The Leeds Teaching Hospitals NHS Trust

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Study Title: Ketone Supplementation to Improve Cardiac Energetics and Function

Short title: KICK-Energy Study

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No potential conflicts of interest

Confidentiality Statement

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

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1 Key Study Contacts

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2 SYNOPSIS

Study Title	**Ketone supplementation to Improve Cardiac Energetics and Function**	
Study Design	Single centre, prospective case control study.	
Study Participants	Adult T2D patients, adult heart failure patients and healthy adults.	
Planned Sample Size	30 T2D patients 30 healthy individuals with no diabetes 30 adults with non-ischemic heart failure with systolic heart failure (ejection fraction <50%)	
Planned Study Period	07/08/2022 to 01/08/2025 for 36 months	
Primary Aim	To advance our understanding of the physiological effects of ketosis on myocardial energetic impairment, perfusion and cardiac contractile function in T2D and heart failure patients.	

Objectives	**Primary objective:** Change in myocardial rest and dobutamine stress PCr/ATP following 2 weeks of oral ketone ester supplementation (delta G three times daily). **Secondary objectives:** Following oral ketone ester supplementation, changes in: 1) myocardial perfusion, 2) LVEF at rest; and during dobutamine stress; 3) Myocardial strain (systolic strain and diastolic strain rate).
Primary Endpoint	Change in myocardial energetics during rest and dobutamine stress before and after oral ketone ester supplementation.
Secondary Endpoints	Following oral ketone ester supplementation, changes in: i) myocardial rest PCr/ATP, ii) myocardial perfusion, iii) left ventricular ejection fraction (LVEF) at rest; iv) LVEF during dobutamine stress.

3 Abbreviations

AE	Adverse event
AR	Adverse reaction
ATP	Adenosine triphosphate
CF	Consent form
CI	Chief Investigator
CRF	Case Report Form
CMR	Cardiac Magnetic Resonance scan (MRI scan of the heart)
CT	Computed Tomography
DM	Diabetes Mellitus
ECG	Electrocardiogram
GCP	Good Clinical Practice
GP	General Practitioner
HbA1c	Glycated hemoglobin
FA	Fatty Acid
ICF	Informed Consent Form
LV	Left ventricle
MRS	Magnetic resonance spectroscopy
MS	Mass Spectroscopy
NHS	National Health Service
PCr	Phosphocreatine
^{31}P-MR	Phosphorus MR
PI	Principal Investigator
PIL/S	Participant/ Patient Information Leaflet/Sheet
R&I	NHS Trust R&I Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TMF	Trial Master File
TSC	Trial Steering Committee

4 Introduction

4.1 Background

Heart failure and diabetes: 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.

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[9]. Efficient matching of energy supply to demand in the heart is therefore essential for maintaining cardiac function. We have previously shown that cardiac energy levels are reduced in T2D patients and discovered that simple exercise activity exacerbates this energy starved state further[10]. Energy starvation in asymptomatic T2D patients preceded other abnormalities such as reductions in left ventricular (LV) ejection fraction or increase in LV mass [10-12]. 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[13, 14]. However, the precise etiology 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[13].

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[15]: reduced myocardial blood supply[10, 16], loss of flexibility in myocardial fuel selection[17] and impaired mitochondrial function[18]. There are also potentially beneficial metabolic alterations in the diabetic heart ameliorating this energy deficit such as enhanced ketone metabolism, which are a lot less researched but could be transformative for the prevention and treatment of heart disease in T2D.

Metabolic flexibility of the heart and the hierarchy of fuels in energy production efficiency: 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[19, 20]. 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. These fuels have different efficiencies in generating ATP and in their oxygen need during this process[21]. Consequently, altered cardiac metabolism may contribute to the development of cardiac dysfunction by affecting the myocardial oxygen demand and impairing the metabolic flexibility of the heart[21]. FAs yield more ATP per

mol when oxidized than glucose[22, 23], but this is at a greater oxygen cost, with approximately 15% more oxygen per mol of ATP synthesized[22, 24, 25]. An increased dependence on FAs relative to carbohydrates therefore decreases cardiac efficiency[26]. Moreover, ketone oxidation yields more ATP per oxygen consumption than FA oxidation (as has been shown in the case of palmitate), ketones therefore representing a more energy-efficient fuel than FAs[27]. Ketones are also more chemically reduced than pyruvate, and result in increased ETC redox span and greater ATP generation[28].

Ketosis: Friend or foe for the heart in patients with diabetes? The ketone bodies are generated by the liver from non-esterified FAs in response to energy depletion or starvation to maintain ATP generation in vital organs. By virtue of their evolutionary role, ketones conserve protein and carbohydrate stores, by substituting themselves as energy fuels[23]. Ketosis was previously feared in type 1 diabetes, being associated with life-threatening acidosis. However, in a study of T2D patients presenting with hyperglycaemic crisis, those with ketosis (but not acidosis) had lower all-cause mortality than those without[29], suggesting that ketosis may potentially be protective in diabetes. Moreover, sodium–glucose-cotransporter-2 inhibitors(SGLT2i) which induce mild ketosis as a class effect, are associated with a lower risk of HF hospitalisation in T2D patients[30],[31]. The mild ketosis associated with SGLT2i is assumed to contribute to the cellular energy restoring effects of these drugs[32-35]. Because the energetic properties of ketones are favourable, increased myocardial ketone oxidation could mitigate myocardial energy starvation in diabetes and compensate for defects in mitochondrial energy generation[27, 36].

