

**The role of Magnetic Resonance Imaging and  
Spectroscopy in the Assessment of Disease  
Pathophysiology in Patients with Type 2  
Diabetes Mellitus**

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Submitted in accordance with the requirements for the degree of Doctor of  
Philosophy



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# **Declarations**

I confirm that the work submitted is my own, except where work which has formed part of jointly authored publications has been included. My contribution and that of other authors to this work has been explicitly indicated below. I confirm that appropriate credit has been given within the thesis where reference has been made to the work of others. The following chapters are based on work from jointly authored publications-

## **Chapter 3: Prospective longitudinal characterization of the relationship between diabetes and cardiac structural and functional changes**

Prospective Longitudinal Characterization of the Relationship between Diabetes and Cardiac Structural and Functional Changes. Chowdhary A, Jex N, Thirunavukarasu S, MacCannell A, Haywood N, Almutairi A, Athithan L, Jain M, Craven T, Das A, Sharrack N, Saunderson CED, Sengupta A, Roberts L, Swoboda P, Cubbon R, Witte K, Greenwood J, Plein S, Levelt E. *Cardiol Res Pract.* 2022 Feb 8; 2022:6401180. doi: 10.1155/2022/6401180.

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## **Chapter 4: Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes**

Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes. Chowdhary A, Thirunavukarasu S, Jex N, Coles L, Bowers C, Sengupta

A, Swoboda P, Witte K, Cubbon R, Xue H, Kellman P, Greenwood J, Plein S, Levelt E. *Obesity* (Silver Spring). 2022 May;30(5):1079-1090. doi: 10.1002/oby.23413.

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*Chapter 5: Cardiac adaptations to acute hemodynamic stress in function, perfusion and energetics in type 2 diabetes with overweight/obesity*

Cardiac Adaptations to Acute Hemodynamic Stress in Function, Perfusion, and Energetics in Type 2 Diabetes with Overweight and Obesity. Chowdhary A, Javed W, Thirunavukarasu S, Jex N, Kotha S, Kellman P, Swoboda, P, Greenwood J, Plein S and Levelt, E. *Diabetes Care*. 2022 Dec 1;45(12):e176-e178. doi: 10.2337/dc22-0887.

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*I dedicate this work to my wonderful children Viraj and Vanya.*

*You have made me stronger, happier and more patient.*

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# **COVID-19 Impact Statement**

Unfortunately, the COVID-19 pandemic had an adverse impact on my research. As part of the response to the pandemic, I was redeployed back to my clinical duties as a Cardiology registrar from April 2020 to August 2020. Due to the nationwide lockdown, study visits also did not take place and research recruitment was halted for five months from March 2020 to August 2020. There was a Trust-wide pause on all non-COVID-19 research which was lifted gradually in stages from August 2020. My wife was pregnant at that point, and she had to go into shielding to protect her health and that of our then unborn child.

As a result of this, many participants could not be called back for their second visits for chapter 3. In addition, there was a significant delay in the approvals required from the Trust and ethics committees for chapter 6. This resulted in a delay in the start of the recruitment and research visits for this study. Once the lockdown was lifted and study visits were permitted, the T2D population remained apprehensive about visiting hospitals for research visits in view of the risks of contracting the infection. As a research group we tried various methods to mitigate the impact of this on our research. During the lockdown I was able to get remote access to my data enabling me to keep up to date with the statistical and scan analysis of my participants. I started writing up papers and parts of the thesis to enable efficient usage of time. The research department was also reconfigured to allow the presence of only one patient at a time during the visits to reduce cross-infection risks. Scan slots were prolonged to account for increased cleaning and decontamination requirements pre and post study visits. As the lockdown was lifted and many of our diabetes

participants remained in shielding, we optimally utilized scanner time by scanning healthy volunteers and athletes who were not shielding.

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# **Abbreviations**

ACEI	Angiotensin converting enzyme inhibitor
ACR	Albumin creatinine ratio
ADA	American diabetes association
ADP	Adenosine diphosphate
AF	Atrial fibrillation
AHA	American Heart Association
AIDS	Adult Immunodeficiency Syndrome
ALT	Alanine transferase
AMARES	Advanced method of accurate,robust and efficient spectroscopy
ANOVA	One-way analysis of variance
ARB	Angiotensin receptor blocker
AS	Aortic stenosis
ASCVD	Atherosclerotic cardiovascular disease
ATP	Adenosine Triphosphate
BMI	Body mass index
BP	Blood pressure
C	Carbon
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
CANVAS	Canagliflozin Cardiovascular Assessment Study
CK	Creatinine kinase
CMD	Coronary microvascular dysfunction
CMR	Cardiovascular magnetic resonance imaging

CO	Cardiac output
CO <sub>2</sub>	Carbon dioxide
CoA	Coenzyme A
CNN	Convolutional neural network
CPET	Cardiopulmonary Exercise testing
CSI	Chemical shift imaging
CT	Computed tomography
CTIMP	Clinical trial of an investigational medicinal product
CVI	Circle cardiovascular imaging
DCCT	Diabetes control and complications trial
DDP-4i	Dipeptidyl peptidase-4 inhibitor
DPG	Diphosphoglycerate
EASD	European association for the study of diabetes
EAT	Epicardial adipose tissue
ECG	Electrocardiogram
ECV	Extracellular volume fraction
EDV	End-diastolic volume
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESC	European society of cardiology
ESV	End-systolic volume
FA	Fatty acids
FBC	Full blood count
FDA	Food and drugs administration
FFA	Free fatty acids

FLASH	Fast low angle shot
FOV	Field of view
FPG	Fasting plasma glucose
GIP	Glucose dependent insulin tropic polypeptide
GLP-1R	Glucagon like peptide-1 receptor
GLP-1RA	Glucagon like peptide-1 receptor agonist
GLS	Global longitudinal strain
GRE	Gradient echo
HBA1c	Glycated haemoglobin
HCM	Hypertrophic cardiomyopathy
HDL	High-density lipoprotein
HF	Heart failure
HIV	Human immunodeficiency virus
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-cTnT	High-sensitivity cardiac troponin T
HV	Healthy volunteers
IQR	Interquartile range
LA	Left atrial
LAEF	Left atrial ejection fraction
LAV	Left atrial volume
LDL	Low-density lipoprotein
LGE	Late gadolinium enhancement
LnHV	Lean healthy volunteers
LnT2D	Lean type 2 diabetes
LV	Left ventricular

LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVOT	Left ventricular outflow tract
MACE	Major adverse cardiovascular events
MAPSE	Mitral annulus planar systolic excursion
MBF	Myocardial blood flow
MEN-2	Multiple endocrine neoplasia-2
MI	Myocardial infarction
MOCO	Motion corrected
MOLLI	Modified look locker inversion recovery
MPRI	Myocardial perfusion reserve index
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance Spectroscopy
MV	Mitral valve
NADH	Nicotinamide adenine dinucleotide + hydrogen
NAFLD	Non-alcoholic fatty liver disease
NGFP	National glycohaemoglobin standardisation programme
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NOE	Nuclear Overhauser effect
NT-proBNP	N-terminal pro hormone b type natriuretic peptide
O-HV	Overweight healthy volunteers
O-T2D	Overweight type 2 diabetes

ObT2D	Obese type 2 diabetes
OGTT	Oral glucose tolerance test
PCR	Phosphocreatinine
PCSS	Peak circumferential systolic strain
PDE	Phosphodiesterase
PDH	Pyruvate dehydrogenase
PEDSR	Peak early diastolic strain rate
PET	Positron Emission Tomography
PG	Plasma glucose
<sup>31</sup> P-MRS	Phosphorus magnetic resonance spectroscopy
PPAR	Peroxisome proliferator-activated receptor
PWV	Pulse wave velocity
REC	Research Ethics committee
RPP	Rate pressure product
RVEF	Right ventricular ejection fraction
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SGLT2	Sodium-glucose co-transporter-2
SGLT2i	Sodium-glucose co-transporter-2 inhibitor
SPECT	Single photon emission computed tomography
SSFP	Steady state free precession
SU	Sulphonylurea
SubWAT	Subcutaneous white adipose tissue
SV	Stroke volume
T1D	Type 1 diabetes

T2D	Type 2 diabetes mellitus
TAG	Triacylglycerol
TE	Echo time
TG	Triglycerides
TLR	Toll like receptor
TNF $\alpha$	Tumour necrosis factor alpha
TR	Repetition time
TTE	Transthoracic Echocardiography
TyG-I	Triglyceride index
TZD	Thiazolidinediones
VA	Veteran athletes
VAT	Visceral adipose tissue
VisWAT	Visceral white adipose tissue

# **Conference Abstracts**

Oral presentation:

1. **Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes.** November 2021  
American Heart Association - Young Investigator Award Finalist

Moderated poster presentation:

1. **Prospective longitudinal characterisation of the relationship between diabetes and cardiac remodelling.** May 2021  
EuroCMR Conference
2. **Coronary Microvascular Dysfunction is detectable in type 2 diabetes in the presence of obesity.** August 2021  
European Society of Cardiology Conference
3. **Cardiac Adaptation to Acute Haemodynamic Stress in Patients with Type 2 Diabetes.** November 2021  
American Heart Association Conference
4. **Body mass index-associated differences in cardiac stress energetics in type 2 diabetes.** June 2022  
British Cardiovascular Society Conference
5. **The rest and stress relationships between myocardial function, blood flow and energetics in type 2 diabetes.** June 2022  
British Cardiovascular Society Conference

# **Publications**

## **Manuscripts that have been published**

- 1. Prospective longitudinal characterization of the relationship between diabetes and cardiac structural and functional changes.** Amrit Chowdhary, Nicholas Jex, Sharmaine Thirunavukarasu, Amanda MacCannell, Natalie Haywood, Altaf Almutairi, Lavanya Athithan, Manali Jain, Thomas Craven, Arka Das, Noor Sharrack, Christopher E.D. Saunderson, Anshuman Sengupta, Lee Roberts, Peter Swoboda, Richard Cubbon, Klaus Witte, John Greenwood, Sven Plein, Eylem Levelt. *Cardiology Research and Practice*. 2022 Feb 8;2022:6401180. Doi:10.1155/2022/6401180.
- 2. Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes.** Amrit Chowdhary, Sharmaine Thirunavukarasu, Nicholas Jex, Lauren Coles, Charles Bowers, Anshuman Sengupta, Peter Swoboda, Klaus Witte, Richard Cubbon, Hui Xue, Peter Kellman, John Greenwood, Sven Plein, Eylem Levelt. *Obesity (Silver Spring)*. 2022 May;30(5):1079-1090. doi:10.1002/oby.23413.
- 3. Cardiac adaptations to acute hemodynamic stress in function, perfusion and energetics in type 2 diabetes with overweight/obesity.** Amrit Chowdhary, Wasim Javed, Sharmaine Thirunavukarasu, Nicholas Jex, Sindhoora Kotha, Peter Kellman, Peter Swoboda, John P. Greenwood, Sven

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# **Thesis Abstract:**

Type 2 diabetes mellitus (T2D) is associated with an increased risk of heart failure and cardiovascular mortality even in the absence of coronary artery disease. Although the reasons for this are not clear, candidate mechanisms are impaired cardiac high energy phosphate metabolism and coronary microvascular dysfunction. Cardiac magnetic resonance imaging (CMR) and magnetic resonance spectroscopy (MRS) are powerful tools for the non-invasive assessment of the functional, structural and the metabolic status of the heart. The overarching aims of this thesis were to combine advanced CMR and MRS techniques to study disease mechanisms in asymptomatic patients with uncomplicated T2D who had no prior diagnosis of cardiovascular disease.

In chapter 3, prospective longitudinal cardiac structural and functional changes were studied in T2D patients over a six-year follow-up period. In this study T2D patients who have not experienced any cardiovascular events and remained asymptomatic over the follow-up period showed significant reductions in cardiac size and biventricular systolic function over time.

The work represented in chapter 4 showed that while T2D is associated with greater visceral adiposity in overweight patients, accumulation of visceral fat was evident even in T2D patients with a normal body weight at levels similar to overweight non-diabetic controls. Secondly, in T2D patients, all measures of adiposity strongly correlated with one another, and visceral adipose tissue, body mass index and waist circumference were each related to abnormalities in cardiac systolic and diastolic strain, and insulin resistance. Thirdly, myocardial stress perfusion was only reduced in overweight T2D participants,

with no reduction in global stress myocardial blood flow or myocardial perfusion reserve index in the normal body weight T2D patients or in overweight healthy volunteers compared to normal body weight healthy volunteers.

The work represented in Chapter 5 was set out to establish the links between myocardial perfusion, systolic and diastolic function and energetics in the healthy and the diabetic human heart, at rest and during pharmacological stress combining <sup>31</sup>phosphorus-MRS (<sup>31</sup>P-MRS) and CMR techniques. It is well established that the diabetic heart is characterised by reductions in myocardial phosphocreatine to ATP ratio which is a sensitive indicator of myocardial energetic status that can be noninvasively measured in vivo using <sup>31</sup>P-MRS. This reduction is among the earlier subclinical cardiac changes in T2D patients. This work showed that in response to dobutamine stress, patients with T2D as well as healthy volunteers and age-matched veteran athletes show decrements in myocardial energetics, similar increments in global longitudinal shortening and left ventricular ejection fraction (LVEF), but with a blunted increment in stress perfusion in T2D patients. This work also showed that rest and stress myocardial blood flow are associated with rest and stress LVEF, while rest and stress energetics are associated with rest and stress diastolic parameters respectively, suggesting that diastolic function is a more energetically sensitive process than global systolic function.

Chapter 6 was a randomised, phase-2, single centre, open-label, cross-over design mechanistic drug trial of glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide compared to peroxisome proliferator-activated receptor (PPAR)-gamma agonist pioglitazone. T2D is characterized by dysregulated insulin secretion and resistance to insulin action. Both the insulin secretion and

insulin resistance are amenable to pharmacological intervention. GLP-1 receptor activation promotes insulin secretion and causes weight loss. Pioglitazone is a peroxisome proliferator activated receptor gamma agonist which targets peripheral insulin sensitivity. This work was set out to compare the efficacies of two distinct glycaemic control strategies of targeting beta-cell dysfunction (liraglutide) or insulin resistance (pioglitazone) in improving subclinical cardiac energetic, structural, functional and perfusion alterations in T2D patients with no known prior cardiovascular disease. This randomised cross-over study showed that four months treatment with incretin mimetic liraglutide results in significant improvements in myocardial perfusion and energetics, while the insulin sensitiser pioglitazone shows no effect in modulation of these parameters. Both treatments led to improvements in insulin sensitivity. Pioglitazone results in significant increases in LV mass and an isolated improvement in rest diastolic function.

In conclusion, the work in this thesis demonstrates the power of CMR and MRS in elucidating the changes in patients with T2D who are overweight/obese, and of studying biological effects of novel treatment agents on the heart.

# **Chapter 1**

## **Introduction**

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Diabetes mellitus is a syndrome of chronic hyperglycaemia due to relative insulin deficiency, insulin resistance or a combination of both. The prevalence of diabetes has been rising exponentially specially in the low- and middle-income countries and it is estimated to affect over 400 million people across the world (1). Between 2000 and 2019, there was a 3% increase in the age-standardized mortality rates from diabetes with an even more significant increase of 13% in the mortality rates in the lower-middle income countries. This is in contrast to a 22% reduction in the probability of dying from any one of the four main non-communicable diseases (cardiovascular disease, cancer, chronic respiratory disease or diabetes) between 2000 and 2019. This further emphasises the huge burden on public health caused by diabetes (2).

## **1.1 History of Diabetes**

The first reference of this condition goes back to 1500 before Christ (BC) when historians have documented an Egyptian papyrus mentioning a disease that causes excessive thirst, frequent urination and potentially treated by plant extracts (3).

Apollonius of Memphis is considered the first one to mention the term 'diabetes' in the year 250 BC. In the year 5 Anno Domini (AD), Sushruta, the Indian surgeon, remarked on the sweetness of urine, its ability to attract ants and the increased incidence of this condition in the wealthy who usually had an increased intake of rice and sweet food products (4). In the 11<sup>th</sup> century AD, the Persian physician Avicenna (980-1037) described diabetes and mentioned gangrene and sexual dysfunction

amongst its complications. A century later, the medieval scholar Moises Maimonides (1138-1204) described the symptoms of diabetes in detail (4).

Matthew Dobson (1732-1784), who was an English Physician working in Liverpool was the first to experimentally demonstrate the presence of sugar in the urine of patients affected by this condition. Dobson also observed that this condition caused rapid mortality in some patients whereas for others, it did not have the same implications- potentially leading to the first suggestions of two different types of diabetes (5). Claude Bernard (1813-1878), a French Physician concluded via animal experiments that the liver was storing a water insoluble starchy substance that he named glycogen which was further converted into glucose and secreted into the blood. He assumed that it was an excess of this compound that caused diabetes (6).

Minowski and von Mering in 1889 were able to demonstrate in their landmark work that the pancreas was a gland of internal secretion that was essential to maintain glucose homeostasis (7). A crucial turning point in the treatment of this condition was brought about by Frederick Banting, Charles Best, John MacLeod and James Collip who in the year 1921 discovered Insulin. Banting and MacLeod went on to be awarded a Nobel Prize in Medicine in 1923 for this discovery. Over the next few decades, significant work was done on insulin purification methods and new insulin formulations were developed such as a long acting insulin in the 1930s, neutral protamine Hagedorn in the 1940s and Lente series in the 1950s (8).

## **1.2 Classification of Diabetes Mellitus**

Diabetes can be broadly classified into four categories:

- Type 1 diabetes (due to  $\beta$ -cell destruction, usually resulting in an absolute deficiency of insulin)
- Type 2 diabetes (due to a progressive loss of adequate  $\beta$ -cell insulin secretory capacity usually also accompanied with insulin resistance)
- Gestational diabetes mellitus (diabetes diagnosed in the second or third trimester of pregnancy)
- Specific types of diabetes due to other causes
  - Monogenic diabetes syndromes (neonatal diabetes, maturity onset diabetes of the young)
  - Disease of the exocrine pancreas (cystic fibrosis, pancreatitis)
  - Drug/chemical induced (glucocorticoid, post-transplant, HIV/AIDS treatment)

### **1.3 Type 2 diabetes**

The prevalence of type 2 diabetes (T2D) has been increasing at an alarming rate and has more than doubled in the past decade. T2D is associated with central obesity, hypertension, hypertriglyceridaemia and an increase in pro-inflammatory markers.

T2D is characterized by insulin resistance, reduced insulin production and eventual pancreatic  $\beta$ -cell failure. This in turn results in a decrease in the glucose uptake in the liver, myocytes and adipose tissue. As a consequence of this, glucagon and hepatic glucose levels are not suppressed after intake of food. This coupled with low levels of insulin and increased insulin resistance results in hyperglycaemia. Obesity

also tends to be a common risk factor in T2D and the presence of adipose tissue promotes insulin resistance through inflammatory processes such as upregulation of free fatty acid (FFA) release and adipokine dysregulation.

The development of T2D is seen as a result of the interaction between the environmental factors and inheritance. Genetics has also been shown to play a role in the development of T2D with previous studies in identical twins of patients with T2D demonstrating a greater than 50% chance of developing the condition (9). Environmental factors in addition to obesity that are known to play a role in the development of T2D include sedentary lifestyle, small or large body weight, stress and nutrition (10).

T2D most commonly presents sub-acutely in patients with thirst, polyuria and weight loss. It can also present with more non-specific symptoms such as malaise, visual disturbance or recurrent infections.

T2D is a chronic condition which may lead to the development of multi-systemic complications. Cardiovascular complications remain the major cause of morbidity and mortality, however renal and infectious complications also are relatively common. The complications related to T2D can be broadly classified into:

- macrovascular complications (myocardial infarction, heart failure, cerebrovascular accident and gangrene)
- microvascular complications (diabetic eye disease, diabetic nephropathy and diabetic neuropathy)

## 1.1 Heart disease in type 2 diabetes

For many decades, diabetes has been recognized as a condition causing disruption of fuel homeostasis and in turn leading to devastating consequences on the body including the heart. In fact, this condition has a special predilection for the heart with cardiovascular disease as the leading cause of mortality in patients with type 2 diabetes mellitus (T2D) (11, 12). Not only does T2D cause an increase in the atherosclerotic event rate, it also increases the risk of developing heart failure (HF) by a factor of two to four(13). Once HF develops in the setting of T2D, the mortality risk increases by 4-6 folds in patients over the age of 65 years compared to individuals without T2D and HF combination(14, 15). Large epidemiological studies support the existence of a diabetic cardiomyopathy(12, 13). In the absence of flow limiting epicardial coronary artery disease, the causality link between T2D and HF rests on several structural, functional, and metabolic observations.

At the macroscopic level, the structural and functional changes in T2D include, concentric left ventricular (LV) hypertrophy (16-18), concentric LV remodelling, reductions in LV systolic(19) and diastolic performance(20). Histological studies have demonstrated that at the microscopic level, T2D is associated with myocyte hypertrophy, perivascular fibrosis, and increased quantities of matrix collagen and cellular triglyceride deposition(21).

Several factors have been implicated in the pathogenesis of diabetic cardiomyopathy.

The major factors include:

- Increased fatty acid oxidation- early studies on substrate metabolism of the human heart have revealed that myocardial glucose uptake is decreased in association with insulin resistance, whereas free fatty acid (FFA) uptake is increased in T2D(22, 23). These structural and functional changes may be

related to the nonenzymatic glycation of vascular and membrane proteins, increased cellular fatty acid uptake and oxidative stress, which are characteristic of the diabetes state(22, 23).

- Myocardial energy impairment- the metabolic phenotype of diabetic heart disease is characterized by impaired myocardial energetics(24, 25) as indicated by reductions in phosphocreatine (PCr) to adenosine triphosphate (ATP) (PCr/ATP) ratio assessed by <sup>31</sup>phosphorus magnetic resonance spectroscopy (<sup>31</sup>P MRS) noninvasively.
- Coronary microangiopathy- which is represented by endothelial scarring in the coronary microvasculature. Histological studies have demonstrated thickening of the capillary basement membrane, medial thickening of the arteriole, and perivascular fibrosis (26, 27).
- Cardiovascular autonomic neuropathy- results in damage to the autonomic nerve fibres that innervate the heart and blood vessels. The autonomic neuropathy results from a complex interaction between glycaemic control, duration of T2D, blood pressure and age-related neuronal death (28). Hyperglycaemia can induce multiple pathways that increase oxidative stress, toxic glycosylation products and free reactive oxygen species leading to neuronal dysfunction or death(28, 29)

### **Cardiac structural and functional changes in type 2 diabetes mellitus**

Although Leyden in the year 1881(30) suggested the link between diabetes and heart failure, it was Rubler in 1972 who observed ventricular hypertrophy with diffuse fibrotic strands extending between bundles of muscle fibres and myofibrillar hypertrophy on histopathology in a series of post-mortem studies of four diabetic cases and coined the term “diabetic cardiomyopathy”(26).

Several studies have shown alterations in LV geometry in patients with type 2 diabetes. Although an increased LV mass is independently associated with type 2 diabetes, often this increase was shown to be modest(31-33). LV mass can increase from either increased wall thickness or chamber dilation, i.e., the spectrum of LV hypertrophy (LVH) ranges from concentric to eccentric hypertrophy, and there is a variation in both the degree and pattern of hypertrophy observed in patients with type 2 diabetes(33, 34). However, LV concentric remodelling represents the main structural characteristic of non-ischemic heart disease in type 2 diabetes(18, 33). There is less evidence that type 2 diabetes itself can cause LV dilatation and eccentric remodelling in the absence of CAD, obesity or hypertension(21, 33). Further, LV concentric remodelling was shown to be more strongly predictive of cardiovascular mortality than eccentric remodelling(18, 33, 35).

The pathogenesis of LVH especially in the early stages of diabetic cardiomyopathy was shown to be myocyte hypertrophy as opposed to fibrosis (36, 37). As the condition progresses, interstitial fibrosis becomes more prevalent(26). Extracellular volume (ECV) quantification using CMR has been demonstrated to correlate closely with collagen proportionate area on histology samples obtained from patients with HF(38). Using ECV and native T1 mapping, two studies failed to demonstrate a rise in ECV and native T1 in patients with well controlled T2D even in the presence of LV concentric remodelling- indicating the absence of significant extra cellular matrix expansion (33, 39, 40).

Diastolic dysfunction has been suggested as the earliest functional alteration secondary to type 2 diabetes with reported prevalence ranging from 15-75% (41). The

Strong Heart study further demonstrated a directly proportional link between the HbA1c level and the extent of diastolic dysfunction (31). Most studies have failed to demonstrate a significant effect of type 2 diabetes on the global LV ejection fraction (LVEF) except for the Strong Heart study which demonstrate a mild reduction in the LVEF (31).

In recent times, the use of more sensitive measures of subclinical cardiac dysfunction such as strain imaging by echocardiography or CMR, has demonstrated reduced longitudinal contractility and impaired circumferential strain in diabetics(42).

Despite the advancements made in the understanding of the myocardial structural and functional changes in T2D, there is a distinct lack of longitudinal studies looking at these changes over a period of time in patients who remain asymptomatic.

### **Myocardial perfusion in type 2 diabetes**

Epicardial CAD and microvascular dysfunction are amongst the pathophysiological changes giving rise to increased cardiovascular morbidity and mortality in T2D (43). A previous study demonstrated that patients with type 2 diabetes without a history of CAD had approximately the same risk of future myocardial infarction (MI) as did patients with prior MI but no type 2 diabetes(44). This gave rise to the concept of patients with type 2 diabetes being established as 'Coronary risk-equivalent'.

A relatively large study including 1123 asymptomatic T2D participants with no known or suspected CAD found a prevalence of silent ischaemia in 22% of the study population using single-photon emission computed tomography (SPECT) (45). Multiple studies using (pharmacological or exercise) stress echocardiography have

demonstrated the modality to be a useful tool for risk stratification and prognostic evaluation in patients with T2D (46-48). Numerous studies have reported a high incidence of adverse cardiac events in people with normal SPECT or stress echocardiography results (49, 50). Again, numerous studies using CMR first pass perfusion have demonstrated impairment in the myocardial perfusion reserve in patients with T2D indicative of microvascular dysfunction (51, 52). A recent study utilizing stress perfusion CMR has shown that the presence of inducible ischaemia was associated with an almost five-fold increased likelihood of cardiac death and non-fatal MI amongst participants with type 2 diabetes, whereas the annual rate of cardiac death and non-fatal MI was low at 1.4% per year in diabetics without inducible ischaemia (53).

### **Ectopic and visceral adiposity**

Adipose tissue produces a wide range of adipocytokines with a diverse range of metabolic functions. In metabolic disease states, a shift between anti-inflammatory and pro-inflammatory adipocytokines occurs, with this imbalance predisposing to chronic, low-grade inflammation which contributes to the development of cardiovascular disease(54).

The role of adipose tissue in CVD pathogenesis is more complex than simple metrics of obesity. Accumulating evidence suggests that adipose tissue distribution is an important determinant of CVD risk [7–14] which influences function and severity of CVD [15]. Subcutaneous white adipose tissue (subWAT) was once thought to have minimal risks for metabolic disease development; however, there is now a growing body of literature that suggests otherwise [16–18]. Visceral white adipose tissue

(visWAT), is located within the abdominal cavity [19] and is associated with a higher risk of metabolic disease, insulin resistance and cardiovascular disease in both men and women [20,21]. visWAT is composed of more adipocytes than subWAT which are generally larger in size, with a higher capacity to hold lipid [22]. Venous blood from visWAT is drained into the liver through the portal vein directing free fatty acids (FFAs), secreted from visceral adipocytes, to the liver [23]. Increased liver fat accumulation can impair liver function, and continued accumulation can lead to non-alcoholic fatty liver disease (NAFLD) [23,24].

Whilst the primary function of adipose tissue was considered to be a storage centre for triacylglycerols (TAGs), recent studies have highlighted adipose tissue to be a prominent endocrine organ, able to influence almost all organs and cell types. Adipose tissue signals can elicit a variety of responses including metabolic, immune, endocrine and cardiovascular. WAT secretes free fatty acids (FFA) and adipokines which include leptin, adiponectin, tumor necrosis factor alpha (TNF $\alpha$ ), Toll-like receptor (TLR) expression and interleukin-6 (IL-6) [36]. These adipokines have the ability to act on organs both centrally and peripherally [37] to regulate food intake, energy balance, and insulin sensitivity [38].

Computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and proton ( $^1\text{H}$ )-MR spectroscopy (MRS) have all been used to quantify adipose tissue amount or lipid content within an organ, and to examine the association of various fat depots with both systemic and local manifestations of disease(55-60). Recently, using these techniques, it was demonstrated that, irrespective of body mass index, type 2 diabetes is related to significant abnormalities in cardiac function, energetics, and

cardiac and hepatic steatosis(61). However, obese patients with T2D were shown to have a greater propensity for ectopic fat deposition that was associated with cardiac contractile dysfunction and fibroinflammatory liver disease than lean T2D patients(61). Moreover, an inverse correlation of epicardial adipose tissue volumes with cardiac systolic strain was demonstrated(61). Similarly, excess liver fat, which is a form of ectopic fat, has been shown to be accompanied by cardiac structural and functional changes(62).

Ectopic and visceral adiposity is linked to insulin resistance and type 2 diabetes (63). Numerous studies back the concept that dysregulated fat tissue is the driver for insulin resistance(63). It is therefore feasible that insulin resistance may be responsible for the increased cardiovascular risk associated with ectopic and visceral adiposity(61). There are many molecular mechanisms that may contribute to the association between insulin resistance and non-ischemic cardiomyopathy(61, 64). These include metabolic inefficiency(21), impaired vascular function(65), inflammation, mitogenic actions of insulin on myocardium leading to changes of left ventricular geometry(66). However, some other studies have raised doubt whether this relationship between adiposity and insulin resistance is of a protective nature(61, 67-69). Although it has been demonstrated that insulin resistance and ectopic adiposity are associated with an even greater cardiovascular risk(70, 71), some authors argued that insulin resistance protects the heart from nutrient-induced damage(69). It has been proposed that insulin resistance is an antioxidant defence mechanism(67). Therefore, there has been a substantial change in the thinking regarding the role of insulin resistance in type 2 diabetes associated cardiovascular risk(33).

## **Myocardial High Energy Phosphate Metabolism**

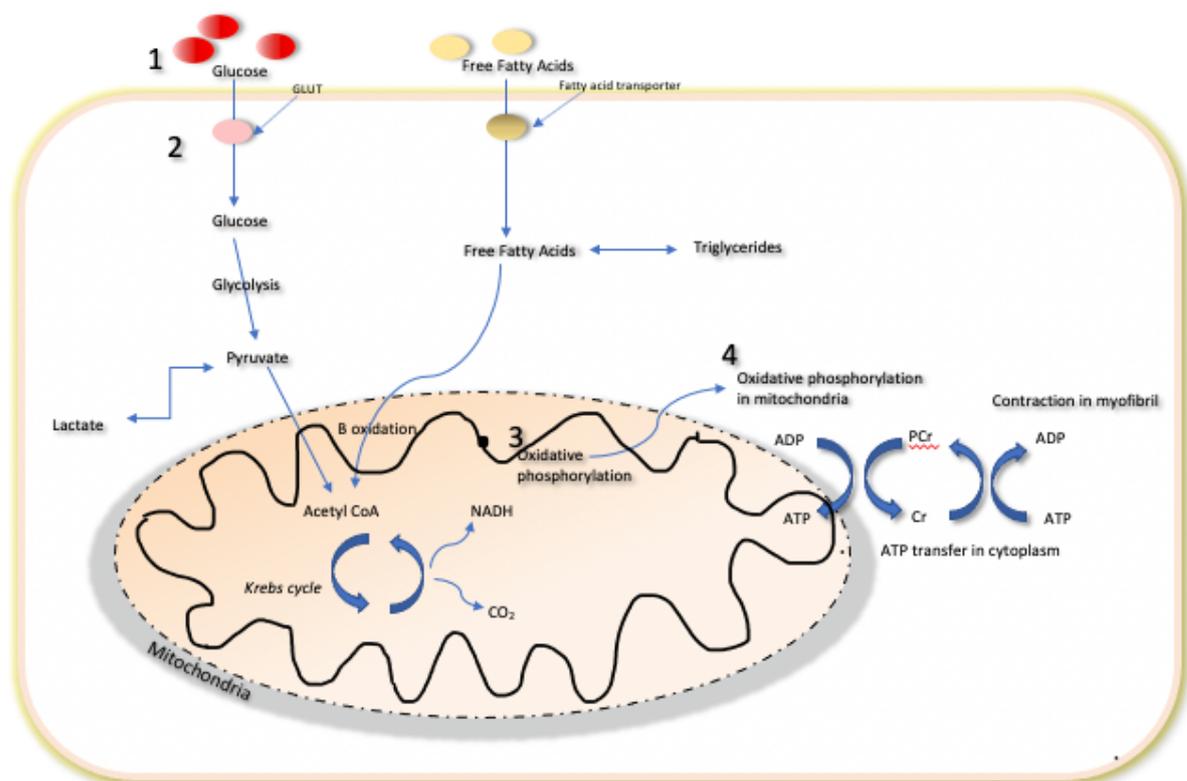
Per gram of tissue, the heart is the most energy intensive organ in the body. Whilst the myocardium has less than 1 gram of ATP in total, on an average, it consumes approximately 6 kilograms of ATP a day to function(72). Conservation of adequate levels of cardiac high-energy phosphate metabolites, ATP, the energy source for contraction, and PCr, the major energy storage compound, are of vital importance for normal heart function(72). Even in the absence of proof of a direct causal link between energy deficit and contractile dysfunction, it is appealing to contemplate that impairment in cardiac energetics can contribute substantially to the pathophysiology and progression of the disease. Supporting this notion, many cardiovascular pathologies including but not limited to diabetic heart disease(24, 25), hypertrophic cardiomyopathy(73) (74) (75, 76), non-ischaemic HF(77) (78) (79), ischaemic heart disease(80)·(81), hypertension(82, 83) and valvular disease(84)·(80) have been shown to demonstrate abnormal resting cardiac energetics. However, despite the multitude of studies, a causal relationship between energy starvation and disease progression has yet to be established in humans.

