The Leeds Teaching Hospitals NHS
NHS Trust

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Study Title: Improving the diabetic heart's energy efficiency

Short title: IDEE

Version: 2.0

Date: 23 Mar 2023

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Sponsor:	University of Leeds
Funder:	Wellcome Trust (221690/Z/20/Z)
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1 Key Contacts

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2 Synopsis

Full Study Title	Improving the diabetic heart's energy efficiency	
Short Title	IDEE	
Study Design	A single center, open label, randomized, parallel design mechanistic study aiming to understand impact of antioxidant product Mitoquinone on myocardial energy levels	
Study Participants	Type 2 diabetes (T2D) patients	
Planned Sample Size	70 patients (35 T2D patients per drug arm)	
Treatment duration	16 week period of Mito-Q supplementation or no new supplements.	
Follow up duration	None	
Planned Study Period	16 week study period.	
	Objectives	Outcome Measures
Primary	To explore and compare the impact of Mito-Q supplementation on myocardial rest energetics in diabetes compared to no new supplement.	Change in myocardial resting PCr/ATP ratio after treatment.
Secondary	To explore and compare the impact of Mito-Q supplementation on: • Myocardial stress energetics (Dobutamine stress myocardial PCr/ATP ratio) • Myocardial rest and stress function (biventricular ejection fraction, left ventricular global longitudinal strain, mitral inflow E/A ratio); • Myocardial perfusion (rest and stress myocardial blood flow); • Myocardial oxygenation (oxygenated CMR);	After Mito-Q supplementation change in: • Percent difference in myocardial PCr/ATP from rest to stress; • Myocardial blood flow (rest and stress myocardial blood flow); • Global longitudinal strain; • Biventricular ejection fraction; • Diastolic function (mitral inflow E/A ratio)
Formulation, Dose, Route of Administration	Mito-Q 40mg od as prescribed in previous studies using MitoQ	

3 Abbreviations

AE	Adverse event
AR	Adverse reaction
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary Artery Disease
CI	Chief Investigator
CMD	Coronary microvascular dysfunction
CMR	Cardiovascular Magnetic Resonance
CRF	Case Report Form
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
GCP	Good Clinical Practice
GP	General Practitioner
HbA1c	Glycated haemoglobin
HF	Heart failure
HRA	Health Research Authority
ICF	Informed Consent Form
LV	Left Ventricle
Mito-Q	Mitoquinone
MPR	Myocardial Perfusion Reserve
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NRES	National Research Ethics Service
PCr	Phosphocreatine
PIS	Participant/ Patient Information Sheet

REC	Research Ethics Committee
RUSAE	Related and Unexpected Serious Adverse Event
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
T2D	Type 2 diabetes
UAR	Unexpected Adverse Reaction

4 Introduction

4.1 Background

Heart failure (HF) is the most common initial presentation of cardiovascular disease in diabetes[1-3]. The risk of developing HF is increased 2.5-fold in patients with type 2 diabetes (T2D), and 1.7-fold in patients with prediabetes[4, 5]. Once HF is established, the risk of death increases 4- to 6-fold[6, 7], with fewer than one in five T2D patients with HF over the age of 65 surviving beyond five years[8] – an outcome worse than for the most aggressive cancers. There is a clear and urgent need for better strategies to prevent the development of HF in T2D patients.

Diabetic cardiomyopathy and subclinical cardiac damage in patients with diabetes: The prevalence of diabetic cardiomyopathy (defined in a person with diabetes and any systolic or at least moderate diastolic dysfunction without a history of coronary disease, hypertension, significant valvular disease or congenital heart disease) was addressed epidemiologically in a large study [9]. Among patients with diabetes, aged 45 years or older, 17% met criteria for diabetic cardiomyopathy, and 54% had diastolic dysfunction. Of those with diabetic cardiomyopathy, 31% developed HF at 9 years or had died. Indeed, the incidence of HF continues to increase in diabetes [10], despite a substantial reduction in the incidence of myocardial infarction (by 25%) in patients with diabetes over the last 10 years. In addition, the increasing prevalence of T2D in the community [11] is also increasing the population-attributable risk of T2D to HF. Accordingly, the understanding of the pathophysiological determinants of HF in diabetes as well as the early detection of subclinical cardiac damage of diabetic patients is an important goal. Cardiac imaging has been extremely useful in responding to these challenges.

