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**Study Title:** The effect of altering myocardial lipid content on cardiac physiology in patients with **A**ortic **St**enosis

**Short title: Ox- FAST Study** (**Ox**ford – **F**ibrates in **A**ortic **St**enosis Study)

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| **Sponsor:** | University of Oxford |
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| **Chief Investigator Signature:** | |

There are no conflicts of interest to declare.

**Confidentiality Statement**

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

## TABLE OF CONTENTS

## 1. SYNOPSIS

| | | |
|---|---|---|
| **Study Title** | The effect of altering myocardial lipid content on cardiac physiology in patients with aortic stenosis | |
| **Internal ref. no. / short title** | Ox-FAST Study | |
| **Study Design** | Prospective randomised, double-blind, placebo-controlled | |
| **Study Participants** | Asymptomatic moderate to severe aortic stenosis patients | |
| **Planned Sample Size** | 62 | |
| **Planned Study Period** | 36 months | |
| | **Objectives** | **Outcome Measures** |
| **Primary** | To assess change in myocardial triglyceride content (MTG) before and after 6 months of treatment with fenofibrate/placebo | Change in myocardial lipid content (lipid/water) measured by Proton magnetic resonance spectroscopy ($^{1}$H-MRS) |
| **Exploratory** | To assess the following changes in cardiac physiology before and after treatment with fenofibrate/placebo<br>– LV strain<br>– LV mass<br>– Myocardial energetics<br>– Peak oxygen consumption ($VO_2$ max)<br>– Pericardial fat | – Change in left ventricular strain measured by tagged CMR<br>– Change in left ventricular mass measured by CMR<br>– Change in Phospho-creatinine/ATP ratio (PCr/ATP) measured by Phosphorous magnetic resonance spectroscopy ($^{31}$P-MRS)<br>– Change in peak oxygen consumption measured by CPET<br>– Change in pericardial fat volume measured by CMR |

## 2. ABBREVIATIONS

| | |
|---|---|
| $^{1}$H-MRS | Proton Magnetic Resonance Spectroscopy |
| $^{31}$P-MRS | Phosphorous Magnetic Resonance Spectroscopy |
| ALP | Alkaline Phosphatase |
| ALT | Alkaline Transaminase |
| AS | Aortic Stenosis |
| AVR | Aortic Valve Replacement |
| BNF | British National Formulary |
| CI | Chief Investigator |
| CK | Creatinine Phosphokinase |
| CMR | Cardiac Magnetic Resonance scan (MRI scan of the heart) |
| CPET | Cardiac Pulmonary Exercise Test |
| CRF | Case Report Form |
| DNA | Deoxy ribonucleic Acid |
| ECG | Electrocardiogram |
| FA | Fatty Acids |
| GCP | Good Clinical Practice |
| GP | General Practitioner |
| HRA | Health Research Authority |
| ICF | Informed Consent Form |
| ISF | Investigator Site File |
| LV | Left Ventricular |
| MedDRA | Medical Dictionary for Drug Related Activities |
| MRS | Magnetic Resonance Spectroscopy |
| MTC | Myocardial Triglyceride Content |
| MTG | Myocardial triglyceride content |
| NHS | National Health Service |
| NRES | National Research Ethics Service |
| NUH | Nottingham University hospital |
| OCMR | University of Oxford centre for Clinical Magnetic Resonance Research |
| OUH | Oxford University hospitals |
| PCr/ATP | Phosphocreatinine to ATP ratio |

| | |
|---|---|
| PI | Principal Investigator |
| PIL | Participant/ Patient Information Leaflet |
| PPARα | Peroxisome Proliferator-Activated Receptor Alpha |
| R&D | NHS Trust R&D Department |
| REC | Research Ethics Committee |
| SOP | Standard Operating Procedure |
| TAVI | Transcatheter Aortic Valve Implantation |
| VO2 max | Peak oxygen consumption |
| WOCP | Women of child bearing potential |
| ZDF | Zucker Diabetic fatty |

## 3. BACKGROUND AND RATIONALE

Aortic valve disease is common and its prevalence increases with age.[1] As the population ages, this condition becomes an ever more important public health problem.[2]

***Altered myocardial substrate metabolism and steatosis in aortic stenosis***

Hypertrophied hearts, such as in aortic stenosis (AS) undergo a shift in substrate metabolism with a preference towards glucose utilisation, as glucose is a more efficient fuel compared to fatty acids (FAs).[3-6] This results in imbalance between fatty acid uptake and oxidation, giving rise to myocardial lipid/fat accumulation (steatosis). This accumulation provides a source for non-oxidative metabolism of excess FAs to diacylglycerol and ceramide, potentially resulting in lipotoxicity, cell death and cardiac dysfunction.[7]

Although in humans there is no direct evidence as to whether this constitutes cause or effect, preclinical studies, as summarised below, have demonstrated a causal link between steatosis and left ventricular (LV) dysfunction.

Chiu *et al* [8] generated transgenic mice that overexpressed long-chain acyl-CoA synthetase in the heart and showed that mismatch between myocardial FA uptake and utilisation leads to cardiac lipotoxicity. Haematoxylin & Eosin staining revealed vacuolated myocytes, consistent with intracellular accumulation of lipid. There was evidence of DNA fragmentation and increased cardiac ceramide content consistent with stimulation of apoptotic pathways. These were observed after lipid accumulation and before development of significant LV dysfunction, suggesting that lipid-induced programmed cell death contributes to the development of cardiomyopathy.

Similarly, Zhou *et al* showed a causal relationship between abnormal FA metabolism and reduced myocardial contractility in obese Zucker Diabetic Fatty (ZDF) rats. Myocardial triacylglycerol was higher in ZDF rats due to under expression of FA oxidative enzymes and PPARα. Ceramide levels were increased compared to controls causing cell death and lipotoxic cardiomyopathy. A causal relationship was further supported by the fact that antisteatotic agent, Trioglitazone, which reduced cardiac triacylglycerol, prevented both cardiac apoptosis and loss of myocardial function.[8-10]

There is increasing evidence implicating altered myocardial substrate utilisation and consequent steatosis in hypertrophy and heart failure.[5, 11, 12] Our research group at University of Oxford centre for Clinical Magnetic Resonance research (OCMR) has shown that steatosis is present in both symptomatic and asymptomatic severe AS and that it independently correlates with impaired myocardial deformation (strain), a measure of early LV contractile dysfunction.[13] Furthermore, in

symptomatic severe AS, positive oil red O (ORO) staining of lipid deposition (a marker of steatosis) in myocardial biopsies was associated with systolic strain abnormalities.[13] This is supported by a previous study by Marfella *et al* demonstrating a significant association between myocardial lipid staining and LV dysfunction in AS patients with the metabolic syndrome.[12]