In the healthy heart, despite limited capacity to store ATP, cardiac pump work can be ramped up to threefold(85). The healthy myocardium has response mechanisms to address the acute changes in energy demand (85). These mechanisms include enhanced contribution of carbohydrates to energy production,(86, 87) and increased phosphotransferase reaction rate(88).

There are four major factors necessary for effective cardiac metabolism(72) (Figure 1.1).

1. Perfusion: A healthy myocardial blood supply with an appropriate hyperaemic response during physiological stress is essential to deliver both substrate and oxygen under resting and stress condition and avoid demand supply mismatch.
2. Substrate utilisation: The heart is a metabolic omnivore able to utilize multiple substrates including fatty acids (FA), glucose, lactate, branched chain amino acids and ketone bodies. While metabolic flexibility is an essential cardiac attribute, under normal physiological conditions, energetic demand is primarily met by glucose and FA. This process therefore involves uptake of FA and glucose and their subsequent breakdown via beta oxidation and glycolysis to form acetyl coenzyme A (CoA). This is then fed into the Krebs cycle and yields nicotinamide adenine dinucleotide + hydrogen (NADH) and carbon dioxide (CO<sub>2</sub>). This is an extremely important step in myocardial energy metabolism and even led to Krep and Lipmann being awarded the Nobel prize for medicine or physiology in 1953.
3. Energy production: High energy phosphate bonds are made in the form of ATP via a chain of electron transferrals in the mitochondria. Respiratory-chain complexes I through IV transfer electrons from NADH to oxygen, thereby creating a proton electrochemical gradient across the inner mitochondrial membrane as well as nicotinamide adenine dinucleotide (NAD) and water. This gradient drives ATP synthase, which produces ATP by phosphorylating adenosine diphosphate (ADP). Uncoupling proteins cause mitochondria to produce heat rather than ATP. This is another crucial step in cardiac metabolism and led to the award of the Nobel prize in chemistry to Dr Mitchell in 1978.

4. Energy transfer and utilisation: The heart's energy transfer mechanism is the creatine kinase energy shuttle. Mitochondrial creatine kinase catalyses the transfer of the high energy phosphate bond in ATP to creatine to form PCr. This molecule is smaller and less polar and hence diffuses out of the mitochondria into the cytoplasm. At the site of energy usage, mainly at the sarcomere and for ion pump function, ATP is reformed in the reverse reaction. Creatine, which is not produced in the heart, is taken up by the creatine transporter.



**Figure 1.1** Cardiac energy metabolism 1. Adequate blood supply; 2. Substrate utilisation; 3. Oxidative phosphorylation; 4. Energy transfer and utilisation. GLUT- glucose transporter, PCr- phosphocreatine, Cr-free creatine

## **1.2 Imaging the heart in type 2 diabetes**

### **Echocardiography**

Transthoracic echocardiography (TTE) is an easily accessible and cost effective investigation which provides both diagnostic and prognostic information in patients with T2D (89). Multiple studies using TTE have demonstrated left ventricular hypertrophy (90-92) and also increased back scatter (93, 94) which is an indicator of myocardial fibrosis. In addition, diastolic dysfunction remains one of the hallmark characteristics of diabetic cardiomyopathy and multiple studies have demonstrated this in the population with T2D and have also linked it to worse prognostic outcomes (95, 96). Longitudinal and circumferential strain has also been shown to be reduced in T2D (97). However, TTE is unable to quantify myocardial perfusion or fibrosis or look at myocardial metabolism.

### **Cardiac Computed Tomography (CT)**

Due to recent advancements in CT technology and significant reductions in radiation dose, CT has been used in assessment of coronary artery disease in T2D. A recent study using CT for screening demonstrated coronary atheroma in well over 50% of the participants, despite aggressive risk factor management, there was no difference in CAD events thus CT is not recommended for routine screening in T2D (98).

### **Myocardial Perfusion Scintigraphy (MPS)**

Stress MPS is widely used in patients with and without T2D to detect inducible ischaemia as a sign for hemodynamically significant coronary artery disease.

However due to the complexity of its protocols, radiation and high costs, it has not been widely used to evaluate the myocardium in T2D.

## **Coronary Intervention and microvascular assessment**

Coronary microvascular disease is an early feature of diabetic cardiomyopathy. In the absence of epicardial coronary artery disease, continuous intracoronary thermodilution and adenosine can be used to assess the maximal coronary flow, coronary flow reserve and microvascular resistance reserve. Studies have shown a reduction in these parameters in T2D (99, 100)

## **Cardiovascular magnetic resonance imaging**

Cardiovascular magnetic resonance imaging (CMR) allows a comprehensive investigation of structural, functional, and ischaemic changes in the heart. T2D affects cardiac mass, strain, systolic and diastolic function and perfusion all of which can be accurately measured with cine CMR(40, 101-103). CMR allows for quantification of myocardial perfusion and perfusion reserve during pharmacological stress(104), offering insight into coronary arterial and microvascular integrity. 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 (PET) and microspheres(105). Combined with MRS, quantitative perfusion CMR has previously been used to describe the association between exercise energetics (PCr/ATP) and myocardial perfusion reserve in T2D(42). Late gadolinium enhanced (LGE) MRI is an excellent test to visualize focal replacement fibrosis and in addition, interstitial diffuse fibrosis can be detected and quantified by the myocardial ECV from pre- and post-contrast T1 maps(106, 107). CMR T1 mapping for ECV quantification correlates closely with collagen proportionate area on histology(38). Table 1.1 outlines observational studies done in participants with T2D using CMR as the main imaging technique.

**Table 1.1** *Observational cardiovascular imaging studies in type 2 diabetes*

<b>Study</b>	<b>Journal/year</b>	<b>Cohort</b>	<b>Primary objective</b>	<b>Results</b>
Haffner, S., et al. (44)	N Engl J Med; 1998	1059 T2D vs 1373 non T2D	Assess risk of CAD in patients with and without T2D	T2D patients without previous MI have equal risk of MI as non-T2D patients with MI.
Diamant, M. et al (108)	J Am Coll Cardiol; 2003	12 well controlled and recently diagnosed T2D patients	Myocardial function in relation to high-energy phosphate metabolism in asymptomatic patients with uncomplicated T2D	Altered myocardial energy metabolism may contribute to LV diastolic functional changes in patients with recently diagnosed, well-controlled and uncomplicated type 2 diabetes
Van der Meer, J R.W. (109)	J Cardiovasc Magn Resonan; 2007	14 well controlled and uncomplicated T2D patients and 16 HV	Assessment of LV function, PWV and aortic distensibility	Abnormal PWV and aortic distensibility in T2D patients
Kwong, K.Y., et al. (110)	Circ; 2008	187 T2D patients	Prognostic significance of myocardial scar in patients with T2D without any clinical evidence of MI	Myocardial scar demonstrated strong association with major adverse cardiovascular outcome (MACE) and mortality hazards that was incremental to clinical, ECG, and LV function combined
Rijzewijk, L.J. et al (111)	J Am Coll Cardiol; 2008	38 T2D patients and 28 HV	Compare myocardial triglyceride (TG) content and function between patients with uncomplicated T2D and HV	Myocardial TG content is increased in T2D this is associated with impaired LV diastolic function

Ng, A.C.T., et al.(112)	Circ; 2010	42 T2D male	Quantify myocardial TG content and relationship to LV and RV function	High myocardial TG content is associated with pronounced LV and RV impairment
Rijzewijk, L.J. et al (113)	J Am Coll Cardiol; 2010	61 T2D participants	Relationship between hepatic TG content and myocardial function and metabolism	High liver TG content was associated with decrease in myocardial perfusion, glucose uptake and high-energy phosphate metabolism
Wilmot, E.G. et al (114)	Diabet Med; 2014	20 young T2D participants and 10 lean HV and 10 obese HV	Phenotyping young adults with T2D	Young T2D participants and obese HV had higher LV mass and trend towards concentric remodelling. In addition, T2D participants demonstrated diastolic dysfunction
Khan, J.N., et al (115)	Eur Heart J Cardiovasc Imaging; 2014	20 young adults with T2D and 20 HV	Assess the cardiac, vascular, anthropometric, and biochemical determinants of subclinical diastolic dysfunction in young T2DM	In young adults with T2DM, diabetes duration and aortic distensibility were associated with diastolic dysfunction.
Larghat, A.M., et al (116)	Eur Heart J Cardiovasc Imaging; 2014	19 participants with T2D, 30 prediabetics and 16 HV	To investigate the cardiac structure, function, and perfusion in patients with and without T2D	Patients with T2D have increased LV mass, LV torsion, and decreased MPR. There is a significant association between decreased MPR and increased LV torsion
Levelt, E. et al (117)	Eur Heart J; 2016	31 participants with T2D and 17 HV	To assess if pre-existing energetic deficit is exacerbated by exercise, and if the impaired myocardial perfusion causes deoxygenation and further energetic	The pre-existing energetic deficit is exacerbated by exercise; stress PCr/ATP correlates with impaired perfusion and oxygenation.

derangement during exercise stress,

Levelt, E., et al (118)	J Am Coll Cardiol; 2016	27 obese T2D, 15 lean T2D and 12 HV	To investigate if lean patients with T2D exhibit increased ectopic and visceral fat deposition and whether these are linked to cardiac and hepatic changes.	Irrespective of BMI, T2D is related to significant abnormalities in cardiac structure, energetics, and cardiac and hepatic steatosis. Obese patients with T2D show a greater propensity for ectopic and visceral fat deposition.
Heydari, B. et al (53)	Circ. Imag; 2016	173 participants with T2D	Evaluate the prognostic value of stress perfusion CMR in T2D cohort with suspected myocardial ischemia	Stress perfusion CMR provided independent prognostic utility and effectively reclassified risk in patients with T2D
Levelt, E., et al (119)	J Cardiovasc Magn Resonance; 2017	31 patients with T2D and 16 HV	To investigate if patients with T2DM but without obstructive CAD will have microvascular dysfunction detectable using adenosine stress and rest T1-mapping.	Patients with well controlled T2DM, exhibit blunted maximal non-contrast T1 response during adenosine vasodilatory stress, likely reflecting coronary microvascular dysfunction.
Swoboda, P.P., et al (102)	J Am Heart Assoc; 2017	100 participants with T2D and 30 HV	To investigate why patients with T2D and high urinary ACR have increased risk of heart failure	Asymptomatic patients with T2D and high ACR have markers of diffuse cardiac fibrosis including elevated ECV, high-sensitivity cardiac troponin T, and diastolic dysfunction.
Storz, C. et al (120)	Eur Heart J Cardiovasc		To characterize changes in the myocardium in subjects with	Subjects with prediabetes and type 2 diabetes but preserved LVEF had higher LV

	Imaging; 2018		prediabetes, type 2 diabetes, and healthy controls with preserved LVEF	remodelling indices, suggesting early detectable changes in the disease process, while diffuse myocardial fibrosis appears to be less relevant at this stage.
Vukomanovic, J V., et al. (121)	Hypertens; 2019	70 participants with uncomplicated T2D	To evaluate the association between functional capacity and LV mechanics in the patients with uncomplicated T2D.	A significant relationship was found between endocardial LV longitudinal and circumferential strains with peak aerobic capacity (VO <sub>2</sub> max), oxygen pulse and ventilation/carbon dioxide slope.
Athithan, L., et al (122)	Ther Adv Endocrinol Metab; 2020	62 male and 67 female participants with T2D	To determine the effect of sex on the phenotypic expression of diabetic heart disease in patients with T2D	Male sex adversely affects the phenotypic expression of diabetic heart disease.
Gulsin, G. et al (20)	Diabetes Care; 2020	247 participants with T2D	To assess the relationship between cardiac dysfunction and aerobic exercise capacity in adults with T2D	MPR and diastolic function are key determinants of aerobic exercise capacity, independent of age, sex, ethnicity, smoking status, or blood pressure.
Sørensen, M.H., et al. (123)	Eur Heart J Cardiovasc Imaging; 2020	193 patients with T2D and 25 HV	To examine the relationship between diabetic complications, LV function and structure and MPR as indicators of CMD	Patients with uncomplicated T2D have reduced MPR compared with HV. Patients with T2D and albuminuria or retinopathy have reduced MPR and increased LV mass and E/e.

Sørensen, M.H., et al. (124)	Diabetes Care; 2020	193 patients with T2D and 20 HV	To examine differences in MBF at rest and during stress between patients with T2D and HV.	Patients with T2D have higher global MBF at rest and lower maximal MBF during vasodilator-induced stress than control subjects.
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*T2D- Type 2 Diabetes, CAD- Coronary artery disease, MI- myocardial infarction, LV- left ventricle, HV- healthy volunteer, PWV- pulse wave velocity, MACE- major adverse cardiovascular events, ECG- electrocardiogram, TG-triglycerides, RV- right ventricle, MPR- myocardial perfusion reserve, ACR- albumin creatinine ration, ECV- extracellular volume, LVEF- left ventricular ejection fraction, CMD- coronary microvascular dysfunction and MBF- myocardial blood flow.*

## Measuring cardiac energy metabolism

Magnetic resonance spectroscopy (MRS) is an ideal tool for the non-invasive study of metabolism, and it is frequently used to interrogate cardiac energy metabolism in preclinical and clinical studies. In the heart, ATP delivery can occur through the creatine kinase (CK) system, which catalyzes the following reversible reaction: Phosphocreatine + ADP + H<sup>+</sup> ↔ Creatine + ATP. The relative concentration of phosphocreatine 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. Phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS) allows non-invasive assessment of the myocardial PCr/ATP ratio (29) as well as absolute levels of high-energy phosphates (30). Using this technique, studies have shown myocardial energetic compromise to be a feature of diabetic cardiomyopathy (31–33) inherited cardiomyopathies (34,35), valvular heart disease (36), cardiac transplant rejection (37).

Over the last decade significant advances have been made in characterizing the cardiac metabolic phenotype non-invasively, and in defining the relationship between the myocardial metabolic remodelling and the structural and functional changes (125) (Table 1.2). Due to the constantly varying cardiac workloads, efficient matching of energy supply to demand is essential for maintaining normal cardiac function (38) and myocardial metabolism is profoundly affected by changes in cardiac workload. The onset of exercise triggers a rapid increase in demand for substrate, and oxygen (39). The healthy myocardium has rapid response mechanisms to deal with acute changes in energy demand (40), including increased rates of phosphotransferase reactions

(41,42). Assessing cardiac energetic response to exercise by  $^{31}\text{P}$ -MRS, exacerbation of the pre-existing energetic deficit in patients with T2D was shown during increased workload (33). Further, despite having no significant obstructive CAD, mean myocardial perfusion reserve index (MPRI) was significantly reduced in these patients (43,44). Pointing to the importance of an appropriate hyperaemic response during exercise to maintain cellular energy metabolism, significant correlations between MPRI with exercise energetics were demonstrated in patients with T2D (43). While similar reductions in myocardial PCr/ATP during exercise was detected in patients with hypertrophic cardiomyopathy (HCM), in HCM this exacerbation of the energetic impairment was independent of perfusion reserve, as well as the degree of myocardial fibrosis or hypertrophy (34). No significant changes in myocardial energetics with exercise activity was detected in comparison to the rest values in patients with dilated cardiomyopathy (45).

In addition to the creatine kinase (CK) shuttle function, the CK system can also act as a buffer to dampen changes in ATP and ADP levels and this system is important to preserve the free energy of ATP hydrolysis in the cytosol. More recently, turnover rates of high-energy phosphates (CK Flux) in humans have also been measured with the saturation transfer method (46), and these dynamic turnover rates seem to be even stronger predictors of outcome in heart failure than steady-state concentrations (47). Utilising this method, in a cohort of obese individuals and no other cardiac comorbidities, the myocardial CK reaction rate at rest was shown to be increased, maintaining ATP delivery despite reduced PCr/ATP (48). However, during increased workload, ATP delivery through CK was not increased in the obese cohort in contrast with what is detected in non-obese controls suggesting that lower ATP delivery during stress in obesity. This finding was shown to be associated with reduced systolic

augmentation and exercise tolerance in patients with obesity. Highlighting the importance of myocardial energy delivery through CK as a potential therapeutic target to improve symptoms in obesity-related heart disease, weight loss has reversed these energetic changes. Similarly, the total CK flux capacity was recently shown to be reduced in patients with aortic stenosis (AS), with a reduced resting CK flux evident already in patients with moderate AS earlier with preserved LV systolic function (49). These findings suggest that significant energetic impairment is already established in patients with moderate AS and that a fall in CK flux is not by itself a necessary cause of the transition to systolic failure.

Finally, the recent development of hyperpolarized  $^{13}\text{C}$  MRS has made it possible to measure cellular metabolism *in vivo*, in real-time. Rider et al successfully utilised hyperpolarized  $[1-^{13}\text{C}]$  pyruvate MRS to assess downstream metabolism of  $[1-^{13}\text{C}]$  pyruvate via PDH (pyruvate dehydrogenase,  $[^{13}\text{C}]$  bicarbonate), lactate dehydrogenase ( $[1-^{13}\text{C}]$  lactate), and alanine transaminase ( $[1-^{13}\text{C}]$  alanine), in 5 patients with T2D and 5 controls at baseline, and repeated these measurements in 5 of these participants (3 T2D, 2 controls) 45 minutes after a 75 g oral glucose challenge (50). They showed metabolic flux through cardiac pyruvate dehydrogenase (PDH) was significantly reduced in the patients with T2D compared to controls. In addition, they have also detected a significant increase in metabolic flux through PDH 45 minutes after the oral administration of 75 g of glucose in patients with T2D and in controls. This study was the first demonstration of the ability of hyperpolarized pyruvate to noninvasively assess physiological and pathological changes in PDH flux in the human heart, highlighting the potential of the technique to assess metabolic alterations in a range of cardiovascular diseases.

Therefore, CMR is a technique that can non-invasively thoroughly interrogate the myocardium by using various techniques as is summarised in table 1.3.

**Table 1.2** Studies using Magnetic Resonance Spectroscopy (MRS) to investigate the cardiac phenotype in T2D.

<b>Study</b>	<b>Journal/year</b>	<b>Cohort</b>	<b>Primary objective</b>	<b>Results</b>
Rijzewijk, L.J. et al (113)	J Am Coll Cardiol; 2010	61 T2D participants	Relationship between hepatic TG content and myocardial function and metabolism	High liver TG content was associated with decrease in myocardial perfusion, glucose uptake and high-energy phosphate metabolism
Levelt, E., et al (118)	J Am Coll Cardiol; 2016	27 obese T2D, 15 lean T2D and 12 HV	To investigate if lean patients with T2D exhibit increased ectopic and visceral fat deposition and whether these are linked to cardiac and hepatic changes.	Irrespective of BMI, T2D is related to significant abnormalities in cardiac structure, energetics, and cardiac and hepatic steatosis. Obese patients with T2D show a greater propensity for ectopic and visceral fat deposition.
Levelt, E. et al (117)	Eur Heart J; 2016	31 participants with T2D and 17 HV	To assess if pre-existing energetic deficit is exacerbated by exercise, and if the impaired myocardial perfusion causes deoxygenation and further energetic derangement during exercise stress,	The pre-existing energetic deficit is exacerbated by exercise; stress PCr/ATP correlates with impaired perfusion and oxygenation.
Rider, O.J., et al (126)	Circ Res; 2020	13 participants with T2D and 12 HV	First case-control study to use hyperpolarized <sup>13</sup> C magnetic resonance spectroscopy to record changes in cardiac metabolism in the healthy and diseased human heart.	Metabolic flux through cardiac pyruvate dehydrogenase was significantly reduced in the people with T2DM. Impaired myocardial energetics, myocardial lipid content, and diastolic function were also demonstrated in the wider study cohort

T2D- Type 2 Diabetes Mellitus, TG- triglyceride, HV- healthy volunteer, BMI- body mass index, PCr/ATP= Phosphocreatinine to adenosine triphosphate ratio, C- Carbon.

**Table 1.3** CMR modalities and features specific to diabetic cardiomyopathy that are assessed.

<b>CMR modality</b>	<b>Features assessed</b>
Cine CMR	Structural changes (volumes and mass)
Perfusion CMR	Functional changes (strain and ejection fraction)
Contrast CMR	Qualitative and quantitative analysis of perfusion and perfusion reserve at rest and stress
MRS	Focal replacement fibrosis (LGE) Interstitial diffuse fibrosis (ECV) Myocardial energetic status

*CMR- cardiovascular magnetic resonance imaging, LGE- late gadolinium enhancement, ECV- extracellular volume, MRS- magnetic resonance spectroscopy*

### 1.3 Type 2 Diabetes diagnosis and treatment guidelines

HF is the most common initial presentation of cardiovascular disease in T2D(1, 16, 127). To prevent catastrophic consequences of T2D, American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), European Society of Cardiology (ESC) and National Institute for Clinical Excellence (NICE) have established diagnosis and treatment guidelines. The past 2 decades have seen exciting developments in treatment of type 2 diabetes with the discovery of new classes of glucose lowering therapies such as the glucagon like peptide-1 receptor agonists (GLP-1RA) and sodium glucose cotransporter-2 inhibitors (SGLT2i).

Diabetes mellitus is a group of metabolic disorders in which persistent hyperglycaemia (random plasma glucose >11.1 mmol/L) is caused by deficient insulin secretion, resistance to the action of insulin, or both (128).

### Diagnostic criteria for type 2 diabetes:

1. Glycated haemoglobin (HbA1c) of 48 mmol/mol (6.5%) or more;
2. Fasting plasma glucose of 7.0 mmol/L or more;
3. Random plasma glucose of 11.1 mmol/L or more in the presence of signs or symptoms of diabetes.

T2D can be diagnosed based on the fasting plasma glucose levels (FPG), or the 2-hour plasma glucose (2h-PG) value during an oral glucose tolerance test (OGTT) or HbA1c criteria (Table 1.4).

**Table 1.4** *Criteria for diagnosis of Diabetes*

<b>Method</b>	<b>Values</b>	<b>Comments</b>
FPG	≥126 mg/dl or 7 mmol/l	fasting defined as no caloric intake for at least 8h
2-h PG	≥200mg/dl or 11.1 mmol/l during OGTT	the test should be performed as described by the WHO, using a glucose load equivalent to 75g of anhydrous glucose dissolved in water
HbA1c	≥6.5% or 48mmol/mol	the test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.

Random plasma glucose >200mg/dl or 11.1 mmol/l only in the presence of classic symptoms of hyperglycaemia or hyperglycaemic crisis.

*FPG- fasting plasma glucose, PG- post glucose, OGTT- oral glucose tolerance test, NGSP- National glycohaemoglobin standardisation programme, DCCT- Diabetes control and complication trial.*

### Glycaemic targets

Glycaemic control is assessed by HbA1c measurement, continuous glucose monitoring (CGM) and blood glucose monitoring. Glycaemic recommendations for non-pregnant adults with T2D is shown in table 1.5 Recent evidence also suggests that CGM in pregnant women with diabetes is associated with normal birth weight babies thus highlighting the importance of this modality in the monitoring of diabetes (129), Glycaemic control should be assessed at least twice a year in patients who are meeting their treatment goals and at least quarterly in those who are not meeting their treatment goals as per NICE guidelines (130).

*Table 1.5 Summary of glycaemic recommendations for many nonpregnant adults with type 2 diabetes*

HbA1c	<7.0% (53 mmol/mol)
Pre-prandial capillary plasma glucose	80–130 mg/dL (4.4–7.2 mmol/L)
Peak post-prandial capillary plasma glucose	<180 mg/dL (10.0 mmol/L)

All three guidelines (ESC, ADA and NICE) divide the treatment based on atherosclerotic cardiovascular disease (ASCVD) (table 1.6). In patients with T2D and high ASCVD risk the ADA and ESC guidelines both advocate the use of either GLP1-RA or SGLT2i as first line therapy (fig 1.2). If the HbA1c remains above the treatment target, the ESC advocate adding in metformin (if the patient is not already receiving this treatment) whereas the ADA suggests addition of SGLT2i or GLP1-RA (if not already receiving this treatment) or drugs such as dipeptidyl peptidase-4 inhibitor (DPP4i) if not on GLP1-RA, basal insulin or sulphonylurea (SU). The NICE guidelines differ as they suggest commencing treatment with metformin as first line even in patients with ASCVD and if the treatment targets are still not met then to add in an SGLT2i. If further treatment escalation is required, then NICE advocates using a DPP4i or pioglitazone or a SU. NICE guidelines state that a GLP-1RA should be used if triple therapy with metformin and two other drugs is not effective and the patient's BMI  $\geq 35$  kg/m<sup>2</sup> or the BMI  $< 35$  kg/m<sup>2</sup> and the patient would benefit considerably from weight loss or insulin therapy would have serious occupational implications. NICE also recommend the institution of basal insulin therapy when dual therapy has failed.

In patients who do not have a high ASCVD risk the ADA guidelines categorise the treatment based on the need to minimize hypoglycaemia, need to minimize weight gain and the cost implications (fig 1.2). If minimizing incidences of hypoglycaemia is vital then either a DPP4i, GLP1-RA, SGLT2i or TZD are recommended. If treatment targets are not met then adding in one of the previous agents is advocated failing which, treatment with basal insulin or SU should be instituted. If the priority is to minimize weight gain, then commencing therapy with a SGLT2i or GLP1-RA is recommended followed by adding on one those two drugs to the treatment. If treatment is still sub optimal then ADA recommends adding in a DPP4i (if not on

SGLT2i) followed by SU, thiazolidinediones (TZD) or insulin. However, if cost implications are the major concern, then treatment with either a SU or TZD should be commenced. If further treatment is required then the recommendation for the addition of the agent that the patients is not currently receiving followed by either DPP4i, insulin or SGLT2i. On the other hand, NICE guidelines recommend commencing therapy with metformin and if treatment targets are not met then adding in a DPP4i/TZD or SU dual therapy followed by triple therapy. The indications for GLP1-RA or insulin use remain the same as that for patients who have a high ASCVD risk. The ESC guidelines advocate the use of metformin followed by either a DPP4i or GLP1-RA or SGLT2i or TZD. If treatment targets are not met, then the advice is to add in another one of these agents followed by SU or insulin.

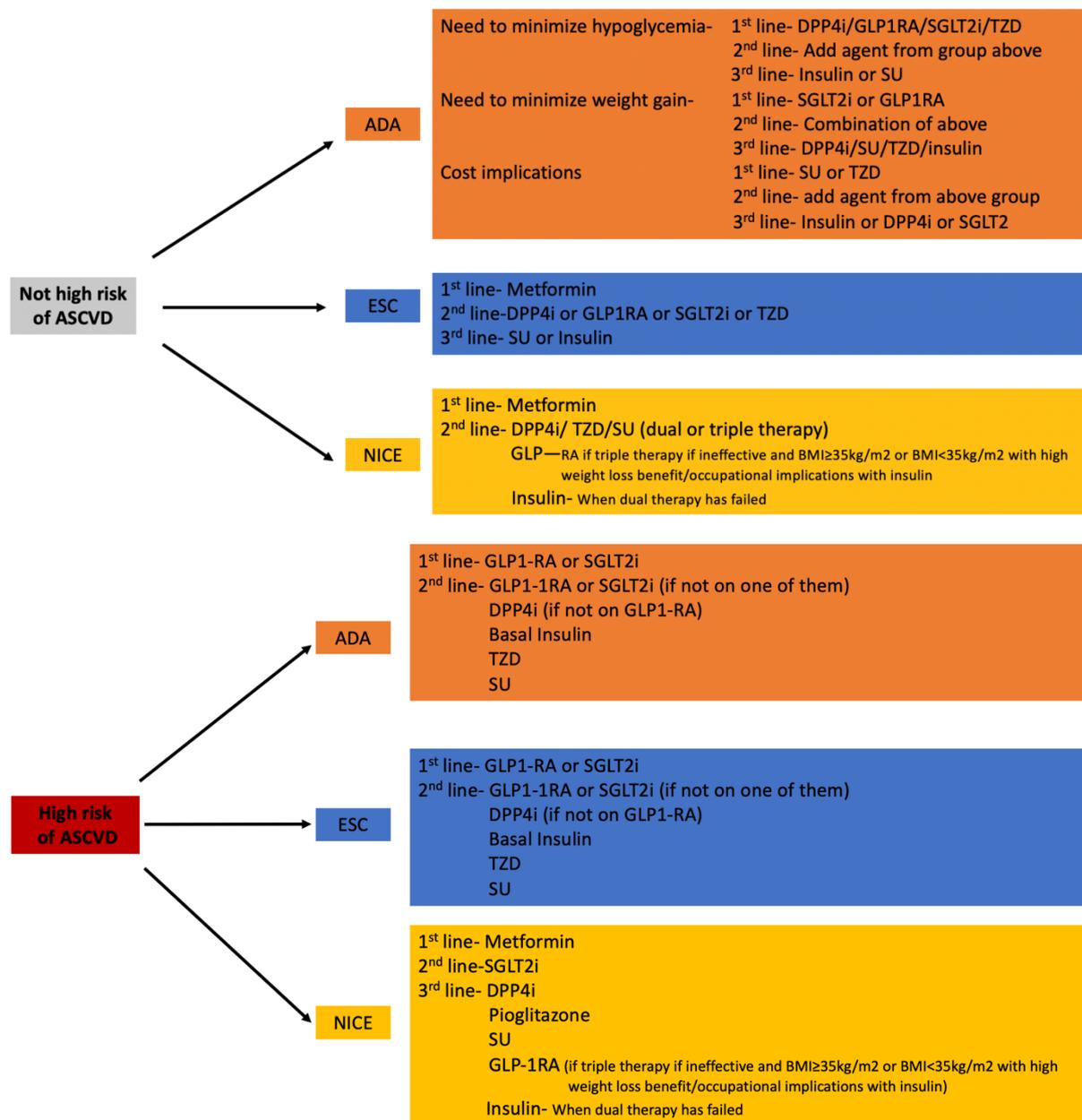


Figure 1.2 Treatment of patients with T2D with and without high risk of ASCVD according to the American Diabetes Association (ADA), European society of Cardiology (ESC) and National Institute for Clinical Excellence (NICE)

### Emerging novel glucose lowering therapies and the guidelines

Type 2 Diabetes is now widely seen as a cardiovascular condition. As a result, over the last two decades the European Society of Cardiology (ESC) has been providing guidance on type 2 diabetes treatment with regular updates. The last decade has been a period in which there has been an unprecedented increase in the evidence base

available for the field of type 2 diabetes. New classes of glucose lowering therapies, such as GLP-1 analogues(131), and inhibitors of SGLT2(132) have shown exciting results with improved glycaemic control as well as reduced cardiovascular mortality in patients with T2D. Reflecting this, the treatment algorithms especially with consideration of cardiovascular risk and comorbidities have changed dramatically over the last decade.

*Table 1.6 Stratification of cardiovascular risk in individuals with diabetes*

Very high risk	Patients with T2D and established CVD or other target organ damage <sup>a</sup> or three or more major risk factors <sup>b</sup> or early onset T1D of long duration (>20 years)
High risk	Patients with diabetes duration ≥10 years without target organ damage plus any other additional risk factor
Moderate risk	Young patients (T1D <35 years or T2D aged <50 years) with diabetes duration <10 years, without other risk factors

<sup>a</sup>Proteinuria, renal impairment (eGFR <30 ml/min/1.73m<sup>2</sup>), left ventricular hypertrophy or retinopathy. <sup>b</sup>Age, hypertension, dyslipidaemia, smoking, obesity

### **Sodium–glucose-cotransporter-2 inhibitors**

A significant breakthrough in contemporary cardiology was the finding that SGLT2 inhibitors are associated with a lower risk of HF hospitalisation in patients with or at high risk of CVD(132). A selective inhibitor of SGLT2, Empagliflozin, reduces rates of hyperglycaemia in T2D patients by decreasing renal glucose reabsorption, thereby increasing urinary glucose excretion(133). In addition, SGLT2 inhibition causes a

modest rapid reduction in weight, haemoconcentration, and reduced blood pressure, consistent with a diuretic effect (2,3).

In the EMPAREG OUTCOME Trial, Empagliflozin reduced cardiovascular death and hospitalization for HF by 38% and 35%, respectively, with an almost immediate beneficial effect despite only a modest difference in glycaemic control, comparing two study arms over 94 weeks(132). The reductions in CV death were not accounted for by the reductions in atherothrombotic outcomes, as the rates of myocardial infarction and stroke remained unchanged with therapy(132). The proposed theory that HF is the outcome most sensitive to SGLT2 inhibition was confirmed in the Canagliflozin Cardiovascular Assessment Study (CANVAS) Program and Dapagliflozin DECLARE–TIMI 58 trials(134, 135). More recently, the EMPEROR trial showed that SGLT2 inhibition reduces the risk of hospitalization for HF in patients regardless of the presence or absence of type 2 diabetes(136).