Before HF develops, patients with diabetes often exhibit adverse subclinical changes on imaging. Although conventional indices (such as ejection fraction) are useful in some patients with diabetes and HF, the majority of presentations are of preserved left ventricular ejection fraction. In the subclinical stage, diabetes-induced remodelling including left ventricular (LV) concentric remodelling and hypertrophy (LVH) are observed in the presence of a normal EF [12-14]. In addition to LV mass changes, patients with diabetes often exhibit reductions in subtle contractile function including in global longitudinal strain (GLS) and diastolic function. In addition to functional and structural changes in diabetes, previous studies have identified impaired myocardial perfusion and oxygenation even in the absence of significant coronary artery disease in patients with diabetes[15].

Energy metabolism in T2D: A major cause of cardiac dysfunction in T2D is impaired cardiac energy metabolism. The heart has a very high energy demand, while having minimal energy storing capacity[16]. Efficient matching of energy supply to demand in the heart is therefore essential for maintaining cardiac function. Cardiac energy levels are reduced in T2D patients and even simple exercise activity exacerbates this energy starved state further[17]. Energy starvation in asymptomatic T2D patients precedes other abnormalities such as reductions in left ventricular (LV) ejection fraction or increase in LV mass [17-19]. These data suggest that myocardial energy metabolism offers both early diagnostic and therapeutic opportunities to prevent or modulate diabetic HF. Metabolic intervention strategies that modulate fuel uptake and utilization or mitochondrial metabolism have already been proposed as therapeutic options[20, 21]. However, the precise aetiology of energy starvation and how the alterations of cardiac energy metabolism differ in the development of HF in T2D remain unknown. Such knowledge is critical for determining the most appropriate type of metabolic intervention in individual patients, in particular as some forms of metabolic intervention may be impractical, inefficient or potentially even harmful for some patient groups[20].

Causes of myocardial energy deficiency in diabetes: Diabetes is a disorder of metabolic dysregulation. There are 3 key potential deleterious alterations in cardiac metabolism ultimately resulting in energy deficiency and impaired contractility in patients with T2D[22]: reduced myocardial blood supply[17, 23], loss of flexibility in myocardial fuel selection[24] and impaired mitochondrial function[25].

Myocardial mitochondrial dysfunction in diabetes: The healthy heart shows metabolic flexibility and consumes a wide range of energy fuels such as fatty acids (FAs), glucose, lactate, ketones, and some amino acids, to generate energy via mitochondrial oxidation[26, 27]. The conservation of energy from the oxidation of these energy fuels is performed by the electron transport chain (ETC), where electrons from the redox reactions of fuel oxidation are trapped to create an electrochemical gradient across the inner-mitochondrial membrane to generate ATP. It is likely that the capacity to oxidize any type of fuel in the diabetic heart reduces during transition to failure, when progressive metabolic remodelling occurs, and this reduction in oxidative capacity perpetuates the contractile dysfunction [28, 29]. Assessment of myocardial mitochondrial function in heart biopsy samples from patients undergoing coronary artery bypass graft (CABG) surgery showed significant evidence for mitochondrial dysfunction and oxidative stress in patients with diabetes[25]. Moreover, even pre-diabetic experimental models show impaired myocardial mitochondrial respiration and ATP production.[30]

A key component of cardiac mitochondrial dysfunction in diabetes is increased generation of radical oxygen species (ROS) and defects in antioxidant defenses leading to oxidative damage.

This occurs in mitochondria as a result of ETC dysfunction and outside mitochondria via various enzyme systems[31]. ROS perpetuate mitochondrial dysfunction by multiple pathways[31]. They inhibit ATP synthase and enzymes involved in the citric acid cycle, damage mitochondrial DNA which codes for ETC complexes and reduce creatine kinase shuttle activity[31]. Wider effects include activation of fibrotic and apoptotic pathways[32].

Therapies targeting mitochondrial oxidative stress - Mitoquinone: Abnormal mitochondria are a major source of reactive oxygen species (ROS) production, which can induce cellular damage[27]. Abnormal mitochondria can promote programmed cell death. Therefore, mitochondria directly influence ongoing cell injury and death[27].Therapies targeting mitochondrial oxidative stress may therefore promote reverse remodelling, reduce myocyte hypertrophy and fibrosis and improve energetics and contractile function in patients with diabetes.