***Role of the PPARα agonist, Fenofibrate in steatosis regression***

The capacity to produce energy via the utilisation of fats by the human heart is controlled in part at the level of expression of nuclear genes encoding enzymes involved in mitochondrial fatty acid β-oxidation (FAO).[5, 6] The principal transcriptional regulator of FAO enzyme genes is the peroxisome proliferator-activated receptor α (PPARα), a member of the ligand-activated nuclear receptor superfamily.[14] PPARα is highly expressed in the heart.[5] Among the ligand activators of PPARα are long-chain fatty acids, therefore, increased uptake of fatty acid substrate into the cardiac myocyte induces a transcriptional response leading to increased expression of FAO enzymes.[5, 14] PPARα-mediated control of cardiac metabolic gene expression is activated during postnatal development,[14] short-term starvation[15] and in response to exercise training.[5, 16] In contrast, certain pathophysiological states, such as pressure overload-induced hypertrophy, result in deactivation of PPARα and subsequent dysregulation of FAO enzyme gene expression, which sets the stage for abnormalities in cardiac lipid homeostasis and energy production.[5, 6, 14] Thus, PPARα not only serves a critical role in normal cardiac metabolic homeostasis, but also alterations in PPARα signalling likely contribute to the pathogenesis of a variety of disease states including pressure-overload hypertrophy in AS.[17-19]

Fibrate drugs such as fenofibrate, gemfibrozil, and bezafibrate, which are PPARα activators are used for treating dyslipidaemia. Various animal and clinical studies have shown fenofibrate to inhibit LVH[25] and phenotypic changes in cardiac gene expression[26] as well as to reduce excessive myocardial lipid accumulation.[27] Myocardial lipid accumulation (steatosis) can be directly cardiotoxic and cause unfavourable LV remodelling.[28]

***Cardiac Magnetic Resonance imaging and spectroscopy***

Cardiac Magnetic Resonance (CMR) is a scanning technique, which does not use ionising or other forms of radiation. It allows the acquisition of moving images of the heart and heart valves. CMR is an invaluable tool in assessing the progression of patients with AS. It is accurate, reproducible and well validated for measuring cardiac volumes and mass.[29, 30] Assessment of change in myocardial shape and dimensions during cardiac cycle (strain) using CMR tissue tagging can provide accurate quantification of global and regional deformation.[31]

Magnetic Resonance Spectroscopy (MRS) provides fundamental insights into in vivo cardiac metabolism. Proton ($^{1}$H) magnetic resonance spectroscopy (MRS) allows non-invasive measurement of myocardial lipid (triglyceride) content[7] by measuring lipid (based on the specific resonance of methylene -CH2) as a percentage of water signal in the same region of myocardium and is also able to distinguish between extracellular and intracellular lipids.[32] This method has previously been validated in several body organs and animal models.[32-34] Our research group at OCMR has recently validated an ECG gated breath-hold technique with excellent reproducibility.[35] The development of this rapid breath hold acquisition has rendered the measurement of myocellular lipid feasible and reliable in a clinical research setting.

$^{31}$P-MRS allows measurement of high energy phosphate metabolism in the myocardium in response to substantial changes in cardiac workload and provides new insights into physiological and pathological cardiac metabolism. By using the non-invasive in vivo technique $^{31}$P-MRS, we and others have demonstrated that AS shows impaired myocardial energetics, indicated by a reduced ratio of phosphocreatine to adenosine triphosphate (PCr/ATP), which is a hallmark of energetic derangement in cardiac hypertrophy.[38-40] While PCr/ATP has been shown to improve post AVR,[38] the effect of a PPAR alpha agonist on myocardial energetics has never been studied before.

***Evidence for a link between steatosis and myocardial deformation (strain) in AS***

Our group has previously examined the relationship between myocardial triglyceride content (MTC) and myocardial deformation. By using $^{1}$H-MRS, we and others have shown that cardiac steatosis occurs in AS, metabolic syndrome, obesity, type 2 diabetes mellitus and HIV.[13, 36, 37] Our group has also successfully validated in vivo MTG determined by $^{1}$H-MRS with histological analysis of myocardial fat in human biopsies obtained intraoperatively[13] in our study of 39 patients with severe AS and normal LV ejection fraction.[13] 25 symptomatic, 14 asymptomatic and 20 age- and sex-matched healthy controls underwent cardiac $^{1}$H-MRS for the determination of MTC and CMR imaging for determination of circumferential strain (measured by tagging). Strain was significantly lower in both symptomatic and asymptomatic AS when compared to controls. Myocardial steatosis was detected in both symptomatic and asymptomatic patients with AS, with significantly higher myocardial triglyceride content when compared to controls. Importantly, the study showed that steatosis was an independent correlate of impaired LV strain. Eight months after AVR, steatosis and strain improved significantly, suggesting that myocardial steatosis is modifiable.

***Study population***

This study will recruit asymptomatic moderate to severe AS patients with at least two of the following: aortic valve area <1.5 cm2, peak pressure gradient >36 mmHg or mean pressure gradient >25 mmHg and not yet eligible for aortic valve replacement (AVR) or transcatheter aortic valve implantation (TAVI).

If fenofibrate is successful in modulating steatosis and strain, this will not only be a breakthrough result with potential to slow the progression of AS in asymptomatic patients, thus delay and in some cases even avoid the need for aortic valve surgery altogether, but also offer a potential alternative treatment strategy for those symptomatic patients who refuse surgery or are not candidates for either AVR or TAVI.

## 4. OBJECTIVES AND OUTCOME MEASURES

The main objective is to study the effects of a fibrate drug, fenofibrate (a PPARα agonist, a key regulator of lipid metabolism) on myocardial triglyceride content (MTG) and cardiac physiology including circumferential strain in patients with asymptomatic moderate-severe AS. We understand from various previous human [24] and animal studies [25,27] that the effect of fenofibrate on cellular lipid metabolism becomes evident within six to fourteen weeks after treatment. In our pilot study[13] eight months post AVR, both myocardial steatosis and strain were found to be significantly reduced. Based on this evidence, we believe that a study duration of 6 months will show expected physiological effect. Thus, we hypothesise that fenofibrate will result in steatosis regression and may result in changes in cardiac physiology in these patients over a period of 6 months.

| **Primary objective** | **Outcome measure** | **Timepoint(s) of evaluation** |
|---|---|---|
| To assess change in myocardial triglyceride content (MTG) before and after 6 months of treatment with fenofibrate/placebo | Change in myocardial lipid content (lipid/water) measured by $^{1}$H-MRS | At baseline and 6 months |
| **Exploratory objectives** | **Outcome measures** | **Timepoint(s) of evaluation** |

| | | |
|---|---|---|
| To assess the following changes in cardiac physiology before and after treatment with fenofibrate/placebo<br>– LV strain<br>– LV mass<br>– Myocardial energetics<br>– Peak oxygen consumption ($VO_2$ max)<br>– Pericardial fat | – Change in left ventricular strain measured by tagged CMR<br>– Change in left ventricular mass measured by CMR<br>– Change in PCr/ATP ratio measured by $^{31}$P-MRS<br>– Change in peak oxygen consumption measured by CPET<br>– Change in pericardial fat volume measured by CMR | At baseline and 6 months |

## 5. STUDY DESIGN (*Appendix A and B)*

This research project is a single-centre, proof-of-concept cardiac magnetic resonance study to investigate the effect of altering MTG using fenofibrate on cardiac physiology in patients with asymptomatic moderate-severe AS. All patients will participate in a randomised, double-blind, parallel design placebo-controlled study for 6 months. The patients will be divided into two groups.

- Group 1: This will include forty-nine patients who will receive treatment with fenofibrate for six months duration.
- Group 2: This group will act as controls, comprising of thirteen patients who will be randomly assigned to receive placebo for six months duration.