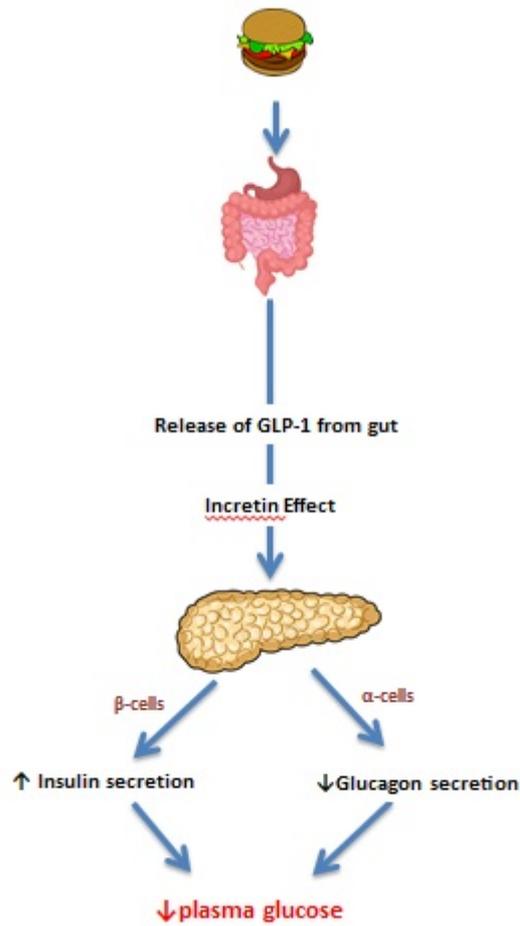
The mechanisms by which SGLT2 inhibitors cause the reduction in HF admissions and cardiovascular mortality are as yet unknown, however recently suggested theories include their impact on coronary microvascular function and pleiotropic anti-fibrotic effects(137). A recent study performed in our centre has suggested that empagliflozin treatment is associated with improved myocardial energetics, regression of the adverse myocardial cellular remodelling, and improvement of cardiac function(138).

### **Glucose like peptide-1 receptor agonists**

In the year 1964, it was first reported that compared to an intravenous glucose load, there was a greater and more sustained insulin response when glucose was administered orally (139). This enhanced release of insulin was secondary to a hormone called the glucose dependent insulin tropic polypeptide (GIP) and the response came to be known as the 'incretin effect' (140). In 1986 it was discovered that patients with T2D had a blunted incretin effect despite similar levels of GIP (141). Shortly after this, GLP-1 was discovered and was found to be more effective than GIP in stimulating insulin secretion and reducing peak glucose concentrations (142).

The GLP-1 receptor (GLP-1R) is expressed in pancreatic islet  $\alpha$  and  $\beta$  cells and in the nervous system, heart, kidney, lung, and gastrointestinal tract. Activation of these receptors on  $\beta$  cells leads to an accelerated rise in the concentration of cAMP and intracellular calcium, followed by insulin exocytosis, in a glucose-dependent manner (143). More sustained incretin receptor signalling is associated with activation of protein kinase A, induction of gene transcription, enhanced levels of insulin biosynthesis, and stimulation of  $\beta$ -cell proliferation (144). GLP-1R activation also promotes resistance to apoptosis and enhanced  $\beta$ -cell survival, in both rodent (145) and human islets (146).

GLP-1 exerts many effects on glucose metabolism such as enhancing glucose-dependent insulin production and secretion, decreasing glucagon secretion, increasing glucose uptake and glycogen synthesis in peripheral tissues and delaying gastric emptying and increasing satiety (143) (fig 1.3). These features make it an ideal therapeutic target for T2D.



*Figure 1.3 The incretin effect and mechanism of action of GLP-1RA*

The first GLP-1R agonist (GLP-1RA) to be approved for the treatment of T2D by the United States Food and Drug administration (FDA) was Exenatide in 2005 (147). Since then, prospective cardiovascular outcome trials have been performed on multiple members of the GLP-1RA family as outlined in the table below (Table 1.7). However, these recent trials were designed to assess the specific effects of these novel drugs on clinical outcomes, and therefore the mechanisms behind the observed cardiovascular benefits remained speculative. As a result, multiple investigators took up the task of assessing mechanisms of action utilising in particular cardiovascular imaging. Table 1.8 lists cardiovascular imaging studies investigating the mechanisms which give GLP-1RA class their beneficial cardiovascular actions. However, despite the growing popularity of this class, only a few studies have used CMR to assess the

impact of GLP1RA treatment on cardiac systolic and diastolic function, perfusion or myocardial metabolism (Table 1.8).

Despite the growing evidence of cardiovascular benefit of this group of medications, no studies have been performed till date to look at the mechanisms behind this benefit. In particular there is a distinct lack of studies examining the changes in the cardiac structure, function and energy utilization when the beta-cells of the pancreas are stimulated as opposed to the changes when the insulin sensitivity is improved by other medications such as peroxisome proliferator activated receptor gamma agonist (PPAR- $\gamma$ ).

**Table 1.7** GLP-1RA cardiovascular outcome trials

<b>GLP-1RA</b>	<b>Study Name; Year</b>	<b>No. of patients</b>	<b>Median follow-up (years)</b>	<b>% with CV disease*</b>	<b>Baseline HbA1c</b>	<b>Primary composite CV outcome HR (95% CI)</b>	<b>P value</b>
Lixisenatide	ELIXA; 2015	6068	2.1	100%	7.7%	1.02 (0.89 to 1.17)	0.81
Liraglutide	LEADER; 2016	9340	3.8	81%	8.7%	0.87 (0.78 to 0.97)	0.01
Semaglutide	SUSTAIN-6; 2016	3297	2.1	60%	8.7%	0.74 (0.58 to 0.95)	0.02
Exenatide QW	EXSCEL; 2017	14752	3.2	73.1%	8.0%	0.91 (0.83 to 1.00)	0.06
Albiglutide	Harmony; 2017	9463	1.6	100%	8.7%	0.78 (0.68 to 0.90)	0.0006
Dulaglutide	REWIND; 2019	9901	5.4	31.5%	7.2%	0.88 (0.79 to 0.99)	0.026
Oral semaglutide	PIONEER 6; 2019	3183	1.3	84.7%	8.2%	0.79 (0.57 to 1.11)	0.17
Efpeglenatide	AMPLITUDE-O; 2021	4076	1.8	89.6%	8.9%	0.79 (0.65 to 0.96)	0.02

**Table 1.8** GLP-1RA studies involving CMR imaging biomarkers

<b>Study</b>	<b>Journal/Year</b>	<b>Recruited cohort</b>	<b>Drugs</b>	<b>Primary objective</b>	<b>Results</b>
Bizino, M.B., et al (148)	Cardiovasc Diabet; 2019	23 T2D patients received Liraglutide and 26 received placebo	Participants on metformin and/or SU and or insulin were also included	To test efficacy of liraglutide in improving diabetic cardiomyopathy in patients with T2D without cardiovascular disease.	Liraglutide reduced early LV diastolic filling, LV filling pressure, and improved LV systolic function thereby unloading LV.
Chen, W.G.Y. et al (149)	Cardiovasc Diabet; 2017	26 patients with T2D and LV systolic dysfunction along with 10 HV	Participants were excluded if they were on insulin or incretin-based therapies	-To investigate myocardial perfusion and oxidative metabolism in T2D patients with LV systolic dysfunction as compared to HV. -To compare the effects of exenatide vs. insulin glargine on cardiac function, perfusion and oxidative metabolism in T2D patients with LV dysfunction	T2D patients with LV systolic dysfunction did not have altered myocardial efficiency as compared to healthy controls. Neither exenatide nor insulin glargine had an effect on cardiac function, perfusion or oxidative metabolism.

Webb, D.R., et al (150)	Diabet, Obes and Metab; 2020	76 participants with T2D and obesity	Patients prescribed insulin, SGLT-2 inhibitor, GLP-1 receptor agonist or DPP-4 inhibitor therapies were excluded.	To compare the GLP-1RA liraglutide with the DPP-4 inhibitor sitagliptin using change in CMR imaging-derived subclinical diastolic dysfunction as the primary outcome measure in younger asymptomatic adults with T2D	The use of the liraglutide did not affect PEDSR compared with sitagliptin, with both groups showing a small decrease over time. There were no significant between-group differences in any CMR imaging-measured markers of structure and function.
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## 1.4 The lean type 2 diabetes variant

Excess adiposity reflected by BMI is a strong risk factor for diabetes. However, a small but significant proportion of patients with T2D are not overweight or obese. The proportion of adults with ideal body weight defined as a BMI between 18.5 to 25kg/m<sup>2</sup> at the time of incident type 2 diabetes ranges from 9 to 21%(151, 152). A previous study performed in India looking at around 10000 patients with T2D revealed that around 4% of them had a BMI even less than 18.5 kg/m<sup>2</sup> and 63% had ideal body weight at time of diagnosis (153). In this study, even though a difference in the age of diabetes diagnosis or smoking habits were not noted, there was a male preponderance in the lean and ideal body weight participants with T2D. A further study performed in the United States looking at 18000 participants with T2D showed that around 13% of the study population were of ideal body weight (BMI 17-25 kg/m<sup>2</sup>) at the time of diagnosis and also corroborated the finding of male preponderance in the lean cohort (154). In the latter study, Asians were found to have a five-fold higher prevalence in the lean cohort. As confirmed by various other studies, glycaemic control was worse among lean diabetics and coronary complications were more prevalent among the T2D patients with obesity with no significant difference noted among micro-vascular complications (154).

Studies examining the relationship of BMI with mortality in patients with type 2 diabetes suggested a paradox: the rate of total, cardiovascular and non-cardiovascular mortality was shown to be similar to or higher in normal weight participants than overweight/obese participants, across strata of gender, age and race(152, 155).

An observational case control study, comparing T2D patients who were lean (Ln-T2D) and T2D patients who were obese (ObT2D) patients with similar diabetes duration and no significant difference in blood pressure, diabetes treatment or glycaemic control

showed no significant difference in degree of cardiac concentric remodeling, myocardial steatosis or PCr to ATP ratio between LnT2D and obese T2D patients(40, 42, 61).

The major pathophysiology in lean or normal body weight patients with T2D was suggested to be rapid  $\beta$ -cell failure. Confirming this, in an autopsy study, a 10-fold increase in the frequency of  $\beta$ -cell apoptosis in lean and a 3-fold increase in obese T2D cases were shown compared with their respective nondiabetic control group(156). Peripheral and hepatic insulin resistance are also present in lean T2D patients, but to a lesser degree(157). Using homeostasis model assessment of insulin resistance (HOMA-IR) index, higher degree of insulin resistance was shown in T2D with obesity compared to lean T2D patients(61). An indirect marker of insulin resistance, triglyceride to HDL ratio, was also shown to be lower among lean patients with T2D(158).

$\beta$ -cell dysfunction is characterized by a decreased insulin gene expression, blunted glucose-stimulated insulin secretion as well as increased  $\beta$ -cell apoptosis rates(159, 160). There is a progressive deterioration in  $\beta$ -cell function and mass in patients with T2D(161) and the reduction of  $\beta$ -cell mass is attributable to accelerated apoptosis(156). There is growing evidence that therapeutic interventions that slow or delay the progression of  $\beta$ -cell failure can lead to more durable glycaemic control(162)

Although  $\beta$ -cell dysfunction has been proposed as a distinct pathophysiology for the lean T2D phenotype, the current state-of-the-art prevention and treatment regimens do not address T2D of the lean cohort differentially. As described earlier, incretin-mimetic agents have favorable effects on  $\beta$ -cell morphology and volume and a

demonstrated CV safety profile, thereby may represent an opportunity to improve cardiac outcomes specifically for the lean T2D variant.

Despite recent progress in the understanding of the lean phenotype of T2D, there remains a deficiency of studies interrogating and comparing the myocardial structure, function and adipose tissue distribution between the lean and overweight/obese phenotypes of T2D.

As mentioned previously, T2D leads to microvascular dysfunction. However, if this dysfunction remains in patients with T2D who are of ideal body weight remains largely unknown. Up till now, studies have not compared microvascular dysfunction in T2D in the presence and absence of overweight/obesity.

### **1.5 Aims and objectives of the thesis**

This work aims to investigate the structural and functional myocardial changes due to T2D in the absence of significant cardiovascular disease and compares the impact of overweight and obesity on this.

The following were then explored:

1. Identification of prospective longitudinal cardiac structural and functional changes in T2D patients in T2D patients who underwent baseline CMR and blood biomarker testing over a 6-year follow-up period. (Chapter 3)
2. Comparison of adipose tissue distribution, and cardiac structural and functional alterations between asymptomatic LnT2D and overweight T2D patients (O-T2D) without established cardiovascular disease and lean and overweight healthy volunteers. (Chapter 4)

3. Establish if diabetes affects coronary microvascular function in T2D even in the absence of overweight/obesity. (Chapter 4)
4. Understand the relative associations of impaired cardiac energetics and perfusion with the systolic and diastolic subclinical functional changes at rest and in response to acute haemodynamic stress in T2D patients (Chapter 5).
5. Compare the efficacies of two distinct glycaemic control strategies of targeting beta-cell dysfunction (liraglutide) or insulin resistance (pioglitazone) in improving subclinical cardiac energetic, structural, functional and perfusion alterations in T2D patients with no known prior cardiovascular disease.  
(Chapter 6)

## **Chapter 2**

### **General methods**

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## 2.1 General methods

The studies conducted in chapters 3, 4 and 5 were approved by the Yorkshire & The Humber - Leeds East Research Ethics Committee (REC Ref: 18/YH/0168) (appendix 1). The study conducted in chapter 6 was approved by the West Midlands-Black Country Research Ethics Committee (REC ref: 19/WM/0365) (appendix 2). Each participant gave written informed consent to be involved. Subjects were recruited from the National Health Service (NHS) Leeds and NHS Wakefield Clinical Commissioning Group General Practice Surgeries. Healthy volunteers were recruited via word of mouth and study posters emailed to staff members of the University of Leeds and various cycling, running and golf clubs in the West Yorkshire region.

General practice surgeries performed a search of their database for the inclusion and exclusion criteria in 2021 and 2022. Potentially suitable participants were then posted a letter detailing the aims and objectives of the study with a self-addressed and stamped envelope for them to reply to the research team (Appendix 3 and 4). Participants who responded favourably to the letters and agreed to be contacted by the research team were contacted via telephone and the study was explained in detail. If agreeable and if they met all the inclusion criteria with none of the exclusion criteria, they were invited for a study visit.

## 2.2 Eligibility criteria

Subjects were eligible to participate in the study if:

1. Aged 18 years and above
2. Willing to participate in a research study on a voluntary basis

### Type 2 diabetes group

1. Volunteers with T2D and no hypertension, willing and able to give informed consent for participation in the study.
2. Male or Female aged 18 years or above.
3. Participants who are either drug naïve (i.e., treated with exercise and diet control) or on oral glucose lowering therapies for at least 12 weeks prior to screening.
4. HbA1c between 6.5% and 10% at screening.
5. Agreement to maintain prior diet and exercise habits for the duration of the study.

Subjects were **excluded** if any of the following were present:

1. Any type of diabetes other than T2D;
2. Pregnancy, or lactating mothers;
3. Past history of significant CAD;
4. Known HF;
5. Significant renal impairment (eGFR<30ml/min/m<sup>2</sup>);
6. Participation in a clinical trial of an investigational medicinal product in the preceding 12 weeks;
7. Known hypersensitivity to dobutamine or gadolinium or any other contraindications to MRI;
8. Participants with obesity where their girth exceeds the scanner bore;

### 2.3 Clinical assessments

On the first visit, all subjects underwent a clinical assessment. This included history for:

1. The presence of exclusion criteria as outlined above;
  2. Medical history and drug history including allergies;
  3. Diabetes history, including duration since the diagnosis, symptoms, presence of diabetic complications;
  4. Cardiovascular examination was also performed to assess for the presence of ventricular hypertrophy and valvular heart disease.
- 
1. Blood pressure measurements using a manual sphygmomanometer (an average of three supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Inc., Tampa, Florida));
  2. Height (cm) and weight (kg) using calibrated scales;
  3. Hip circumference (cm) and waist circumference (cm) measurements
  4. 12-lead resting electrocardiogram (ECG);
  5. Fasting blood samples for assessments of full blood count, estimated glomerular filtration rate, glucose, insulin, HbA1c, total cholesterol levels, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), free fatty acids, aminotransferase (ALT), bilirubin and albumin. For chapter 6 (Liraglutide treatment improves myocardial energetics and stress perfusion in type 2 diabetes- a randomised, single-centre, open label, cross-over drug trial) additional bloods including liver function tests, N-terminal pro b-type natriuretic peptide, high sensitivity troponin I, free fatty acids, beta

hydroxybutyrate, c-peptide levels and anti-glutamic acid decarboxylase antibodies were checked.

## 2.4 Scan Protocol

### Cardiac Volumes, Function and Mass

CMR is accurate, reproducible and well validated for measuring left ventricular volumes and mass(163). Cardiac volumes were acquired using Steady State Free Precession (SSFP) imaging. Pilot, horizontal long axis, vertical long axis, left ventricular outflow tract (LVOT) views and left and right ventricular short axis stack of contiguous images were acquired with the patient in the supine position. Each slice was 8mm thick with no interslice gap and was prospectively gated with echo time (TE), 1.4 ms; repetition time (TR), 3.9 ms; flip angle, 52°. The slices were obtained during a breath-hold at the end of normal expiration to minimize the effects of respiratory motion LV and RV short axis epicardial and endocardial borders were manually contoured from base to apex at end diastole and the endocardial border was again traced at end systole, (figure 2.1) for determining end diastolic volume (EDV); end systolic volumes (ESV); stroke volume (SV) using CVI42© (Circle Cardiovascular Imaging Inc., Calgary, Canada). The basal slice was selected for the left ventricle when at least 50% of the blood volume was surrounded by myocardium in both end-diastole and end-systole. The apical slice was defined as the final slice showing intracavity blood pool at both end-diastole and end-systole. For the right ventricle, volumes below the pulmonary valve were included (figure 2.1). From the inflow tract, RV volumes were excluded if the surrounding muscle was thin and not trabeculated, suggestive of the right atrium.

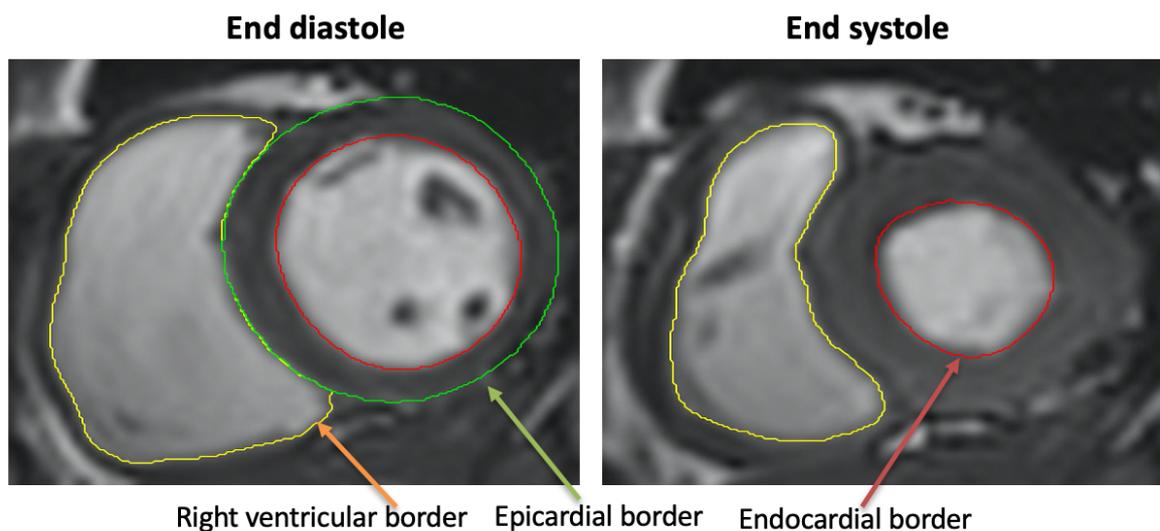
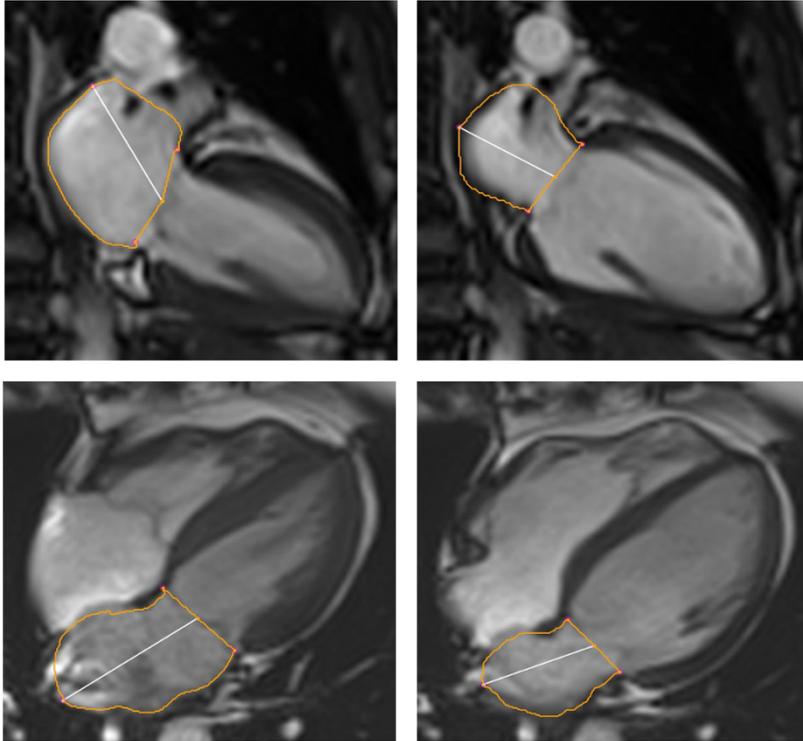


Figure 2.1 Left and right ventricular contours in end-diastole and end-systole

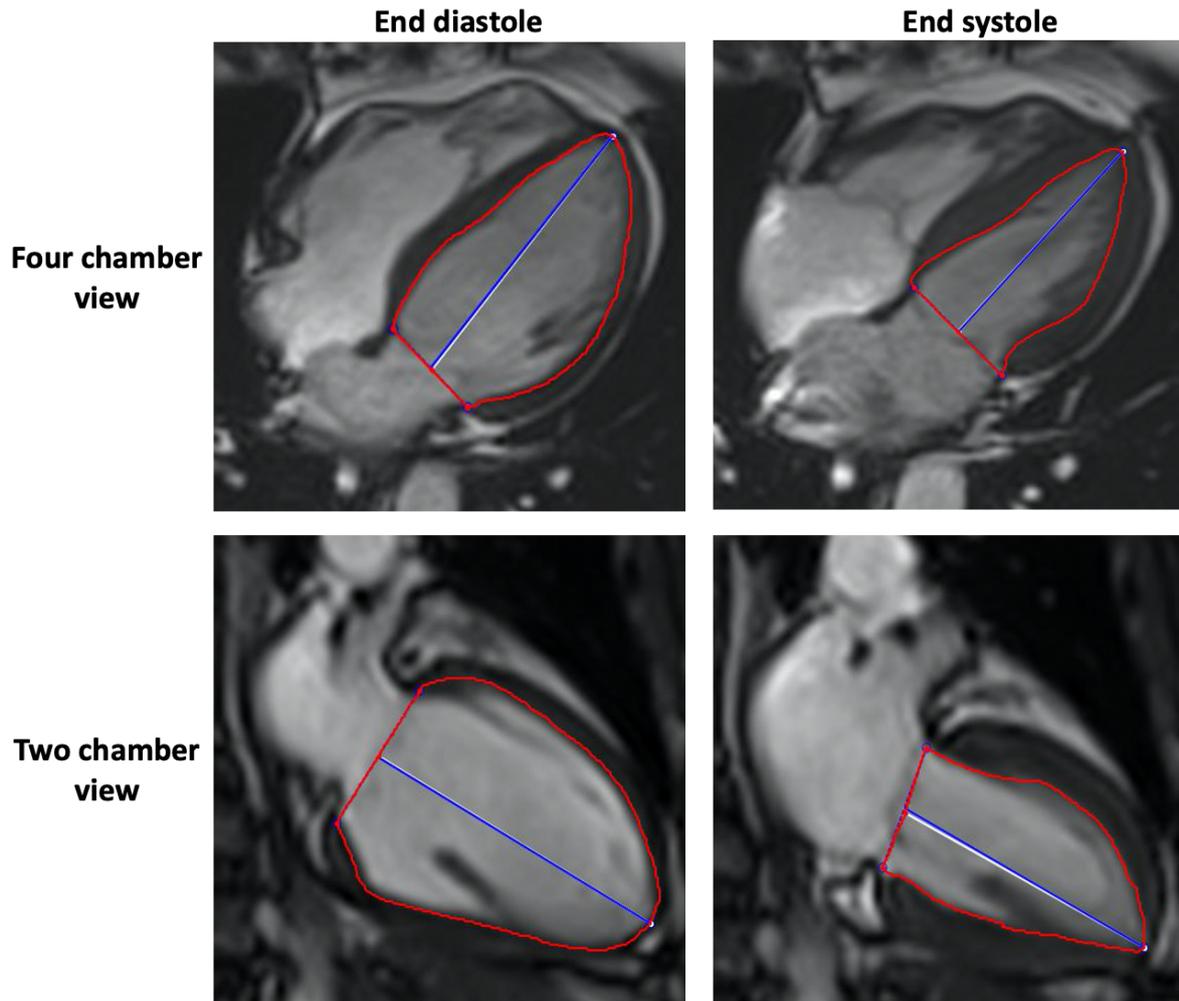
Ejection fraction (EF) and cardiac output (CO) are calculated ( $EF = SV/EDV$ ,  $CO = SV \times \text{heart rate}$ ). Myocardial mass is also calculated by subtracting the endocardial volume from the epicardial volume. Left ventricular mass is calculated based on prior knowledge of myocardial specific gravity ( $1.05 \text{ g/cm}^3$ ).

The LA volume and LA EF were calculated using the biplane area-length method in the horizontal and vertical long axes (figure 2.2). The LA endocardial border was manually contoured in both the horizontal and vertical long axes views with the mitral annulus serving as the division between the LA and LV. The maximum LA area was contoured in the frame immediately prior to mitral valve opening. The minimum LA area was contoured in the frame immediately after mitral valve closure. LA volumes (LAV) were calculated using the area-length method, where:  $\text{volume} = (0.85 \times \text{area}^2) / \text{length}$ . LAEF was derived as follows:  $\text{LAEF} = (\text{LAV}_{\text{max}} - \text{LAV}_{\text{min}}) / \text{LAV}_{\text{max}}$ .



*Figure 2.2 Two chamber and 4 chamber views of the LA in end-diastole and end-systole*

Further horizontal and vertical long axes views identical as those obtained at rest were acquired at peak dobutamine stress. LV function at peak stress was calculated by contouring the LV endocardial contours in the horizontal and vertical long axis views in end diastole and end systole (figure 2.3). LV volumes were calculated using the area-length method, where:  $\text{volume} = (0.85 \times \text{area}^2) / \text{length}$ . LV EF was derived as follows:  $\text{LVEF} = (\text{LVmax} - \text{LVmin}) / \text{LVmax}$ . The LA volumes were also contoured at peak stress as described above and LA EF was calculated in a similar manner as described above for rest.



*Figure 2.3 LV volumes by biplanar method in four chamber and two chamber view at end-diastole and end-systole*

### **Strain and mitral annular plane systolic excursion (MAPSE):**

In chapter 3 and 4, a convolutional neural network (CNN) model (164) was used for the automated measurement of Global Longitudinal Shortening (GL-Shortening) and mitral annular plane systolic excursion (MAPSE) in the 2-chamber and 4-chamber cine images implemented directly on the CMR scanners. The inferoseptal and anterolateral mitral annular hinge points were detected from the 4-chamber view. From the 2-chamber view, the anterior and inferior points were detected. The apex was marked

for all views. The detection was performed for every cine phase covering the entire cardiac cycle (figure 2.4).

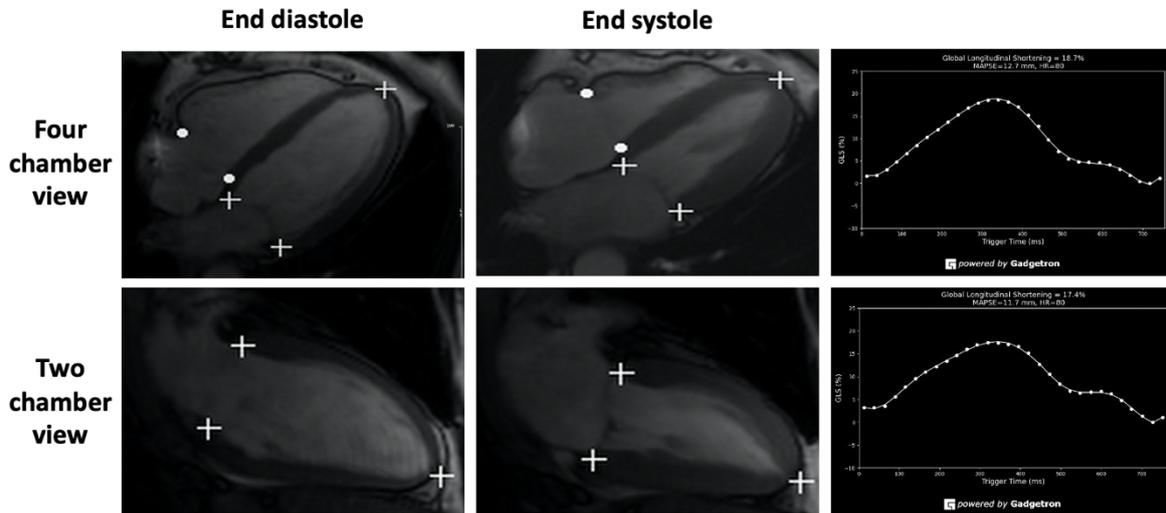
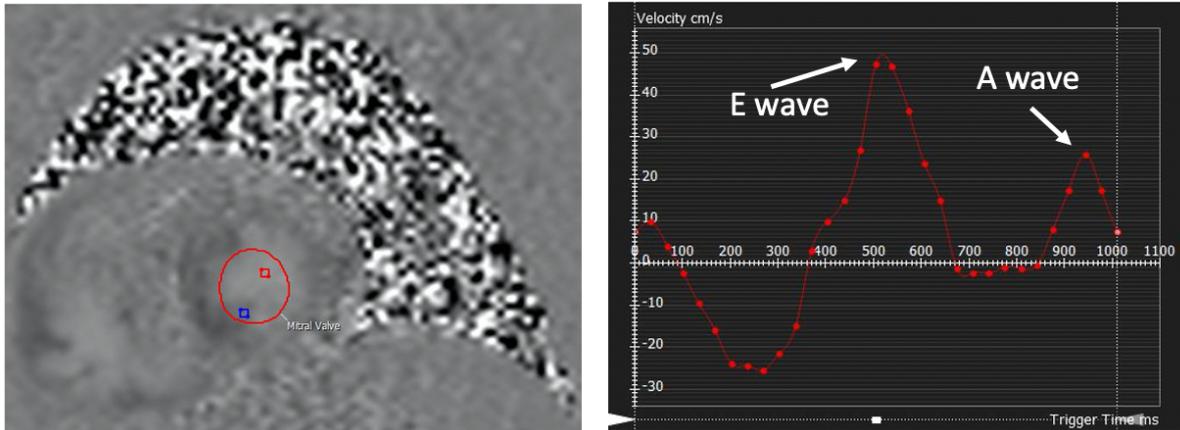


Figure 2.4 Global longitudinal shortening and MAPSE in two chamber and four chamber views

### Diastolic function assessment

To perform assessments of the diastolic function, phase contrast imaging of the mitral valve was performed (figure 2.5). The phase contrast slice was positioned at the tip of the mitral valve leaflets as seen in systole avoiding the left ventricular outflow tract and checked in the horizontal long axis, vertical long axis and left ventricular outflow tract views. A free breathing, time-resolved acquisition with velocity-encoding perpendicular to this plane was acquired. Post processing was done using CVI42© by contouring round the mitral valve (MV) leaflets in end systole and propagating the contour to all acquired phases followed by manual correction of the contours as required. Using this method peak E and A waves were obtained.



*Figure 2.5 Phase contrast imaging of the mitral valve inflow and the E and A waves obtained*

All the above imaging was performed at rest and repeated during peak stress once target heart rate (THR) was achieved using a dobutamine infusion.