Exogenous ketone ester: The ketone bodies, D-beta-hydro- xybutyrate (βHB) and acetoacetate, are not readily available in the human diet, but are produced in the liver from free fatty acids in response to low blood glucose and insulin levels, as an alternative to glucose as an energy supply for the brain. Until recently, blood ketones could only be raised over several days by fasting or by strictly following low-carb, high-fat diets. Similar blood βHB concentrations can now be attained within 30 min by drinking the ketone monoester, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate, which introduces a new metabolic state, termed exogenous nutritional ketosis. Interventions that induce ketosis simultaneously lower blood glucose, as a result a recent study has assessed safety, tolerability, and effects of an exogenous ketone ester supplementation on glycaemic control in 21 patients with T2D [37]. The participants consumed 25 ml of ketone ester product 3 times daily for 4 weeks. This study assessed serum electrolytes, acid-base status, and beta hydroxybutyrate concentrations weekly and cardiovascular risk markers were measured before and after the intervention. The investigators have shown that exogenous ketosis using a ketone ester product was capable of inducing fasting-like elevations in serum β-hydroxybutyrate without the need for caloric or carbohydrate restriction. Importantly constant ketone ester consumption over 1 month was safe, well tolerated, and improved glycaemic control in patients with T2D. Safety of the same ketone ester product has also been shown in healthy volunteers[38]. The only side effect detected in both studies was mild nausea.

While oral supplementation was not assessed in heart failure patients previously, another study assessed higher doses of ketone infusion in heart failure patients and demonstrated its safety[39]

^{31}Phosphorus Magnetic Resonance Spectroscopy and Myocardial Energetics Magnetic resonance spectroscopy (MRS) is the only noninvasive, non-radiation exposure technique for the investigation of

cardiac metabolism *in vivo*, without the use of an external tracer. Maintenance of adequate levels of cardiac high-energy phosphate metabolites, ATP, the energy source for contraction, and phosphocreatine (PCr), the major energy storage compound, are of vital importance for normal heart function. The relative concentration of PCr to ATP (PCr/ATP) 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]. ^{31}P-MRS yields peaks for PCr and the three phosphorus atoms of ATP, all of which are proportional to the cellular concentration of the metabolites. The primary energy reserve compound in the heart is PCr and the enzyme creatine kinase allows the transfer of the high-energy phosphate bond between ATP and PCr, through the phosphotransferase reactions, in order to diffuse energy from the mitochondria to the myofibrils as PCr[41]. The CK energy shuttle ensures thermodynamic control of the cell, maintaining a low concentration of free adenosine diphosphate near the mitochondria, and providing ATP to the myofibrils for muscle contraction. When demand for ATP outweighs ATP synthesis, PCr levels fall, resulting in a low PCr to ATP ratio. Decreased PCr/ATP ratio is a predictor of mortality[42], linked to contractile dysfunction[41, 42], and is a recognized complication of diabetes[43, 44].

Cardiovascular Magnetic Resonance (CMR) and Myocardial Perfusion and Function CMR allows detailed characterization of the cardiac phenotype, and it is the reference standard technology for assessment of cardiac structural and functional changes. CMR is frequently utilised to determine disease pathophysiology, and it is the only imaging modality that can assess non-invasively cardiac function, ischaemia, perfusion and fibrosis[11]. The latest technology for myocardial perfusion CMR allows fully automated analysis with perfusion values that are in close correlation with the reference standards of Positron Emission Tomography and microspheres[45].

4.2 Hypothesis

This study will test the hypothesis that ketones improve myocardial energetics and contractile function.

5 Objectives and Outcome Measures

Overall aim: This study aims to advance our understanding of the physiological effects of ketosis on myocardial energetic impairment, perfusion, and cardiac contractile function in T2D patients and in heart failure patients.

5.1 Primary Objectives:

Change in myocardial rest and dobutamine stress PCr/ATP after 2 weeks of oral ketone ester supplementation.

5.2 Secondary Objectives:

Following 2 weeks of oral ketone ester supplementation, change in:

1) Myocardial perfusion,

2) LVEF at rest; and during dobutamine stress;

3) Myocardial strain (systolic strain and diastolic strain rate).

6 Study Design

Study design: Single centre, prospective case controls study.

Study duration: The study will run over 36 months. Each participant will remain in the study for a maximum of 4 weeks. We will schedule the second hospital visit (which is also the final hospital visit) within 2 week from the first hospital visit.

Setting: Leeds General Infirmary, University of Leeds

Study population: 30 T2D patients, 30 adults with systolic heart failure diagnosis, 30 healthy adults.

7 Study Participants

30 adult T2D patients and 30 adults with systolic heart failure diagnosis of non-ischemic etiology with LV ejection fraction <50% will be recruited. In addition, 30 healthy individuals will be recruited for the control group. Diabetes patients may receive any type of glucose lowering treatment or be treated with diet and exercise alone for at least 12 weeks prior to study.

7.1 Inclusion and Exclusion Criteria- T2D Participants

Inclusion criteria:

1) Men and women > 18 years of age;
2) 6.0≤HBA1c≤10% at screening;
3) Ability and willingness to provide written informed consent and to comply with the requirements of the study.

Exclusion criteria:

1) History of coronary artery disease, previous CABG, angioplasty or myocardial infarction;

2) Known HF or reduced LVEF on baseline CMR (<50%);
3) Female participants who are pregnant, lactating or planning pregnancy during the course of the study;
4) Renal impairment (eGFR<30ml/min/m2);
5) Participation in a clinical trial in the preceding 12 weeks;
6) Contra-indications to CMR or to dobutamine or gadolinium;
7) Any type of diabetes other than T2D.