The study will be conducted in a single centre specialising in cardiac imaging. Both groups of patients will be identified from the large pool of outpatient clinics at John Radcliffe Hospital (Oxford) and district general hospitals in the region. Patients with asymptomatic moderate to severe aortic stenosis not planned for AVR or transcatheter aortic valve implantation (TAVI) will be reviewed against the eligibility criteria and invited to participate in the study.

Based on previous studies it is estimated that approximately 100 patients will need to be reviewed in total in order to identify 62 eligible patients. Vetting will continue until eligible patients have been randomised into both groups. A log of all participants recruited into the study will be maintained in the investigator site file (ISF) at the investigational site irrespective of whether they have been treated with investigational drug or not. Re-screening of patients who do not meet the inclusion/exclusion criteria will not be permitted, however assessments may be repeated within the screening period and the latest assessment prior to randomisation must always be used to assess eligibility.

Subsequently, all eligible patients will undergo informed consent and baseline tests - bloods will be drawn for lipid profile, renal and liver function, glucose and free fatty acids, and urine for pregnancy test in women of child bearing potential. $^{1}$H-MRS to assess MTG, $^{31}$P-MRS to assess myocardial energetics (PCr/ATP), standard cardiac magnetic resonance imaging to assess LV strain and LV mass, late gadolinium enhancement (fibrosis), physiological exercise assessments to measure $VO_2$ max and 6 minute walking distance. Patients will then be randomly assigned in a double blind fashion to either receive fenofibrate 200mg once daily or placebo. Randomisation will be done by local pharmacy using randomisation code list provided by Nottingham pharmacy. The subject and study investigators will be blinded to the randomisation schedule.

At three months, patient will be asked to re-visit for evaluation via history taking and drug review to check for any issues and compliance to the study. At the end of six months, patients will attend for another study visit at which all baseline investigations will be repeated. Additional blood test for creatinine phosphokinase may be undertaken at this visit in patients at risk of/or with symptoms of myopathy.

Appendix A (page 40) provides a schematic overview of the study design.

## 6. PARTICIPANT IDENTIFICATION

### 6.1. Study Participants

Sixty-two asymptomatic moderate to severe aortic stenosis patients.

### 6.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or Female, aged 18 years or above
- Asymptomatic with diagnosed moderate to severe AS (at least two of the following: aortic valve area <1.5 cm2, peak pressure gradient >36 mmHg or mean pressure gradient >25 mmHg)
- Not planned for AVR or TAVI
- No other significant valvular pathology
- No contraindication to magnetic resonance imaging
- Able (in the investigator's opinion) and willing to comply with all study requirements
- Female participants of child bearing potential ensure effective use of contraception by themselves or their partner during the study and six months thereafter.

- Participant on stable doses of current regular medications for at least 6 weeks prior to entry to the study

### 6.3. Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Known coronary artery disease, history of angina, myocardial infarction or presence of regional wall motion abnormalities
- Presence of other underlying cardiomyopathy
- Left ventricular EF<50%
- Presence of uncontrolled hypertension
- Presence of type 1 or type 2 diabetes mellitus
- Presence of liver impairment
- Female participant who is pregnant, lactating or planning pregnancy during the course of the study
- BMI >40 kg/m2
- Significantly impaired renal function (eGFR<30ml/min)
- Intolerance to or concurrent use of fibrates or PPARα agonists.
- Contraindications to CMR imaging (implantable devices or other metal implants, internal cardioverter-defibrillator, cranial aneurysm clips, metallic ocular foreign bodies, hypersensitivity to gadolinium)
- Inability to tolerate MRI scanning (claustrophobia, inability to lie flat)
- Any other significant disease or disorder which, in the opinion of the investigator, may either put the participant at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate.

## 7. STUDY PROCEDURES

### 7.1. Recruitment

Participants will be recruited via the following routes:

1. Those attending general cardiology/valve clinic at John Radcliffe Hospital, Oxford and general cardiology clinics in regional district hospitals identified by their routine clinical team. A member of the clinical team will also identify potential participants directly from cardiology and echocardiography clinics.
2. Through advertisement via REC approved study posters and leaflets placed in University of Oxford and Oxford University Hospital locations as well as REC approved social media adverts/posters.

The research team will approach a potential participant only after either their clinical team or primary care GP has confirmed that he/she is both eligible (as checked against pre-defined inclusion/exclusion criteria) and willing to further discuss the study with the research team. Clinical care team or GP will hand over the study information to the potential participant for a brief overview of the study. Verbal agreement to be approached for the study will be recorded in the patient's notes by their clinical/primary care team. For those identified via referral letter, a letter of invitation will be sent along with the study leaflet and PIL. The invitation letter will indicate the contact details of the research team for the participant to respond. Research team will also contact them by telephone in the near future to discuss the study and answer any questions they may have. If the potential participant is interested in participating then the research team will arrange a convenient time for the first study visit where informed consent will be obtained.

Recruitment of potential participants will be completed in the OCMR as part of the informed consent process at the baseline visit.

### 7.2. Informed Consent

Interested participants will be presented with written and verbal versions of the Participant PIL and Informed Consent form (ICF) detailing no less than the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The potential participant will be allowed as much time as they require to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study.

Written Informed Consent will then be obtained by the investigators and study team who are familiar with the study protocol and procedures and have been delegated the role by the Chief investigator. The participant must personally sign and date the informed consent form before any study procedures are performed. A copy of the signed Informed Consent will be given to the participant. The original signed form will be placed in the participants medical records/ notes and a copy retained at the study site.

## 7.3. Study Assessments

### 7.3.1. Visit 1 - Screening and Baseline assessments (0 months)

This will include the following:

1. *Eligibility check and informed consent*

2. *History*

3. *Physical Examination (5 mins)*
   Anthropometric measurements including height, weight, BMI and waist to hip ratio will be established. Brief clinical examination including blood pressure and heart rate will be undertaken.

4. *Resting 12 lead ECG*

5. *Venous Cannulation and blood collection (15-20 minutes)*
   Prior to undergoing CMR scanning, each participant will be cannulated to prepare the participant for the administration of contrast agent gadolinium, and at the same time baseline blood samples (25mls) will be taken as detailed in section 7.4.

   Patients will be instructed to fast at least six hours before the scheduled visit as fasting blood samples will be required (refer to section 7.4). We will also ask participants to refrain from strenuous exercise for 24 hours prior to the study visit to decrease the potential for spuriously elevated biomarkers that reflect the influence of vigorous physical activity.

   The venous cannulation and blood collection will be performed in an appropriate University of Oxford clinical research facility environment meeting OUHFT clinical and infection control standards, by appropriately trained study investigators.

   The study visits will be scheduled with consideration to allow the option of early light breakfast or lunch to avoid unnecessarily long fasting periods, especially in the case of elderly patients. The study visit timing will be discussed with the patients on an individual basis depending on their convenience as well as the availability of the scanner.

6. *Cardiac Magnetic Resonance (60-75 mins with short breaks) (please see appendix C)*
All participants will undergo CMR scanning on a 3Tesla MR scanner.

All participants will undergo CMR scan and participants will be asked for their consent to have their de-identified images stored at the time of initial consent. Participants will lie in a supine position and a dedicated CE marked cardiac coil placed around their chest. Images are obtained using breath hold and ECG gating.