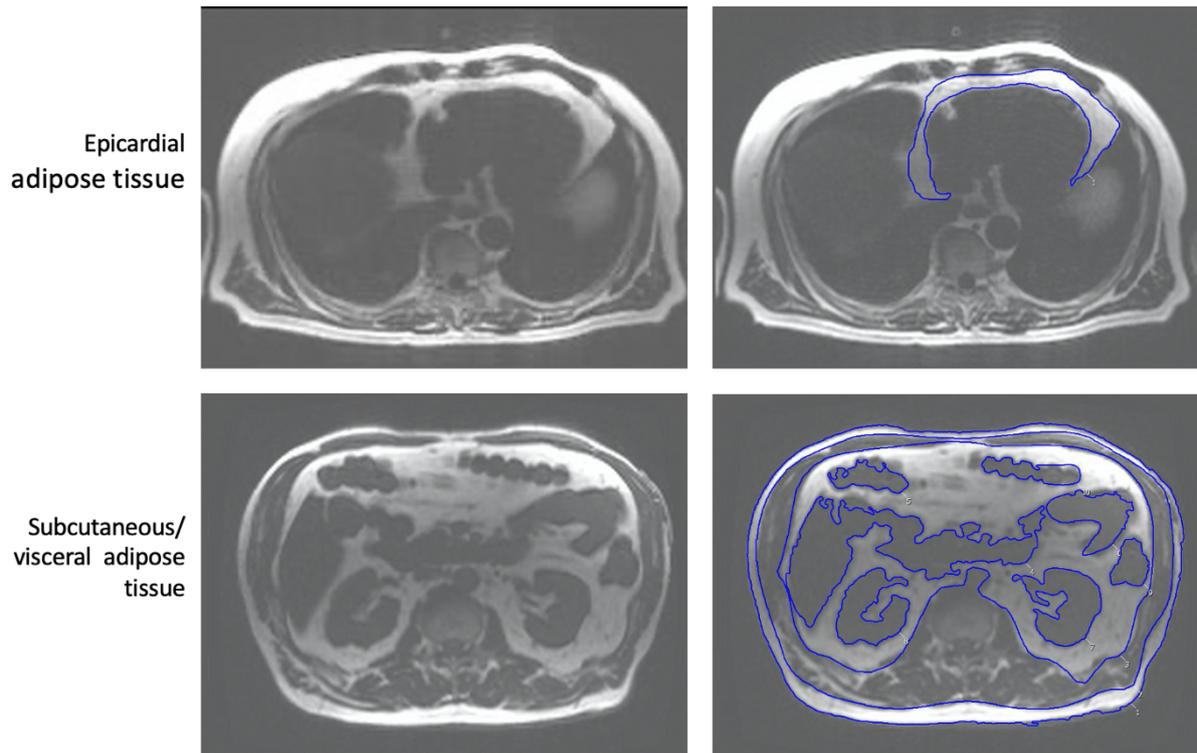
For strain analysis (Chapter 3) semi-automated feature tracking analysis in Circle cvi<sup>42</sup> software (Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) was used. Left ventricular epicardial and endocardial borders were manually traced in the 2-, 3-, and 4-chamber views at end-diastole, which were then propagated throughout the cardiac cycle. Automatic feature tracking yielded GLS measures. All contour tracings were inspected to ensure fidelity with manual adjustments as necessary. GLS measures included at least 2 long-axis images for strain assessment and were expressed as a percentage in which higher (less negative) values indicated worse contractile function.

### **Epicardial and visceral fat analysis**

For epicardial and abdominal visceral fat area measurements, single-shot acquisition of thoracic and abdominal non-breath-hold images was performed using a multi-echo

GRE sequence with gradient flyback for monopolar readout to acquire three echoes for each phase encode (parallel imaging rate 3) using GRAPPA with separate reference line acquisition, 32-channel cardiac array; bandwidth = 1,184 Hz/pixel; TE = 1.32, 2.34 3.35, and 4.37 milliseconds; TR= 9.77 milliseconds; readout flip angle = 20°; matrix = 192 × 108; single-shot duration = 242 milliseconds; field of view (FOV)= 300 × 225 mm<sup>2</sup>; section thickness = 6 mm), as previously described (165).

Abdominal VAT area was measured at the level of the third to fourth lumbar vertebral body from the single shot performed using multi-echo GRE sequence abdominal images. Adipose tissue was categorized into VAT and SAT through manual division, which was accomplished by drawing a line following the abdominal wall to separate intra- and extra-abdominal compartments (figure 2.6). The EAT area was traced from the thoracic multi-echo GRE sequence images acquired on transaxial orientation of a four-chamber view on a single section at maximal adiposity (figure 2.6). The VAT and SAT areas were segmented separately using cvi42 software and VAT over SAT area was calculated.



*Figure 2.6 Images of epicardial adipose tissue followed by visceral and subcutaneous adipose tissue acquisitions with their contouring*

### **Native T1 and post contrast T1 measurements**

Native T1 mapping was acquired in three slices using a breath-held modified Look-Locker inversion recovery acquisition, as previously described (166) (precontrast 5 seconds [3 seconds] 3 seconds and postcontrast 4 seconds [1 second] 3 seconds [1 second] 2 seconds schemes) and were planned using the 3 of 5 method (167). Postcontrast T1 mapping acquisition was performed exactly 15 minutes after the last contrast injection using identical planning as the native T1 map in the same three slices (figure 2.7).

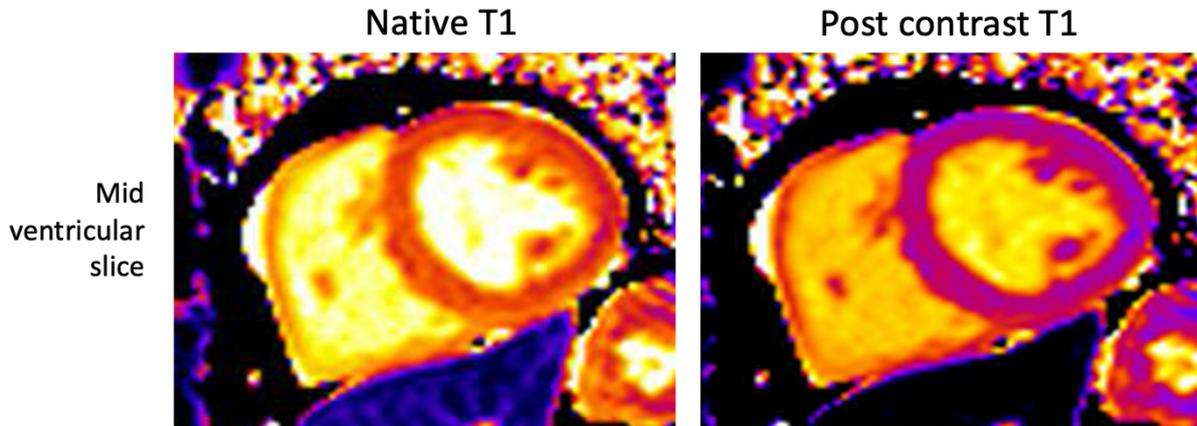


Figure 2.7 Native T1 and post-contrast T1 images at the mid-ventricular level

### Quantitative perfusion

Perfusion imaging used a free-breathing, fast low-angle shot (FLASH) MR protocol with motion-corrected (MOCO) automated in-line perfusion mapping using the Gadgetron streaming software image reconstruction framework, as previously described (168). An intravenous bolus of 0.05 mmol/kg gadobutrol (Gadovist®, Bayer Pharma, Berlin, Germany) was administered for each stress and rest perfusion imaging sequence (figure 2.8).

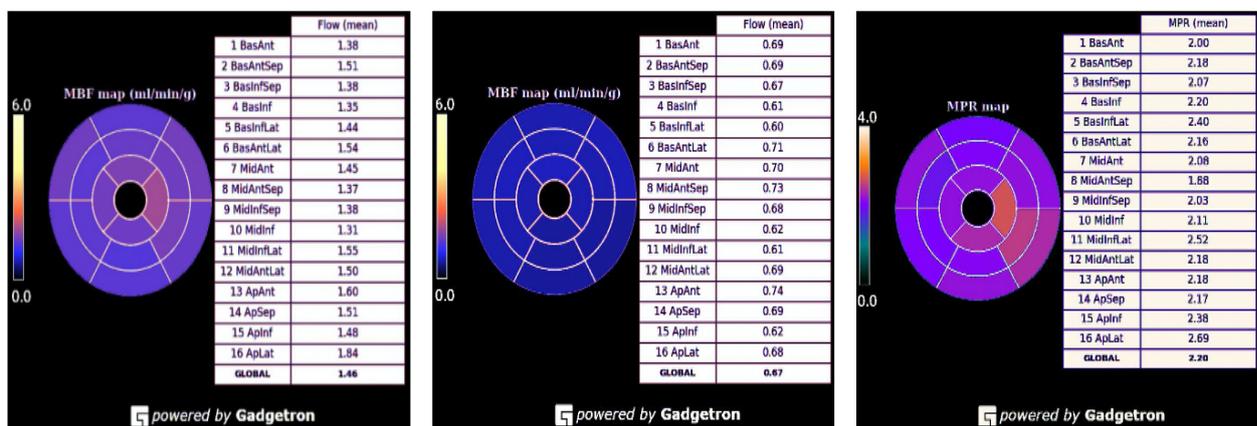


Figure 2.8 Representative quantitative perfusion maps of MBF at stress, MBF at rest and MPR

For Chapter 3 (Prospective longitudinal characterization of the relationship between type 2 diabetes and cardiac structural and functional changes) and chapter 4 (Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes) , stress perfusion imaging was performed with adenosine which was infused at a rate of 140  $\mu\text{g}/\text{kg}/\text{min}$  and increased up to a maximum of 210  $\mu\text{g}/\text{kg}/\text{min}$  according to hemodynamic and symptomatic response (a significant hemodynamic response to adenosine stress was defined as a  $>10$ -beats/min increase in heart rate or a BP drop  $>10$  mm Hg and  $>1$  adenosine-related symptom, e.g., chest tightness, breathlessness). Participants had continuous ECG and HR monitoring with BP monitoring at 90 second intervals.

For chapter 5 (Cardiac adaptations to acute hemodynamic stress in function, perfusion and energetics in type 2 diabetes with overweight/obesity) and chapter 6 (Liraglutide treatment improves myocardial energetics and stress perfusion in type 2 diabetes- a randomised, single-centre, open label, cross-over drug trial) stress perfusion imaging was performed with dobutamine in order to elicit the mechanistic changes in the myocardium secondary to its inotropic effect. The dobutamine infusion was started at a dose of 10  $\mu\text{g}/\text{kg}/\text{min}$  and titrated to a maximum dose of 40  $\mu\text{g}/\text{kg}/\text{min}$  to achieve a target heart rate of 65% of the age-predicted maximum, with continuous ECG and HR monitoring along with blood pressure (BP) monitoring at 90 second intervals. Mean rate pressure product was recorded at rest and stress. Target heart rate was maintained for the perfusion acquisitions.

A minimum 10-minute interval was kept between perfusion acquisitions to ensure equilibration of gadolinium kinetics and resolution of all hemodynamic effects of adenosine. For each perfusion acquisition, an intravenous bolus of 0.05 mmol/kg of gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5 mL/s followed by

a 20-mL saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer, Leverkusen, Germany).

### **Late Gadolinium Enhancement**

For late gadolinium enhancement (LGE) CMR, a top-up bolus of 0.05 mmol/kg of body weight of a gadolinium-based contrast agent- gadobutrol (Gadovist®, Bayer Pharma, Berlin, Germany) followed by a 20-mL saline flush were administered through an intravenous cannula inserted into the antecubital fossa. Electrocardiographically gated images were acquired at least 5mins after contrast administration in matching short axis plane slices as T1 images to exclude the presence of previous silent myocardial infarction or regional fibrosis.

Areas of LGE were visually scored as absent or present.

### **<sup>31</sup>P-MR Spectroscopy Protocol**

All scans were performed on a 3.0 Tesla MR system (Prisma, Siemens, Erlangen, Germany). <sup>31</sup>P-MRS was performed to obtain the PCr/ATP from a voxel placed in the mid-ventricular septum, with the subjects lying supine with the <sup>31</sup>P transmitter/receiver cardiac coil (Rapid Biomedical GmbH, Rimpfing, Germany) placed over their heart, in the iso-centre of the magnet. Coil position was standardised to be placed above the mid ventricular septum (figure 2.9). A series of inversion-recovery free induction decay signals were acquired, in order to allow for correction of transmit efficiency differences between subjects (due to variable coil loading) during analysis(169). Four fiducial markers were positioned on the anterior coil surface to allow spatial localisation of the coil relative to the patient anatomy and spectroscopy acquisition. These data allowed

determination of study-specific flip angle maps to allow correction for the differential radiofrequency saturation of metabolites.  $^{31}\text{P}$ -MRS data were acquired with a non-gated 3-D acquisition-weighted chemical shift imaging (CSI) sequence(170). The acquisition matrix was 16 x 8 x 8 for the protocol. Field of view was 240 x 240 x 200 mm. The acquisition was run with a fixed TR of 720ms. Two 50mm saturation bands were placed over the chest wall muscle and an additional 50mm saturation band was placed over the liver.

$^{31}\text{P}$ -MRS spectra were processed with a custom Matlab (The Mathworks Inc., Natick, MA) implementation of the Advanced Method of Accurate, Robust, and Efficient Spectroscopic (AMARES) fitting algorithm(171), using prior knowledge(172) specifying 11 Lorentzian peaks ( $\alpha,\beta,\gamma$ -ATP multiplet components, PCr, PDE, and 2x2,3-DPG), fixed amplitude ratios and scalar couplings for the multiplets, and a fixed begin time(173). Peak areas were corrected for Nuclear Overhauser Effects (NOE) using the following empirical correction factors(169): PCr 0.80,  $\beta$ -ATP 0.88,  $\alpha$ -ATP 0.88,  $\gamma$ -ATP 0.79, 2,3-DPG 0.70. Partial saturation was corrected the excitation flip angle at the centre of the chosen voxel and literature  $T_1$  values(174): PCr 3.8 s,  $\gamma$ -ATP 2.4 s,  $\alpha$ -ATP 2.5 s,  $\beta$ -ATP 2.7 s, 2,3-DPG 1.39 s, and PDE 1.1 s. The resulting ATP amplitudes were averaged and corrected for blood contamination by subtracting 11% of the total 2,3-DPG amplitude(175) (figure 2.9)

Acquisition time was 9 minutes during rest and a further 9 mins once the target heart rate had been achieved. The rate pressure product (RPP) was calculated using the product of the heart rate and systolic blood pressure, providing a measure of cardiac work.  $^{31}\text{P}$ -MRS post processing analysis was performed as previously described.(176, 177)

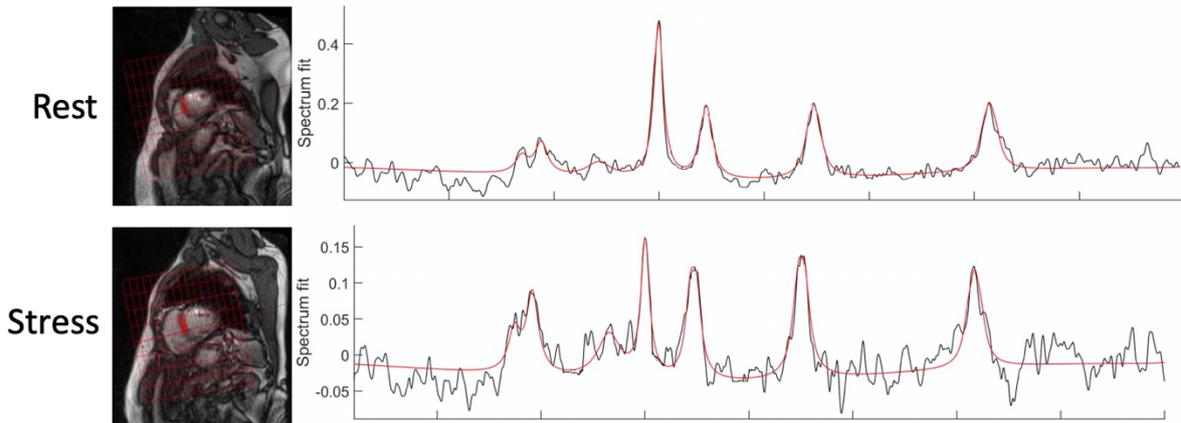


Figure 2.9 MRS voxel position and PCr/ATP spectra obtained at rest and stress

## 2.5 Statistical analysis

Statistical analysis was performed using SPSS (IBM SPSS statistics, version 26.0). All data were checked for normality using Shapiro-Wilk test and presented as mean  $\pm$  standard deviations, mean [95% confidence intervals] and median (interquartile range) as appropriate. Normally distributed data sets were analysed with the independent Student *t* test. Categorical data were compared with Pearson's chi-square test. Comparisons between more than two groups were performed by 1-way analysis of variance with post hoc Bonferroni corrections. Bivariate correlations were performed using the Pearson correlation coefficient. For these tests a p-value of  $\leq 0.05$  was considered statistically significant. However, a p-value of  $< 0.003$  was applied as indicating statistical significance, based on the Bonferroni correction, where 6 comparisons or more between 4 or more groups were performed.

## **Chapter 3**

# **Prospective longitudinal characterization of the relationship between type 2 diabetes and cardiac structural and functional changes**

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### **3.1 Abstract**

#### **Objectives:**

In a cohort of type 2 diabetes (T2D) patients who underwent baseline cardiac magnetic resonance (CMR) and biomarker testing, during a median follow-up of 6-years we aimed to determine longitudinal changes in the phenotypic expression of heart disease in diabetes; report clinical outcomes; and compare baseline clinical characteristics and CMR findings of patients who experienced major adverse cardiovascular events (MACE) to those remaining MACE free.

#### **Background:**

T2D increases the risk of heart failure (HF) and cardiovascular mortality. The long-term impact of T2D on cardiac phenotype in the absence of cardiovascular disease and other clinical events is unknown.

#### **Methods:**

T2D patients (n=100) with no history of cardiovascular disease or hypertension were recruited at baseline. Biventricular volumes, function, and myocardial extracellular volume fraction (ECV) were assessed by CMR and blood biomarkers taken. Follow-up CMR was repeated in those without interim clinical events after 6-years.

#### **Results:**

Follow-up was successful in 83 participants. Of those, 29 experienced cardiovascular/clinical events (36%). Of the remaining 59, 32 patients who experienced no events received follow-up CMR. In this cohort, despite no significant

changes in blood pressure, weight, or glycated-haemoglobin, significant reductions in biventricular end-diastolic-volumes and ejection fractions occurred over time. The mean ECV was unchanged. Baseline plasma high-sensitivity cardiac-troponin-T (hs-cTnT) was significantly associated with change in left ventricular (LV) ejection fraction. Patients who experienced MACE had higher LV mass and greater LV concentricity than those who remained event-free.

**Conclusions:**

T2D results in reductions in biventricular size and systolic function over time even in the absence of cardiovascular/clinical events.

## 3.2 Introduction

Cardiovascular disease represents the primary cause of death in type 2 diabetes patients (T2D)(1). Although T2D is recognized as a strong risk factor for atherosclerosis-related events, heart failure (HF) is the commonest initial presentation of cardiovascular disease in T2D(1, 16, 127). The risk of developing HF is increased 2.4-fold in men and 5-fold in women with T2D compared with age-matched controls(178), and the combination of T2D and HF is associated with a 4- to 6-fold higher mortality(179, 180). The early detection of adverse subclinical myocardial structural and functional alterations associated with progressive myocardial dysfunction might offer the opportunity of early initiation of disease modifying pharmacological therapies prior to the onset of overt HF(181).

Cardiac magnetic resonance imaging (CMR) is the reference standard for assessment of cardiac volumes, mass and function(182). Using CMR, patients with T2D have been extensively phenotyped with a nuanced description of disease burden(37, 183). However, to our knowledge no CMR study to date has examined longitudinal changes in biventricular structure and function in T2D patients with no prior cardiovascular disease.

In this longitudinal observational study, we tested the hypothesis that T2D would be associated with a progressive decline in biventricular systolic function even in a cohort of diabetes patients with no prior cardiovascular disease, or interim major adverse cardiovascular events (MACE) during the follow-up period. We also sought to report clinical outcomes, and compare demographic, clinical, biochemical variables, and

CMR and plasma biomarkers measured at baseline between those patients who experienced MACE and those who remained free of MACE during the follow-up period.

### **3.3 Methods**

Using CMR at two time points: baseline visit and end of the study, we performed a prospective longitudinal study in a cohort of ethnically diverse, asymptomatic T2D patients with no history or evidence on examination of cardiovascular disease. Participants who remained asymptomatic and free of MACE or any other new clinical comorbidity were invited for a second CMR scan after 6-years.

#### **Participants**

Recruitment was performed from primary care health centers in Leeds, United Kingdom. One hundred participants with T2D were recruited at baseline(102). The results of this initial study have previously been published(102, 184). For the current study all surviving T2D participants who could be contacted and remained eligible were offered a follow-up research visit for a repeat CMR scan.

#### **Inclusion and exclusion criteria**

Asymptomatic adult patients with a diagnosis of T2D (diagnosed according to the World Health Organization criteria)(185) with the ability to provide informed written consent were recruited at baseline. Patients were excluded if they had a previous diagnosis of cardiovascular disease [previous cardiac surgery, angioplasty,

myocardial infarction, angina, moderate or above valvular heart disease, atrial fibrillation (AF)], hypertension (resting systolic blood pressure [BP] >140mmHg and diastolic BP >90mmHg on 24 hour ambulatory BP monitoring), contraindications to CMR, ischemic changes on 12-lead electrocardiogram (ECG), renal impairment (estimated glomerular filtration rate [eGFR] below 30 mL/min/1.73m<sup>2</sup>), or if they were using insulin. After 6-years, surviving participants who remained asymptomatic, with no MACE, other diabetes complications, or important co-morbidity (such as inflammatory disease or malignancy) were invited for a second CMR study.

### **Baseline clinical assessment**

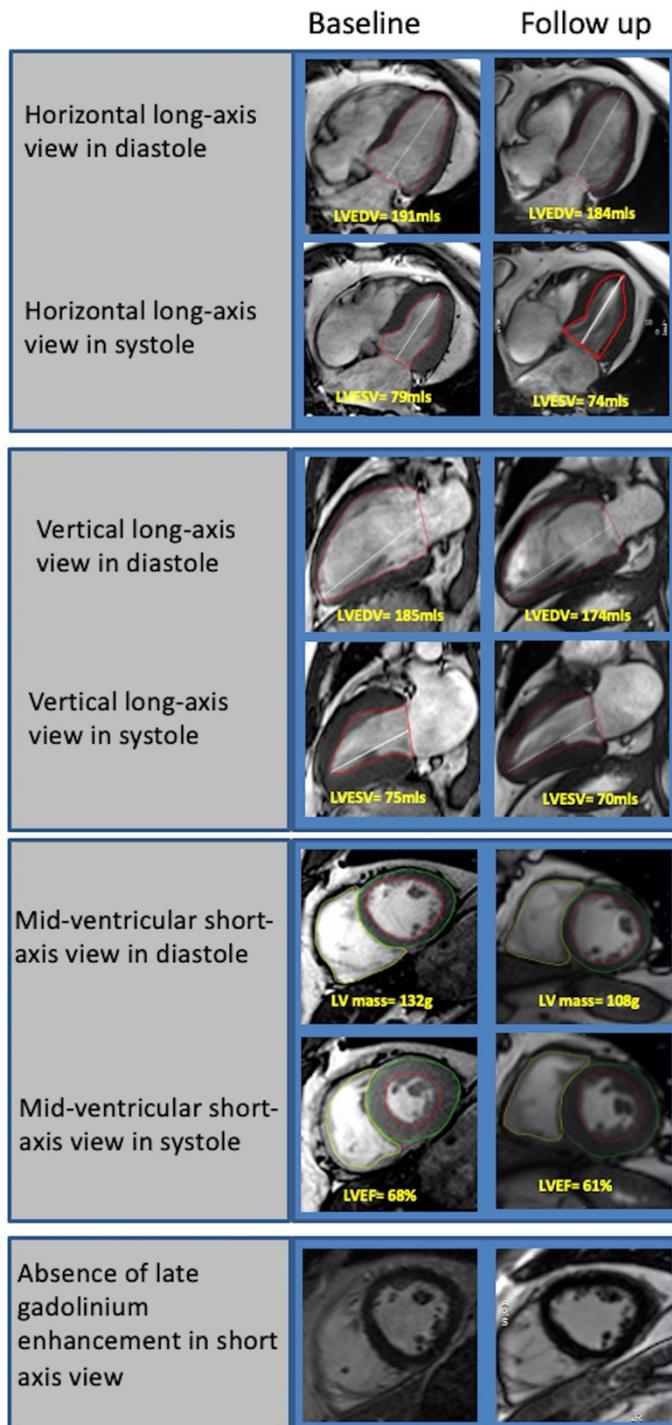
At baseline and at the follow-up visit, height and weight were recorded and body mass index (BMI) was calculated. A fasting blood sample was taken from each participant at baseline for assessments of full blood count (FBC), eGFR, fasting glucose, glycated haemoglobin (HbA1c), high-sensitivity cardiac-troponin-T (hs-cTnT) and N-terminal pro hormone B-type natriuretic peptide (NT-proBNP) levels. All participants underwent resting ECG and all had 24-hour BP monitoring at baseline to exclude undiagnosed hypertension(102). At the follow-up study visit, brachial BP was recorded as an average of 3 supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Corp), a fasting blood sample was obtained for repeated assessments of FBC, eGFR, glucose, HbA1c and lipids, and a resting ECG was recorded.

### **Cardiac magnetic resonance imaging**

Imaging at baseline was performed on a 3.0 Tesla Philips Achieva Magnetic Resonance system and at year-6 follow up, this was performed on a 3.0 Tesla Siemens Prisma system. The baseline CMR protocol has previously been described(102). Follow-up scans were performed using a matching imaging protocol. Images for biventricular and left atrial (LA) volumes and function were acquired using a steady state free precession (SSFP) sequence with breath holding at end expiration in multiple orientations (Figure-3.1). Adenosine stress myocardial-perfusion CMR was performed to rule out significant epicardial coronary artery stenosis(105). Pharmacological stress was achieved with adenosine infusion at 140mcg/kg/min for a minimum of 3 mins and an intravenous bolus of 0.075 mmol/kg gadobutrol (Gadovist®, Bayer Pharma, Berlin, Germany) was administered for each stress and rest perfusion imaging sequence. Visual analysis of the perfusion images was performed by one reporter (EL, with >8 years of CMR experience and level 3 accreditation). Ischemia was defined as a territory with a perfusion defect during stress(105). Late gadolinium enhancement (LGE) imaging was performed in matching LV short-axis planes >8 minutes after contrast administration to exclude the presence of previous silent myocardial infarction or regional fibrosis.

All image analysis was performed off-line by AC (with 2 years of CMR experience) in a blinded fashion and all scan contours were subsequently reviewed by EL using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada). Baseline and follow-up images were analyzed in a random order after the second visit by investigators blinded to any other data. Biventricular volumes and ejection fraction (EF) were obtained from contouring the endocardial and epicardial borders in diastole and systole on the SSFP short axis stacks. The LA volume and EF were calculated using the biplane area-length method in the horizontal and vertical long axes as previously described(182).

Using cvi42 Tissue Tracking software, global longitudinal strain (GLS) and as a marker of diastolic function, LV diastolic strain measurements were performed from balanced SSFP short-axis and 2 chamber and 4 chamber long axis cine images, to calculate circumferential peak early diastolic strain rate (PEDSR) and longitudinal PEDSR(183).



*Figure 3.1: Representative examples of CMR imaging (horizontal long axis, vertical long axis, mid-ventricular short axis in diastole and systole) and late-gadolinium enhancement imaging (mid-ventricular short axis) in a patient with T2D at baseline and follow-up.*

## **Statistical analysis**

Statistical analysis was performed using SPSS (IBM SPSS statistics, version 26.0). Categorical data were compared with Pearson's chi-square test. Continuous variables were checked for normality using the Shapiro-Wilks test and are presented as mean  $\pm$ SD. Comparisons of CMR data between baseline and follow-up were performed with two-tailed paired t-test. Bivariate correlations were performed using Pearson's correlation coefficient. The relationships of change in left ventricular ejection fraction (LVEF) and right ventricular ejection fraction (RVEF) ( $\Delta$ LVEF and  $\Delta$ RVEF respectively) with age, BMI, HBA<sub>1c</sub>, fasting glucose, resting BP and heart rate (HR), NT-proBNP and hs-cTnT were analyzed using multiple logistic regression. A p-value of  $\leq 0.05$  was considered statistically significant.

## **Ethical considerations**

The study was approved by the National Research Ethics Committee (Ref:13/YH/0098) and informed written consent was obtained from each participant. The follow-up assessment was given additional ethical approval (Ref:18/YH/0168). Participants were asked to sign a second consent form for the follow-up scan.

### 3.4 Results

#### Participant characteristics and clinical outcomes

Clinical outcomes of the baseline cohort were determined after a median follow-up of 6.3years (interquartile range [IQR]: 6.05–6.53years) using electronic health records systems, and symptom status was determined by phone assessments (Figure-3.2).

Demographics, clinical and biochemical data are shown in Table 3.1. Of the hundred participants with T2D recruited at baseline (82 male, mean age  $61 \pm 11$  years, median diabetes duration 4.1 years (IQR: 1.4–7 years), 17 participants were uncontactable (Figure-3.2). The healthcare records of the remaining 83 participants revealed that 5 participants (6%) had died during the follow-up period (one due to acute coronary syndrome [ACS]), 8 participants (9.6%) had survived an ACS, 3 participants (3.8%) had a cerebrovascular accident, 6 participants (7.7%) developed a malignancy, 1 participant (1.3%) had a permanent pacemaker implanted for a high-grade atrioventricular block and 1 participant (1.3%) developed significant renal dysfunction (Figure-3.2). These participants were not invited back for a follow-up CMR scan. Of the remaining 59 participants (76%) with T2D who remained free of MACE invited for a repeat CMR scan, 16 participants declined, and a further 6 participants were unable to attend for their research visit due to the coronavirus pandemic (Figure 3.2). Hence 37 participants completed a second CMR scan. Of these, 5 were found to have suffered a silent MI as evidenced by subendocardial hyperenhancement on LGE and were excluded from further analysis leaving a study population of 32 participants. 25% of the original study population had suffered a major adverse cardiovascular event (MI, angina, revascularisation, stroke, cardiovascular mortality) during the 6-year follow-up period (Figure-3.3) with an overall clinical event rate of 35%.

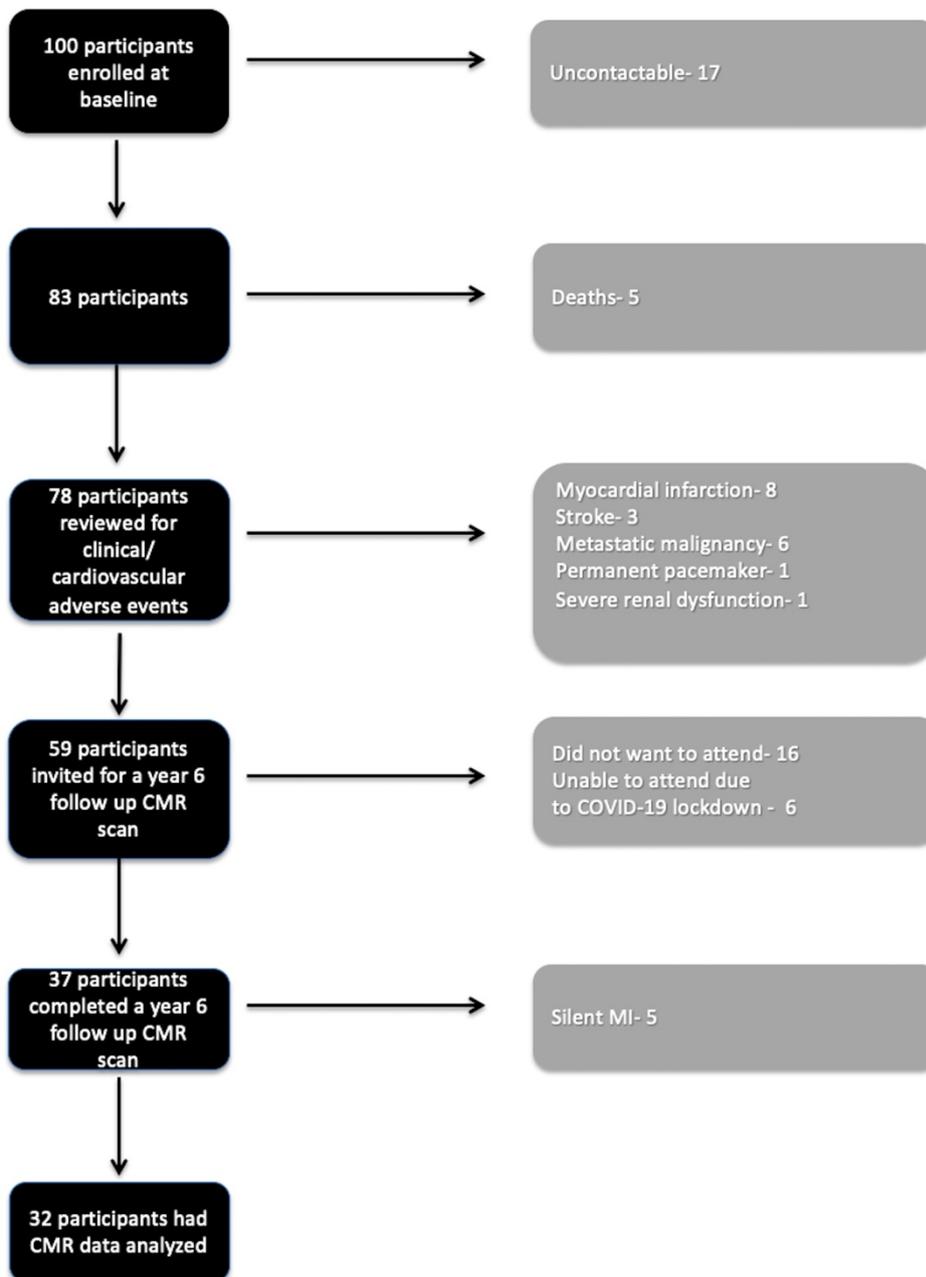


Figure-3.2: Recruitment flowchart demonstrating recruitment and follow-up pathway for the participants (CMR- Cardiac magnetic resonance; COVID-19- Coronavirus disease; MI- myocardial infarction)

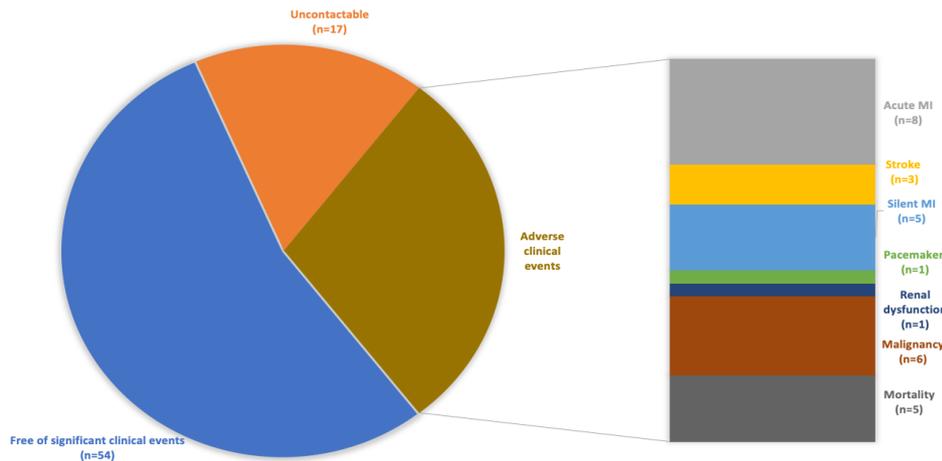


Figure-3.3: The major adverse cardiovascular event rate (MI, angina, revascularisation, CVA, death) during the 6-year follow-up period, including the patients with a silent MI, amounted to 25% in this study with an overall clinical event rate of 35%.