CoQ10, or ubiquinone, is an endogenous antioxidant found in mitochondrial membranes. Mitoquinone (Mito-Q) is a ubiquinone derivative conjugated to a lipophilic cation[33]. Mito-Q is considered a supplement and is available over the counter. Due to the large mitochondrial membrane potential, it is targeted to the mitochondria[33]. Following adsorption to the mitochondrial inner membrane it is reduced to the antioxidant, ubiquinol. After acting as an antioxidant, ubiquinol is oxidised to ubiquinone, which is rapidly recycled back to ubiquinol by complex II[33, 34]. The reduced form is not oxidised by complex III; therefore, it acts purely via ROS scavenging. These compounds protect mitochondria from damage following oral delivery and may therefore form the basis for mitochondria-protective therapies. In animal models of hypertensive heart disease and doxorubicin-induced cardiotoxicity, Mito-Q had antioxidant and cardioprotective effects[35, 36]. In a randomised trial of older adults, it had anti-oxidative effects, reducing oxidised LDL and improving endothelial function[37]. In another study Mito-Q decreased liver damage in patients with hepatitis C[38]. It has thus been safely studied in human trials in other diseases with excellent compliance[37,38]. These data support studies using Mito-Q to investigate the effects of reducing mitochondrial oxidative stress in patients with type 2 diabetes.

Measuring myocardial energy metabolism non-invasively - Phosphorus magnetic resonance spectroscopy (^{31}P-MRS): The relative concentration of phosphocreatine to ATP (PCr/ATP) measured with ^{31}P-MRS is a marker of the myocardium's ability to convert substrate into ATP for active processes, and a sensitive index of the energetic state of the myocardium[40]. Impaired ATP transfer may limit contractile function by means of a decrease in the average ATP concentration, a reduction in the ATP transfer capacity through creatine kinase so that insufficient high energy phosphate bonds are transported from the mitochondria to myofibrils, or an increased

concentration of free ADP[40]. Myocardial energetic impairment is a key process in T2D that can be assessed non-invasively by ^{31}P-MRS.

Cardiovascular magnetic resonance (CMR): CMR is the only imaging modality that can non-invasively assess cardiac function, strain, ischaemia, perfusion, oedema/inflammation, fibrosis and oxygenation. Blood-oxygen level-dependent (BOLD) CMR can assess myocardial tissue oxygenation with no need for exogenous contrast, by measuring BOLD signal intensity (SI) differences, reflecting deoxygenated hemoglobin concentration during adenosine stress[41-43]. Measuring myocardial perfusion is vital for interpretation of the findings of energetics and substrate metabolism as substrate utilization by the heart is dependent on substrate presentation to the heart and can now be quantified using CMR[44].

4.2 Purpose of the study

We hypothesize that Mito-Q offers specific benefits in treatment of subclinical cardiovascular disease in T2D through its beneficial effects on myocardial energy metabolism. These distinct mechanisms include improved mitochondrial energy production with Mito-Q. Thus we aim to understand the impact of antioxidant product Mitoquinone on myocardial energy levels.

In this study we will test the primary hypothesis by measuring myocardial energetics utilizing ^{31}P-MRS scans. We will further test whether treatment with these drugs each improve subclinical abnormalities in myocardial perfusion, oxygenation and contractile function in T2D patients.

This study will thus underscore the relevance of myocardial energy metabolism as a novel treatment target in diabetes for preventing diabetic cardiomyopathy by reversing adverse subclinical cardiac features such as abnormalities in cardiac strain, and reductions in myocardial perfusion and oxygenation on CMR.

In a parallel study design, patients with T2D and no known cardiovascular disease will be randomized to receive either Mito-Q or no additional supplementation to their usual treatment and undergo serial assessments, including CMR imaging of quantitative dobutamine stress perfusion imaging, oxygenation and function, ^{31}P-MRS assessment of rest and dobutamine stress myocardial energetics at the initial phase. The study will not be blinded but analysed by blinded operators. The results of these investigations will help determine if the novel strategy of targeting myocardial energy metabolism could offer specific cardiovascular benefits to T2D patients.

5 HYPOTHESES, OBJECTIVES AND ENDPOINTS

5.1 Original Hypotheses

1. Mito-Q will restore the energy balance of the heart;
2. Mito-Q will promote improvements in myocardial perfusion, oxygenation and contractile function in the diabetic heart, and thus reverse the subclinical cardiomyopathic process in T2D.

5.2 Aim, Objectives and Endpoints

Overall aim: To evaluate, *in vivo*, the efficacy of modulating myocardial energetics through mito-Q supplementation in patients with diabetes to reverse subclinical diabetic heart disease process.

Primary objective:
To explore and compare the impact of Mito-Q supplementation on myocardial rest energetics in diabetes.

Secondary objectives:
To explore and compare the impact of Mito-Q on:

- Myocardial stress energetics (Dobutamine stress myocardial PCr/ATP ratio)
- Myocardial rest and stress function (biventricular ejection fraction, left ventricular global longitudinal strain, mitral inflow E/A ratio);
- Myocardial perfusion (rest and stress myocardial blood flow);
- Myocardial oxygenation (oxygenated CMR);

Primary endpoint:
Change in myocardial resting PCr/ATP ratio after Mito-Q supplementation.