Cine images will be acquired for cardiac volume analysis using a 3T MR System using steady state free precession cine imaging as previously described.[43] Analysis of cardiac volumes, function and mass will be performed using cvi[42] post-processing software (Circle Cardiovascular Imaging Inc., Calgary, Canada). Aortic valve area will be measured with direct planimetry of aortic valve cine.[44] Late gadolinium enhancement (LGE) will be performed and analysed as previously described.[45, 46] Tagged MR images will be acquired and analysed as previously described.[13]

*Magnetic Resonance Spectroscopy (MRS) at 3T*

$^{1}$H-MRS

Myocardial $^{1}$H-MR spectra will be obtained from the mid-interventricular septum. Spectroscopic acquisitions will be performed using ECG trigger at end-expiration to minimize motion artefacts [47].

$^{31}$P MRS

$^{31}$P-MR spectroscopy will be performed to obtain the PCr/ATP ratio from a voxel placed in the midventricular septum, with the subjects lying prone with their heart over the centre of the phosphorus coil in the iso-centre of the magnet[48].

Magnetic Resonance Spectroscopy at 7 Tesla Magnetic Field strength (optional): The 7 Tesla (7T) MRI scanner, operating at a higher magnetic field strength than typical clinical MRI systems will be used for additional $^{31}$P MR spectroscopy assessment. This will take approximately 90 minutes. There will not be any need for a cannula insertion, or blood assessments. Participants will not be given any contrast agent for this test. Participants will be asked to lie in the scanner for the duration and resting images will be acquired. This study can be carried out either on a separate day or the same day as the baseline MR assessments visit, depending on the participants' preference. Up to 15 patients will be invited to undergo scanning at 7T MR scanner as part of the

study. It will be made clear to the participants that this additional part of study assessment is optional.

7. *6 minute walk test*

   Participants will perform a 6 minute walk test (6MWT) by walking at their own pace backwards and forwards along a 30 metre length for 6 minutes. The standard validated protocol will be followed which is established for use in clinical and research practice. Total distance covered will be recorded along with Borg scores for dyspnoea and fatigue.

8. *Cardiopulmonary Exercise Testing*

   Cardiopulmonary exercise testing (CPET) will be undertaken using an upright bicycle ergometer protocol with simultaneous respiratory gas analysis as previously described.[50] An incremental ramp protocol will be utilised whereby workload will be gradually increased with continual heart rate, blood pressure and ECG recording. Subjects will be exercised to volitional fatigue with a corresponding adequate respiratory exchange ratio (RER) achieved as a requirement for satisfactory effort defined as respiratory exchange ratio (RER) of >1.1. Peak oxygen consumption (peak VO2) will be determined by averaging VO2 measures over 30 seconds of peak exercise. The exercise protocol is a validated incremental protocol with established use in clinical and research practice. During the exercise testing we will measure the oxygen uptake of the exercising thigh muscles. This is recorded using near-infrared spectroscopy which measures the refraction of light from circulating haemoglobin to measure the oxygen concentration. The device measures the change in oxygen concentration to provide a measure of the muscle oxygen uptake via two sticky sensors attached to one of the thigh muscles of the participant.

After the baseline assessments, patients will be randomised to either receive fenofibrate or placebo. At this visit, study medication sufficing for the first three-month treatment period will be dispensed to participants in a double-blinded manner as described in section 5 (Study design)

### 7.3.2. Visit 2 - Safety and compliance assessment (3 months ± 7 days)

This will include the following:

1. *Evaluate the participant's safety by history taking*
2. *Check medication packaging and compliance (refer to section 7.3.9)*
3. *Dispense study medication for the remaining three-month study period*

### 7.3.3. Visit 3 – End of treatment assessments (6 months)

Same assessments as baseline visit 1 will be undertaken:

1. *History and physical examination*
2. *Resting ECG*
3. *Venous cannulation and blood collection*
4. *CMR and MRS at 3.0T and 7.0T MRS (optional)*
5. *6 minute walk test*
6. *Cardiopulmonary exercise testing (CPET)*

Any excess medication will be returned during this visit.

### 7.3.4. Visit 4 – Study completion (1 month after end of treatment)

Visit 4 will mark the completion of the study for the individual participant. It is expected that this follow up will be executed via telephone call although a clinic visit may be scheduled where the patient reports any symptoms or if there are any study related concerns.

## 7.4. Sample Handling

Blood samples will be sent to the John Radcliffe Oxford University Hospitals (OUH) laboratory for analysis. Plasma and serum samples will be pseudo-anonymised and stored in the Division of Cardiovascular Medicine under the custodianship of the CI for the duration of the study and will be accessible to the study team for this project. De-identified samples may be used in collaboration with other organisations which may be outside the EU. No identifying information would be shared or leave the research site. De-identified samples may also be used in future ethically approved research with the consent of the participant.

*Laboratory tests:*

Using a cannula venous blood samples (approximately 25mls) will be collected at the time points indicated in Appendix B. Laboratory parameters to be tested are as shown below:

| |
|---|
| Haematology (blood sample)<br>• Full blood count |
| Clinical Chemistry (fasting blood samples)<br>• Liver function tests (Albumin, ALP, ALT, bilirubin)<br>• Renal function profile (Sodium, potassium, urea, creatinine)<br>• Creatinine Phosphokinase (CK) – only where clinically indicated |
| Lipid Profile (fasting blood samples)<br>• Total cholesterol<br>• HDL cholesterol<br>• Calculated LDL cholesterol<br>• Triglycerides<br>• Free fatty acids |
| Pregnancy test in women of child bearing potential (WOCP)<br>• Urine sample for pregnancy test |

## 7.5. Investigational Medicinal Product (IMP)

### 7.5.1. IMP description

**Fenofibrate**

| | |
|---|---|
| Substance | Micronised Fenofibrate |
| Pharmaceutical formulation | Gelatine capsules |
| Source | Nottingham University hospital pharmacy |
| Unit strength | 200mg and 67mg |
| Posology | 1 tablet, once daily |
| Route of administration: | Oral |

**Placebo matching Fenofibrate**

| | |
|---|---|
| Substance | Placebo matching Fenofibrate |
| Pharmaceutical formulation | Gelatine capsules containing lactose powder |
| Source | Nottingham University hospital pharmacy |
| Unit strength | Not applicable |
| Posology | 1 tablet, once daily |
| Route of administration: | Oral |

### 7.5.2. Selection of dose for the study

Fenofibrate 200 mg once daily is effective in reducing levels of triglycerides, total cholesterol, and LDL-C and increasing levels of HDL-C in patients with dyslipidemias. Its efficacy and tolerability in the treatment of hypertriglyceridemia and combined hyperlipidemia have been demonstrated in numerous clinical trials.[22,23.] As a PPARα agonist, it has been shown to regulate cellular lipid metabolism and have protective cardiovascular effects.[24-27] These modes of action support the scientific rationale of using fenofibrate in this study and 200mg once daily is the recommended initial adult dose. In elderly patients without renal impairment, the normal adult dose is recommended. In renal dysfunction, the dose of fenofibrate may need to be reduced. Our recruitment criteria will exclude patients with eGFR<30ml/min. In patients with eGFR 30-59ml/min, the BNF recommended dose of 67mg daily will be administered. Nottingham University hospital (NUH) pharmacy will supply both 200mg and 67mg dose preparations to the local pharmacy. Patients will be randomised in a double blind fashion to receive either placebo or active drug (fenofibrate dose based on eGFR) by the local pharmacy as explained in section 7.5.3. Patients renal functions will be assessed at the beginning and end of the study as mentioned in section 7.4.