**Table-3.1** Clinical and Biochemical Characteristics

Variable	Baseline total participants (n = 100)	Follow up (n = 32)	P value
Age, years	61 ± 11	64 ± 11	0.2
BMI, kg/m <sup>2</sup>	29 ± 4	27 ± 4	0.02*
Male, n (%)	82 (82)	29 (91)	0.2
Ethnicity, n (%)			
Caucasian	67 (67)	22 (69)	0.7
Asian	29 (29)	8 (25)	0.4
Afro-Caribbean	4 (4)	2 (6)	0.1
Diabetes duration, years	5.0 ± 4.4	10.9 ± 1.3	0.0001*
Smoking, n (%)	6 (6)	2 (6)	0.9
Heart rate, bpm	71 ± 12	68 ± 12	0.2
Systolic blood pressure, mmHg	131 ± 15	129 ± 16	0.5
Diastolic blood pressure, mmHg	73 ± 9	74 ± 7	0.6
Plasma fasting glucose, mmol/L	9.9 ± 4.1	9.4 ± 3.8	0.5
Glycated haemoglobin, mmol/mol	63 ± 20	64 ± 18	0.7
Total cholesterol, mmol/L	4.4 ± 1.1	4.5 ± 1.2	0.7
HDL, mmol/L	1.19 ± 0.35	1.36 ± 0.38	0.03*
LDL, mmol/L	2.60 ± 0.98	2.59 ± 1.21	0.9
Medications, n (%)			
Metformin	87 (87)	22 (69)	0.01*
Sulphonylurea	33 (33)	13 (40)	0.4
Gliptins	19 (19)	7 (22)	0.7
Thiazolidendiones	5 (5)	2 (6)	0.8

<b>SGLT2-inhibitors</b>	0 (0)	2 (6)	0.08
<b>GLP-1RA</b>	2 (2)	1 (3)	0.7
<b>Aspirin</b>	18 (18)	9 (28)	0.2
<b>Statin</b>	69 (69)	23 (72)	0.7
<b>ACEI</b>	0 (0)	13 (40)	0.001*
<b>ARB</b>	0 (0)	3 (9)	0.0001*

Values are mean  $\pm$  standard deviations or percentages. \*signifies  $P \leq 0.05$ . n-numbers; BMI-body mass index; kg-kilogram; m-metre; bpm-beats per minute; mmHg-millimetres of mercury; mmol-millimoles; L-litres; mol-moles; HDL-high-density lipoprotein; LDL-low-density lipoprotein; SGLT2- Sodium-glucose co-transporter 2; GLP-1RA- Glucagon like peptide-1 receptor agonist; ACEI-angiotensin converting enzyme inhibitor; ARB-angiotensin receptor blocker

### Baseline to follow-up demographics and medical therapy

In the 32 patients comprising the present study cohort, 29 were male, the mean age was  $64 \pm 9$  years, and median diabetes duration was 11.9 years (IQR: 11.8–12.3 years). There were no significant differences in resting HR and BP, glycemic control or BMI between the baseline and follow-up measurements (Table 3.2). Glucose lowering treatment had been altered for the majority of patients between baseline and follow-up. While the proportion of patients on a biguanide reduced from 88% to 69% ( $p=0.01$ ), the proportion taking a sodium-glucose co-transporter-2 (SGLT2) inhibitor increased from none to 12% ( $p=0.03$ ). The number of participants on sulphonylureas, thiazolidinediones, gliptins, aspirin or statins did not change significantly during the follow-up period.

None of the patients were on an angiotensin converting enzyme inhibitor (ACEI) or an angiotensin receptor blocker (ARB) therapy at baseline as per recruitment criteria of the initial study (102), whereas 13 participants (41%) were receiving this therapy at the time of follow-up visit (Table 3.2).

**Table-3.2:** Clinical and biochemical characteristics of the participants who had baseline and year 6 follow up CMR scans

Variable	Baseline (n = 32)	Follow up (n = 32)	P value
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<b>Age, years</b>	58 ± 11	64 ± 11	0.03*
<b>BMI, kg/m<sup>2</sup></b>	28 ± 4	27 ± 4	0.1
<b>Male, n (%)</b>	29 (91)	29 (91)	1.0
<b>Smoker, n (%)</b>	1 (3)	2 (6)	0.6
<b>Diabetes duration, years</b>	5.1 ± 1.2	10.9 ± 1.3	0.0001*
<b>Heart rate, bpm</b>	72 ± 13	68 ± 12	0.3
<b>Systolic blood pressure, mmHg</b>	131 ± 16	129 ± 16	0.7
<b>Diastolic blood pressure, mmHg</b>	73 ± 10	74 ± 7	0.4
<b>Plasma fasting glucose, mmol/L</b>	8.5 ± 3.5	9.4 ± 3.8	0.3
<b>Glycated haemoglobin, mmol/mol</b>	61 ± 15	64 ± 18	0.13
<b>hs-cTnT, ng/ml</b>	7.35 ± 5.14	-	
<b>Total cholesterol, mmol/L</b>	4.5 ± 1.3	4.5 ± 1.2	1.0
<b>HDL, mmol/L</b>	1.24 ± 0.31	1.36 ± 0.38	0.2
<b>LDL, mmol/L</b>	2.86 ± 1.19	2.59 ± 1.21	0.4
<b>Medications n (%)</b>			
<b>Metformin, n (%)</b>	28 (88)	22 (69)	0.01*
<b>Sulphonylurea, n (%)</b>	12 (38)	13 (40)	0.3
<b>Gliptin, n (%)</b>	3 (9)	7 (22)	0.1
<b>Thiazolidinediones, n (%)</b>	0	2 (6)	0.1
<b>SGLT2 inhibitors, n (%)</b>	0	4 (12)	0.03*
<b>Aspirin, n (%)</b>	7 (22)	9 (28)	0.1
<b>Statin, n (%)</b>	22 (69)	23 (72)	0.3
<b>ACEI, n (%)</b>	0 (0)	13 (40)	0.0001*
<b>ARB, n (%)</b>	0 (0)	3 (9)	0.08

Values are mean ± standard deviations or percentages. \*signifies  $P \leq 0.05$ . CMR-cardiovascular magnetic resonance imaging; n-numbers; BMI-Body mass index; kg-kilogram; m-metre; bpm-beats per minute; mmHg-millimetres of mercury; mmol-millimoles; L-litres; mol-moles; hs-cTnT-high sensitivity cardiac troponin-T; ng-nanograms; HDL-high density lipoprotein; LDL-low density lipoprotein; SGLT2-Sodium glucose co-transporter 2; ACEI-angiotensin converting enzyme inhibitor; ARB-angiotensin receptor blocker

### Cardiac geometry, function and myocardial scarring

The CMR results of the 32 participants at baseline and follow-up are shown in Table-3.3. At follow-up there was a reduction in cardiac size with reduced biventricular end diastolic volumes (Figures-3.4A & 3.4B) and a deterioration of biventricular systolic function (mean LVEF 60±7% vs. 55±8%,  $p=0.0001$ ; mean RVEF 55±5% vs. 51±7%,  $p=0.003$ )(Figures-3.4C & 3.4D) with reductions in stroke volumes.

**Table-3.3: CMR findings**

<b>Variable</b>	<b>Baseline (n = 32)</b>	<b>Follow up (n = 32)</b>	<b>P value</b>
<b>LV end diastolic volume (ml)</b>	159 ± 29	145 ± 22	0.005*
<b>LV end diastolic volume index (ml/m<sup>2</sup>)</b>	78 ± 12	73 ± 10	0.02*
<b>LV end systolic volume (ml)</b>	64 ± 16	65 ± 19	0.5
<b>LV end systolic volume index (ml/m<sup>2</sup>)</b>	31 ± 7	33 ± 9	0.3
<b>LV stroke volume (ml)</b>	95 ± 20	80 ± 14	0.001*
<b>LV ejection fraction (%)</b>	60 ± 7	55 ± 8	0.0001*
<b>ΔLVEF (%)</b>		- (5.66 ± 4.38)	
<b>LV mass (gm)</b>	102 ± 17	94 ± 16	0.01*
<b>LV mass index (gm/m<sup>2</sup>)</b>	51 ± 8	47 ± 8	0.04*
<b>LV mass to LV end diastolic volume (gm/ml)</b>	0.65 ± 0.12	0.66 ± 0.14	0.8
<b>Global longitudinal strain (% , negative)</b>	13.06 ± 2.05	11.74 ± 2.54	0.8
<b>Peak diastolic circumferential strain rate (1/s)</b>	0.98 ± 0.28	1.04 ± 0.23	0.4
<b>Peak diastolic longitudinal strain rate (1/s)</b>	0.86 ± 0.19	0.69 ± 0.17	0.1
<b>RV end diastolic volume (ml)</b>	166 ± 33	142 ± 25	0.03*
<b>RV end diastolic volume index (ml/m<sup>2</sup>)</b>	82 ± 14	71 ± 12	0.0001*
<b>RV end systolic volume (ml)</b>	76 ± 18	70 ± 16	0.05*
<b>RV end systolic volume index (ml/m<sup>2</sup>)</b>	37 ± 8	35 ± 8	0.1
<b>RV stroke volume (ml)</b>	91 ± 20	72 ± 15	<0.0001*
<b>RV ejection fraction (%)</b>	55 ± 5	51 ± 7	0.003*
<b>ΔRVEF (%)</b>		- (6.69 ± 4.15)	
<b>LA maximum volume (ml)</b>	88 ± 17	67 ± 21	0.0001*
<b>LA ejection fraction (%)</b>	58 ± 6	56 ± 9	0.4
<b>Extra-cellular volume (%)</b>	24.96 ± 3.02	24.10 ± 2.66	0.3

Values are mean±standard deviations or percentages. \*signifies P≤0.05. CMR-cardiac magnetic resonance imaging; n-numbers; LV-left ventricle; ml-milliliters; m-metre; ΔLVEF-change in LV ejection fraction; gm-grams; s-seconds; RV-right ventricle; ΔRVEF-change in RV ejection fraction; LA-left atrium.

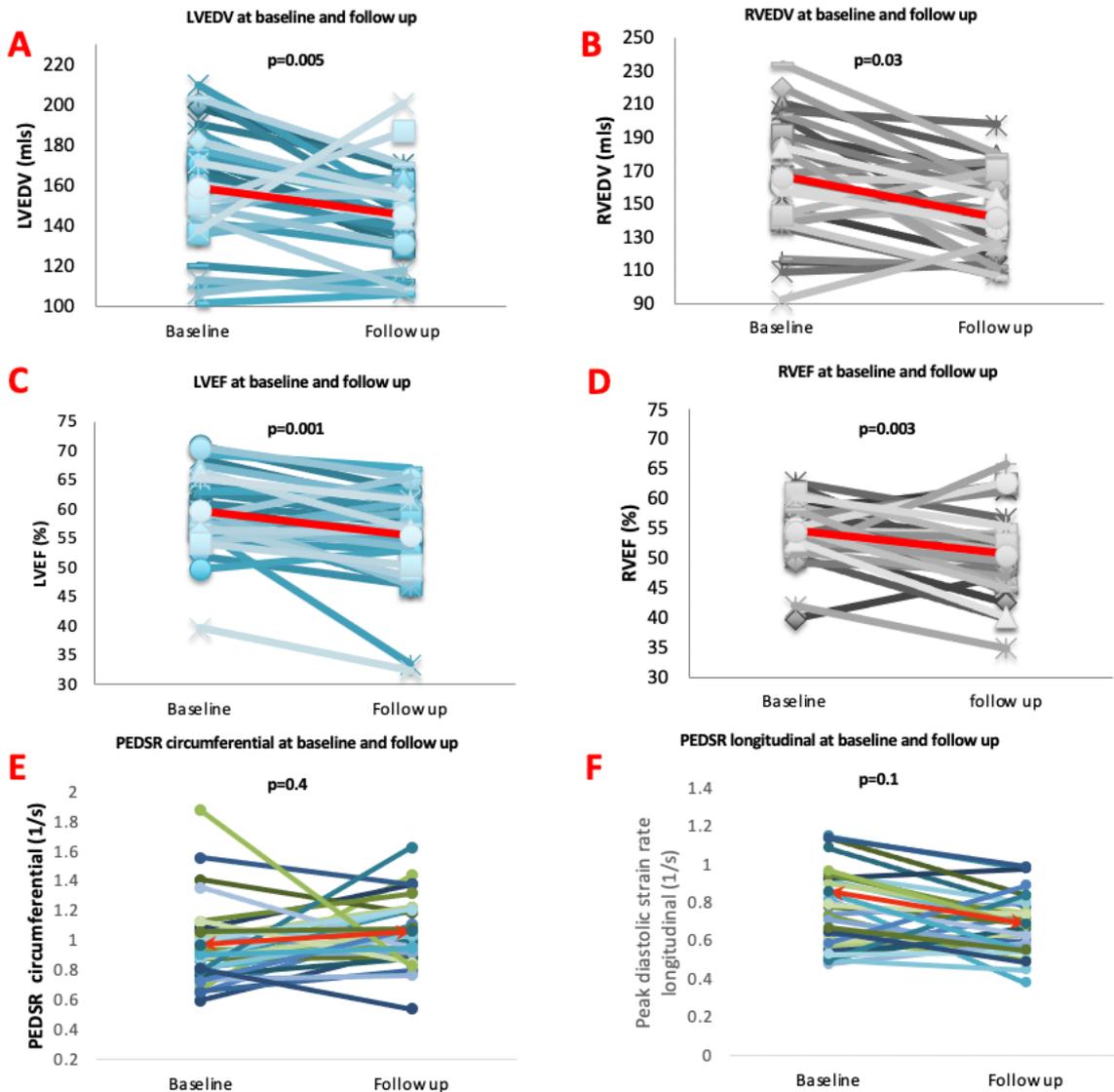


Figure-3.4: Comparison of imaging parameters at baseline and follow-up. Comparison between the left ventricular end diastolic volume (LVEDV), right ventricular end diastolic volume (RVEDV), left ventricular ejection fraction (LVEF), right ventricular ejection fraction (RVEF), peak diastolic strain rate (PEDSR) circumferential and PEDSR longitudinal at baseline and year 6 follow-up scans (line in red indicates mean values for each variable)

### Comparison of CMR features, plasma biomarkers and biochemistry at baseline between patients who experienced cardiovascular events and those who remained asymptomatic

There were no differences in baseline hs-cTnT and NTproBNP biomarker levels or clinical and biochemical variables at baseline in participants who experienced MACE (angina, myocardial infarction, revascularization, cerebrovascular accident and

cardiovascular mortality) compared to those who did not (Table-3.4). However, patients who experienced MACE during the follow-up period had higher prevalence of cigarette smoking, higher LV mass, LV mass indexed to body surface area, and a higher LV mass to LV EDV ratio indicating a greater concentric remodeling of the LV at baseline compared to those remaining asymptomatic and event free during the follow-up (Table-3.5).

### Associations of the change in myocardial function and baseline variables

There were no associations between change in cardiac function and the baseline clinical variables (table 3.6). Although there were also no associations between laboratory variables of glucose management or NT-proBNP, there was a significant correlation between the change in LVEF and baseline plasma hs-cTnT ( $R=-0.44$ ,  $p=0.01$ ). There was no such association for change in RVEF.

The relationships of change in LVEF and RVEF with a model including age, BMI, HBA<sub>1c</sub>, fasting glucose, resting BP, HR, NT-proBNP and hs-cTnT did not show a significant correlation ( $R^2=0.11$ ,  $p=0.9$  and  $R^2=0.39$ ,  $p=0.3$  respectively).

**Table 3.4:** *Clinical and biochemical characteristics at baseline of the participants with and without MACE (angina, myocardial infarction, revascularization and cerebrovascular accident) at follow-up*

Variable	No MACE (n=65)	MACE (n=18)	P value
Age, years	66 ± 11	65 ± 9	0.7
BMI (baseline), kg/m <sup>2</sup>	29 ± 4	28 ± 3	0.3
Male, %	55 (85)	17 (94)	0.2
Ethnicity, n (%)			
Caucasian	42 (64)	11 (67)	0.6
Asian	20 (31)	6 (33)	0.7

<b>Afro-Caribbean</b>	3 (5%)	1 (6%)	0.6
<b>Diabetes duration, years</b>	9.9 ± 4.6	10.6 ± 3.8	0.6
<b>Smoker, n (%)</b>	6 (10)	6 (33)	0.01*
<b>Systolic blood pressure, mmHg</b>	131 ± 15	132 ± 14	0.8
<b>Diastolic blood pressure, mmHg</b>	72 ± 9	74 ± 9	0.4
<b>Glycated haemoglobin, mmol/mol</b>	62 ± 21	71 ± 20	0.1
<b>Troponin T, ng/L</b>	7.6 ± 5.8	7.0 ± 3.6	0.7
<b>NT-pro BNP, pg/ml</b>	72 ± 129	39 ± 42	0.3
<b>Total cholesterol, mmol/L</b>	4.3 ± 1.1	4.6 ± 1.3	0.3
<b>LDL, mmol/L</b>	2.6 ± 0.9	2.7 ± 1.6	0.7
<b>Medications, n (%)</b>			
<b>Metformin, n (%)</b>	57 (88)	15 (83)	0.6
<b>Sulphonylurea, n (%)</b>	21 (32)	6 (33)	0.9
<b>Gliptins, n (%)</b>	7 (11)	4 (22)	0.2
<b>Thiazolidinediones, n (%)</b>	0 (0)	0 (0)	-
<b>SGLT2 inhibitors, n (%)</b>	0 (0)	0 (0)	-
<b>Aspirin, n (%)</b>	15 (23)	3 (17)	0.6
<b>Statin, n (%)</b>	47 (72)	13 (72)	0.9
<b>ACE-I, n (%)</b>	0 (0)	0 (0)	-
<b>ARB, n (%)</b>	0 (0)	0 (0)	-
<b>Beta blockers, n (%)</b>	3 (4)	1 (5)	0.9
<b>Calcium channel blockers, n (%)</b>	6 (8)	3 (17)	0.4

Values are mean ± standard deviations or percentages. \*signifies  $P \leq 0.05$ . MACE-major adverse cardiovascular events; n-numbers; BMI-body mass index; kg- kilograms; m-metres; mmHg-millimetres of mercury; mmol-millimoles, mol-moles; ng-nanograms; L-litres; NT-pro BNP-N-terminal prohormone B type natriuretic peptide; pg-picograms; ml-millilitres; LDL-low density lipoprotein; SGLT2-sodium glucose co-transporter 2; ACE-I-angiotensin converting enzyme inhibitor; ARB-angiotensin receptor blocker.

**Table-3.5:** CMR findings at baseline of the participants with and without MACE (angina, myocardial infarction, revascularization and cerebrovascular accident) at follow-up.

	<b>No MACE (n=65)</b>	<b>MACE (n=18)</b>	<b>P value</b>
<b>LV end diastolic volume (ml)</b>	146 ± 35	147 ± 35	0.9
<b>LV end diastolic volume index (ml/m<sup>2</sup>)</b>	73 ± 15	72 ± 14	0.8
<b>LV end systolic volume (ml)</b>	59 ± 21	58 ± 22	0.9
<b>LV end systolic volume index (ml/m<sup>2</sup>)</b>	29 ± 9	28 ± 9	0.9
<b>LV stroke volume (ml)</b>	87 ± 20	89 ± 16	0.7
<b>LV ejection fraction (%)</b>	60 ± 7	61 ± 6	0.6
<b>LV mass (gm)</b>	102 ± 23	116 ± 25	0.02*
<b>LV mass index (gm/m<sup>2</sup>)</b>	51 ± 9	57 ± 10	0.01*
<b>LV mass to LV end diastolic volume (gm/ml)</b>	0.72 ± 0.13	0.79 ± 0.15	0.05*
<b>RV end diastolic volume (ml)</b>	151 ± 37	147 ± 31	0.6

<b>RV end diastolic volume index (ml/m<sup>2</sup>)</b>	76 ± 17	72 ± 14	0.3
<b>RV end systolic volume (ml)</b>	68 ± 21	67 ± 17	0.8
<b>RV end systolic volume index (ml/m<sup>2</sup>)</b>	34 ± 10	33 ± 7	0.7
<b>RV stroke volume (ml)</b>	83 ± 19	80 ± 19	0.5
<b>RV ejection fraction (%)</b>	55 ± 5	54 ± 7	0.5

Values are mean ± standard deviations or percentages. \*signifies  $P \leq 0.05$ . CMR-cardiovascular magnetic resonance; MACE-major adverse cardiovascular events; LV-left ventricle; ml-millilitres, m-metre; gm-grams; RV-right ventricle.

Table-3.6: Simple linear regression analysis to check association between change in LVEF and RVEF and baseline clinical and biochemical variables

	Change in LVEF		Change in RVEF	
	Pearson Correlation coefficient (r)	P value	Pearson Correlation coefficient (r)	P value
<b>Age (years)</b>	-0.17	0.2	0.16	0.2
<b>Systolic blood pressure (mmHg)</b>	-0.14	0.2	-0.25	0.09
<b>Diastolic blood pressure (mmHg)</b>	0.13	0.5	-0.16	0.19
<b>Heart rate (bpm)</b>	0.12	0.26	-0.11	0.28
<b>Body mass index (kg/m<sup>2</sup>)</b>	0.04	0.4	-0.23	0.11
<b>Glucose (mmol/L)</b>	-0.09	0.3	-0.16	0.11
<b>HbA1c (mmol/mol)</b>	-0.02	0.4	-0.18	0.16
<b>NT-proBNP (pg/ml)</b>	0.15	0.7	0.16	0.5
<b>HS-cTnT (ng/L)</b>	-0.44	0.01*	0.29	0.08

\*signifies  $P \leq 0.05$ , LVEF- left ventricular ejection fraction; RVEF- right ventricular ejection fraction; mmHg- millimetres of mercury; bpm- beats per minute; kg- kilogram, m- metre; mmol- millimoles, L-litre; mol- moles; pg- picogram; HbA1C- glycated haemoglobin; NT-proBNP- N-terminal prohormone b-type natriuretic peptide; Hs-cTnT- high sensitivity cardiac troponin T; ng- nanograms.

### 3.5 Discussion

Despite the epidemiologically established link between T2D and congestive cardiac failure(16), longitudinal cardiac structural and functional changes in asymptomatic T2D patients who remain free of cardiovascular events have not been explored before.

In

a cohort of ethnically diverse, asymptomatic T2D patients with no history of prior cardiovascular disease, this study has shown for the first time that T2D is associated with clinically relevant adverse changes in biventricular function at follow-up after 6-years even in the absence of cardiovascular events, cardiac ischaemia or other predisposing factors such as hypertension. The present data have also shown that baseline glucose control seems to have no effect, although plasma hs-cTnT is associated with the magnitude of the subsequent change in LVEF. Finally, underscoring the prognostic relevance of changes in LV mass and LV geometry in type 2 diabetes, this study has also shown higher LV mass and greater LV concentric remodeling at baseline in patients who experienced MACE during the follow-up compared to those who remained asymptomatic and event free. There were no other significant differences in clinical or biochemical variables between the two groups, suggesting that the adverse cardiovascular events in T2D are not limited to patients with poor glycemic, BP or weight control.

### **Longitudinal morphological alterations in type 2 diabetes**

Our results show that cardiac size and mass decreases over time in patients with T2D, while biventricular function deteriorates. In contrast to our findings in T2D patients, in healthy aging LVEF remains static or increases over time as shown by multiple studies(186-190).

In this study, in 30% of the patients on the year-6 CMR scan, LVEF levels dropped below the normal range (<50%) despite the asymptomatic status of these patients(191). Supporting our findings, multiple studies showed that even in asymptomatic individuals with T2D, there is a high prevalence of LV systolic and

diastolic dysfunction(41, 183). The American Heart Association has classified asymptomatic individuals with impaired cardiac function as having stage B HF(192). These patients remain at risk for significant cardiovascular morbidity and mortality, and experience a 5-fold increase in the risk of subsequent symptomatic HF development(193). As stage B HF is a precursor to clinical HF, earlier identification of the cardiovascular manifestations of stage B HF may permit earlier diagnosis and treatment of patients at higher risk.

### **Relationship of glycemic control, blood pressure control and body weight changes and longitudinal cardiac functional changes in type 2 diabetes**

There were no changes in mean HbA1c, systolic and diastolic BP, resting HR or BMI at follow-up. Moreover, we detected no relationship between the baseline systolic or diastolic BP, HR, BMI, HbA1c, and glucose levels with the change in LVEF and RVEF over time ( $\Delta$ LVEF and  $\Delta$ RVEF respectively). This lack of association between glycemic control and cardiac functional decline supports the notion that there are more central mechanisms to HF pathophysiology in type 2 diabetes than the glycemic control. While a few studies demonstrated a positive impact of metabolic control on ventricular function(194, 195), most previous studies failed to demonstrate any favorable changes in cardiac function despite improvements in glycemic control(196, 197). Interestingly, we have not detected any significant changes in the diastolic function in this cohort despite the aging process. This is likely to be consequence of the normotensive status of the cohort at baseline with no significant changes in systolic or diastolic BP assessments over time despite the aging process.

## **Relationship of plasma biomarkers (high sensitivity cardiac troponin-T and N terminal pro b-type natriuretic peptide) and longitudinal cardiac functional changes in type 2 diabetes**

High sensitivity cardiac troponin isoforms are unique to the cardiac myocyte, and are objective, quantifiable and sensitive biomarkers for detecting cardiac injury(198). They are predictors of cardiovascular morbidity and mortality risk in population-based studies besides their role as the cornerstone for the diagnosis of acute myocardial infarction(199). We show here for the first time that there is a significant association between the plasma hs-cTnT measured at baseline with change in LVEF over time, highlighting a potentially important role for hs-cTnT as a biomarker for assessing HF risk in T2D patients. A recent study has shown that lifestyle factors, such as smoking, diet, and physical activity, are associated with changes in high-sensitivity cardiac troponin levels, suggesting that lifestyle modifications may be able to affect changes in troponin and be beneficial in reducing mortality risk. As an easily obtainable plasma biomarker, hs-cTnT may be of great assistance in the incremental risk stratification of patients with T2D into high risk and low risk sub-groups(198).

Our study does not suggest a similar role for NT-proBNP in asymptomatic T2D patients with no known cardiovascular disease. A previous type 2 diabetes study did however demonstrate an independent correlation of NT-proBNP with short term prognosis of cardiovascular events(200). The discrepancy between the two studies might have resulted from the distinct populations investigated. While Huelsmann et al (200) have not excluded symptomatic patients, patients with ischaemic heart disease, AF or other significant cardiovascular diseases, in order to better characterize the occult heart disease in type 2 diabetes we have excluded these comorbidities and symptomatic patients.

## **Left ventricular geometry and major adverse cardiovascular events in type 2 diabetes**

LV mass is strongly associated with cardiovascular events, including myocardial infarction, HF, and mortality(201). While the precise underlying mechanism of LV hypertrophy and concentric LV remodeling in the absence of significant hypertension remains unclear, it has been suggested that T2D induces LV mass enlargement through metabolic, and not hemodynamic pathways(202). Supporting this, a recent study has shown that treatment with selective SGLT2 inhibitor empagliflozin was associated with significant reductions in LV mass, which may account in part for the beneficial cardiovascular outcomes of empagliflozin(203).

### **Study limitations**

The present data are in a modest number of patients, recruited to a single study site. The study was not powered to assess the potential association of the treatments and the CMR findings with regression analysis. However, the longitudinal nature of the study allowed paired analysis of images which, to minimize bias, were randomized in time and by subject. Moreover, the image data of a random sample of subjects were evaluated by two investigators to demonstrate good inter- and intra-observer reproducibility.

Another limitation is the small number of female participants as only a smaller proportion agreed to return for a second scan. While T2D has been consistently found to be a stronger risk factor for heart disease in women compared to men(16), in this study we show that biventricular reductions in systolic function occur over time even in a predominantly male population.

We also found smoking to be a confounding factor in those who experienced MACE.

## **Conclusions**

Even in the absence of overt clinical CAD, significant valvular disease, uncontrolled hypertension or change in BMI, T2D resulted in a significant reduction in cardiac size and biventricular systolic function over time. Plasma hs-cTnT measured at baseline was associated with the magnitude of change in LV systolic function suggesting that hs-cTnT could play a role in identifying patients with T2D at higher risk for heart failure. Patients who experienced MACE during the follow-up were more likely to be smokers and exhibited higher LV mass and greater LV concentric remodeling at baseline compared to those who remained asymptomatic and event free.

## **Chapter 4**

# **Coronary microvascular function and visceral adiposity in patients with normal body weight and type 2 diabetes**

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## 4.1 Abstract

**Objectives:** We sought to assess if type 2 diabetes mellitus affects coronary microvascular function in individuals with normal body weight.

**Methods:** Seventy-five participants (30 patients with T2D who were overweight [O-T2D], 15 patients with T2D who were lean [LnT2D], 15 healthy volunteers who were lean [LnHV] and 15 healthy volunteers who were overweight [O-HV]) without established cardiovascular disease were recruited. Participants underwent magnetic resonance imaging for assessment of subcutaneous, epicardial and visceral adipose tissue areas (SAT, EAT and VAT respectively), adenosine stress myocardial blood flow (MBF), cardiac structure and function.

**Results:** Stress MBF was reduced only in O-T2D (LnHV:2.07±0.47ml/g/min, O-HV:2.08±0.42ml/g/min, LnT2D:2.16±0.36ml/g/min, O-T2D:1.60±0.28ml/g/min;  $p<0.0001$ ). Accumulation of visceral fat was evident in LnT2D patients at similar levels to O-HV (LnHV:127±53cm<sup>2</sup>, O-HV:181±60cm<sup>2</sup>, LnT2D:182±99cm<sup>2</sup>, O-T2D:288±72cm<sup>2</sup>;  $p<0.0001$ ). Only O-T2D patients showed reductions in left ventricular ejection fraction (LnHV:63±4%, O-HV:63±4%, LnT2D:60±5%, O-T2D:58±6%;  $p=0.0008$ ) and global longitudinal strain (LnHV:(-)15.1±3.1%, O-HV:(-)15.2±3.7%, LnT2D:(-)13.4±2.7%, O-T2D:(-)11.1±2.8%;  $p=0.002$ ) compared to both control groups.

**Conclusions:** Patients with T2D and normal body weight do not show alterations in global stress myocardial blood flow; but show significant increases in visceral adiposity. Overweight patients with T2D and no prior cardiovascular disease show increased visceral adiposity and significant reductions in stress myocardial blood flow.

## 4.2 Introduction

Driven predominantly by the obesity epidemic, the prevalence of T2D continues to rise(1), with heart failure as the leading cardiovascular complication(204) even in patients with good cardiovascular risk-factor management(205). Coronary microvascular dysfunction (CMD) has emerged as a candidate mechanism of heart disease in type 2 diabetes(19, 91), preceding clinical HF manifestation (206, 207) and carrying important prognostic information(18, 208). However, coronary microvascular dysfunction has also been demonstrated in individuals with obesity without type 2 diabetes(209, 210). The proportion of adults with normal body weight at the time of incident type 2 diabetes ranges from 9 to 21%(151, 152). Whether or not type 2 diabetes affects coronary microvascular function in the absence of obesity is uncertain.