Secondary endpoints:

After Mito-Q supplementation, changes in:

- Percent difference in myocardial PCr/ATP from rest to stress;
- Myocardial blood flow (rest and stress myocardial blood flow);
- Global longitudinal strain;
- Biventricular ejection fraction;

- Diastolic function (mitral inflow E/A ratio)

6 STUDY DESIGN

This is a single centre, prospective, randomized, open label clinical trial. T2D patients will be randomly assigned in a 1:1 ratio to receive either Mito-Q 40mg supplementation od for 16 weeks or no additional supplements after randomization.

Patients will be identified from Yorkshire local GP practices and enrolled and followed up in a single centre at Leeds Teaching Hospitals NHS Trust.

Study duration: 16-week open label study period.

Setting: Tertiary cardiac centre – Leeds General Infirmary and University of Leeds
Study population: 70 adult T2D patients (35 per arm).

7 STUDY PARTICIPANTS

70 adult T2D patients (35 per group) will be recruited. Diabetes patients may receive any type of glucose lowering treatment or treated with diet and exercise alone for at least 12 weeks prior to study.

7.1 Inclusion Criteria

1. Men and women>18 years of age;
2. T2D patients with ability and willingness to provide written informed consent and to comply with the requirements of the study.

7.2 Exclusion Criteria

1. Any type of diabetes other than T2D;
2. Cardioverter defibrillator or a pacemaker implant;
3. Cardiac amyloidosis;
4. Known HF;
5. Established significant renal impairment on routine clinical assessments (eGFR<60ml/min/m2);

6. Participation in a clinical trial of an investigational medicinal product (CTIMP) in the preceding 12 weeks;
7. Known hypersensitivity to dobutamine or gadolinium or any other contra-indications to MRI;
8. Female participants who are pregnant, lactating or planning pregnancy during the course of the study;
9. Known prior allergic reaction to Mito-Q.

7.3 Recruitment

Participants will be recruited via the following pathways:

1. With assistance from the NIHR Yorkshire and Humber Clinical Research Network (CRN), the study team will recruit participants with T2D from local GP practices. These practices will be provided the eligibility criteria for screening. They will also be provided the study protocol and the Participant Information Sheet (PIS). They will have detailed discussion about the study aims with the study investigators (Dr Eylem Levelt and her team). Potential participants will be identified from patient lists by a specialist nurse or GP in the practice. Potential participants identified in this manner will be mailed an invitation letter with reply slip, and a PIS by the practice. These forms will have the contact details for the study investigators. Participants who are willing to participate will this way be able to express their interest by directly contacting the investigators via mail, email or phone. The invitation letter will indicate that when this expression of interest has been received by the research team, the team will contact potential participants by telephone in the near future to answer any questions they may have about the study. If the potential participant is interested in participating, the research team will arrange a convenient time for the first study visit to take place.
2. The study team at the Leeds Teaching Hospital's NHS Trust (LTHT) will also contact those who have participated in previous observational ethically approved studies in the department (University of Leeds, Biomedical Imaging) and who have consented to have their contact details retained to be contacted if eligible to take part in other studies. We will consult the LTHT electronic patient record database (EPRO), Patient Administration System (PAS) and/or Patient Pathway Manager (PPM) containing up-to-date information regarding deceased patients prior to contacting potential participants.

7.4 Informed Consent

Written and verbal versions of the Participant Information Sheet (PIS) and Informed Consent Form (ICF) will be presented to the participants 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 trial 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 participant will be allowed as much time as they wish to consider the information, and the opportunity to discuss with the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written informed consent will then be obtained by means of dated participant signature and dated signature of the person who presented and obtained the informed consent on the ICF. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed ICF will be given to the participant. The original signed and dated form will be retained at the trial site.

The participant must personally sign and date the latest approved version of the Informed Consent Form before any trial specific procedures are performed.

7.5 Contraceptive Protection

Women of childbearing potential participating in the study must use a medically-approved, highly effective acceptable birth control method to prevent pregnancy. Acceptable methods of contraception include the following:

- Barrier-type devices (e.g., female condom, diaphragm, and contraceptive sponge) used only in combination with a spermicide
- Intrauterine Devices (IUDs)
- Oral contraceptive agents started at least 90 days before start of study
- Depo-Provera (medroxyprogesterone acetate)
- Levonorgestrel implants
- Naturally or surgically sterile (amenorrheic for at least 1 year and no record of childbirth for naturally sterile persons)
- Male partner is sterile and is the only sexual partner.