7.2 Inclusion and Exclusion Criteria- Healthy Volunteers

Inclusion criteria:

1) Men and women >18 years of age;
2) Mean HBA1c≤6%;
3) Ability and willingness to provide written informed consent and to comply with the requirements of the study.

Exclusion criteria:

1) Any type of diabetes;
2) Significant renal impairment (eGFR<30ml/min/m2);
3) Female participants who are pregnant, lactating or planning pregnancy during the course of the study;
4) Participation in a clinical trial of an investigational medicinal product in the preceding 12 weeks;
5) Contra-indications to MRI;
6) Known hypersensitivity to dobutamine or gadolinium;
7) History of coronary artery disease, previous CABG, angioplasty or myocardial infarction;
8) Known HF or reduced LVEF on baseline CMR (<50%).

7.3 Inclusion and Exclusion Criteria- Adults with Heart Failure with and without T2D

Inclusion criteria:

1) Men and women >18 years of age;
2) Ability and willingness to provide written informed consent and to comply with the requirements of the study;
3) Prior diagnosis of non-ischemic heart failure

4) LV ejection fraction of <50% on prior CMR or echocardiography scans
5) For participants with T2D: 6.0≤HBA1c≤10% at screening

Exclusion criteria:

1) Significant renal impairment (eGFR<30ml/min/m2);
2) Female participants who are pregnant, lactating or planning pregnancy during the course of the study;
3) Participation in a clinical trial of an investigational medicinal product in the preceding 12 weeks;
4) Contra-indications to MRI;
5) Known hypersensitivity to dobutamine or gadolinium;
6) History of coronary artery disease, previous CABG, angioplasty or myocardial infarction;
7) Any type of diabetes other than T2D.

7.4 Recruitment

There are 3 recruitment pathways in place:

1. With assistance from the NIHR Yorkshire and Humber Clinical Research Network (CRN), the study team will recruit participants with T2D, healthy volunteers and heart failure patients (with or without 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 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 Hospitals 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 Pathway Manager (PPM) and/ or Patient Administration System (PAS)

containing up-to-date information regarding deceased patients prior to contacting in order to be contacted if eligible to take part in other studies.

3. The heart failure volunteers will be recruited by study team at LTHT from the previous ethically approved studies in the department and that have consented to be have their contact details to be retained to be contacted if eligible to take part in further studies. Heart failure patients may also be recruited from appropriate clinics at LTHT.

7.5 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.

8 Methodology

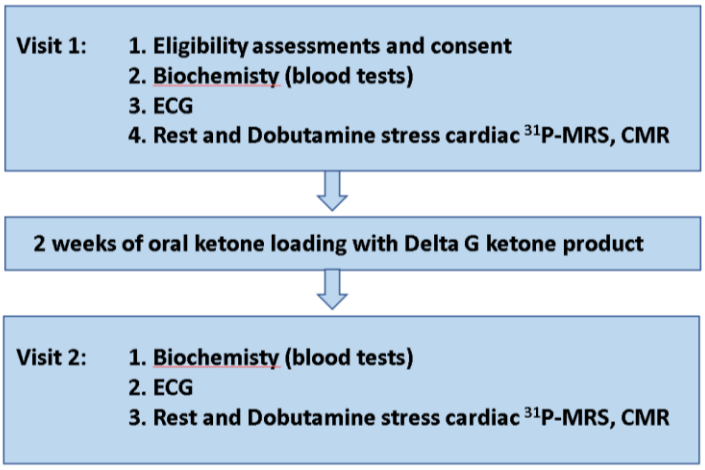
8.1 Study Visits

The assessments listed below will be carried out at each visit in the Advanced Imaging Centre at the Leeds General Infirmary. All participants, including the ones with T2D and heart failure, will continue taking their previously prescribed medications throughout the study.

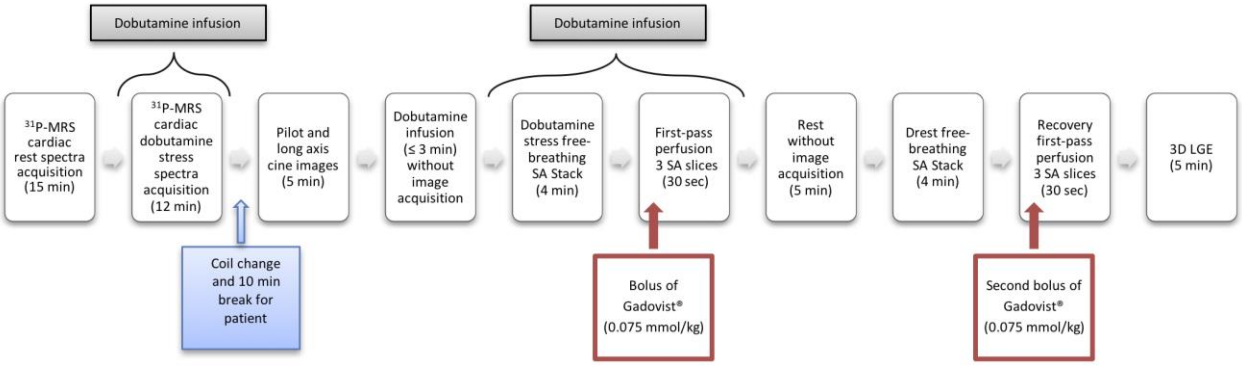
Participants will be asked to attend 2 hospital visits. To minimise the inconvenience of hospital visits we will arrange transport. The first study visit will take up to 2.5 hours and the second visit will take up to 2.5 hours as well but 2 weeks later following adequate oral ketone loading. Participants will not need to fast for the visits.