Moreover, individuals with the same body mass index do not necessarily share the same degree of adiposity and may have a very different fat mass percentage and fat distribution, with diverse metabolic consequences(61). It has been proposed that the visceral adipose tissue (VAT), including the epicardial adipose tissue (EAT), may have a more adverse influence on cardiovascular health compared to subcutaneous adipose tissue (SAT) as it is a metabolically active tissue capable of secreting adipokines and proinflammatory mediators that regulate appetite and insulin action(55, 211). Moreover, highlighting the cardiometabolic relevance of distinct adipose tissue distribution phenotypes, lower amount of lower-body fat mass was shown to be an important determinant of cardiometabolic risk(212, 213). Novel findings suggest an important and independent role of increased gluteofemoral fat

mass to maintain metabolic health (214). While in the context of prediabetes, the role of excess visceral adiposity in individuals with normal body weight has been carefully studied(215, 216), there is sparse evidence to show if type 2 diabetes patients with normal body weight possess excess visceral adiposity compared to their weight-matching counterparts with no diabetes, and if type 2 diabetes and obesity have a combined impact on the alterations of adipose tissue distribution.

First-pass dynamic contrast-enhanced myocardial perfusion cardiovascular magnetic resonance imaging (CMR) can be used to derive quantitative estimates of hyperemic and resting myocardial blood flow (MBF) for assessment of myocardial microvascular function(217). CMR is also the reference technique for comprehensive non-invasive assessment of changes in cardiac structure, function, strain, fibrosis and scar(105). VAT, SAT and EAT can be measured with high accuracy by magnetic resonance imaging (MRI)(218).

Consequently, using CMR we sought to establish if type 2 diabetes affects coronary microvascular function in patients with T2D and normal body weight (LnT2D). We further aimed to compare adipose tissue distribution, and cardiac structural and functional alterations between asymptomatic LnT2D and patients who were overweight with T2D (O-T2D) without established cardiovascular disease and healthy volunteers who were lean and overweight (LnHV and O-HV respectively).

## **4.3 Methods**

### **Study population and design**

This single-center observational study was approved by the National Research Ethics Committee (REC Ref 18/YH/0168) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from each participant.

Thirty O-T2D participants (BMI  $>25$  m/kg<sup>2</sup>), fifteen participants with LnT2D (BMI  $\leq 25$  m/kg<sup>2</sup>), fifteen LnHV with no T2D (BMI  $\leq 25$  m/kg<sup>2</sup>) and fifteen O-HV with no T2D (BMI  $>25$  m/kg<sup>2</sup>) with a similar age and sex distribution were recruited to the study. Patients were recruited from the general practices in Yorkshire, United Kingdom. Controls were recruited from local golf clubs. The data underlying this article will be shared on reasonable request to the corresponding author.

### **Inclusion and exclusion criteria**

Participants were excluded if they had a previous diagnosis of cardiovascular disease (previous coronary artery bypass graft surgery, angioplasty, myocardial infarction, angina, moderate or above valvular heart disease, atrial fibrillation), contraindications to CMR, ischemic changes on 12-lead electrocardiogram (ECG), renal impairment (estimated glomerular filtration rate [eGFR] below 30 mL/min/1.73m<sup>2</sup>), or if they were receiving treatment with insulin. Controls had no overt cardiovascular disease and they had normal glycemic control with glycated haemoglobin (HbA1c) values  $\leq 40$  mmol/mol. For the control cohorts, at the point of recruitment, it was ascertained by verbal questioning that the exercise duration had been  $< 6$  hours/week for the past 12 months.

### **Anthropometric measurements**

Height and weight were recorded, BMI was calculated, blood pressure was recorded as an average of 3 supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Corp) and a resting ECG was recorded. Fasting blood samples were taken from each participant for assessments of full blood count, eGFR, glucose, insulin, HbA1c, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol levels. Homeostasis model assessment of insulin resistance index

(HOMA-IR; fasting serum insulin ( $\mu\text{U/L}$ )  $\times$  fasting plasma glucose ( $\text{mmol l}^{-1}$ )/22.5)(61, 219) and triglyceride to HDL ratio(158) were calculated as validated surrogate markers of insulin resistance from fasting blood samples.

### **Magnetic resonance imaging**

All scans were performed on a 3.0 Tesla MR system (Prisma, Siemens, Erlangen, Germany). Participants were advised to avoid caffeine for 24 hours prior. The MRI protocol (Figure 4.1) consisted of cine imaging using a steady-state free precession (SSFP) sequence, thoracic and abdominal water/fat images using multi-echo GRE sequence, native and post-contrast T1 mapping, stress and rest perfusion and late gadolinium enhancement (LGE).

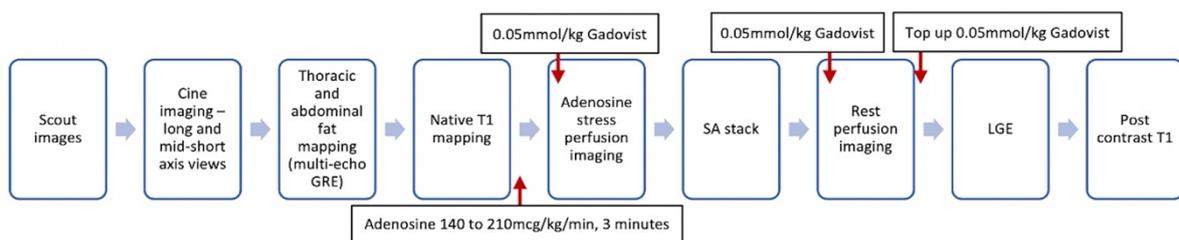
For epicardial and abdominal visceral fat area measurements single-shot acquisition of thoracic and abdominal non-breath-hold images were performed using multi-echo GRE sequence with gradient flyback for monopolar readout to acquire 3 echoes for each phase encode (parallel imaging rate 3 using GRAPPA with separate reference line acquisition, 32 channel cardiac array, bandwidth=1184 Hz/pixel, TE=1.33, 3.35, and 5.37ms, TR=6.71ms, readout flip angle=20°, matrix=192x108, single shot duration=242ms, FOV=300x225mm<sup>2</sup>, slice thickness=6mm) as previously described (220).

Native T1 mapping was acquired in three slices using a breath-held modified look-locker inversion recovery (MOLLI) acquisition as previously described (pre-contrast 5s(3s)3s and post-contrast 4s(1s)3s(1s)2s schemes)(221). Post-contrast T1 mapping acquisition was performed 15 minutes after the last contrast injection using identical planning as the native T1 map.

Perfusion imaging used a free breathing, fast low angle shot (FLASH) magnetic resonance protocol with motion-corrected (MOCO) automated in-line perfusion

mapping using the Gadgetron streaming software image reconstruction framework as previously described(105). For stress perfusion imaging, adenosine was infused at a rate of 140  $\mu\text{g}/\text{kg}/\text{min}$  and increased up to a maximum of 210  $\mu\text{g}/\text{kg}/\text{min}$  according to hemodynamic and symptomatic response (a significant hemodynamic response to adenosine stress was defined as  $>10$  beats/min increase in heart rate, or BP drop  $<10$  mm Hg and  $>1$  adenosine-related symptom, e.g., chest tightness, breathlessness)(222). A minimum 10-minute interval was kept between perfusion acquisitions to ensure equilibration of gadolinium kinetics and resolution of all hemodynamic effects of adenosine. For each perfusion acquisition, an intravenous bolus of 0.05mmol/kg gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5ml/s followed by a 20ml saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer).

Late gadolinium enhancement (LGE) imaging was performed using a phase-sensitive inversion recovery (PSIR) sequence in matching LV short-axis planes, and long axis planes  $>8$  minutes after contrast administration to exclude the presence of previous myocardial infarction or regional fibrosis.



**Figure 4.1: Scan protocol.** The scan protocol included cine imaging, thoracic and abdominal fat/water maps, native precontrast and postcontrast T1 mapping, adenosine stress perfusion imaging, and LGE imaging. SA short axis; LGE, late gadolinium enhancement; GRE, gradient echo

## **Quantitative analysis**

All CMR post-processing analysis was performed off-line blinded to all participant details by AC (with 2 years of CMR experience) after completion of the study. The anonymization codes which were generated using a random number generator were only unlocked once all data analysis was completed.

All CMR image analysis was performed off-line by AC blinded to all participant details and all scan contours were subsequently reviewed by EL (with 8 years of CMR experience; level 3 EACVI accreditation) using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada) who was also blinded to participant details. Images for biventricular volumes and function were analyzed as previously described(223). The LA volume and LA EF were calculated using the biplane area-length method in the horizontal and vertical long axes as previously described(182). Strain measurements were performed using cvi42 Tissue Tracking from balanced SSFP from the short axis images, and the horizontal long axis and vertical long axis views. The peak circumferential systolic strain and peak early diastolic strain rates and global longitudinal strain were measured as previously described(183).

Myocardial perfusion image reconstruction and processing was implemented using the Gadgetron software framework as previously described(105). Rest/stress myocardial blood flow (MBF) were measured for each of the 16 segments using the American Heart Association (AHA) classification. MBF values for all remaining segments were averaged to provide a global value. Native T1 maps and ECV were analyzed using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada) from a region of interest in the mid-wall of the septum using the native pre-contrast and native post-contrast T1 times of myocardium, blood pool and haematocrit as previously described(102). Myocardial cell volume was calculated from native T1

maps by using the following calculation:  $LVM/1.05 \times (1 - ECV)$  as previously described(224).

Abdominal VAT area was measured at the level of the 3-4th lumbar vertebral body from the single-shot performed using multi-echo GRE sequence abdominal images. Adipose tissue was categorized into visceral and subcutaneous fat through manual division, which was accomplished by drawing a line following the abdominal wall to separate intra- and extra-abdominal compartments. Epicardial fat area was traced from the thoracic multi-echo GRE sequence images acquired on transaxial orientation of a four-chamber view on a single slice (Figure 4.2A & 4.2B). Visceral and subcutaneous fat areas were segmented separately using cvi42 software (Figure 4.2C & 4.2D) and visceral fat over subcutaneous fat area were calculated.

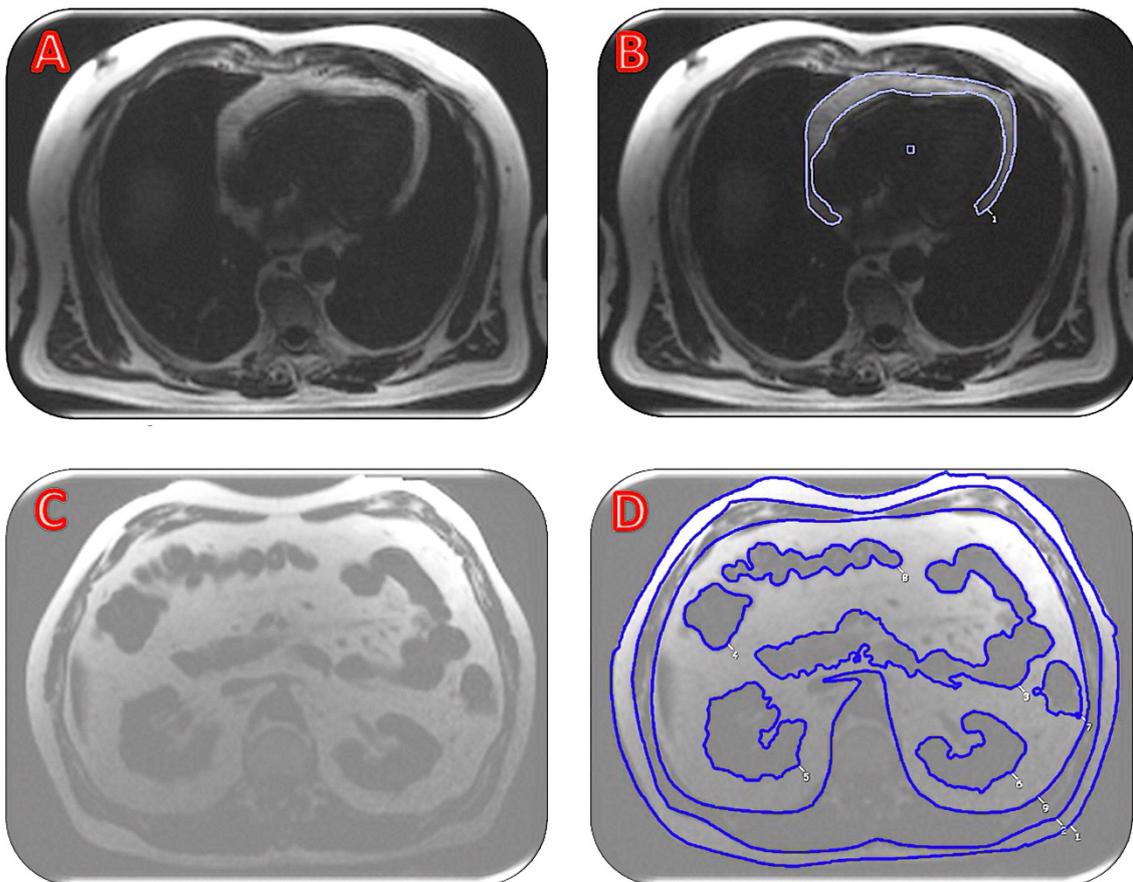


Figure 4.2: Epicardial, visceral, and subcutaneous adipose tissue imaging and analysis. (A) Multi-echo GRE sequence image acquired on transaxial orientation of a

*four-chamber view on a single section showing epicardial adipose tissue. (B) Representative example of contouring of epicardial adipose tissue on a single-section four-chamber view. (C) Multi-echo GRE sequence abdominal imaging used to characterize adipose tissue into visceral and subcutaneous fat through manual division. (D) Representative example of manual contouring and segmentation of the abdominal adipose tissue into subcutaneous and visceral components. GRE, gradient echo*

### **Qualitative perfusion and scar assessment**

The CMR perfusion images were interpreted visually by EL. Rest/stress perfusion images were carefully reviewed for each of the 16 segments using the AHA classification.

For LGE imaging analysis, areas of contrast enhancement were visually scored as absent or present by 2 operators (AC, EL). Hyperenhancement was considered present only if myocardial enhancement was confirmed on both short-axis and perpendicular long-axis locations.

### **Statistical analysis**

Statistical analysis was performed using SPSS (IBM SPSS statistics, version 26.0). Categorical data were compared with Pearson's chi-square test. Continuous variables are presented as mean  $\pm$  SD and were checked for normality using the Shapiro-Wilk test. Comparisons between the 4 groups were performed by 1-way analysis of variance with post hoc Bonferroni corrections. Based on the Bonferroni correction, considering the 6 comparisons between the 4 groups, a p-value of  $<0.003$  was applied as indicating statistical significance.

The student's t-test was used for comparison of normally distributed data sets where data was obtained for only the LnT2D and O-T2D groups. Bivariate correlations were performed using the Pearson correlation coefficient. For these tests a p-value of  $\leq 0.05$  was considered statistically significant.

Priori sample size calculations were performed before the study (T2D:  $1.74 \pm 0.24$  ml/g/min, controls:  $2.12 \pm 0.26$  ml/g/min) which suggested to detect a 25% difference in the stress MBF across the 4 cohorts twelve participants per group would be needed (with 80% power at  $\alpha=0.05$ ). There was no difference in rest MBF data between patients with T2D and controls with no diabetes. In line with the higher prevalence of T2D and overweight/obesity comorbidity compared to T2D in isolation in the general population there were more T2D volunteers who were overweight expressing interest in participating in the study. All eligible participants expressing interest in the study during the predetermined recruitment phase were included. Consequently, more participants were recruited into the O-T2D group while the other 3 groups were matched in sample size. Overall recruitment goals were exceeded in the study and 15 participants per Ln-T2D, lean and overweight control groups and 30 O-T2D were recruited.

## 4.4 Results

### **Participant demographics and biochemical characteristics:**

Demographic, clinical, and biochemical data of the four study groups are shown in Table 4.1.

A total of 30 O-T2D (age  $65 \pm 11$  years, BMI  $30 \pm 3$  kg/m<sup>2</sup>, mean HbA1c  $63 \pm 19$  mmol/mol), 15 LnT2D (age  $63 \pm 12$  years, BMI  $23 \pm 1$  kg/m<sup>2</sup>, mean HbA1c  $58 \pm 8$  mmol/mol), 15 LnHV (age  $63 \pm 7$  years, BMI  $23 \pm 2$  kg/m<sup>2</sup>, mean HbA1C  $37 \pm 3$  mmol/mol) and 15 O-HV (age  $66 \pm 8$  years, BMI  $29 \pm 2$  kg/m<sup>2</sup>, mean HbA1C  $38 \pm 2$  mmol/mol) were recruited. Age and sex distribution were similar between the cohorts. The two T2D groups were matched for diabetes treatment and duration. There was no significant difference in the smoking

history amongst the four cohorts. The two lean groups and the two overweight groups were matched for BMI. Controls were not receiving any medications.

There was no significant difference in systolic BP or heart rate across the groups. Waist circumference and waist/hip circumference ratios were similarly increased in both overweight groups (O-T2D and O-HV) compared to the lean groups (LnT2D and LnHV). Plasma triglyceride, insulin levels, HOMA-IR calculations and triglyceride/HDL ratio were higher in the O-T2D compared to the other three cohorts.

### Multiparametric MRI results

CMR results for cardiac volumes and function, perfusion, native T1 maps, ECV and thoracic and abdominal MRI results for epicardial, visceral and subcutaneous fat areas are summarized in Table 4.2.

**Table 4.1:** Demographics, biochemical characteristics and medications

	<b>LnHV (n=15)</b>	<b>O-HV (n=15)</b>	<b>LnT2D (n=15)</b>	<b>O-T2D (n=30)</b>	<b>ANOVA/C hi-square</b>
Age (yrs)	63±7	66±8	63±12	65±11	0.8
Sex (M, %)	10 (67)	8 (53)	9 (60)	23 (69)	0.3
Duration of T2D (yrs)	-	-	13±6	11±3	0.2
Smokers (n, %)	2 (13)	3 (20)	2 (13)	5 (17)	0.8
Systolic BP, mmHg	130±13	133±13	126±17	132±14	0.6
Diastolic BP, mmHg	77±7	76±7	72±8	76±6	0.08
Heart rate, bpm	63±10	67±11	69±13	67±10	0.7
Height, cm	171±9	169±8	168±10	169±10	0.5
Weight, kg	70±10€§	83±8€#	68±10 #	86±10§	<b>&lt;0.0001</b>
BMI, kg/m <sup>2</sup>	23±2€†	29±2#	23±1§	30±3	<b>&lt;0.0001</b>
Waist circumference, cm	90±7 €†	105±6#	92±11§	109±8	<b>&lt;0.0001</b>
Waist over hip ratio	0.95±0.08€†	1.01±0.06#	0.94±0.10 §	1.01±0.05	<b>0.0001</b>
Haemoglobin, g/L	141±9	148±8	140±11	149±17	0.07
Creatinine, umol/L	71±11	71±11	64±14	73±17	0.3
eGFR, ml/min/1.73m <sup>2</sup>	82±8	81±6	87±7	82±11	0.5
Total cholesterol, mmol/L	5.85±0.65†	5.17±1.35	4.71±1.19	4.72±1.22	0.04
HDL, mmol/L	1.94±0.58€††	1.53±0.38	1.61±0.45§	1.26±0.22	<b>&lt;0.0001</b>
LDL, mmol/L	3.35±0.38	2.99±1.10	2.41±0.97	2.73±1.31	0.1

TG, mmol/L	1.22±0.65†	1.49±0.64	1.60±0.82	2.50±1.57	<b>0.002</b>
Fasting glucose, mmol/L	4.9±0.6¶†	5.2±0.4#*	8.7±2.9	9.3±3.9	<b>0.0002</b>
HbA1c, mmol/mol	37±3¶†	38±2 #*	58±8	63±19	<b>&lt;0.0001</b>
Insulin, pmol/L	25±15†	67±35*	41±29 §	173±115	<b>&lt;0.0001</b>
C peptide, pmol/L	432±205†	745±300	553±265	945±695	0.01
HOMA-IR	0.79±0.49†	2.21±1.23*	2.40±2.02 §	10.21±6.99	<b>&lt;0.0001</b>
TG/HDL	0.76±0.63†	1.05±0.48*	1.14±0.84§	2.38±1.75	<b>0.002</b>
<b>Medications, n (%)</b>					
ACE inhibitor	-	-	4 (20)	14 (47)	0.2
ARB	-	-	3 (20)	5 (17)	0.1
Beta blocker	-	-	0 (0)	5 (17)	<b>0.02</b>
Calcium Channel blocker	-	-	3 (20)	4 (13)	0.6
Aspirin	-	-	6 (40)	7 (23)	0.6
Statin	-	-	10 (67)	22 (73)	0.1
Metformin	-	-	10 (67)	15 (50)	0.3
Sulphonylurea	-	-	4 (27)	8 (27)	1.0
GLP-1RA	-	-	0 (0)	0 (0)	-
Gliptins	-	-	4 (27)	6 (20)	0.6
SGLT2i	-	-	1 (6)	2 (7)	0.4
Thiazolidinediones	-	-	3 (20)	1 (3)	0.06

Values in bold signify € signifies  $p < 0.05$  between LnHV and O-HV, § signifies  $p < 0.05$  between LnT2D and O-T2D, ¶ signifies  $p < 0.05$  between LnHV and LnT2D, \* signifies  $p < 0.05$  between O-HV and O-T2D, † signifies  $p \leq 0.05$  between LnHV and O-T2D, # signifies  $p \leq 0.05$  between O-HV and LnT2D. Values are mean  $\pm$  SD or median (IQR) for continuous variables and number (%) for categorical variables. T2D indicates type 2 diabetes; LnHV, lean healthy volunteer; O-HV, overweight HV; LnT2D, lean type 2 diabetes patients; O-T2D, overweight type 2 diabetes patients; yrs, years; BP, blood pressure; BMI, Body mass index; kg, kilograms; n, number; bpm, beats per minute; mmHg, millimeters of mercury; g/l, gram per Liter; mmol/L, millimoles per litre; umol/L, micromoles per Litre; eGFR; estimated glomerular filtration rate, ml/min/1.73m<sup>2</sup>, millilitre per minute per (1.73 square meters); pmol/L, picomoles per litre; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; GLP-1RA, Glucagon like peptide 1 receptor agonists; SGLT2i, sodium glucose cotransporter-2 inhibitors.

**Table 4.2: MRI parameters**

	<b>LnHV</b> (n=15)	<b>O-HV</b> (n=15)	<b>LnT2D</b> (n=15)	<b>O-T2D</b> (n=30)	<b>ANOV</b> <b>A</b>
LV end-diastolic volume (ml)	152±33	140±30	125±28	143±29	0.09
LV end diastolic volume index (ml/m <sup>2</sup> )	82±13	72±13	70±12	72±15	0.06
LV end systolic volume (ml)	56±13	52±16	50±14§	61±17	0.1
LV end systolic volume index (ml/m <sup>2</sup> )	31±6	27±7	29±7	30±8	0.3
LV stroke volume (ml)	95±22	88±16	75±17	82±16	0.01
LV ejection fraction (%)	63±4†	63±4*	60±5	58±6	<b>0.0008</b>
LV mass (g)	93±27	95±29	79±19	96±20	0.1
LV mass index (g/m <sup>2</sup> )	52±12	52±11	48±10	48±10	0.2

LV mass /LV end diastolic volume(mg/ml)	0.65±0.12	0.72±0.15	0.69±0.21	0.68±0.14	0.5
RV end diastolic volume (ml)	161±36	153±43	122±30	142±30	0.02
RV end diastolic volume index (ml/m <sup>2</sup> )	87±14¶†	78±18	69±12	70±14	<b>0.001</b>
RV end systolic volume (ml)	67±22	61±24	54±17	63±17	0.6
RV stroke volume (ml)	93±19	92±21	69±17	79±18	0.7
RV ejection fraction (%)	60±7	60±5	56±6	56±6	0.06
Native T1 (ms)	1207±81	1166±84	1148±111	1197±69	0.09
Extracellular volume (%)	23±3	22±3	23±2	22±3	0.5
Cell Volume (ml/m <sup>2</sup> )	70±18	74±17	64±20	73±17	0.8
Peak circumferential strain, negative (%)	21.3±2.7†	21.9±2.2*	21.7±3.3§	18.3±3.0	<b>0.0005</b>
Global longitudinal strain, negative (%)	15.1±3.1†	15.2±3.7*	13.4±2.7	11.1±2.8	<b>0.002</b>
Peak diastolic strain rate, (1/s)	1.18±0.25	1.15±0.18	1.22±0.29 §	1.00±0.21	0.01
LA maximum volume (ml)	57±24	61±20	61±16	66±24	0.6
LA maximum volume indexed (ml/m <sup>2</sup> )	33±13	32±11	34±8	31±12	0.8
LA ejection fraction (%)	57±9	63±13	55±8	54±11	0.09
<b>Myocardial Perfusion</b>					
Stress myocardial blood flow (ml/g/min)	2.07±0.47 †	2.08±0.42 *	2.16±0.36 §	1.60±0.28	<b>&lt;0.0001</b>
Rest myocardial blood flow (ml/g/min)	0.64±0.08	0.72±0.15	0.74±0.13	0.67±0.15	0.1
Myocardial perfusion reserve index	3.18±0.84	3.17±0.59	2.98±0.66	2.47 ± 0.62	0.009
Stress rate pressure product (bpm*mmHg)	11942±32 56	11764±31 02	11464±21 00	11672±25 15	0.7
Rest rate pressure product (bpm*mmHg)	8339±158 5	8265±172 3	8645±159 9	8934±190 2	0.5
Increase in rate pressure product (%)	39	38	37	32	0.7
<b>Adipose tissue measurements</b>					
Subcutaneous adipose tissue (cm <sup>2</sup> )	106±31	169±55	147±71	171±80	0.06
Visceral adipose tissue (cm <sup>2</sup> )	127±53†	181±60*	182±99§	288±72	<b>&lt;0.0001</b>
Epicardial adipose tissue (cm <sup>2</sup> )	14±6†	17±5*	18±8§	31±13	<b>&lt;0.0001</b>
Visceral/Subcutaneous adipose tissue	1.23±0.43 †	1.15±0.49 *	1.56±1.24 §	2.05±1. 00	<b>0.0007</b>

Values in bold signify  $p < 0.05$ . € signifies  $p < 0.05$  between LnHV and O-HV, § signifies  $p < 0.05$  between LnT2D and O-T2D, ¶ signifies  $p < 0.05$  between LnHV and LnT2D, \* signifies  $p < 0.05$  between O-HV and O-T2D, † signifies  $p \leq 0.05$  between LnHV and O-T2D, ‡ signifies  $p \leq 0.05$  between O-HV and LnT2D. Values are mean  $\pm$  SD for continuous variables and number (%) for categorical variables. LnHV indicates lean healthy volunteers; O-HV, obese/overweight healthy volunteers; LnT2D, lean type 2 diabetes; O-T2D, overweight type 2 diabetes; ANOVA, analysis of variance; LV, left ventricular; ml, milliliter; ml/m<sup>2</sup>, milliliters per square meter of body surface area; g, gram; g/m<sup>2</sup>, gram per

## Cardiac geometry and function

Only O-T2D patients showed reductions in LV ejection fraction (LVEF) compared to both control groups (LnHV:63±4%, O-HV:63±4%, LnT2D:60±5%, O-T2D:58±6%;

$p=0.0008$ ). While significant reductions in peak circumferential systolic strain (PCSS) and global longitudinal strain (GLS) were detected in the O-T2D group compared to both the weight-matching and the lean control groups, PCSS was not reduced in Ln-T2D patients. The numeric differences in peak diastolic strain rates (PDSR) across the groups did not reach statistical significance.

The LnT2D group had the lowest LV end diastolic volumes (LVEDV), mass, and myocardial cell volumes compared to the other three groups, and there was no significant difference in ECV between the four groups.

There was no difference in LA volumes or function across the groups.

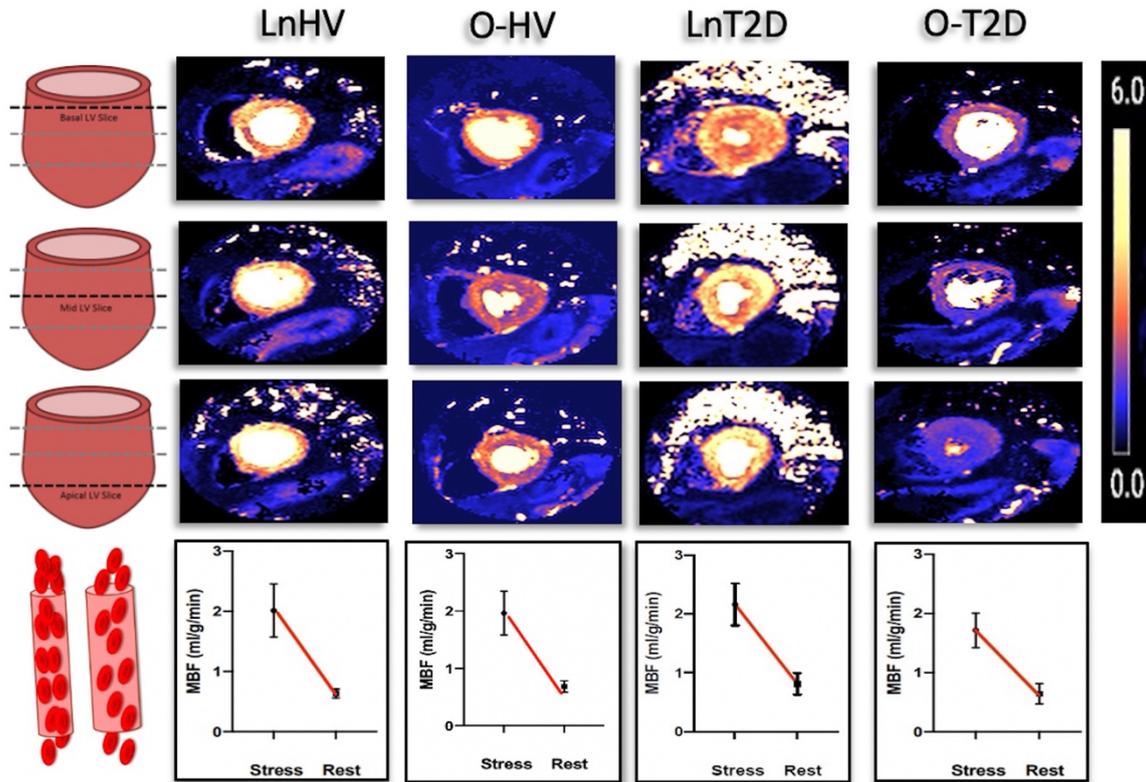
### **Myocardial blood flow**

Rest and stress rate pressure product (RPP) values, MBF and MPR measurements are summarized in Table 4.2. Participants from all groups demonstrated a similar increase in RPP during adenosine stress.

Only the O-T2D group showed significant reductions in stress MBF (LnHV  $2.07\pm 0.47$  ml/g/min, O-HV  $2.08\pm 0.42$  ml/g/min, LnT2D:  $2.16\pm 0.36$  ml/g/min, O-T2D:  $1.60\pm 0.28$  ml/g/min;  $p<0.0001$ ). There were no significant differences in rest MBF or in MPR between the four groups (Figure 4.3).

### **Qualitative assessment of myocardial perfusion and late gadolinium enhancement imaging**

Image quality for first-pass perfusion was rated as good in all participants with none of the participants demonstrating visual stress-induced perfusion defects. None of the participants showed subendocardial hyperenhancement to indicate the presence of a chronic silent myocardial infarction.



**Figure 4. 3:** Representative MBF maps acquired at basal, midventricular, and apical levels during peak stress, along with graphs showing the changes in MBF from peak stress to rest in lean healthy volunteers and healthy volunteers with overweight, as well as lean patients with T2D and patients with overweight and T2D. MBF, myocardial blood flow; T2D, type 2 diabetes; HV, healthy volunteers; LnHV, lean HV; LnT2D, lean patients with type 2 diabetes; O-HV, HV with overweight; O-T2D, patients with overweight and type 2 diabetes

### Visceral and subcutaneous adipose tissue

Numerically, the LnHV had the lowest SAT area (LnHV:106±31cm<sup>2</sup>, O-HV:169±55, LnT2D:147±71cm<sup>2</sup>, O-T2D:171±80cm<sup>2</sup>; p=0.06), however this did not reach statistical significance. The VAT area was significantly higher in the O-T2D compared to the other three groups (LnHV:127±53cm<sup>2</sup>, O-HV:181±60, LnT2D:182±99cm<sup>2</sup>, O-T2D:288±72cm<sup>2</sup>; p<0.0001). This was also numerically increased in O-HV and LnT2D compared to LnHV although it did not reach statistical significance (Figure-4.4). The

O-T2D patients had the highest VAT/SAT ratios; however, this was also significantly higher in LnT2D patients compared to the HV groups who were lean and overweight.

### Epicardial adipose tissue

O-T2D had higher EAT area compared to LnT2D, LnHV and O-HV (LnHV:14±6cm<sup>2</sup>, O-HV:17±5cm<sup>2</sup>, LnT2D:18±8cm<sup>2</sup>, O-T2D:31±13cm<sup>2</sup>; p<0.0001).