Note that true or periodic abstinence, the rhythm method, or contraception by the partner only are not acceptable methods of contraception.

Male participants with female partners of childbearing potential do not have to use birth control methods.

There are no reports of teratogenicity with Mito-Q however as this was not studied in depth in previous studies in this study participants will be advised that if they were to become pregnant during the study, they should stop taking the Mito-Q supplement and contact the research team. The participants initial data will be retained, but they would not attend for any subsequent visits, and would be withdrawn from the study. These patients would be followed through to birth by the research team. The sponsor will be informed in the event of any pregnancy report.

8 Methodology

8.1 Randomisation

Eligible patients will be randomized in a 1:1 ratio to receiving Mito-Q or no additional study treatment. Randomisation to one of the two study arms will be performed by the investigators using an online block randomization tool. They will use a validated randomisation programme and will securely backup both the randomisation seed and the randomisation allocation. We will use randomization to reduce the risk of our findings being related to chance. Otherwise as this mechanistic study is not a drug trial randomization would not be required.

8.2 Study Visits

The assessments listed below will be carried out at each visit in the Advanced Imaging Centre at the Leeds General Infirmary. Participants will continue taking their previously prescribed medications throughout the study. At their first visit, they will be given the PIS to read through, and given the opportunity to ask questions. If they are interested in participating, their consent will be taken in written form. Each participant will then have a series of non-invasive tests. Study assessments are described in detail in Section 8.3.

There will be three visits. Visits 1 and 3 will include heart imaging using multiparametric Magnetic Resonance Imaging, and visit 2 will be a phone call to participants in the Mito-Q arm to assess their tolerance of the Mito-Q and their adherence. Participants will need to fast overnight for visits 1 and 3; they will not need to fast for visit 2.

Visit 1 (Baseline; week 0)

- Review of medical history and concomitant medications
- Review of history of diabetes and complications
- Review of inclusion/exclusion criteria
- Collection of demographic data
- Height and weight
- Urine pregnancy test in women of childbearing potential
- Written informed consent

<u>Baseline assessments</u>

- Vital signs
- Physical examination
- 12-lead ECG

- Blood pressure
- Venepuncture (fasting sample): 20mls for assessment of NTproBNP, and HbA1c
- Multiparametric MRI
- Randomization
- Dispense study medication and issue patient diary

Visit 2 (phone visit for drug tolerance assessments; week 8, +/- 5 days)

- Check current medication list and patient clinical status
- Check study medication compliance (diary review)

Visit 3 (final visit; week 16, +/- 5 days)

Final assessments

- Vital signs
- Height and weight
- Physical examination
- 12-lead ECG
- Blood pressure
- Venepuncture (fasting sample): 20mls for assessment of NTproBNP, and HbA1c
- Multiparametric MRI
- Review and collect patient diary

End of the study

8.3 Study Assessments

8.3.1 Blood Tests and Urine Pregnancy Test

Blood will be taken via venepuncture at each visit and tested for the following: N-terminal pro–B-type natriuretic peptide (NTproBNP), lipids and glycated haemoglobin (HbA1c).

Participants will be administered a urine pregnancy test (stick test at the research center) at their first study visit (if applicable).

Blood and urine samples will be destroyed as soon as analysis is complete. Blood samples will be sent to labs at the University of Leeds for analysis and only be stored until they can be analysed on the day they are taken. Urine samples will be destroyed as soon as the test is read and the result recorded in the CRF. No samples will be kept for long term storage.

8.3.2 Multiparametric MRI

Multiparametric MRI assessments will consists of 2 parts:

Magnetic Resonance Spectroscopy (MRS): The relative concentration of phosphocreatine to ATP (PCr/ATP) by ^{31}P-MRS.

^{31}P-MRS (3-Tesla): ^{31}P-MRS will be performed to obtain the rest and dobutamine stress PCr/ATP from a voxel placed in the mid-ventricular septum, with the subjects lying supine with the ^{31}P coil placed over their heart, in the iso-centre of the magnet[45-47].

After the resting 3-dimensional chemical shift imaging ^{31}P-MRS and resting short-axis stack are acquired, dobutamine will be infused intravenously at incremental rates from 5 to a maximum of 40 µg/kg with a target of 65% of age maximal heart rate. During this time, blood pressure will be measured every minute. Heart rate, blood pressure, and pulse oximetry will be monitored continuously during both dobutamine infusion studies. Heart rate will then be maintained at target for the duration of the scans (8 minutes 29 seconds for ^{31}P-MRS, 5 minutes for short-axis cine imaging).

