy volunteer studies. 5. Updated name of the Clinical Research Facility (CRF) to Cambridge Clinical Research Centre (CCRC) to include the whole facility. 6. Extended duration of study to coincide with the funding from the CRUK Fellowship Grant to 31st December 2024.
5.0	6.0	06/05/2022	Prof Ferdia Gallagher, Mathew Locke, Dr Mary McLean, Alixander Khan	1. Removal for the requirement of enhancing/inflammatory MS lesions seen over 3 consecutive proton scans and only image with on ^{13}C-MRI scan. 2. Addition of DMI substudy for MS patients. 3. Removal of formal screening requirements in the Clinical Research Facility for C13 healthy volunteers. 4. Removal of the stroke and blood sampling substudies. 5. Minor typographical changes to the protocol and other study documentation.