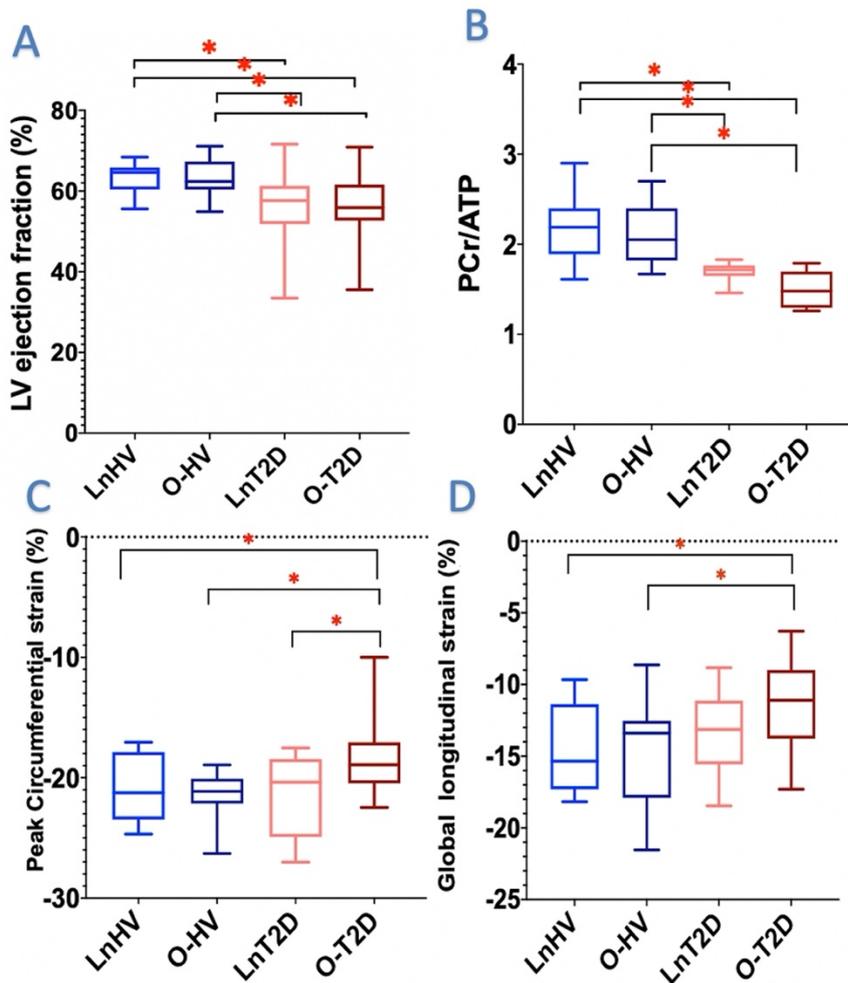


Figure 4.4: Box plot comparisons of epicardial, visceral, and subcutaneous adipose tissue. \*p < 0.05. HV, healthy volunteers; LnHV, lean HV; LnT2D, lean patients with type 2 diabetes; O-HV, HV with overweight; O-T2D, patients with overweight and type 2 diabetes

### Correlations between adipose tissue measurements

The correlations between metabolic parameters and visceral adiposity are provided in Table 4.3. There were significant positive correlations among all quantitative measures

of adiposity (BMI and waist circumference, EAT and VAT.) among each of the measures.

**Table 4.3:** Correlations of body mass index, visceral adipose tissue, epicardial adipose tissue, and waist circumference.

	BMI		VAT		EAT		Waist circumference	
	r	p	r	p	r	p	r	p
BMI	-	-	0.47	<b>0.0002</b>	0.30	<b>0.01</b>	0.72	<b>&lt;0.0001</b>
Waist circumference	0.72	<b>&lt;0.0001</b>	0.76	<b>&lt;0.0001</b>	0.63	<b>0.0002</b>	-	-
VAT	0.47	<b>0.0002</b>	-	-	0.73	<b>0.01</b>	0.76	<b>&lt;0.0001</b>
EAT	0.30	<b>0.01</b>	0.73	<b>0.01</b>	-	-	0.63	<b>0.0002</b>

Values in bold signify  $p < 0.05$ ; BMI, body mass index; VAT, visceral adipose tissue; EAT, epicardial adipose tissue.

### Correlations of perfusion with functional parameters

The stress or rest MBF did not correlate with LVEF. There were significant but weak correlations of the stress MBF with the strain parameters: PDSR ( $r=0.29$ ,  $p=0.03$ ) and GLS ( $r=0.30$ ,  $p=0.02$ ).

### Subgroup analyses

The study had inadequate power to assess subgroup analyses, nor were such tests planned. Nevertheless, the VAT data spread suggested heterogeneity within the LnT2D groups suggesting two groups of LnT2D patients: those with VAT similar to O-T2D and those similar to LnHV. When the LnT2D cohort is divided into two groups around the mean value for VAT ( $182\text{cm}^2$ ), the stress MBF was numerically higher in the participants with lower VAT; however, this trend did not reach statistical significance, likely due to small numbers (Low VAT Ln-T2D stress MBF:  $2.25 \pm 0.40$  ml/g/min vs High VAT LnT2D stress MBF:  $2.06 \pm 0.30$  ml/g/min,  $p=0.3$ ). When the O-T2D cohort is divided into two groups around the mean value for VAT for this cohort

(288 cm<sup>2</sup>), the stress MBF was numerically higher in the participants with lower VAT. However, this trend again did not reach statistical significance (Low VAT O-T2D stress MBF:  $1.59 \pm 0.33$  ml/g/min vs high VAT O-T2D stress MBF:  $1.61 \pm 0.20$  ml/g/min,  $p=0.8$ ).

Moreover, when the two T2D groups were subdivided based on their metformin treatment status, these subgroups showed no significant differences in any of the cardiac structural, functional or perfusion parameters except for the significant difference in circumferential strain in the O-T2D group. O-T2D subjects who were receiving metformin treatment (metformin +,  $n=15$ ) exhibited significantly higher circumferential strain compared to 15 O-T2D subjects who were not receiving metformin (GCS Metformin (+) O-T2D= $-19.43 \pm 1.79\%$  vs GCS Metformin (-) O-T2D= $-16.60 \pm 3.84$ ;  $p=0.02$ ). No such difference was detected in the Ln-T2D group based on metformin treatment status for any of the cardiac parameters.

#### **4.5 DISCUSSION**

The results of the present study provide several new findings. Firstly, myocardial stress perfusion was only reduced in O-T2D, with no reduction in global stress MBF or MPR in LnT2D and in O-HV compared to LnHV. Secondly, while O-T2D showed greater visceral adiposity, accumulation of visceral fat was evident even in patients with T2D and normal body weight at levels similar to controls who were overweight without diabetes. Thirdly, in patients with T2D, all measures of adiposity strongly correlated with one another, and VAT, BMI and waist circumference were each related to abnormalities in systolic and diastolic strain.

These results add further evidence for increased body weight as an important integrating determinant of myocardial perfusion in type 2 diabetes. Although capillary

rarefaction has been proposed among the mechanisms of coronary microvascular dysfunction in type 2 diabetes and obesity(225, 226), in this study, the reduction in stress MBF was detected in O-T2D patients despite no significant structural alterations, such as increases in LV mass, native T1, ECV or myocardial cell volume calculations. We have not detected any association between insulin resistance (HOMA-IR) and global rest or stress MBF or MPR. However, O-T2D showed striking increases in visceral adiposity with 40% higher VAT area compared to LnT2D. The mechanistic link between increased body weight and impairment of the total vasodilator capacity might therefore include etiologies such as altered adipokine profile associated with visceral fat accumulation in type 2 diabetes patients. Supporting this, adipokine profile in patients with T2D was shown in a previous study to depend on degree of adiposity, with no alterations in plasma adiponectin levels detected in LnT2D(227). The latter study also provided further evidence for significant associations between adipokine levels and plasma markers of systemic inflammation(227).

In a previous study, Sørensen and colleagues have also explored the alterations in rest and stress MBF in patients with T2D (228) and also showed a significant reduction in stress MBF in the type 2 diabetes cohort compared to controls. The investigators in that study included mainly patients with T2D who were overweight or obese (mean BMI  $31.1 \pm 4.6 \text{ kg/m}^2$ ) with a significant difference in the mean BMI compared to the control cohort (mean BMI:  $25.3 \pm 3.4 \text{ kg/m}^2$ ) which supports our finding that patients with T2D who are overweight or obese show significant alterations in myocardial perfusion. However, Sørensen and colleagues have not explored alterations in myocardial perfusion in Ln-T2D or weight matched lean and overweight controls with no diabetes. To our knowledge ours is the only study addressing this specific question. Our study

has also differed in the perfusion analysis methodology. While Sørensen and colleagues have used an in-house developed Matlab tool for their manual perfusion analysis, the data in our study were analyzed via a machine learning algorithm developed and extensively validated by study collaborators(105, 229, 230). In this study a deep neural network-based computational workflow for inline myocardial perfusion analysis automatically delineated the myocardium. This computational neural network is capable of cardiac perfusion mapping and integrated an automated inline implementation on the MR scanner, enabling instant data analysis and reporting without manual assessment(229). These automated methods for MBF estimation from CMR investigations may soon provide new opportunities for screening of coronary microvascular disease in type 2 diabetes and obesity in routine clinical care.

LVEF was significantly lower only in the O-T2D group compared to the control groups, while remaining still within normal range. Moreover, systolic and diastolic strain parameters were also only significantly lower in the O-T2D group. Among the global and segmental strain parameters, GLS has been shown to be the most reproducible strain parameter(231). While not yet resolved, it is possible that the identification of subclinical LV dysfunction by the means of reductions in GLS may lead to management changes that will alter cardiac outcomes in obesity and type 2 diabetes(232).

Body composition analysis based on the characterization of different tissue compartments is increasingly used for both clinical and research questions. MRI with its optimal soft tissue resolution and inherently high contrast between fat and water is an ideal modality for the assessment of adipose tissues with high accuracy and precision, and without the use of ionizing radiation. Neeland and colleagues have previously assessed the associations of abdominal VAT and SAT mass on MRI with

markers of cardiac and metabolic risk in a population-based cohort of obese adults(233). They showed that VAT associated with an adverse metabolic, dyslipidaemic, and atherogenic obesity phenotype, while SAT associated with a more benign phenotype, characterized by modest associations with inflammatory biomarkers and leptin, but no independent association with dyslipidaemia, insulin resistance, or atherosclerosis in obese individuals. While in our study visceral fat accumulation was also evident in Ln-T2D patients, O-T2D patients exhibited the highest level of visceral adiposity among the study groups. In a longitudinal population-based study, Kouli and colleagues confirmed the prognostic significance of excess visceral adiposity(234). They showed that visceral adiposity index derived from waist circumference, BMI, triglyceride and HDL levels was independently associated with elevated 10-year CVD risk, particularly in men(234). In our study as well as the higher visceral adiposity, O-T2D patients showed lower HDL and higher triglyceride levels, and higher waist circumference compared to Ln-T2D patients or lean and overweight controls with no diabetes suggesting a worse metabolic phenotype in the O-T2D group.

### **Study limitations**

Plasma adipokine levels or markers of systemic inflammation were not measured; however, large studies have previously reported the differences in adipokine profile and markers of systemic inflammation in patients who have T2D and are lean or obese which complemented our study by providing valuable insights for the interpretation of our findings(209, 227, 235, 236).

A complete characterization of coronary microvascular function also requires assessment of the response to vasoconstrictor stimuli during an invasive coronary

angiography procedure. Subjecting our participants to invasive coronary angiography was deemed unacceptable for asymptomatic patients and healthy controls.

For the control cohort, at the point of recruitment, it was ascertained by verbal questioning that the exercise duration had been < 6 hours/week for the past 12 months which is similar to the T2D cohorts participating in this study. However, this was not objectively assessed by requesting participants to wear an exercise activity monitor in our attempt to minimize the overall burden of the study to participants. However, the control groups showed numerically higher cholesterol measurements, similar or higher systolic and diastolic blood pressure measurements and resting heart rates, they have not shown any cardiac features which could be regarded as athlete's heart, further confirming the point that these controls were not uncommonly healthy and athletic individuals, but representing the local retiree population with an activity level similar to the T2D cohort.

Even though a minority of participants were smokers, they were spread almost equally between the four cohorts.

## **Conclusion**

Patients with type 2 diabetes and normal body weight do not exhibit reductions in myocardial blood flow, which suggests that increased body weight is an important integrating determinant of myocardial perfusion in patients with T2D. While patients with type 2 diabetes who were overweight show greater visceral adiposity, accumulation of visceral fat is evident even in LnT2D at levels similar to controls who are overweight and non-diabetic.

## **Chapter 5**

**Cardiac adaptations to acute**

**hemodynamic stress in function,**

**perfusion and energetics in type**

**2 diabetes with**

**overweight/obesity**

---

## 5.1 Abstract

### Background

The relative contribution of coronary microvascular dysfunction (CMD) and compromised cardiac energy production to the subclinical functional alterations in type 2 diabetes (T2D) has not been reported. We aimed to assess changes in cardiac energetics, perfusion, global longitudinal shortening (GLS), systolic and diastolic function in response to increases in cardiac workload with dobutamine stress in T2D patients with overweight/obesity (O-T2D) and also the correlation of systolic and diastolic function with the above parameters.

### Methods

36 patients with T2D, 12 veteran athletes (VA) and 20 healthy volunteers (HV) were recruited. All participants had dobutamine stress <sup>31</sup>P-MRS and CMR and achieved a target heart rate of 65% of the age-predicted maximum which was maintained for the <sup>31</sup>P-MRS and dobutamine stress CMR cine, mitral in-flow and perfusion acquisitions.

### Results

We confirmed that O-T2D participants show reductions in myocardial energetics, GLS, and the diastolic function at rest. With dobutamine stress all groups show decrements in energetics and diastolic function and similar increment in GLS and LVEF, but with a blunted increment in stress MBF in T2D patients with overweight/obesity. We also demonstrate that rest and stress MBF are independently associated with rest and stress LVEF, while rest and stress energetics were independently associated with rest and stress diastolic parameters respectively.

## Conclusions

This study gives important insights into the distinct associations between energetics and perfusion with diastolic and systolic function in T2D with overweight/obesity and supports development of patient-specific therapies and monitoring strategies.

## 5.2 Introduction

Heart failure is the leading cardiovascular complication of type 2 diabetes. Compromised myocardial energy production(237, 238) and coronary microvascular dysfunction (239) have been proposed as pivotal features underpinning myocardial dysfunction. The mechanism of compromised energy production in patients with diabetes is a complex one and involves limitations in substrate uptake and utilization (240), mitochondrial dysfunction (241) and impaired energy transfer from mitochondria to myofibrils (242). These metabolic alterations may therefore attenuate the capacity of the myocardium to adapt to increased workloads such as exercise (117). This could therefore serve as an indicator of poor myocardial contractile reserve eventually leading to heart failure.

Phosphorus magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) allows non-invasive assessment of the myocardial phosphocreatine to ATP concentration ratio (PCr/ATP), which is a sensitive indicator of the myocardial energy status (79). The diabetic heart has been demonstrated to be energetically impaired with a decreased PCr/ATP at rest (237, 238)and with exercise stress(117). However, to the best of our knowledge, the effect of catecholamine stress on the myocardial energy production in T2D has not yet been studied.

First-pass dynamic contrast-enhanced myocardial perfusion cardiovascular magnetic resonance imaging (CMR) can be used to derive quantitative estimates of hyperaemic and resting myocardial blood flow (MBF) as well as the myocardial perfusion reserve index (MPRI) (168). Abnormal MPRI in the absence of significant epicardial artery stenosis is likely to represent coronary microvascular dysfunction. CMR is also the reference technique for a comprehensive, non-invasive assessment of changes in cardiac structure, function, strain, fibrosis, and scar.

Thus, CMR facilitates a comprehensive investigation of the intricate relationship between metabolic, functional, and ischaemic adaptations in the diabetic heart.

The objectives of this study were two-fold.

1. To study the effect of acute haemodynamic stress on myocardial energy production in T2D
2. To investigate the relative associations of impaired cardiac energetics, and perfusion to systolic and diastolic subclinical functional changes at rest and in response to acute haemodynamic stress in T2D.

### 5.3 Methods

The study complies with the Declaration of Helsinki and was approved by the National Research Ethics Committee (Ref: 18/YH/0168), and informed written consent was obtained from each participant. Thirty-six participants who had T2D with overweight/obesity ( $\text{BMI} \geq 25 \text{ kg/m}^2$ ), twenty non-athletic healthy volunteers and twenty veteran athletes were recruited for the study. Veteran athletes were included in the study due to the known increased work demands of athletic training. It has been previously demonstrated that athletes have a higher PCr/ATP ratio at rest (243). It was thought that due to the regular increase in work demands of athletic training, the athletes would have a better adaptability to acute increases in haemodynamic stress and would serve as an excellent comparator group.

Participants were excluded if they had a previous diagnosis of cardiovascular disease (previous coronary artery bypass graft surgery, angioplasty, myocardial infarction, angina, moderate or above valvular heart disease, and atrial fibrillation), contraindications to CMR, ischemic changes on 12-lead electrocardiogram (ECG), or renal impairment (estimated glomerular filtration rate below  $30 \text{ mL/min/1.73 m}^2$ ) or if they were receiving treatment with insulin. Control participants had no overt cardiovascular disease, and they had normal glycaemic control with glycated haemoglobin (HbA1c) values  $\leq 40 \text{ mmol/mol}$ . For the control cohorts, at the point of recruitment, it was ascertained by verbal questioning that the exercise duration had been  $<3 \text{ h/week}$  for the past 12 months. Whereas for the veteran athletes, average training duration was confirmed to be  $>5 \text{ h/week}$ .

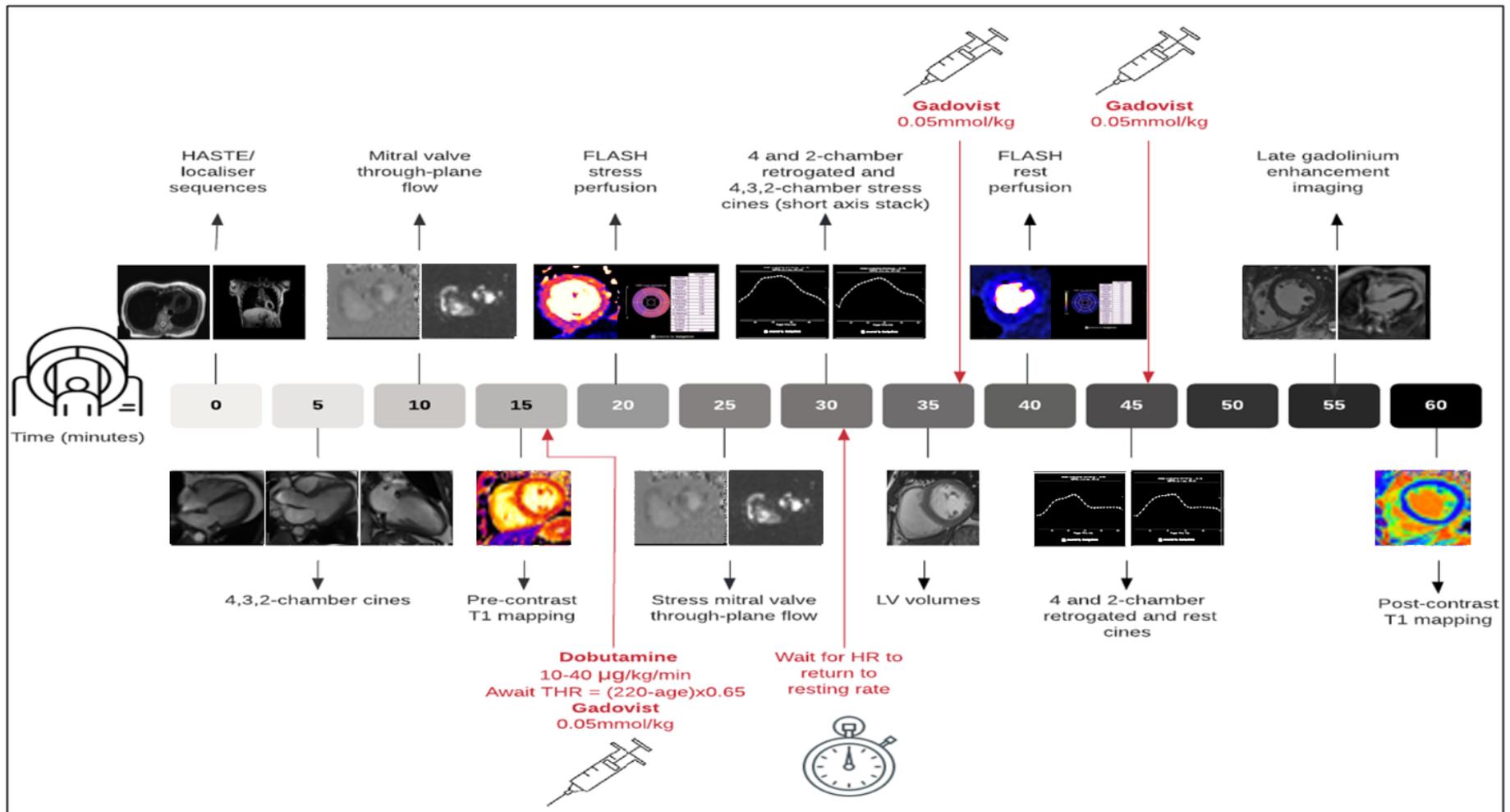
### **Anthropometric measurements**

Height and weight were recorded, BMI was calculated, blood pressure (BP) was recorded as an average of three supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Inc., Tampa, Florida), and a resting ECG was recorded. Fasting blood samples were taken from each participant for assessments of full blood count, estimated glomerular filtration rate, glucose, insulin, HbA1c, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol levels. Triglyceride index ( $\ln [\text{fasting triglycerides (mg/dL)} \times \text{fasting plasma glucose (mg/dL)} / 2]$ ) was calculated as a validated surrogate markers of insulin resistance from fasting blood samples (244, 245).

### **Magnetic Resonance Spectroscopy**

$^{31}\text{P}$ -MRS was performed to obtain the rest and dobutamine stress PCr/ATP with the participants lying supine in the scanner and the  $^{31}\text{P}$  transmitter/receiver cardiac coil (Rapid Biomedical GmbH, Rimpfing, Germany) positioned above the mid-ventricular septum of the heart. A series of inversion-recovery free induction decay signals were acquired. Four fiducial markers were positioned on the anterior coil surface to allow localization of the coil relative to the patient.  $^{31}\text{P}$ -MRS was acquired with a non-gated 3D acquisition-weighted chemical shift imaging sequence. The acquisition matrix was  $16 \times 8 \times 8$  with a field of view of  $240 \times 240 \times 200$  mm. The acquisition ran with a fixed TR of 720 ms. Two 50mm saturation bands were placed over the chest wall muscle and an additional 50mm saturation band placed over the liver. Acquisition time was 9 mins at rest and 9 mins once the target heart rate had been achieved.

Haemodynamic measurements were taken and recorded every minute and the mean rest and dobutamine stress rate pressure product (RPP) was calculated ( $RPP = \text{systolic BP} \times \text{HR}$ ). Participants were maintained at a steady RPP level during the 9 min acquisition of spectra.  $^{31}\text{P}$ -MRS post-processing analysis was performed as previously described (246, 247).



HASTE, Half-fourier Single-Shot Turbo-spin Echo; THR, target heart rate; FLASH, Fast Low Angle Shot; HR, heart rate

Figure 5.1: MRI protocol for this study

## **Magnetic resonance imaging (MRI)**

All scans were performed on a 3.0-T MR system (Prisma, Siemens, AG, Erlangen, Germany). The MRI protocol (Figure 5.1) consisted of cine imaging using a steady-state free precession (SSFP) sequence, thoracic and abdominal water/fat images using a multi-echo gradient echo (GRE) sequence, native and postcontrast T1 mapping, stress and rest perfusion, and late gadolinium enhancement (LGE).

Native T1 mapping was acquired in three sections using a breath-held modified Look-Locker inversion recovery acquisition, as previously described (precontrast 5 seconds [3 seconds] 3 seconds and postcontrast 4 seconds [1 second] 3 seconds [1 second] 2 seconds schemes) (248). Postcontrast T1 mapping acquisition was performed 15 minutes after the last contrast injection using identical planning as the native T1 map. Perfusion imaging used a free-breathing, fast low-angle shot (FLASH) MR protocol with motion-corrected (MOCO) automated in-line perfusion mapping using the Gadgetron streaming software image reconstruction framework, as previously described (168). For stress perfusion imaging, dobutamine was infused at a rate of 10 $\mu$ g/kg/min and increased up to a maximum of 40 $\mu$ g/kg/min to reach an individual target heart rate for the patient, calculated as  $(220 - \text{age}) \times 0.65$ . A minimum 10-minute interval was kept between perfusion acquisitions to ensure equilibration of gadolinium kinetics and resolution of all hemodynamic effects of dobutamine. For each perfusion acquisition, an intravenous bolus of 0.05 mmol/kg of gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5 mL/s followed by a 20-mL saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer, Leverkusen, Germany).

LGE imaging was performed using a phase-sensitive inversion recovery sequence in matching left ventricle (LV) short-axis planes and long-axis planes >8 minutes after

contrast administration to exclude the presence of previous myocardial infarction or regional fibrosis.

### **Quantitative analysis**

All CMR postprocessing analysis was performed offline and blinded to all participant details after completion of the study. The anonymization codes, which were generated using a random number generator, were unlocked only after all data analysis was completed.

Images for biventricular volumes and function were analyzed as previously described (182). The left atrial (LA) volume and LA ejection fraction were calculated using the biplane area-length method in the horizontal and vertical long axes, as previously described (249). Strain measurements were performed using cvi42 Tissue Tracking (Circle Cardiovascular Imaging) from balanced SSFP from the short-axis images and the horizontal long-axis and vertical long-axis views. The peak circumferential systolic strain and peak early diastolic strain rates and global longitudinal strain (GLS) were measured as previously described (250)

Myocardial perfusion image reconstruction and processing were implemented using the Gadgetron software framework as previously described (168). Rest/stress MBF was measured for each of the 16 segments using the American Heart Association (AHA) classification. MBF values for all remaining segments were averaged to provide a global value. Native T1 maps and extracellular volume (ECV) were analyzed using cvi42 software from a region of interest in the midwall of the septum using the native precontrast and native postcontrast T1 times of myocardium, blood pool, and haematocrit, as previously described (250).

For LGE imaging analysis, areas of contrast enhancement were visually scored as absent or present. Hyperenhancement was considered present only if myocardial enhancement was confirmed on both short-axis and perpendicular long-axis locations.

### **Statistical analysis**

Statistical analysis was performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, New York). Categorical data were compared with the Pearson  $\chi^2$  test. Continuous variables are presented as mean [upper limit of 95% confidence interval-lower limit of 95% confidence interval] and they were checked for normality using the Shapiro–Wilk test. Comparisons between the three groups were performed by one-way analysis of variance (ANOVA) with post hoc Bonferroni corrections. The diastolic function, stress MBF and resting PCr/ATP were corrected for body mass index (BMI) by using the one-way analysis of co-variance (ANCOVA). Bivariate correlations were performed using the Pearson correlation coefficient. For these tests,  $p \leq 0.05$  was considered statistically significant.

## **5.4 RESULTS**

Demographic, biochemical and rest and stress CMR and  $^{31}\text{P}$ -MRS data are shown in Table-5.1.

A total of twenty participants in the healthy volunteer group (mean age= 57 [51-62] years, BMI= 25 [23-26] kg/m<sup>2</sup>, HbA1C= 35[34-38]mmol/mol), twelve participants in the athletes group ( mean age= 58 [52-64] years, BMI= 24 [23-26] kg/m<sup>2</sup>, HbA1c= 35 [33-37] mmol/mol) and 36 participants in the T2D group (mean age= 59 [57-62] years,

BMI= 28 [26-19] kg/m<sup>2</sup>, HbA1C= 66 [58-69] mmol/mol) were recruited. Age and sex distribution was similar across the three cohorts. The healthy volunteers and athletes were not receiving any medications. There was no significant difference in the systolic and diastolic blood pressures between the three cohorts. However, the athletes had a significantly lower resting heart rate. Markers of diabetes and insulin resistance (fasting glucose, HbA1c and triglyceride index) were significantly elevated in the T2D cohort.

### Multiparametric MRI results

CMR results for cardiac volumes and function, strain, perfusion, native T1 maps and ECV are summarised in Table 5.2.

**Table 5.1:** Demographic and biochemical characteristics on the healthy volunteers

	<b>HV</b> (n=20)	<b>Veteran athletes</b> (n=12)	<b>T2D</b> (n=36)	<b>ANOVA</b>
Age (yrs)	57 [51-62]	58 [52-64]	59 [57-62]	0.6
Male (n,%)	12 (60)	7 (58)	23(64)	0.7
Body mass index (kg/m <sup>2</sup> )	25 [23-26]	24 [23-26]†	28 [26-29]Ω	<b>0.006</b>
Systolic blood pressure (mmHg)	124 [122-136]	112 [105-121]	130 [127-137]	0.2
Diastolic blood pressure (mmHg)	77 [73-82]	69 [62-75]	77 [75-81]	0.06
Heart rate (beats/min)	60 [54-62]	49 [43-54]	68 [64-71]	<b>0.0001</b>
Fasting glucose (mmol/L)	4.9 [4.8-5.2]	4.9 [4.7-5.1]†	9.1 [8-12]Ω	<b>0.001</b>
Glycated haemoglobin (mmol/mol)	35 [34-38]	35 [33-37]†	66 [58-69]Ω	<b>&lt;0.0001</b>

NTproBNP (pg/ml)	59 [41-75]	50 [36-64]†	114 [58-171]Ω	<0.0001
Triglyceride Index	3.7 [3.6-3.8]	3.6 [3.5-3.7]†	4.2 [4.1-4.3]Ω	<0.0001

(HV), Veteran athletes and patients with type 2 diabetes (T2D). Values are mean [LL of 95% confidence interval – UL of 95% confidence interval]; § indicates  $p < 0.05$  between HV and athletes; † indicates  $p < 0.05$  between athletes and T2D; Ω indicates  $p < 0.05$  between HV and T2D

### Cardiac Geometry and function

The athletes showed significantly higher LV end-diastolic (HV= 151[134-167] mls, athletes= 186[150-186] mls and T2D= 128[119-137] mls;  $p=0.001$ ) end-systolic (HV= 57[48-65] mls, athletes= 66[55-76] mls and T2D= 51[46-56] mls;  $p=0.05$ ) and stroke volumes (HV= 94[84-104] mls, athletes= 102[91-113] mls, T2D= 77[71-83] mls;  $p=0.0003$ ) compared to the T2D cohorts. However, there was no significant difference in the LV ejection fraction between the three groups. The T2D cohort showed has a higher LVEDV:LV mass ratio (HV= 0.64[0.59-0.70], athletes= 0.64[0.57-0.70] and T2D= 0.79[0.74-0.85];  $p=0.0008$ ). Similarly, the RV end-diastolic volume (HV= 152 [131-173] mls, athletes= 183[156-209] mls, T2D= 139[128-150] mls;  $p=0.001$ ) and RV stroke volume (HV= 92[82-102] mls, athletes= 99[83-115] mls and 78 [72-85] mls;  $p=0.01$ ) was significantly higher in the athletes compared to the T2D group.