CMR (3-Tesla): CMR will include pilot and cine imaging to assess LV volumes, mass and ejection fraction, myocardial strain parameters. Dobutamine (5 to 40 µg/kg/min rate increased in a staged incremental fashion) will then be infused again for at least 3 minutes. Subsequently, gadolinium-based contrast (Gadovist®, Bayer Pharma, Berlin, Germany) will be injected for first-pass perfusion imaging(39). Dobutamine will then be discontinued and, after at least 20 minutes to allow washout, another bolus of gadolinium (0.075 mmol/kg) will be given for rest perfusion imaging. Data acquisition will use a multi-slice, free-breathing, saturation recovery pulse sequence with fast low angle shot (FLASH) readout, acquired over 60 heartbeats. In the first three beats proton density weighted images (without saturation preparation) will be acquired. Arterial input function (AIF) data will be obtained from interleaved low- resolution images (dual-sequence method) in a single slice with dual-echo acquisition to allow correction of T2* related signal loss. Late gadolinium enhancement (LGE) in matching LV short- and long-axis planes will be carried out more than 8 minutes after rest perfusion imaging. Total scan time for the CMR is approx. 1 hour.

With the participant's permission, CMR results will be shared with the patient's GP for the patients with T2D. If the CMR detects any unexpected abnormalities (such as previous myocardial infarction, features of cardiomyopathy etc.), urgent cardiology review will be organised as an outpatient.

8.4 Expenses and Benefits

Reasonable travel expenses for research visits will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

8.5 Discontinuation/Withdrawal of Participants from Trial Treatment

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 trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Disease progression which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Withdrawal of Consent
- Loss to follow up

Withdrawal from the study will 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. If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilized.

8.6 Definition of End of Study

The end of study will be the date of the last visit of the last participant.

9 Study Drug Treatment

9.1.1 Frequency and duration

Mito-Q 40mg once daily po.

This dose was chosen based on previous research which has used doses of 40 to 80mg daily, with no significant side-effects noted. The manufacturer suggests 10mg daily, but does report that "studies have shown MitoQ to be safe well beyond the recommended dose".

9.2 Mitoquinone

Mito-Q is an antioxidant product and available over the counter. The only side effects with Mito-Q in the trials were mild gastrointestinal symptoms. Participants will be educated on these symptoms and given a written action plan on how to manage them in the unlikely event that they occur. Moreover, participants in this study will be monitored closely and given 24-hour access to a study or on-call doctor.

9.2.1 Contraindications

Hypersensitivity to the active substance or to any of the other ingredients.

9.3 Administration / handling of the study drugs

Mito-Q will be prescribed and dispensed by the University of Leeds study investigators who are also doctors at the Leeds Teaching hospitals NHS Trust. Instructions as to how and when to take the prescribed medication will be issued at the time of enrolment. A diary will be issued at the time of enrolment for subjects to record taking the medication to assess compliance with treatment.

9.4 Monitoring

There are no specific monitoring recommendations for Mito-Q. However, we will be contacting participants for an over the phone assessment of tolerance to Mito-Q at week 8. Participants will be advised regarding potential side effects listed above (mild gastrointestinal symptoms) as per usual clinical practice.

9.5 Drug Supply

The study supplements will be prescribed and dispensed by the investigators. There will be no blinding.

9.6 Concomitant Medications

There are no contraindicated medications for Mito-Q.

9.7 Withdrawal of Treatment

Treatment will be withdrawn if contra-indications to continued administration develop. Unexpected reactions to the medication will be recorded and may lead to the patient being withdrawn from the study.

9.8 Post-study Treatment

There will be no provision of the study drugs beyond the study period.

10 Serious Adverse Events Procedures

10.1 General Definitions

An adverse event (AE) is any untoward medical occurrence in a patient which does not necessarily have a causal relationship with this supplement and can include:

- any unintentional, unfavourable clinical sign or symptom
- any new illness or disease or the deterioration of existing disease or illness
- any clinically relevant deterioration in any laboratory assessments or clinical tests.

An adverse reaction (AR) or adverse drug reaction (ADR) is any untoward or unintended responses to a study product related to any dose administered to that subject. All AEs judged by either the reporting investigator or the Sponsor as having reasonable causal relationship to a supplementary product qualify as adverse reactions. The expression “reasonable causal relationship” means to convey in general that there is evidence or argument to suggest a causal relationship.

An unexpected adverse reaction (UAR) is any adverse reaction the nature and severity of which is not consistent with the information about the study product in question set out in the listed side effects.