### 7.5.3. Randomisation, blinding and code-breaking

- Randomisation and Blinding: Nottingham University hospital (NUH) pharmacy will supply both the active drug and placebo bottles, with annex 13 (EU guidelines to Good Manufacturing Practice) compliant labels containing a sequential pack number according to randomisation list, to the site pharmacy. The small tear off section on the bottle stating whether the product is active or placebo will be removed at the point of dispensing by the site pharmacy and attached to an accountability log as proof of which product the patient has been administered. The local pharmacy will also be made aware of patient's eGFR thus enabling them to dispense the correct dose of the active drug. Hence, with the exception of the site pharmacy, all patients, investigators and everyone else involved in study conduct/analysis or with any other interest in this double-blind study will remain blinded with regard to the randomised treatment cohort until all patients have completed the study and database lock has taken place. The randomisation code will be kept secret by the site pharmacy until the database lock.

- Generation of the allocation sequence: This will be generated by the Nottingham university pharmacy using website randomization.com and they will supply a list to the site pharmacy,

who will be unblinded. Participants will be enrolled and assigned to the intervention/study arm by site pharmacy based on this randomisation list.

- Code-breaking: Code break envelopes will be provided by Nottingham University Pharmacy for each participant number according to the randomisation list to the site pharmacy. Emergency unblinding will be available to study investigators or clinical teams via site pharmacy 24 hours a day, 7 days a week, but will only be used in an emergency situation when the identity of the study drug must be known to the investigator/participant's clinical team in order to provide appropriate medical treatment or otherwise assure safety of study participants. Whenever possible and if time allows, the need for unblinding will be discussed with the CI before the unblinding of study medication takes place. The reason for unblinding will be documented in the source documents along with the date and the initials of the person who broke the code. If unblinded, the participant may continue in the study as planned, unless in the opinion of the investigator there is a reason why the participant must discontinue.

### 7.5.4. Supply of IMP

Study medication will be assigned via flagged labels at randomisation and dispensed in a double-blind manner. At Visit 1 the patient will be dispensed sufficient medication for three-month treatment period. Further dispensing for rest of the treatment period will occur on visit 2. In the event that the patient requires replacement medication, extra dispensing will be arranged.

From the start of the treatment period patients will be instructed to take the study medication once daily with a glass of water. Fenofibrate should be taken with food. To ensure a dose interval of about 24 hours, the medication should be taken in the morning at approximately the same time every day. If a dose is missed by more than 12 hours, that dose should be skipped and the next dose should be taken as scheduled. No double doses should be taken. On the day of Visit 2 and Visit 3 the dose should be taken at the usual scheduled time, regardless of the timing of assessments. Daily dosing should continue until the day of Visit 3, when the last dose is taken.
To allow for any delay in scheduling Visit 3 whilst avoiding bias (since half-life of fenofibrate ranges from 10 to 35 hours) patients will be provided with sufficient extra medication.

In extenuating circumstances, IMP may be provided to participants by courier or other secure delivery method for example via a nominated person. Participants will be asked to

provide verbal consent to providing contact details for shipping purposes and for a nominated person to deliver. Participants may also return IMP to the research team this way, for example if they are withdrawn from the study.

### 7.5.5. Packaging, labelling and re-supply

Active 200mg Fenofibrate capsules will be removed from their original blister pack and placed into an empty hard shell gelatine capsule. The capsule will then be back filled with lactose powder to prevent the capsule moving inside the capsule shell. These capsules will then be packed into bottles, each containing 30 capsules.

Matching placebo capsules will be manufactured using the matching capsule shells and packed into bottles in the same quantities described above. 294 bottles containing 30 X 200mg fenofibrate capsules over-encapsulated into suitably sized capsules and back filled as required for the 49 treatment group patients, and 78 bottles containing 30 X matching colour placebo capsules adequate for the 13 patients in the placebo group will be supplied. This is sufficient for the duration of the study.

The investigational products will be provided by Nottingham University pharmacy. They will be manufactured, packaged and labelled in accordance with the principles of Good Manufacturing Practice (GMP). Re-supply to the site will be managed by the study team in collaboration with site pharmacy, which will also monitor expiry dates of supplies available at the site.

### 7.5.6. Storage Conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area in accordance with site pharmacy SOPs and the recommended storage conditions on the medication label. A temperature log will be maintained for documentation. If the storage conditions are found to be outside the specified range, the process outlined in site pharmacy SOPs will be followed.

### 7.5.7. Accountability of trial treatments

The pharmacist and/or investigational drug storage manager at site pharmacy will receive the investigational drugs delivered by the sponsor or delegate when the following requirements are fulfilled:

- Approval of the clinical study protocol by the ethics committee;
- Availability of a signed and dated study contract between the sponsor and the investigational site,
- Approval/notification of the regulatory authority, e.g. competent authority,
- Availability of the curriculum vitae of the Principal Investigator,
- Availability of a signed and dated study protocol,
- Availability of the proof of a medical license for the Principal Investigator

Investigational drugs are not allowed to be used outside the context of this protocol. Patients should be instructed to return unused investigational drug.

The investigator and/or pharmacist and/or investigational drug storage manager will maintain records of the product's delivery to the study site, the inventory at the site, the use by each patient, and the disposal of unused products. If applicable, the sponsor or drug distribution centre will maintain records of the disposal. These records will include dates, quantities, batch/serial numbers, expiry ('use-by') dates, band the unique code numbers assigned to the investigational product and study patients. The investigator/ pharmacist/investigational drug storage manager will maintain records that document adequately that the patients were provided the doses specified by the study protocol and reconcile all investigational products received from the sponsor. At the time of returning investigational product to the site pharmacy pharmacist/investigational drug storage manager must verify that all unused or partially used drug supplies have been returned by the study patients and that no supplies will remain in the investigator's possession.

### 7.5.8. Concomitant medication

Fenofibrate is approved for treatment of hypercholesterolemia and is relatively safe with most of the medications. Common known drug interactions include those with warfarin and statins. Patients already on warfarin may need their dose adjusted based on their INR. Such patients and their GP's will be given all relevant information and advice. Concomitant use of statins and fenofibrate can increase risk of myopathy in certain patients. Patients will be monitored throughout the study for any such symptoms and blood levels for CK will be checked where indicated. Accordingly, necessary dose adjustment/action will be taken. Other drug interactions, if any, with participant's concomitant medications will be checked to ensure safety. To eliminate confounding bias, only those participants who have been stable on their current regular medications for at least six weeks prior to the entry to the study will be recruited.

Participants will be provided with study specific telephone number to contact if they have any issues or concerns.