Asymptomatic T2D patients with no history of heart disease, non- ischemic heart failure patients with LV ejection fraction <50% and healthy controls will undergo ^{31}P-MRS and CMR scans over two visits (see study

flowchart below).



Visit 1: All participants will undergo CMR and ^{31}P-MRS studies at 3.0Tesla for evaluation of rest and dobutamine stress myocardial energetics, perfusion and function (see scan protocol below). In order to study the impact of ketosis on the myocardial energetic response to acute increases in cardiac workload, MR experiments will be conducted at rest and during haemodynamic stress (dobutamine stress). Blood samples will be obtained for measuring the levels of plasma beta-hydroxybutyrate, acetoacetate, and glucose.



Visit 2: ^{31}P-MRS and CMR studies will be repeated after oral ketone supplementation to assess the impact of ketosis on myocardial energy metabolism, perfusion and function.

The blood ketone level will be assessed and recorded on arrival. Ketosis will then be achieved via oral supplementation with ketone ester product Delta- G (BHB,TΔS® Ltd), for 2 weeks three times daily between the 2 study visits. This product has been developed for commercial use by a University of Oxford spinoff company, TΔS® Ltd and is available for use over the counter. The kinetics, safety and tolerability study of this product in healthy adults and in T2D patients suggested favourable results apart from mild gastrointestinal upset at higher doses[37, 38]. While oral supplementation was not assessed in heart failure patients previously, another study assessed higher doses of ketone infusion in heart failure patients and demonstrated its safety[39].

Baseline Assessments/ Visit 1:

- Review of medical history and concomitant medications
- Review of history of diabetes and complications
- Review of inclusion/exclusion criteria
- Collection of demographic data (sex, ethnicity, age)
- Urine pregnancy test in women of childbearing potential
- Written informed consent
- Height, weight, waist, and hip circumferences
- 12-lead ECG
- Resting heart rate and blood pressure
- Venepuncture: 20mls of blood sample will be taken while inserting 2 venous lines (one line for dobutamine infusion and the other one for MRI contrast injection). This sample will be used for assessing blood ketone and glucose levels
- Multiparametric MRI
- Distribute 2 weeks supply of 25ml three times daily oral ketone ester product (Delta- G) supplementation
- Issue diaries and ketone finger prick test kits. The investigators will demonstrate how to perform the finger prick tests during this visit. In addition, the investigators will discuss how to keep the diary and answer any questions participants may have with regards to these tasks.

Study Visits:

Visit 2:

- 12-lead ECG
- Resting heart rate and blood pressure
- Venepuncture: 20mls and 2 venous lines (one line for dobutamine infusion and the other for MRI contrast injection).
- Small blood sample will be taken assess blood ketone and glucose levels
- Multiparametric MRI
- Collect and review diaries

End of the study.

Participants will be asked to self-administer a ketone finger prick test once a week at home while taking the ketone ester drink. They will be instructed to do this 1 week after starting

the drinks, taking a reading immediately before and 30 minutes following the third drink on their least active day.

8.2 Study Assessments

8.2.1 Blood Tests and Urine Pregnancy Test

Blood will be taken via venipuncture at visit 1 and visit 2. At Visits 1 and 2, the levels of plasma lipids, insulin, beta-hydroxybutyrate, acetoacetate, and glucose will be measured. At Visit 1, U&Es and HbA1c will also be measured. These blood tests are purely for research purposes. No additional interventions should be needed due to the results and participants will adhere to their usual treatment plans (if applicable).

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

8.2.2 Multiparametic MRI

^{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[46-48].

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, 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 c. 1 hour.

With participant's permission, CMR results will be shared with the participant's GP for the T2D and heart failure patients and the healthy volunteers. 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.

Any other incidental findings picked up on the scans not concerning the heart will be reported to the GP for further investigation if required.

8.2.3 Ketone Ester Drink Storage & Distribution

The ketone ester drinks are stored at room temperature. Stocks of the drink will be kept in a storage area in the Advanced Imaging Centre. Participants will be given a 2 week supply of the drink at their first study visit.

8.2.4 Potential risks and hazards

MR is safe, with no known risks. The contrast medication is safe, but rarely reactions may occur. The department is equipped to cope with allergic reactions if they happen. Dobutamine can cause breathlessness and chest discomfort, subsiding within minutes of the medication being stopped. Oral Ketone supplements are safe and should not cause any significant side effects at the recommended doses. Mild nausea might be experienced by the participants, they will therefore be fully informed of this potential side effect. Participants will be provided 24hour access to investigators and will be asked to contact the study team directly should they experience this side effect. The investigators, who are medically qualified NHS doctors, will organise an urgent review (within 24 hours) and assess the participants with physical examination. Participants will be asked to hold the next dose of ketone ester until they have been reviewed. Patients will be monitored and cardiology team will be present during the CMR studies. The oral ketones have been safely administered to patients with diabetes. While oral supplementation was not assessed in heart failure patients previously, another study assessed higher doses of ketone infusion in heart failure patients and demonstrated its safety[39].

Ketones are naturally produced energy fuels by the liver. Ketones have been safely delivered per orally with no complications in healthy volunteers and intravenously even in patients with severe heart failure[39, 49]. Ketone will be delivered via per oral administration for a course of 2 weeks in the form of a ketone ester drink three times daily. No side effects are expected at this dose and duration in the patients or controls.