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study and will be classified as expected adverse reactions. The only reported side-effect of Mito-Q in the manufacturer's documentation is nausea and a mild stomach upset.

In addition, the following criteria may be used in order to collect protocol-defined reportable adverse events which do not meet the criteria for serious (below):

- requires medical or surgical intervention to prevent permanent impairment of function or permanent damage to body structure.

A serious adverse event (SAE) or serious adverse reaction (SAR) is defined in general as "any untoward medical occurrence or effect that:

- results in death,
- is life-threatening*,
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- consists of a congenital anomaly or birth defect,
- may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

*the term life-threatening refers to an event in which the patient 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 was more severe.

An SAE/SAR occurring to a research participant, where in the opinion of the Chief Investigator the event is Related and Unexpected will be reported to the main Research Ethics Committee (REC) and to the Sponsor. The National Research Ethics Service (NRES) defines Related and Unexpected SAEs (RUSAEs) as follows:

- Related: that is, it resulted from administration of any research procedures; and
- Unexpected: that is, the type of event is not listed in the protocol as an expected occurrence.

10.2 Causality

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below. All adverse events judged as having a reasonable suspected causal relationship to the study medications (i.e. definitely, probably or possibly related) are considered to be adverse reactions. In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the REC and other bodies will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).

Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

10.3 Severity

Severity of all AEs and ARs will be graded on a three-point scale of intensity (mild, moderate, severe):

Mild	Discomfort is noticed, but there is no disruption of normal daily activities.
Moderate	Discomfort is sufficient to reduce or affect normal daily activities.
Severe	Discomfort is incapacitating, with inability to work or to perform normal daily activities.

Note: An AE or AR may be severe but not serious

10.4 Operational definitions and reporting for AEs and SAEs

10.4.1 Expected AEs/SAEs – Not reportable

The patient populations being studied here may have co-morbid disease along with type 2 diabetes. However, we expect these patient populations to be generally healthy. Because these patients have been diagnosed with type 2 diabetes, we expect some adverse events related to this condition.

In recognition of this, events fulfilling the definition of an adverse event or serious adverse events will not be reported in this study unless they are classified as 'expected' or 'unexpected' and 'related' (section 10.2).

10.4.2 Expected AEs/SAEs – Reported within standard CRFs

The following SAEs are expected within the study population and will be reported by the clinical research team using standardised tests and follow-up CRFs including:

- Complications related to any study test that requires a specific treatment or hospital admission

- Other known side effects of study medications
- Any pre-planned hospitalisations (e.g. elective surgery) not associated with clinical deterioration
- Elective or scheduled treatment for pre-existing conditions that did not worsen during the study

These events are expected within the study population and will not be subject to expedited reporting to the main REC. All non-serious or expected adverse events will be recorded on the study CRF at visits 2 and 3. They will also be included in the annual safety report provided to the main REC.

10.4.3 Related and Unexpected SAEs – Expedited Reporting

In keeping with HRA guidelines, reports of Serious Adverse Events (SAEs) or Serious Adverse Reactions (SARs) that are:

- related to the study (i.e. they resulted from administration of any of the research procedures) and
- unexpected (i.e. not listed in the protocol as an expected occurrence)

will be submitted to the REC within 15 days of the chief investigator becoming aware of the event and will be reported to the sponsor within 1 working day of the research team becoming aware of the event. This will be via email to governance-ethics@leeds.ac.uk.

Events will be followed up until the event has resolved or a final outcome has been reached.

11 STATISTICS

11.1 Description of Statistical Methods

For this randomized study, outcome measures at follow-up will be compared between groups using linear regression models, adjusted for baseline measures of the outcome. Data will be transformed if necessary to meet model distributional assumptions. Differences between randomised groups will be tested overall, and then pairwise differences between groups will be estimated separately. All analyses will be pre-specified in a detailed Statistical Analysis Plan, to be finalised prior to any data being analysed.

11.2 The Number of Participants

Previous work found the mean (SD) myocardial PCr/ATP ratio for T2D patients to be 1.58 (0.42), compared to 2.01 (0.40) for healthy controls, a difference of 0.42. We proposed that increasing the PCr/ATP for T2D patients by half of this difference (0.21) would constitute a biologically

significant effect. Assuming that both study drugs are associated with an increase of this size, and assuming the SD to be 0.42, a study with 28 participants in each group would have 90% power, based on a one-way analysis of variance at a 5% significance level. Allowing for a dropout of up to 20%, 35 patients will be recruited per group (70 in total) and randomized.

11.3 The Level of Statistical Significance

Statistical significance will be considered at P value of 5% significance and a power of 90%.