### 7.5.9. Compliance with study medication

Patients will be requested to bring all remaining study medication including empty package material with them when attending visits. Based on tablet counts, treatment compliance will be calculated as shown in the formula below.

$$\text{Treatment compliance } (\%) = 100 \text{ x} \frac{\text{Number of tablets actually taken}}{\text{Number of tablets which should have been taken}}$$

Compliance should be between 80% and 120%. If the number of doses taken is not between 80-120%, site staff will explain to the patient the importance of treatment compliance. If the assessment of compliance at Visit 3 indicates a compliance of less than 80%, the investigator may decide to perform limited Visit 3 assessments as the patient will not be used for the primary analysis.

### 7.5.10. Post study treatment

There is no indication or requirement for any post study treatment in this study

## 7.6. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- Withdrawal of Consent
- Loss to follow up

Withdrawal from the study will not necessarily result in exclusion of the data for that participant from analysis. Withdrawn participants will be replaced. The reason for withdrawal will be recorded in the CRF.

Patients will receive follow-up phone call one month post completion of study to check for any side-effects from the study medication or any other issues.

### 7.7. Definition of End of Study

The end of study is the date of the last analysis of the last sample.

## 8. INTERVENTIONS

The study will investigate the effect of fenofibrate on myocardial lipid content in patients with asymptomatic moderate to severe aortic stenosis. All subjects will be randomised such that 49 patients receive fenofibrate 200mg once daily and 13 patients receive matching placebo tablets for 6 months +/- 1 month in a double-blind randomized parallel design. Outcome measures will be assessed at baseline and at the end of 6 months. For detailed information on IMP, refer to section 7.5.

## 9. SAFETY REPORTING

Vital signs, physical examinations, or other safety-relevant data observed at screening, baseline, during the course of the study and at the end-of-study evaluation will be assessed with regard to possible changes compared to findings before start of treatment.

Laboratory data will be analysed both quantitatively as well as qualitatively. The latter will be done via comparison of laboratory data to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be summarised.

### 9.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

### 9.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' - resulting from administration of any of the research procedures and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form.

All related AEs that result in a patient's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs. It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the patient's removal from treatment. A patient may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the patient would undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

## 10. STATISTICS AND ANALYSIS

### 10.1. Description of Statistical Methods

Power and sample size calculations are based on our previous data[13] on asymptomatic AS with MTG of 0.75 ± 0.36% vs 0.45 ± 0.17% in normal controls. In this current study, we acknowledge that pressure overload will not be reduced as patients will not undergo surgical intervention along with the uncertainty of change in MTG in moderate-severe AS patients.

Accordingly, we have chosen a sample size of 62 (n=49 in treatment group, n=13 in placebo group) to detect a mean difference in MTG of 0.15% (compared to normals) instead of 0.3%, with SD 0.36 and 80% power.

In our proposed study, the primary focus is to detect physiological effect i.e. change in MTG before and after fenofibrate in the treatment group. Thus, our power calculations aim to detect expected changes from baseline in the treatment group alone, and the comparison between treatment group and placebo is not included in the calculation. The purpose of including the placebo group is to blind the study team during the data analysis process. Hence, we have higher number of patients in the treatment group compared to placebo.

### 10.2. The Number of Participants

To detect a mean difference in MTG of 0.15% in the treatment group (i.e half the difference to normals when compared to our previous study) with a SD of 0.36, power of 80% and a p <0.05, a minimum sample size of 38 will be required. We have allowed for 23% dropouts based on data from a previous randomised controlled trial on similar patient cohort [42]. Taking into account the 23% dropouts, the number of subjects required in the treatment group will be 49, and 13 in the placebo group, thus, taking the total number of recruited patients to 62 (n=49 for treatment group and n=13 for placebo).

### 10.3. Analysis of Outcome Measures

Based on literature data, the primary endpoint of change from baseline in MTG is regarded as normally distributed. The change from baseline of MTG after 6 months of treatment will therefore be analysed by paired student's t-test. The primary objective will be addressed by a one-sided test at level alpha=0.05. For continuous exploratory endpoints, the same model as the primary one will be used.

## 11. DATA MANAGEMENT

### 11.1. Access to Data

Direct access will be granted to authorised representatives from University of Oxford and Oxford University Hospital Trust for monitoring and/or audit of the study to ensure compliance with regulations.

### 11.2. Data Recording and Record Keeping

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic

database, with the exception of the CRF, where participant initials may be added. The code-break document which links participant ID with participant name and identifying information will be held on the medical sciences high security server, which is specifically designed for the storage of sensitive information. All documents will be stored securely and only accessible by study staff and authorised personnel.

All study data will be entered onto an Excel spreadsheet using the de-identified subject ID number in accordance with SOPs for data entry and validation. The name and any other identifying detail will not be included. Data will be analysed on Medcalc 9.5.2.0, SPSS or similar by delegated study investigators. As explained above, the participants will be identified only by a participant ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by research staff and authorised personnel.

The Consent forms will be retained with the study master file and archived for up to 10 years after which they will be destroyed along with the study master file.

### 11.3. Data Blinding

We have delegated responsibilities within the study team such that the investigator analysing images will be blinded to blood test results and vice-versa so that any risk of unblinding through corelation of findings by a study team member is eliminated.

## 12. QUALITY ASSURANCE PROCEDURES

Data collected for the study may be reviewed for auditing and monitoring by authorised persons from the Sponsor (University of Oxford), regulatory authorities or the local host institution to make sure that the study is being carried out correctly. All investigators have a duty of confidentiality to research participants and nothing that could reveal their identity would be disclosed outside the research study team or site.

## 13. ETHICAL AND REGULATORY CONSIDERATIONS

### 13.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

### 13.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

### 13.3. Approvals

The protocol, informed consent form, participant information leaflet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

### 13.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

## 14. PARTICIPANT CONFIDENTIALITY

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR) and the Data Protection legislation 2018, which requires data to be de-identified as soon as it is practical to do so.

### 14.1. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate. On those visits where patients are asked to fast participants will be offered light refreshments or reimbursed the cost of light refreshments.

### 14.2. Other Ethical Considerations

***Venous Cannulation***

*Risk to Participants*: Common risks associated with phlebotomy are pain during the procedure and bruising (with associated pain afterwards). These risks will be minimised by ensuring that all staff are fully trained in phlebotomy. All participants will be fully informed about these risks in the Participant Information Leaflet. The worry associated with taking blood may cause some participants to feel unwell or faint before, during or after the procedure. The risk associated

with this will be reduced by having an adequately equipped facility for performing the procedure (see above) and having a staff member trained in basic life support. Although phlebotomy is a very safe procedure, it does create a puncture wound on the skin which may very rarely lead to infection around the puncture site. The risk of this will be minimised by ensuring strict hygiene during the procedure and by not recruiting participants who are at increased risk of infection. In the event that a participant reports symptoms of an infection (local redness, swelling, pain or discharge of pus) they should be referred to their GP or to A+E urgently.