During the scan acquisitions, participants will also hold a buzzer in their dominant hand and will be able to use the buzzer to attract attention immediately for any reason while in the scanner. The study investigators are all medically qualified stuff (consultant cardiologists or cardiology trainees and nurses) who will be present at all times during the research visits, which will be conducted in an hospital environment.

The exact nature of the study and the risks involved in taking part will be explained to participants. They will have the option to leave the study at any point, and will be given the contact details of study investigators to share any concerns they may have. The study will be conducted in full conformance with Good Clinical Practice, and NHS indemnity will operate.

8.3 Image Analysis

All imaging will be analysed blinded to patient details. Images will be allocated a computer-generated random number, the key to which will not be accessible to staff involved in the analysis until all analyses have been completed and the database has been locked.

^{31}P-MR Spectroscopy: ^{31}P-MRS post processing analysis will be performed using Matlab version R2012a (Mathworks, Natick, Massachusetts) as previously described[50].

Cardiac Volumes and Function: CMR data will be assessed quantitatively using commercially available software (CVI42, Circle Cardiovascular Imaging Inc, Calgary, Canada). Epicardial and endocardial borders will be traced offline on the LV cine stack at end-diastole and end-systole to calculate end-diastolic, end-systolic LV volumes, stroke volume, ejection fraction, and LV mass, as previously described[51]. Feature tracking analysis will be performed using cvi42. The peak systolic circumferential strain, global longitudinal strain and diastolic strain rate data will be measured.

Myocardial Perfusion: The endo- and epicardial borders for the basal, mid-ventricular and apical short-axis images will be manually delineated both at rest and stress. Similar to oxygenation analysis, the myocardium will be divided into equiangular segments on the basis of the American Heart Association segmentation model. Myocardial perfusion (MP) in ml/min/g will be assessed globally (average myocardial perfusion for 16 segments). MP reserve will also be calculated, defined as the ratio between MP at stress over rest. In-line automatic reconstruction and post-processing of the perfusion data will be implemented within the Gadgetron software framework[45]. Images will be motion corrected and then corrected for surface coil intensity variation based on the proton density weighted images. Signal intensity data will be converted to Gadolinium concentration based on automatically generated look-up tables. AIF data will be extracted from the low-resolution Gadolinium concentration images using automated segmentation of the LV cavity. Myocardial blood flow will be calculated on a pixel-wise basis in the high-resolution images by blood tissue exchange model constrained deconvolution incorporating estimation of the delay time between bolus arrival in the LV cavity and the tissue of interest.

Late gadolinium Imaging: For LGE analysis, areas of contrast enhancement will be visually scored as absent or present by consensus of 2 experienced operators. LGE will be considered present only if myocardial enhancement was confirmed on both short-axis and matching long-axis locations.

8.4 Discontinuation/Withdrawal of Participants

Each participant has the right to withdraw from the trial at any time. In addition, the Investigator may discontinue a participant from the trial 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
- An adverse event to gadolinium contrast or dobutamine injection during the baseline visit
- Withdrawal of Consent

Where patients wish to withdraw from the study, clarification of the extent of withdrawal will be sought and documented in the CRF. Patients who withdraw from the study will be replaced.

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 stabilised.

8.5 Definition of End of Study

The end of trial is the date of the last visit of the last participant.

9 Safety Reporting

9.1 Definitions

Adverse Event (AE)

An AE or adverse event is:

Any untoward medical occurrence in a patient or clinical investigation participants, which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study, whether or not considered related to the study.

Serious Adverse Event

A serious adverse event is any untoward medical occurrence that:

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

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.

*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 patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

An SAE occurring to a research participant, which in the opinion of the Chief Investigator is Related and Unexpected will be reported to the main Research Ethics Committee (REC). Related and Unexpected SAEs are defined as: i) Related: it resulted from administration of any research procedures; and ii) Unexpected: the type of event is not listed in the protocol as an expected occurrence.

9.2 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 causal relationship to study procedures (i.e. definitely, probably or possibly related) are considered to be adverse events. 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 conducting study procedures). 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 conducting study procedures). 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.

9.3 Severity

Severity of all AEs will be graded on a three-point scale of intensity (mild, moderate or 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 may be severe but not serious. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

9.4 Expected AEs & SAEs- Not Reportable

Due to the nature of T2D and heart failure, participants with this condition may experience adverse events during the course of their disease.

9.5 Expected AEs & SAEs- Reportable

The following events are expected within the study population and will be reported by the clinical research team using standardised Case Report Forms (CRFs).

T2D Patients:

- Hypoglycaemia
- Routine treatment or monitoring of the studied indication (T2D) not associated with any deterioration in condition

Heart Failure Patients:

- Exertional breathlessness (these are controlled heart failure patients under the care of heart failure team, therefore they are expected to be well managed by the clinical team)
- Routine treatment or monitoring of the studied indication (HF) not associated with any deterioration in condition

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. In keeping with HRA guidelines, reports of Serious Adverse Events (SAEs) that are related and unexpected will be submitted to the REC using the HRA Non-CTIMP safety report to REC form 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.

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

9.6 Procedures for Recording Adverse Events

The patient information sheets will include all possible adverse events relevant to MR scans and ketone supplementation. Potential risks are outlined in section 8.2.4.

All AEs occurring during the study that are observed by the Investigator or reported by the participant, will be recorded on the CRF, whether or not attributed to study investigations.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the study assessments as judged by a medically qualified investigator or the Sponsor will be followed either until resolution or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

9.7 Reporting Procedures for Serious Adverse Events

In keeping with HRA guidelines, reports of Serious Adverse Events (SAEs) that are related and unexpected will be submitted to the REC using the HRA Non-CTIMP safety report to REC form 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. Events will be followed up until the event has resolved or a final outcome has been reached.