11.4 Procedure for Accounting for Missing, Unused, and Spurious Data

Spurious and missing data will lead to data from that participant being excluded from the study.

12 DATA MANAGEMENT

12.1 Data Collection Methods

All personal data is stored on the NHS server as all the participants will be NHS patients. Anonymised results will be password protected and stored on the University of Leeds server. There will be a master cipher sheet, which will be the only place where participants will be linked to their study number. This will be encrypted and password protected and stored on the NHS server. A separate study spread sheet will contain the anonymised results of the analysis of all study investigations. All data will be anonymised.

Data will be entered into a locally developed electronic database stored on the University of Leeds server. All imaging data, blood results and urine results will be entered into this database. All data will be anonymised on the electronic database.

12.2 Types of data

Clinical measurements such as the following will be collected: demographics, medical history, relevant concomitant medications, 12-lead ECG, clinical laboratory tests, and MR studies as described previously for those that participate. These data will be spread across the baseline and follow-up visits. Safety reporting will be collected as per UK legislation requirements. Other data such as administrative data regarding attendance of visits and patient status in regards to withdrawal will also be collected.

12.3 Methodologies for data collection / generation

Once data is collected, it will be entered into the study specific CRF by study investigators in accordance with the protocol requirements. Data will be held for 15 years. Paper records will be stored in a restricted access room at the University of Leeds Advanced Imaging Centre located at the Leeds General Infirmary. Digital records will be destroyed in accordance with NHS Digital

guidelines (https://digital.nhs.uk/data-and-information/looking-after-information/data-security-and-information-governance/codes-of-practice-for-handling-information-in-health-and-care).

12.4 Formal information/data security standards

Data will be secured in line with Data Protection Act 2018.

12.5 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 trial 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 trial staff and authorised personnel. The study will comply with the Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

13 SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the study protocol which is likely to affect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the study; or

(b) the scientific value of the study".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. This will be via email to governance-ethics@leeds.ac.uk. In collaboration with the Chief Investigator, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC, regulatory authority and the NHS host organisation within seven calendar days.

14 ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

14.2 Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

14.3 Approvals

Once Sponsor authorisation has been confirmed, the protocol, informed consent form,

participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), Health Research Authority (HRA) and host institution(s) for written approval.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.4 Reporting

The CI shall submit once a year throughout the clinical study, or on request, an Annual Progress Report to the REC, host organisation and Sponsor. Safety reports will also be submitted to the REC and HRA on an annual basis. In addition, an End of study notification and final report will be submitted to the REC, host organisation and Sponsor.

14.5 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.

We have found in previous studies that provision of transport and light refreshment enhances recruitment and patient experience. This is particularly important given the need for repeat imaging in this study. We will reimburse travel costs incurred by participants when attending for a MR scans.

14.6 Other Ethical Considerations

For the participants, this study would require their time and would not directly provide any benefit to the participants themselves. Participants will be reimbursed for reasonable study expenses for travel to and from study visits on production of a receipt. There will not be any payments for participating in this study. Light refreshments will be provided following all of the visits.

15 FINANCE AND INSURANCE

15.1 Funding

Funding for this study rewarded from Wellcome Trust.

15.2 Insurance

The University, when acting as Sponsor, has insurance cover in force, which meets claims against it and where those claims arise from the Universities own negligence in its role and activities relating to the study (and which is subject to the terms, conditions and exceptions of the relevant policy). Clinical negligence indemnification will rest with the participating NHS Trust under standard NHS arrangements.

16 PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. Anonymized images of the heart and blood vessels may be used in research publications. Copies of any resultant research publications will be sent to participants on request.

Appendix B: Visit Schedule

	Visit 1 (Baseline; Week 0)	Visit 2 (phone assessment for Mito-Q arm participants) (Week 8, +/- 5 days)	Visit 3 (Week 16, +/- 5 days)
Medical History	X		
Diabetes and complications history	X		
Inclusion/ Exclusion Criteria	X		
Demographic Data	X		
Vital signs	X		X
Blood Pressure	X		X
Physical examination	X		X
Height and Weight[1]	X		X
12-lead ECG	X		X
Urine Pregnancy test (if applicable)	X		
Informed Consent	X		
Blood collection[2]	X		
CMR	X		X
Randomisation	X		
Dispense study medication	X		
Issue patient diary	X		X
Check current medications	X	x	X
Check clinical status (AEs)		x	X
Diary review and collection			X

1= Weight only to be checked at Visits 2 and 3.

2= N-terminal pro–B-type natriuretic peptid (NT proBNP) and glycated haemoglobin (HbA1c).

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