*Risk to Researchers/Other Staff*: Taking blood carries a risk of needle stick injury to the phlebotomist, which in turn carries a risk of exposure to blood borne infections. This risk will be minimised by a) ensuring staff are adequately trained, b) ensuring staff have been vaccinated against, and show immunity to Hepatitis B and c) having a local policy for needle stick injury which describes the process of being assessed for and receiving post exposure prophylaxi***s.***

***Investigational Medicinal Product***

Fenofibrate has been in use since 1975 and is one of the most commonly prescribed fibrates.[20] It is usually safe and well tolerated and is recommended for the treatment of hypertriglyceridemia and hypercholesterolemia.[21, 22].The fenofibrate dose has been comprehensively validated [23,24]in various large studies. Patients can experience a few common side effects with fenofibrate like itchy skin rash or photosensitivity reaction, nasal congestion or runny nose, sneezing, headache, backache, nausea, constipation and abdominal pain which usually do not need medical attention and are likely to go away during treatment as their body adjusts to the medication. Serious side-effects that occur less commonly and require medical attention include increase in liver enzymes, muscle weakness, severe allergic reaction and peripheral neuropathy. Participants will be well informed of these potential side-effects and their frequency in the PIL. Participants with low eGFR will be given reduced dose of fenofibrate as recommended by BNF. Blood tests will be taken at the time points as detailed in section 7.3. They will be provided with a study specific telephone number to contact during working hours and would be advised to contact GP or emergency services as appropriate if they feel unwell outside of the normal working hours. Participants will also be provided with a number for their clinical team to contact should they need to be unblinded in a medical emergency.

Both fenofibrate and placebo will be packaged into similar looking gelatine capsules and back filled with lactose powder. This may be of concern to patients who are vegetarian/ have certain religious beliefs or those who are lactose intolerant. Patients will be accurately informed of the bovine/porcine origin of the gelatine and presence of lactose powder in the capsules before enrolment into the study so that they are able to make an informed decision.

***CMR***

CMR is a safe and non-invasive technique with no known risk when appropriately supervised. It does not involve ionising radiation (X-rays). Potential participants with ferromagnetic objects in their bodies or with implanted devices which can be damaged by the magnet will be excluded by carefully screening all subjects for ferromagnetic objects, metal implants and other metal (e.g. shrapnel injury) every time prior to entering the scanner environment.

The MRI facilities are fully equipped for resuscitation (including defibrillation) in the unlikely event of a medical emergency during scanning and doctors performing and/or supervising the scans are trained in Advanced Life Support.

While most people do not experience discomfort in a MRI environment, the enclosed space of the scanner can potentially feel uncomfortable, especially for more elderly participants. Discomfort from lying still for a long period of time will be minimised with comfortable padding, short break during coil change and positioning. People with a history of claustrophobia would be excluded from participation in the study. Participants will be given a chance to see the scanner before the study starts. Whilst in the scanner, participants are able to use the alarm button or can squeeze a bulb placed in their hands if they wish to communicate with the operator or to interrupt the scanning at any stage of the scanning process. As the MRI scanner is noisy, participants would be given ear-plugs and/or acoustically shielded headphones (3T and 7T) to minimize the noise and aid communication between participants and investigators.

Participants will be asked to wear a gown for the CMR scans that preserves their modesty while remaining loose in the scanner to avoid potential burns from synthetic clothing. To help maintain participant dignity they will be asked to leave their underwear on, so long as it has no metal parts (e.g. zips, bras clasps or studs). Participants will be asked to change in a changing room near to the scanner and will be given a locker to securely store their belongings. If they are unable to change into the gown by themselves a member of staff will be on hand to offer assistance.

The 7 Tesla (7T) MRI scanner operates at a higher magnetic field strength than typical clinical MRI systems. The Health Protection Agency (HPA) and European (ICNIRP) advice has stated that field strengths up to 8 Tesla can be safely used as long as the participant is aware of the possibility of experiencing various effects including dizziness, nausea, phosphenes (flashes of light) and a metallic taste in the mouth. These effects are transitory and no long-term exposure effects were detected. All participants will be informed of the possible effects by the operator in the information sheet at the consent stage and again before they go in the scanner. To further minimise the possibility of experiencing dizziness, the speed by which the participant moves into the scanner will be minimised. If they do experience dizziness, they may choose to temporarily halt the table movement or to stop all procedures.

In the unlikely event of seeing any structural abnormalities on an MRI scan, a designated clinical specialist will discuss the implications with the participant and arrange for further investigations as necessary. However, it is important to note that scans are not carried out for diagnostic purposes, and therefore the scans are not a substitute for a clinical appointment. Rather, the scans are intended for research purposes only.

***Contrast Agent***

Gadolinium contrast is widely used for clinical indications in CMR and is safe to use. Occasionally (< 1 in 1000), it may cause a mild headache, rash and very rarely a more severe allergic reaction. These severe reactions generally respond very well to standard emergency drug treatment, similar to that given for other severe allergic reactions. However, in people with reduced kidney function, it can lead to a rare condition called nephrogenic systemic fibrosis (NSF); hence, as per departmental SOPs based on Royal College of Radiologists guidelines, (2007) only research participants with estimated glomerular filtration rate (eGFR) >30ml/min can be given gadolinium. For this study, all potential participants with eGFR <30ml/min will not be recruited. Participants' consent will be sought to obtain a pre−scan point-of-care blood test to check kidney function if there is no laboratory blood result for creatinine within the last 6 months prior to consent, or if investigators make a clinical judgement that a new creatinine result is needed and in accordance with the MRI facilities SOPs. As explained to the participant in the PIL, no gadolinium will be given before this result is available.

To-date, there have been no specific recommendations for the use of Gadolinium based contrast agents (GBCAs) in research MRI scans, either from the FDA, the Society for Cardiovascular Magnetic Resonance Research (SCMR), or the International Society for Magnetic Resonance in Medicine (ISMRM). The ISMRM presented its views on the clinical

and research use of GBCAs in their publication in Lancet Neurology in July 2017. Recently published reports in 2017 in the peer-reviewed literature showed that subjects who had repeated use of GBCA showed small amounts of GBCA deposited in the brain that can be seen in later non-contrast studies (Lancet Neurology in July 2017). There are two types of GBCAs based on their chemical structures: linear and macrocyclic. It appears that certain GBCAs (with a linear structure) have a higher risk of this than other GBCAs with a macrocyclic structure, although deposition of gadolinium has also been observed with both types of contrast, and the extent of gadolinium deposition varies between agents. Currently, there is no known adverse clinical effect due to the gadolinium deposition in the brain but further investigations are being conducted. It should be emphasized that deposits have primarily been observed only after repeated or very high doses of GBCAs. This study uses lowest required dose of GBCA for the MR scan protocol to minimise risks. To date, the only known adverse health effect related to gadolinium retention is a rare condition called nephrogenic systemic fibrosis (NSF) that occurs in a small subgroup of patients with pre-existing kidney failure.

The Participant Information Leaflets (PILs) includes relevant information on the risks of GBCAs, including allergic reactions, screening and checking for severe impairment of kidney function, and that it is known that small amounts of gadolinium may remain in the body, including the skin, bone and brain. The PIL also explains that in the event that new information becomes available describing adverse biological or clinical effects associated with gadolinium deposition, participants would be informed and it would be appropriate to include that information as part of the consent process.

***Cardiopulmonary exercise testing***

Cardiopulmonary exercise testing is very safe and is even used in people with heart failure. However, as with all forms of hard exercise, very occasionally some people have significant changes in their heart rate and rhythm that requires medical attention. Although the risk of this happening is small, the test is carried out in a room equipped with emergency monitoring, emergency medications and resuscitation equipment. Medical personnel will be available throughout the testing. Participants' heart rate and electrocardiography (ECG) are monitored throughout the exercise testing and the exercise test will be stopped if there are any concerns.