10 Description of Statistical Methods

Statistical Analysis: Non-normally distributed data will be transformed to achieve normality prior to analysis, where possible. Bivariate correlations will be performed using Pearson's or Spearman's method, as appropriate. Two-tailed paired *t*-test will be used for: i) Comparisons between rest and stress energetics in patients with and without oral ketone ester supplementation; ii) Comparisons between rest and stress myocardial flow in patients with and without oral ketone ester supplementation; iii) Comparisons between rest and stress LV EF in patients with and without oral ketone ester supplementation.

Power Calculations: Leading on from this previous work, the current proposal aims to advance our understanding of the link between myocardial insulin resistance and rest and haemodynamic stress energetics, perfusion and contractile function in patients with T2D and in patients with heart failure with reduced ejection fraction. Our previous work(40) showed the mean (SD) PCr/ATP ratio with exercise for

T2D patients to be 1.54 (0.26), compared to 2.07 (0.35) for healthy controls, a difference of 0.53. While we do not have pilot data for the effect of ketone supplementation on myocardial energetics in T2D patients or in HF patients, we propose that increasing the PCr/ATP ratio for T2D patients by one third of this difference (0.18) would constitute a biologically significant effect. Assuming that dobutamine stress energetics with ketone supplementation is associated with an increase from the baseline dobutamine stress energetics of this size, and assuming the SD to be 0.26 as in our previous studies, a group size of 25 would have 90% power, based on a one-way analysis of variance at a 5% significance level. Allowing for a conservative loss to follow-up, we shall recruit 30 T2D patients and 30 patients with heart failure with reduced ejection fraction. 30 healthy controls will be recruited to compare the impact of oral ketone supplementation in patients with diabetes or in patients with heart failure to healthy controls.

10.1 The Level of Statistical Significance

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

10.2 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.

10.3 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any deviation(s) from the original statistical plan will be described and justified in the final report, as appropriate.

10.4 Local Steering Committee

A local study steering committee will be set up (Dr Eylem Levelt, Prof Sven Plein, Prof John Greenwood, Prof Richard Cubbon) responsible for monitoring of screening, recruitment, auditing consent procedures, data collection, study end-point validation. This committee will meet biannually and assess progress against intermediate bench marks, milestones, and time-based deliverables including:

1. Recruitment to date.
2. Multiparametric MR analysis.
3. Data auditing.
4. Dissemination of the findings to academic audiences.
5. Dissemination of research to the diabetes community.

11 DATA MANAGEMENT

All personal data is stored on the NHS server. 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.

11.1 Types of data

Clinical measurements such as the following will be collected: demographics, medical history, relevant concomitant medications, 12-lead ECG, clinical laboratory tests, MR study results for patients with diabetes and heart failure and healthy volunteers as described previously for those that participate. These data will be spread across all 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.

11.2 Methodologies for data collection / generation

Once data is collected, it will be entered into the study specific CRF by site staff in accordance with the protocol requirements. Data will be held for 15 years.

11.3 Formal information/data security standards

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

11.4 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.

12 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. 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, and the NHS host organisation within seven calendar days.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 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).

13.2 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.

13.3 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.

13.4 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. In addition, an End of Trial notification and final report will be submitted to the REC, host organisation and Sponsor.

13.5 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.

13.6 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.

14 FINANCE AND INSURANCE

14.1 Funding

Funding for this study awarded by Wellcome Trust.

14.2 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.

15 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.

16 REFERENCES

References

1. Kannel WB and McGee DL. Diabetes and cardiovascular disease: The framingham study. *JAMA*. 1979;241:2035-2038.

2. WHO. Fact sheet no 310; the top 10 causes of death, updated May 2014. *World Health Organization, Geneva*.

3. Shah A.D. LC, Rapsomaniki E., et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1.9 million people. Lancet Diabetes Endocrinol. 2015;3(2):105–113.

4. Nichols GA, Gullion CM, Koro CE, Ephross SA and Brown JB. The Incidence of Congestive Heart Failure in Type 2 Diabetes. *An update*. 2004;27:1879-1884.

5. Maack C, Lehrke M, Backs J, Heinzel FR, Hulot J-S, Marx N, Paulus WJ, Rossignol P, Taegtmeyer H, Bauersachs J, Bayes-Genis A, Brutsaert D, Bugger H, Clarke K, Cosentino F, De Keulenaer G, Dei Cas A, González A, Huelsmann M, Iaccarino G, Lunde IG, Lyon AR, Pollesello P, Rena G, Riksen NP, Rosano G, Staels B, van Laake LW, Wanner C, Farmakis D, Filippatos G, Ruschitzka F, Seferovic P, de Boer RA and Heymans S. Heart failure and diabetes: metabolic alterations and therapeutic interventions: a state-of-the-art review from the Translational Research Committee of the Heart Failure Association–European Society of Cardiology. *European Heart Journal*. 2018;39:4243-4254.

6. Okin Peter M, Devereux Richard B, Harris Katherine E, Jern S, Kjeldsen Sverre E, Lindholm Lars H and Dahlöf B. In-Treatment Resolution or Absence of Electrocardiographic Left Ventricular Hypertrophy Is Associated With Decreased Incidence of New-Onset Diabetes Mellitus in Hypertensive Patients. *Hypertension*. 2007;50:984-990.

7. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving H-H, Remuzzi G, Snapinn SM, Zhang Z and Shahinfar S. Effects of Losartan on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Nephropathy. *New England Journal of Medicine*. 2001;345:861-869.

8. Hippisley-Cox J and Coupland C. Diabetes treatments and risk of heart failure, cardiovascular disease, and all cause mortality: cohort study in primary care. *BMJ*. 2016;354.

9. Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS and Stanley WC. Myocardial Fatty Acid Metabolism in Health and Disease. *Physiological Reviews*. 2010;90:207-258.

10. Levelt E, Rodgers CT, Clarke WT, Mahmod M, Ariga R, Francis JM, Liu A, Wijesurendra RS, Dass S, Sabharwal N, Robson MD, Holloway CJ, Rider OJ, Clarke K, Karamitsos TD and Neubauer S. Cardiac energetics, oxygenation, and perfusion during increased workload in patients with type 2 diabetes mellitus. *European Heart Journal*. 2015.

11. Levelt E, Mahmod M, Piechnik SK, Ariga R, Francis JM, Rodgers CT, Clarke WT, Sabharwal N, Schneider JE, Karamitsos TD, Clarke K, Rider OJ and Neubauer S. Relationship between Left Ventricular Structural and Metabolic Remodelling in Type 2 Diabetes Mellitus. *Diabetes*. 2015.

12. Levelt E, Pavlides M, Banerjee R, Mahmod M, Kelly C, Sellwood J, Ariga R, Thomas S, Francis J, Rodgers C, Clarke W, Sabharwal N, Antoniades C, Schneider J, Robson M, Clarke K,

Karamitsos T, Rider O and Neubauer S. Ectopic and Visceral Fat Deposition in Lean and Obese Patients With Type 2 Diabetes. *Journal of the American College of Cardiology*. 2016;68:53-63.

13. von Hardenberg A and Maack C. Mitochondrial Therapies in Heart Failure. In: J. Bauersachs, J. Butler and P. Sandner, eds. *Heart Failure* Cham: Springer International Publishing; 2017: 491-514.

14. Pillai VB, Samant S, Sundaresan NR, Raghuraman H, Kim G, Bonner MY, Arbiser JL, Walker DI, Jones DP, Gius D and Gupta MP. Honokiol blocks and reverses cardiac hypertrophy in mice by activating mitochondrial Sirt3. *Nature Communications*. 2015;6:6656.

15. Young ME, McNulty P and Taegtmeyer H. Adaptation and Maladaptation of the Heart in Diabetes: Part II: Potential Mechanisms. *Circulation*. 2002;105:1861-1870.

16. Bratis K, Child N, Terrovitis J, Nanas J, Felekos I, Aggeli C, Stefanadis C, Mastorakos G, Chiribiri A, Nagel E and Mavrogeni S. Coronary microvascular dysfunction in overt diabetic cardiomyopathy. *IJC Metabolic & Endocrine*. 2014;5:19-23.

17. Ungar I, Gilbert M, Siegel A, Blain JM and Bing RJ. Studies on myocardial metabolism: IV. Myocardial metabolism in diabetes. *The American Journal of Medicine*. 1955;18:385-396.

18. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ and Neufer PD. Substrate-Specific Derangements in Mitochondrial Metabolism and Redox Balance in Atrium of Type 2 Diabetic Human Heart. *Journal of the American College of Cardiology*. 2009;54:1891-1898.

19. Heather LC and Clarke K. Metabolism, hypoxia and the diabetic heart. *Journal of Molecular and Cellular Cardiology*. 2011;50:598-605.

20. Taegtmeyer H. Cardiac Metabolism as a Target for the Treatment of Heart Failure. *Circulation*. 2004;110:894-896.

21. Evans RD CK. Myocardial substrate metabolism in heart disease. *Front Biosci (Schol Ed)*. 2012;4:556-80.

22. Stanley WC and Chandler MP. Energy Metabolism in the Normal and Failing Heart: Potential for Therapeutic Interventions. *Heart failure reviews*. 2002;7:115-130.

23. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, leukotrienes, and essential fatty acids*. 2004;70:309-19.

24. Nagoshi T, Yoshimura M, Rosano GMC, Lopaschuk GD and Mochizuki S. Optimization of Cardiac Metabolism in Heart Failure. *Current Pharmaceutical Design*. 2011;17:3846-3853.

25. Mjøs OD. Effect of Free Fatty Acids on Myocardial Function and Oxygen Consumption in Intact Dogs. *The Journal of Clinical Investigation*. 1971;50:1386-1389.

26. Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, Han X, Gross RW, Kozak R, Lopaschuk GD and Kelly DP. The cardiac phenotype induced by PPARα overexpression mimics that caused by diabetes mellitus. *The Journal of Clinical Investigation*. 2002;109:121-130.

27. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2004;70:309-319.

28. Sato K, Kashiwaya Y, Keon C, Tsuchiya N, King M, Radda G, Chance B, Clarke K and Veech R. Insulin, ketone bodies, and mitochondrial energy transduction. *The FASEB journal*. 1995;9:651-658.

29. Kruljac I, Cacic M, Cacic P, Ostojic V, Stefanovic M, Sikic A and Vrkljan M. Diabetic ketosis during hyperglycemic crisis is associated with decreased all-cause mortality in patients with type 2 diabetes mellitus. *Endocrine*. 2016.

30. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC and Inzucchi SE. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *New England Journal of Medicine*. 2015;373:2117-2128.