***Unexpected health related findings***

It is possible that the study investigations could uncover unexpected disease findings on the MRI scan, blood tests or other research procedures. OCMR has an established SOP to be

followed in the instance of an incidental finding as part of a research scan. If any evidence of an unexpected abnormality is detected on the MRI scans the investigators will not attempt to interpret them. They will contact the site MR technologist, consultant or radiologist for confirmation of an abnormality prior to disclosing any problem to the participant. For any abnormalities confirmed, a designated clinical specialist will discuss the implications with the participant and may arrange for further investigations as necessary. Participants will be aware from the PIL that research scans and procedures are not for diagnostic purposes, and therefore are not a substitute for a clinical appointment. Investigators will gain permission from the participant to contact their general practitioner (GP) directly so that the GP can then arrange appropriate management.

## 15. EXCEPTIONAL CIRCUMSTANCES

In exceptional circumstances, including during the COVID-19 pandemic, amendments as detailed below will be made to the study visits in order to minimise participant risk and prevent the risk of loss to follow-up.

The participants will only attend for study visits twice in the 6 month study period. They will attend for visit 1 in the beginning, and then at the end of the study for visit 3. The 3 month follow-up visit (visit 2) will be completed remotely, for example, via telephone.

Going forwards all study medication for the duration of the study will be dispensed on the first visit. This will ensure that visit 2 can be carried out remotely.

The research team will be contactable throughout the study period in case there are any concerns.

## 16. FINANCE AND INSURANCE

### 16.1. Funding

The study is fully funded by The British Heart Foundation. Indemnity is provided by the Research Sponsor, the University of Oxford.

### 16.2. Insurance

***Negligent Harm***

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

## 17. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the British heart foundation. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

## 18. INTELLECTUAL PROPERTY (IP)

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University's technology transfer office, Oxford University Innovations.

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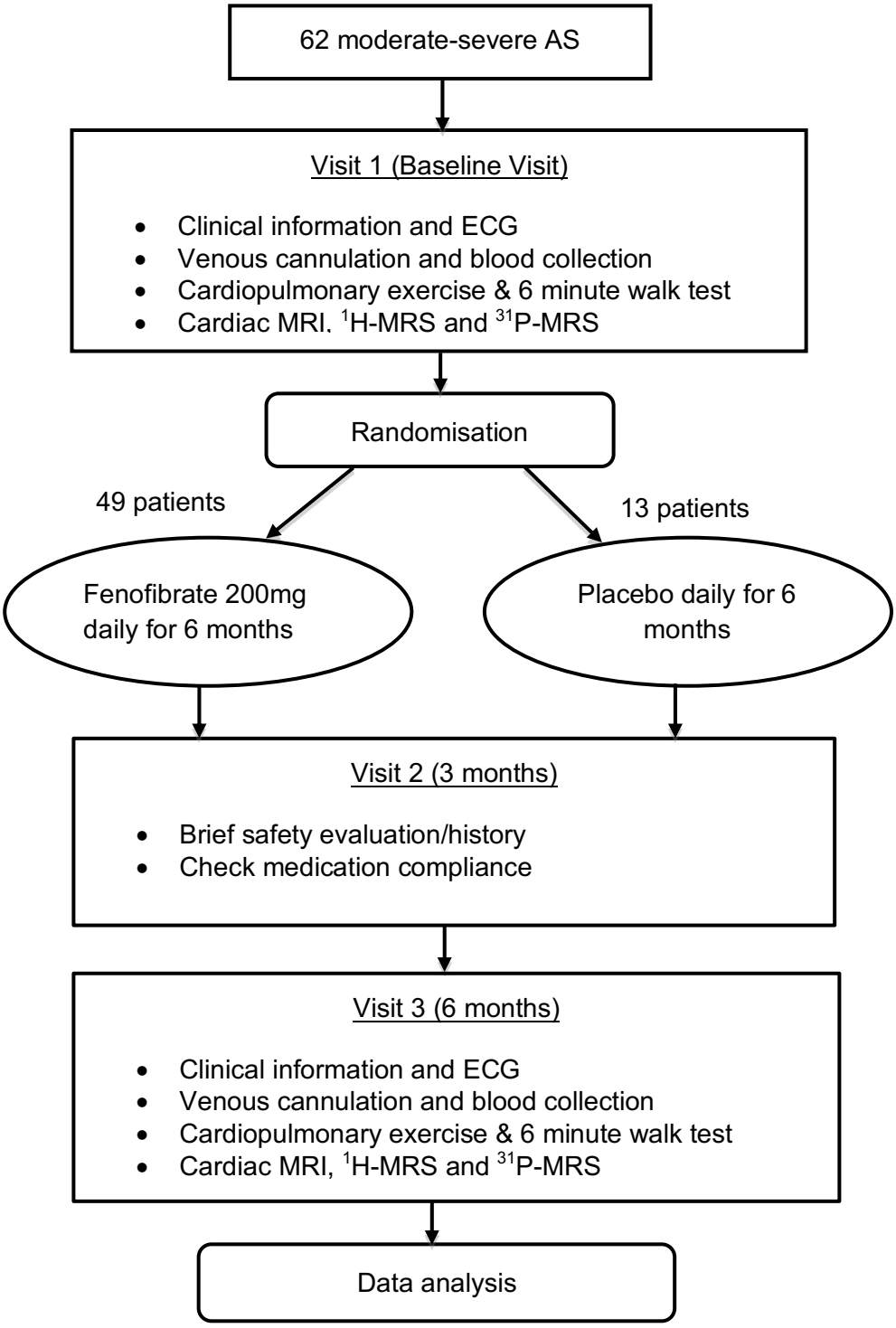
## 20. APPENDIX A: STUDY FLOW CHART



## 21. APPENDIX B: SCHEDULE OF STUDY PROCEDURES

| | Visit 1 Baseline (month 0) | Visit 2 (3 months ± 7 days) | Visit 3 (6 months ± 7 days) | Visit 4 (7months ± 7 days |
|---|---|---|---|---|
| **Informed Consent** | X | | | |
| **Inclusion and exclusion review** | X | | | |
| **History** | X | X | X | |
| **Physical Examination** | X | | X | |
| **Blood tests** | X | | X | |
| **ECG** | X | | X | |
| **Randomisation** | X | | | |
| **CMR & MR spectroscopy (75mins)** | X | | X | |
| **CMR Consumables** | X | | X | |
| **Drug prescribed (Pharmacy)** | X | X | | |
| **Drug review** | | X | X | |
| **Cardiopulmonary Exercise Test (CPET)** | X | | X | |
| **Six minute walk test (6MWT)** | X | | X | |
| **Follow up phone call** | | | | X |

## 22. APPENDIX C: AMENDMENT HISTORY

| Amendment No. | Protocol Version No. | Date issued | Author(s) of changes | Details of Changes made |
|---|---|---|---|---|
| 01 (minor) | V2.0 | 19/03/2020 | P Kemp | Addition of option for the Investigational Medicinal Product (IMP) to be delivered to and sent from participants. |
| 02 minor (COVID-19) | V2.1 | 11/11/2020 | Shveta Monga | During exceptional circumstances visit 2 will be carried out remotely, and medication for the whole study period will be dispensed at visit 1. |