31. Tahara A, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, Takasu T, Imamura M, Li Q, Tomiyama H, Kobayashi Y, Noda A, Sasamata M and Shibasaki M. Effects of sodium-glucose cotransporter 2 selective inhibitor ipragliflozin on hyperglycaemia, oxidative stress, inflammation and liver injury in streptozotocin-induced type 1 diabetic rats. *Journal of Pharmacy and Pharmacology*. 2014;66:975-987.

32. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR and Muscelli E. Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes*. 2016;65:1190-5.

33. Mudaliar S, Alloju S and Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis. *Diabetes Care*. 2016;39:1115-22.

34. Ferrannini E, Mark M and Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A “Thrifty Substrate” Hypothesis. *Diabetes Care*. 2016;39:1108.

35. Mudaliar S, Alloju S and Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis. *Diabetes Care*. 2016;39:1115.

36. Sato K, Kashiwaya Y, Keon CA, Tsuchiya N, King MT, Radda GK, Chance B, Clarke K and Veech RL. Insulin, ketone bodies, and mitochondrial energy transduction. *FASEB J*. 1995;9:651-8.

37. Soto-Mota A, Norwitz NG, Evans R, Clarke K and Barber TM. Exogenous ketosis in patients with type 2 diabetes: Safety, tolerability and effect on glycaemic control. *Endocrinology, Diabetes & Metabolism*. 2021;4:e00264.

38. Soto-Mota A, Vansant H, Evans RD and Clarke K. Safety and tolerability of sustained exogenous ketosis using ketone monoester drinks for 28 days in healthy adults. *Regulatory Toxicology and Pharmacology*. 2019;109:104506.

39. Nielsen R, Møller N, Gormsen Lars C, Tolbod Lars P, Hansson Nils H, Sorensen J, Harms Hendrik J, Frøkiær J, Eiskjaer H, Jespersen Nichlas R, Mellemkjaer S, Lassen Thomas R, Pryds K, Bøtker Hans E and Wiggers H. Cardiovascular Effects of Treatment With the Ketone Body 3-Hydroxybutyrate in Chronic Heart Failure Patients. *Circulation*. 2019;139:2129-2141.

40. Neubauer S. The Failing Heart — An Engine Out of Fuel. *New England Journal of Medicine*. 2007;356:1140-1151.

41. Ingwall JS. Energy metabolism in heart failure and remodelling. *Cardiovascular research*. 2009;81:412-419.

42. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS and Kochsiek K. Myocardial Phosphocreatine-to-ATP Ratio Is a Predictor of Mortality in Patients With Dilated Cardiomyopathy. *Circulation*. 1997;96:2190-2196.

43. Shivu GN, Phan TT, Abozguia K, Ahmed I, Wagenmakers A, Henning A, Narendran P, Stevens M and Frenneaux M. Relationship Between Coronary Microvascular Dysfunction and Cardiac Energetics Impairment in Type 1 Diabetes Mellitus. *Circulation*. 2010;121:1209-1215.

44. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S and Clarke K. Abnormal Cardiac and Skeletal Muscle Energy Metabolism in Patients With Type 2 Diabetes. *Circulation*. 2003;107:3040-3046.

45. Kellman P, Hansen MS, Nielles-Vallespin S, Nickander J, Themudo R, Ugander M and Xue H. Myocardial perfusion cardiovascular magnetic resonance: optimized dual sequence and reconstruction for quantification. *Journal of Cardiovascular Magnetic Resonance*. 2017;19:43.

46. Tyler DJ, Emmanuel Y, Cochlin LE, Hudsmith LE, Holloway CJ, Neubauer S, Clarke K and Robson MD. Reproducibility of 31P cardiac magnetic resonance spectroscopy at 3 T. *NMR in biomedicine*. 2009;22:405-13.

47. Dass S, Cochlin L, Holloway C, Suttie J, Johnson A, Tyler D, Watkins H, Robson M, Clarke K and Neubauer S. Development and validation of a short 31P cardiac magnetic resonance spectroscopy protocol. *Journal of Cardiovascular Magnetic Resonance*. 2010;12:P123.

48. Stoll V, Clarke WT, Levelt E, Myerson SG, Robson MD, Neubauer S and Rodgers C. 7T versus 3T phosphorous magnetic resonance spectroscopy in patients with dilated cardiomyopathy. *Journal of Cardiovascular Magnetic Resonance*. 2015;17:P249.

49. Cameron D, Soto-Mota A, Willis DR, Ellis J, Procter NEK, Greenwood R, Saunders N, Schulte RF, Vassiliou VS, Tyler DJ, Schmid AI, Rodgers CT, Malcolm PN, Clarke K, Frenneaux MP and Valkovic L. Evaluation of Acute Supplementation With the Ketone Ester (R)-3-Hydroxybutyl-(R)-3-Hydroxybutyrate (deltaG) in Healthy Volunteers by Cardiac and Skeletal Muscle (31)P Magnetic Resonance Spectroscopy. *Front Physiol*. 2022;13:793987.

50. Lucian AB Purvis, William T. Clarke, Luca Biasiolli, Robson MD and CT R. Linewidth constraints in Matlab AMARES using per-metabolite T2 and per-voxel ΔB0. *ISMRM*. 2014.

51. Karamitsos T, Hudsmith L, Selvanayagam J, Neubauer S and Francis J. Operator induced variability in left ventricular measurements with cardiovascular magnetic resonance is improved after training. *J Cardiovasc Magn Reson*. 2007:777-783.

Appendix C: Amendment History

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	09 Mar 2023	Kathryn Richards and Eylem Levelt	Addition of U&E, HbA1c, lipid and insulin testing.