**Table 5.2:** CMR and 31P-MRS characteristics of the study population.

	HV (n=20)	Veteran athletes (n=12)	T2D (n=36)	ANOV A
<b>CARDIAC STRUCTURAL CHANGES</b>				
LV end-diastolic volume (ml)	151 [134-167]	168[150-186]†	128 [119-137]	0.001
LV end diastolic volume index (ml/m <sup>2</sup> )	83 [75-92]	91 [84-98]†	66 [62-71] Ω	<0.0001

LV end systolic volume (ml)	57 [48-65]	66 [55-76]†	51 [46-56]	<b>0.05</b>
LV end systolic volume index (ml/m <sup>2</sup> )	31 [27-35]	36 [32-40]†	26 [24-29]	<b>0.002</b>
LV stroke volume (ml)	94 [84-104]	102 [91-113]†	77 [71-83] Ω	<b>0.0003</b>
LV ejection fraction (%)	63 [61-65]	62 [60-65]	60 [59-62]	0.2
LV mass (g)	96 [83-109]	108 [89-127]	99 [92-106]	0.5
LV mass /LV end diastolic volume (mg/ml)	0.64 [0.59-0.70]	0.64 [0.57-0.70]†	0.79 [0.74-0.85] Ω	<b>0.0008</b>
RV end diastolic volume (ml)	152 [131-173]	183 [156-209] †	139 [128-150]	<b>0.001</b>
RV end diastolic volume index (ml/m <sup>2</sup> )	82 [70-93]	99 [88-110]†	72 [67-77]	<b>0.0006</b>
RV end systolic volume (ml)	61 [49-73]	80 [64-95]	61 [55-66]	0.06
RV stroke volume (ml)	92 [82-102]	99 [83-115]†	78 [72-85]	<b>0.01</b>
RV ejection fraction (%)	61 [59-64]	57 [54-60]	57 [55-59] Ω	<b>0.03</b>
Native T1 (ms)	1179 [1154-1203]	1193 [1155-1231]	1155 [1130-1181]	0.1
Extracellular volume (%)	20 [18-21]	21 [20-22]	21 [20-22]	0.5

**REST AND STRESS STRAIN, DIASTOLIC ASSESSMENT, EJECTION FRACTION AND PERFUSION**

Stress (bpm*mmHg)	RPP	16,196 [14,088-18,342]	15,121 [12,976-17,854]	16,907 [14,402-19,524]	0.07
Rest (bpm*mmHg)	RPP	6,583 [4,877-8,421]	5,995 [2,439-7,996]	7,077 [5,142-8,913]	0.09
Delta (bpm*mmHg)	RPP	8,972 [6,335-11,703]	9,566 [6,629-13,101]	8,824 [6,143-11,563]	0.7
Increase in RPP (%)		138%	152%	137%	0.07
Rest GLS, (%)		18 [17-19]	20 [18-21]†	17 [16-18]	<b>0.008</b>
Stress GLS, (%)		25 [22-28]	24 [22-26]	20 [18-22] Ω	<b>0.01</b>

Rest E/A	1.38 [1.13-1.62]	1.53 [1.35-1.98]	1.02 [0.89-1.15] Ω	<b>0.0007</b>
Stress E/A	1.22 [0.95-1.49]	1.25 [1.01-1.37]	0.78 [0.70-0.87] Ω	<b>0.0003</b>
Rest LV EF (biplanar) (%)	65 [63-68]	63 [60-65]	63 [61-65]	0.4
Stress LV EF (biplanar) (%)	77 [74-80]	74 [70-78]	76 [74-78]	0.4
Stress myocardial blood flow (ml/g/min)	1.89 [1.70-2.02]	1.97 [1.56-2.37]†	1.49 [1.34-1.63] Ω	<b>0.006</b>
Rest myocardial blood flow (ml/g/min)	0.68 [0.64-0.74]	0.60 [0.50-0.70]	0.67 [0.62-0.71]	0.2
Myocardial perfusion reserve	2.70 [2.38-3.02]	3.44 [2.54-4.35]†	2.37 [2.11-2.62]	<b>0.01</b>

#### REST AND STRESS MYOCARDIAL ENERGETICS

Stress (bpm*mmHg)	RPP	15,732 [13,786-18,213]	14,738 [12,770-17,214]	16,234 [13,979-18,531]	0.07
Rest (bpm*mmHg)	RPP	6,397 [4,596-8,201]	5,846 [4,078-7,606]	6,983 [5,003-8,901]	0.09
Delta (bpm*mmHg)	RPP	9,416 [6,532-12,059]	9,196 [6,335-11,836]	9,151 [6,500-11,721]	0.7
Increase in RPP (%)		145%	148%	135%	0.2
Rest PCr/ATP		1.98 [1.80-2.16]	2.07 [1.86-2.29]†	1.72 [1.46-1.70] Ω	<b>0.03</b>
Stress PCr/ATP		1.62 [1.40-1.84]	1.61 [1.37-1.85]	1.41 [1.35-1.57]	0.3
Change in PCr/ATP between rest and stress		20 [12-28]	22 [21-38]	16 [11-21]	0.1
P value- Rest and stress PCr/ATP		<b>0.004</b>	<b>0.03</b>	<b>0.001</b>	

Values are mean [LL of 95% confidence interval – UL of 95% confidence interval]; § indicates  $p < 0.05$  between HV and athletes; † indicates  $p < 0.05$  between athletes and T2D; Ω indicates  $p < 0.05$  between HV and T2D

### **Rest and stress strain, diastolic function parameters, ejection fraction and perfusion assessment**

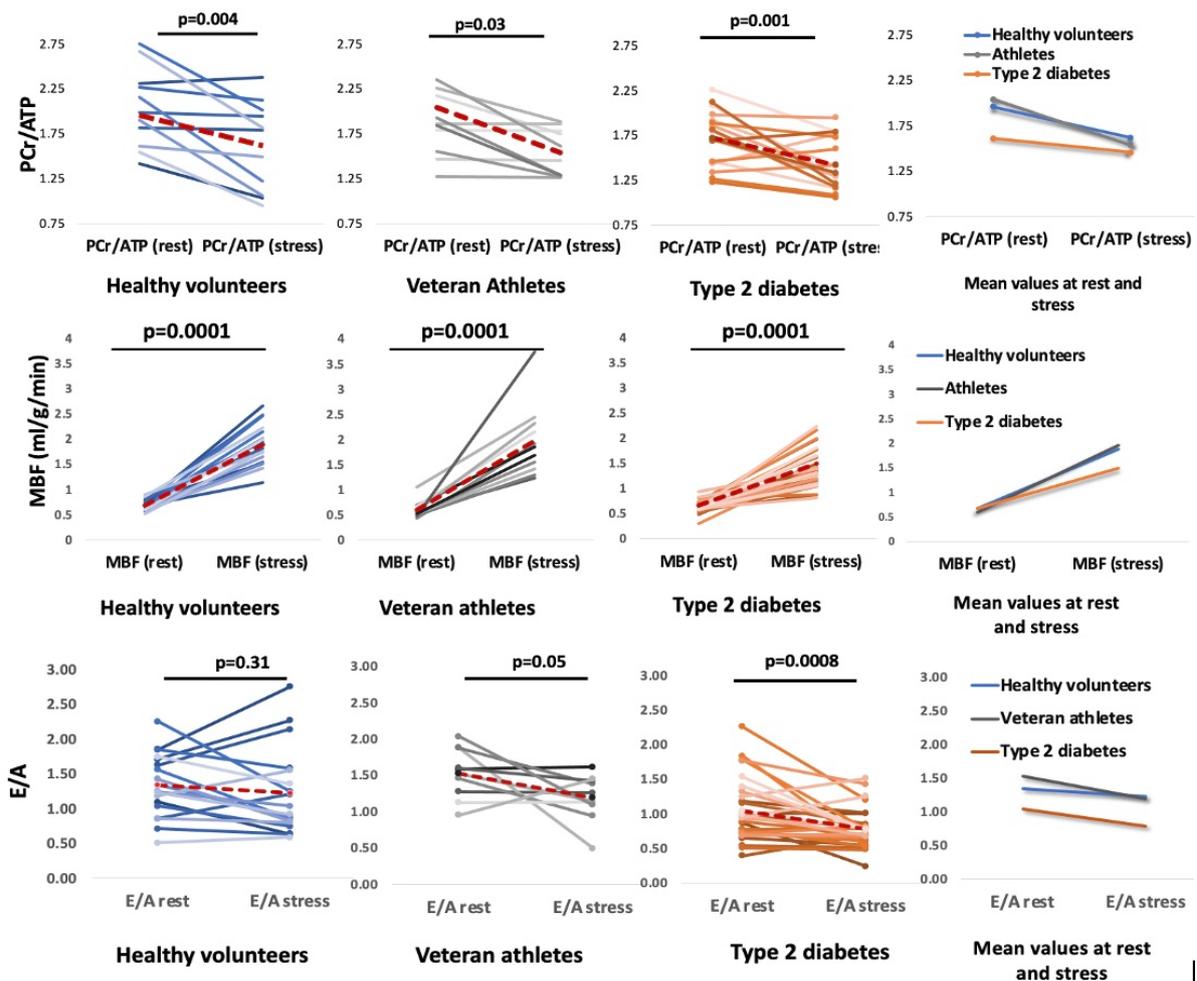
Rest and stress rate pressure product values, strain, E/A ratio, ejection fraction, MBF, and myocardial perfusion reserve (MPR) measurements are summarized in Table 5.2. Participants from all groups demonstrated a similar increase in rest and stress rate pressure products during dobutamine stress. The global longitudinal shortening and E/A was lowest in the T2D cohort at rest (HV= 1.38[1.13-1.62], athletes= 1.53[1.35-1.98] and T2D= 1.02[0.89-1.15];  $p=0.0007$ ) and remained lower during dobutamine stress (HV= 1.22[0.95-1.49], athletes= 1.25 [1.01-1.37] and T2D= 0.78[0.70-0.87],  $p=0.0003$ ) (Figure 5.2).

There was no significant difference in the LV ejection fraction at rest and although the ejection fraction increased with dobutamine stress, the difference remained insignificant. The stress MBF was reduced in the T2D cohort compared to the healthy volunteers and the athletes (HV=1.89[1.70-2.02] ml/g/min, athletes= 1.97[1.56-2.37]ml/g/min and T2D= 1.49[1.34-1.63] ml/g/min;  $p=0.006$ ) (Figure 5.2). In terms of the myocardial perfusion reserve index, it was lowest in the T2D group and highest in the athletes with the healthy volunteers not being significantly different from either of the other two groups (HV= 2.70[2.38-3.02], athletes= 3.44[2.54-4.35] and T2D= 2.37[2.11-2.62];  $p=0.01$ ).

### Rest and stress myocardial energetics

Rest and stress rate pressure product values and PCr/ATP ratios are summarised in Table 5.2. Participants from all groups demonstrated a significant and similar increase in rest and stress rate pressure products during dobutamine stress. The T2D cohort had a significantly lower rest PCr/ATP than the athletes and healthy volunteers (HV= 1.98[1.80-2.16], athletes= 2.07[1.86-2.29] and T2D= 1.72[1.46-1.70]; p=0.03) (Figure 5.2). During dobutamine stress the PCr/ATP for the three groups declined and it remained numerically lower for the T2D cohort (HV=1.62[1.40-1.84], athletes= 1.61[1.37-1.85] and T2D= 1.41[1.35-1.57]; p=0.3) (figure 5.2).

Figure 5.2: Changes in the rest and stress PCr/ATP, myocardial blood flow (MBF) and E/A of study participants





Tyg index	0.59	-	-	-	<b>0.01</b>	<b>-0.45</b>	0.09	-0.48	<b>0.00</b>	<b>0.46</b>	0.66	-3.91
		0.07							<b>2</b>			

LVEF indicates left ventricular ejection fraction; GLS, global longitudinal strain; MBF, myocardial blood flow; PCr/ATP, phosphocreatine to ATP ratio; HbA1c, glycated haemoglobin; Tyg index, triglyceride index. Values in bold signify statistical significance with  $p < 0.05$

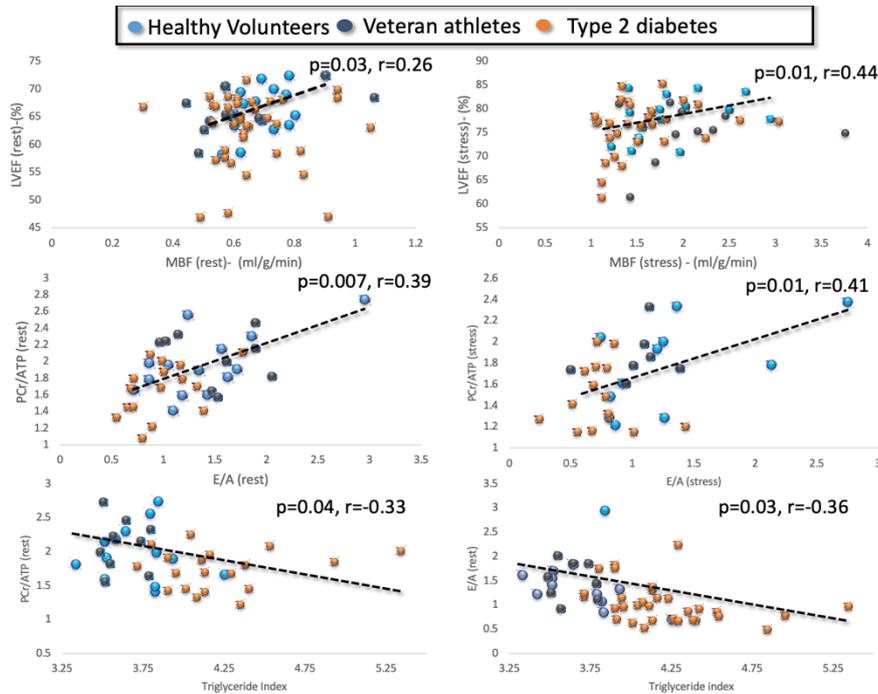


Figure 5.3: Correlations between LVEF and MBF, PCr/ATP and E/A, and PCr/ATP and triglyceride index at rest and stress

## 5.5 Discussion

The results of the present study highlight several important findings. First, in this study we confirmed that T2D patients with overweight/obesity show reductions in myocardial energetics, GLS and diastolic function at rest. In response to dobutamine stress, T2D patients with overweight/obesity as well as healthy volunteers and age-matched veteran athletes show decrements in myocardial energetics and diastolic function, and similar increments in GLS and LVEF, but with a blunted increment in stress MBF in T2D patients with overweight/obesity. We also demonstrate that rest and stress MBF are independently associated with rest and stress LVEF, while rest and stress energetics were independently associated with rest and stress diastolic parameters

respectively, suggesting that diastolic function is a more energetically sensitive process than global systolic function.

In line with previous studies, we confirm that overweight/obesity in patients with T2D confers decline in myocardial energetics (117, 238), GLS (117, 251) and diastolic function (96, 252) at rest.

We have demonstrated that all three cohorts show a decline in the myocardial energetics and diastolic dysfunction with dobutamine stress. Compared with the HV and athletes, the T2D cohorts showed a similar decrease in the PCr/ATP with dobutamine stress while exhibiting similar rest and stress RPPs. The drop in PCr/ATP could be secondary to demand ischaemia which occurs when myocardial energy requirements exceed myocardial oxygen availability in the absence of significant coronary artery stenosis (253). It has been previously demonstrated that these marked metabolic changes occurred despite an increase in the myocardial blood flow (253). In our study, the athletes also demonstrated a higher E/A ratio at rest when compared to the other two cohorts- which is in line with previous studies (254-257). The E/A declined in all three cohorts with stress due to the atrial contribution predominating the diastolic filling pattern as the heart rate increases. Despite the fall, the athletes continued to have a marginally higher E/A ratio when compared to the HV and significantly higher ratio when compared to the participants with T2D.

The blunted increment in stress MBF in T2D compared to the other two cohorts is likely secondary to endothelial dysfunction or coronary microvascular dysfunction (52, 237, 239, 258). Under normal conditions, there is a balance between the endothelium derived relaxing and contracting factors, however type 2 diabetes results in a disruption of this balance resulting in overt endothelial dysfunction (259). The key

processes leading to coronary microvascular dysfunction in type 2 diabetes include impaired endothelial vasodilation, impaired hypoxia induced vasodilation and a blunted myogenic response (260).

## **Chapter 6**

**Liraglutide treatment improves  
myocardial energetics and stress  
perfusion in patients with type 2  
diabetes- A randomised, single  
centre, open label, cross-over  
drug trial**

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## 6.1 Abstract

### Background:

Both the insulin secretion and insulin resistance in Type 2 diabetes (T2D) are amenable to pharmacological intervention. Glucagon-like peptide-1 receptor agonists (GLP-1RA) promote insulin secretion, causes weight loss and is an important pharmacological target in T2D with a proven cardiovascular safety profile and beneficial cardiovascular outcomes(131, 162). Pioglitazone is a peroxisome proliferator activated receptor gamma agonist which targets peripheral insulin sensitivity. In a single center, open-label, randomized, cross-over design trial we sought to compare two distinct glycaemic control strategies of 1) targeting beta-cell dysfunction (liraglutide), 2) insulin resistance (pioglitazone) results in greater improvements in myocardial perfusion, energetics and function in T2D patients.

### Methods:

Forty-one eligible patients with T2D and no known prior cardiovascular disease were randomized in a 1:1 ratio to one of the study drugs for a 16-week treatment period followed by an 8-week washout and a further 16-week treatment period for the second drug. Thirty-five participants completed the pioglitazone treatment period and thirty-two participants completed the liraglutide treatment. Participants have undergone <sup>31</sup>phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS) at rest and dobutamine stress followed by the cardiac magnetic resonance (CMR) scans. The CMR protocol consisted of rest and dobutamine stress cine imaging, perfusion imaging (motion corrected, automated in-line perfusion mapping), velocity-encoded mitral in-flow imaging and late gadolinium enhanced imaging at 3T immediately before commencement and after completion of each treatment arm (four scans per

participant). Intravenous dobutamine was infused, at incremental doses (10 to 40 µg/kg/min to achieve 65% of the age-predicted maximal heart rate).

### **Results:**

Liraglutide therapy resulted in significant reductions in the body mass index, with average weight loss of 1.7kg and significant improvements in glycaemic control. Pioglitazone resulted in an average weight gain of 1.8kg. The improvement in glycaemic control was significantly higher with liraglutide compared to pioglitazone ( $p=0.03$ ) and only liraglutide led to significant reductions in fasting blood glucose. Pioglitazone led to a significant increment in mean LV mass, while no significant effect in myocardial mass or mass index were detected with liraglutide. Liraglutide therapy resulted in increased rest and dobutamine stress energetics, global stress myocardial blood flow and myocardial perfusion reserve (MPR). With pioglitazone treatment, only an isolated improvement in the rest diastolic function was observed. The improvements in rest energetics and MPRI with liraglutide were significantly higher compared to changes with pioglitazone.

### **Conclusions:**

In this randomised cross-over study we showed for the first time that four months treatment with GLP-1RA liraglutide results in significant improvements in myocardial perfusion and energetics, while the insulin sensitiser pioglitazone shows no effect in modulation of these parameters. Pioglitazone results in significant increases in LV mass and an isolated improvement in rest diastolic function.

## 6.2 Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by hyperglycaemia that is associated with a high risk of cardiovascular and other serious health-related consequences. People with T2D are two to three times more likely to die from cardiovascular causes than people with no history of diabetes even after controlling for other cardiovascular risk factors, making it the leading cause of morbidity and mortality in this population. Heart failure (HF) is a leading cardiovascular complication of diabetes(1, 16) with a 2.5-fold increased risk of developing HF in patients with T2D and 1.7-fold in patients with prediabetes(261, 262). Once HF is established in T2D patients survival rates reduces significantly(181). While strict risk factor control was shown to significantly diminish the vascular complications of T2D with no excess risk of death, myocardial infarction, or stroke, as compared with the general population, the same benefit was not seen in heart failure hospitalisations(205). It is therefore imperative to identify effective strategies that prevent heart failure in T2D.

T2D is characterized by dysregulated insulin secretion and resistance to insulin action. Both the insulin secretion and insulin resistance are amenable to pharmacological intervention. It is widely accepted that all patients with T2D exhibit a degree of defective insulin secretion from pancreatic beta cells, making them unable to compensate for the peripheral insulin resistance(263). Glucagon-like peptide-1 (GLP-1) receptor activation promotes insulin secretion, causes weight loss and is an important pharmacological target in T2D with a proven cardiovascular safety profile

and beneficial cardiovascular outcomes(131, 162). GLP-1 is an incretin hormone secreted from the small intestine. An incretin hormone is a gut-derived peptide with important physiological function in augmenting post-prandial insulin secretion in response to ingestion of a meal. GLP-1 has a glucose dependent stimulatory effect on insulin and inhibitory effect on glucagon secretion from the pancreatic islets (i.e., when plasma glucose levels are above normal).

Coupled with defective insulin secretion, insulin resistance is a fundamental aspect of T2D as evidenced by cross-sectional studies demonstrating insulin resistance in all patients with T2D(264). Insulin resistance refers to impaired insulin action in target tissues such as muscle, liver and adipose tissue. Increasing evidence points to a strong association between insulin resistance and non-ischemic heart failure(64, 70, 71), albeit with differing opinions held whether this relationship is of protective or pathological nature(67-69). There are many molecular mechanisms that may contribute to the association between insulin resistance and non-ischemic cardiomyopathy(64). These include metabolic inefficiency(21), impaired vascular function(65), inflammation, mitogenic actions of insulin on myocardium leading to changes of left ventricular geometry(66). However, contrary to these, more recently, insulin resistance has been proposed as a defence mechanism that protects critical tissues of the cardiovascular system from energy fuel overload in dysregulated metabolic states(67, 69, 265). Pioglitazone is a widely used peroxisome proliferator activated receptor gamma agonist which targets peripheral insulin sensitivity. Meta-analyses of prior randomized trials studying the risk of cardiovascular events have demonstrated a significant reduction in the risk of all-cause mortality with pioglitazone use despite an increased incidence of heart failure(266).

T2D contributes to the development of HF through a variety of mechanisms, including disease specific myocardial structural, functional and metabolic changes. Cardiovascular magnetic resonance (CMR) is the only imaging modality that can assess non-invasively cardiac function, strain, ischaemia, perfusion, fibrosis and scar. Utilising CMR, previous studies have identified predictors of adverse CV events in T2D patients including distinct ventricular morphology(183), impaired strain(40, 183), and reduced myocardial perfusion(267). CMR is also established as a tool for quantification of diffuse fibrosis by quantifying the extracellular volume fraction (ECV) by T1 mapping(268).

In addition to these structural and functional changes, compromised cardiac energy production is an important contributor to most forms of heart disease including T2DM(42, 269). The relative concentration of phosphocreatine to ATP (PCr/ATP) is a sensitive indicator of the energetic state of the myocardium and can be assessed non-invasively using <sup>31</sup>phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS)(72). Utilising <sup>31</sup>P-MRS, we have shown that despite normal left ventricular ejection fraction (LVEF) and absence of significant coronary artery disease (CAD) or hypertension, patients with T2D have a decreased myocardial PCr/ATP(40), suggesting that even symptom-free T2D patients are 'cardiac energy-deficient', and this deficiency in type 2 diabetes is exacerbated by simple exercise activity(42). Despite the normal LVEF, this energy deficient state is associated with subtle abnormalities in contractile function of the heart measured by strain(40, 42).

In a single center, open-label, randomized, cross-over design trial we sought to compare the efficacies of two distinct glycaemic control strategies of 1) targeting beta-

cell dysfunction (liraglutide), 2) insulin resistance (pioglitazone) results in greater improvements in myocardial perfusion, energetics and function in T2D patients.

### **6.3 Methods**

The study took place between 25<sup>th</sup> of January, 2021 and the 6<sup>th</sup> of September, 2022 at the Leeds General Infirmary, United Kingdom. The trial protocol was approved by the West Midlands - Black Country REC (19/WM/0365). The trial was conducted in accordance with the principles of the Declaration of Helinski and Good Clinical Practice guidelines.

#### **Study design**

The study took place over four patient visits during a 40-week period. Participants were administered two drugs, pioglitazone which is a peroxisome proliferator activated receptor gamma agonist (PPAR- $\gamma$ ), and Liraglutide, a glucagon like peptide-1 receptor agonist (GLP-1RA), sequentially. Participants were randomised to receive either Pioglitazone or Liraglutide first for a 16-week treatment period, followed by an eight-week washout period to reduce the impact of carry-over effects. After this, participants were administered whichever drug they did not receive first for the remaining 16-week treatment period.

#### **Study Population**

Potential participants were identified through three recruitment pathways:

- With assistance from the NIHR Yorkshire and Humber Clinical Research Network, individuals with T2D were recruited from local GP practices.
- The study team also contacted those who had participated in previous observational ethically approved studies in the department (University of Leeds, Biomedical Imaging) and who had consented to have their contact details retained to be contacted if eligible to take part in other studies.
- A REC-approved poster placed at GP practices and additional locally organised awareness events for GPs and specialist nurses was also used to create awareness and help with recruitment.

The trialled enrolled participants greater than 18 years of age with T2D who were either drug-naïve or were established on treatment with oral glucose lowering therapies for at least 12 weeks. A full list of inclusion and exclusion criteria is provided in table 6.1. All the participants provided written informed consent prior to participation.

**Table 6.1:** *Inclusion and Exclusion criteria.*

Inclusion Criteria	Exclusion Criteria
Participants aged $\geq 18$ years	Any type of diabetes other than T2D
Normal body weight ( $18.5 \leq \text{BMI} \leq 25$ kg/m <sup>2</sup> ) for the lean cohort or increased body weight ( $\text{BMI} > 27$ kg/m <sup>2</sup> ) for the overweight cohort	Past history of significant CAD

Either drug naïve or if on an oral glucose lowering therapy, participants must have been on these treatments for at least 12 weeks prior to screening	Known HF
6.5≤HbA1c≤10% at screening	Significant renal impairment (eGFR <30ml/min/m <sup>2</sup> )
Agreement to maintain prior diet and exercise habits for the duration of the study	Participation in a CTIMP in the preceding 12 weeks
	Known hypersensitivity to dobutamine or gadolinium or any other contra-indications to MRI
	Participants with obesity where their girth exceeds the scanner bore
	History of pancreatitis
	Any history of liver disease
	Patients with MEN-2
	Prior or current use of thiazolidinediones (PPAR-γ agonists), fibrates, GLP-1RA or insulin
	Patients that are pregnant (female participants only)
	Inflammatory bowel disease
	Diabetic gastroparesis

	Women of childbearing potential not using contraception*
--	--

*BMI, body mass index; T2D, type 2 diabetes; CAD, coronary artery disease; HF, heart failure; eGFR, estimated glomerular filtration rate; CTIMP, clinical trial of an investigational medicinal product; MEN-2, Multiple Endocrine Neoplasia syndrome type 2; MRI, magnetic resonance imaging; PPAR- $\gamma$  agonists, peroxisome proliferator activated receptor gamma agonists; GLP-1RA, glucagon-like peptide-1 receptor antagonist. \* Acceptable contraception includes barrier-type devices only in combination with a spermicide, intrauterine devices, oral contraceptive agents started at least 90 days before study start, Depo-Provera, levonorgestrel implants, naturally or surgically sterile, male partner is sterile and is the only sexual partner.*

## Study design

This was a single centre, open-label, randomised cross-over study based in a tertiary care setting. Eligible participants were randomised in a 1:1 ratio to either receive Liraglutide or Pioglitazone first. The randomization was carried out by the clinical trials pharmacy using stratification via a computer-generated program that allocated patients to either treatment after accounting for age, gender and BMI. The study was not blinded following randomisation as this would have been challenging given the different routes of administration of the two drugs. In addition, un-blinding was useful to assess tolerability and side effect profile of the study medications.

As mentioned, recruited participants were randomised to one of two groups:

(1) Liraglutide treatment first or (2) Pioglitazone treatment first.

1. Liraglutide was prescribed at 0.6mg once daily initially, then titrated up to 1.2mg once daily via subcutaneous injection, either by the individual or by a qualified member of the study team if preferred. Treatment was continued for a total of 16 weeks and was followed by an eight-week washout period. Participants were then initiated onto Pioglitazone treatment as detailed in (2) for a period of 16 weeks.

2. Pioglitazone was prescribed at 15mg orally once daily initially, and then the dose was increased to 30mg once daily after two weeks. The dose was further titrated to the target of 45mg once daily after a further two weeks if glucose levels permitted. Treatment was continued for a total of 16 weeks and was followed by an eight-week washout period. Participants were then initiated onto Liraglutide treatment as detailed in (1) for a period of 16 weeks.

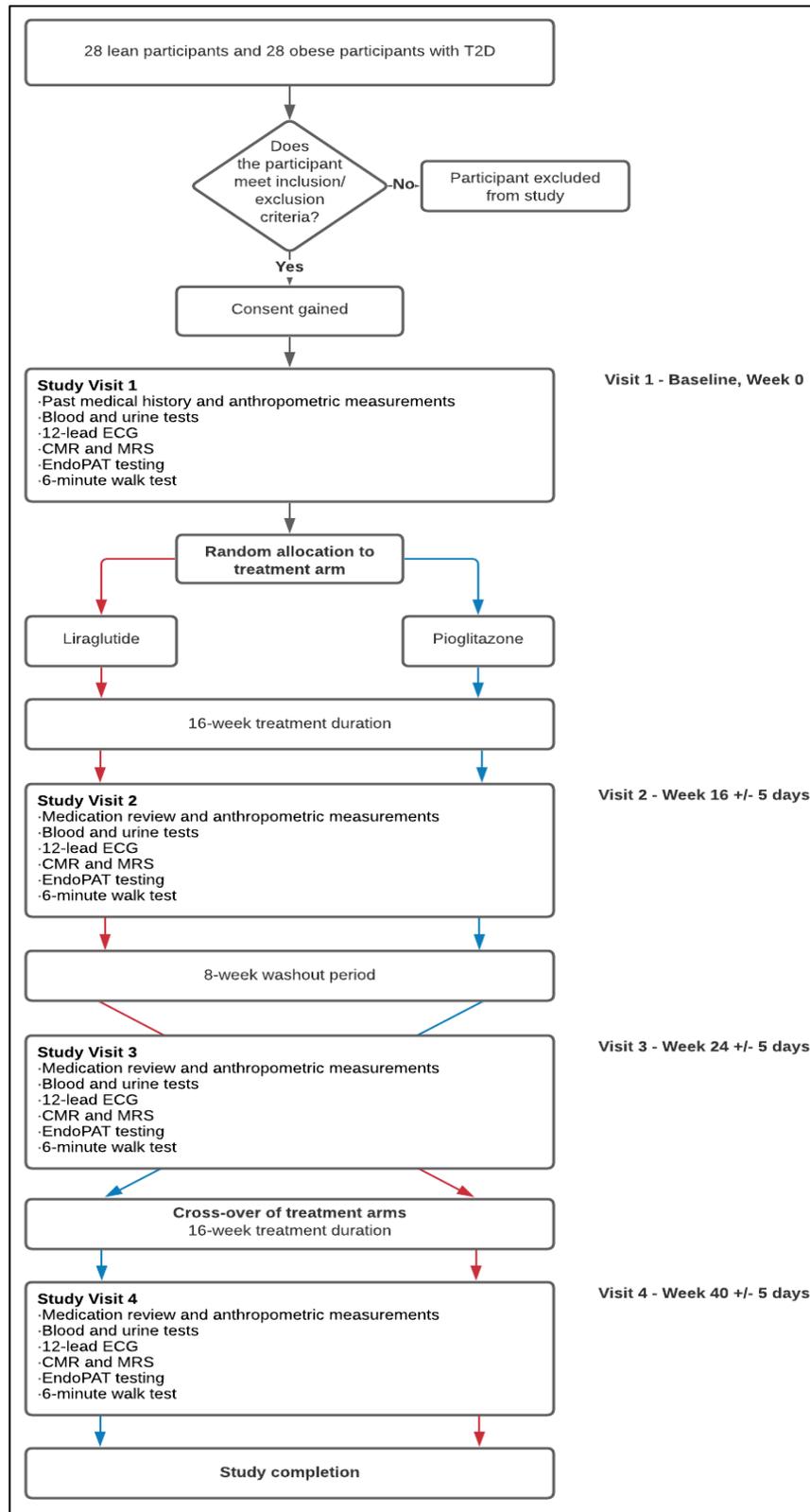
Medication were labelled to make participants aware of which medication they were taking and information on how and when to take the medication was provided by study investigators who were General Medical Council registered practitioners.

### **Study Visits**

A study flow chart detailing study visits can be seen in Figure 6.1.

During the first visit, eligibility criteria of the potential participant was checked and written informed consent was obtained. Baseline demographic data and past medical history was also obtained. At this and each of the three subsequent visits, a medication review was performed and anthropometric measurements (height, weight, hip and waist circumference and blood pressure using DINAMAP-1846-SX, Critikon Corp) was taken. An average of three blood pressure readings in the supine position after resting for 10 minutes was obtained. Urine was inspected for presence of visible haematuria. A urine sample was taken for assessment of urine albumin/ creatinine ratio and a urinary pregnancy test was done for women of childbearing age.

Figure 6.5: LeanDM Study Protocol



T2D, type 2 diabetes; ECG, electrocardiograph; CMR, cardiovascular magnetic resonance; MRS, magnetic resonance spectroscopy.

A 12-lead ECG was also performed. A fasting blood sample was taken for assessment of full blood count, liver and kidney function, lipid profile, glucose, N-terminal pro-B type natriuretic peptide, insulin, free fatty acids, adiponectin, high sensitivity cardiac troponin T and glutamic acid decarboxylase antibodies. Homeostasis model of insulin resistance (HOMA-IR), calculated as  $\text{fasting serum insulin } (\mu\text{U/L}) \times \text{fasting plasma glucose } (\text{mmol l}^{-1})/22.5$  and Triglyceride index ( $\ln [\text{fasting triglycerides } (\text{mg/dL}) \times \text{fasting plasma glucose } (\text{mg/dL})/2]$ ) were calculated as validated surrogate markers of insulin resistance from fasting blood samples (219, 244, 245).

Participants underwent non-invasive assessment of endothelial function using endoPAT™ (Peripheral Arterial Tone) 2000, Itamar Medical, a validated device approved for the assessment of endovascular dysfunction, which measures dilatory response through pulsatile volume changes at the fingertips (270). In addition, participants completed a six-minute walk test (6MWT) for assessment of functional capacity as detailed below.

### **Magnetic Resonance Spectroscopy**

<sup>31</sup>P-MRS was performed to obtain PCr/ATP ratio at 3.0 Tesla. Spectroscopy at 3.0 Tesla was preferred over 1.5 Tesla due to higher field strength increasing signal-to-noise ratio (271). <sup>31</sup>P-MRS is the only non-invasive method capable of quantifying PCr/ATP effectively (272). Participants lied down supine in the scanner with the <sup>31</sup>P transmitter/receiver cardiac coil (Rapid Biomedical GmbH, Rimpar, Germany) positioned above the mid-ventricular septum of the heart. A series of inversion-recovery free induction decay signals were acquired. Four fiducial markers were positioned on the anterior coil surface to allow localization of the coil relative to the patient. <sup>31</sup>P-MRS was acquired with a non-gated 3D acquisition-weighted chemical

shift imaging sequence. The acquisition matrix was 16 x 8 x 8 with a field of view of 240 x 240 x 200 mm. The acquisition was run with a fixed TR of 720 ms. Two 50mm saturation bands were positioned over the chest wall muscle and an additional 50mm saturation band was placed over the liver.

### **Magnetic Resonance Imaging**

Magnetic resonance imaging was performed on a 3.0 Tesla MR system (Prisma, Siemens, Erlangen, Germany). Participants were asked to avoid caffeine for 24 hours in advance of the scan. The MRI protocol as outlined in Figure 6.2. consisted of cine imaging using a steady-state free precession (SSFP) sequence, thoracic and abdominal water/fat images using a multi-echo gradient echo (GRE) sequence, native and postcontrast T1 mapping, stress and rest perfusion, and late gadolinium enhancement.

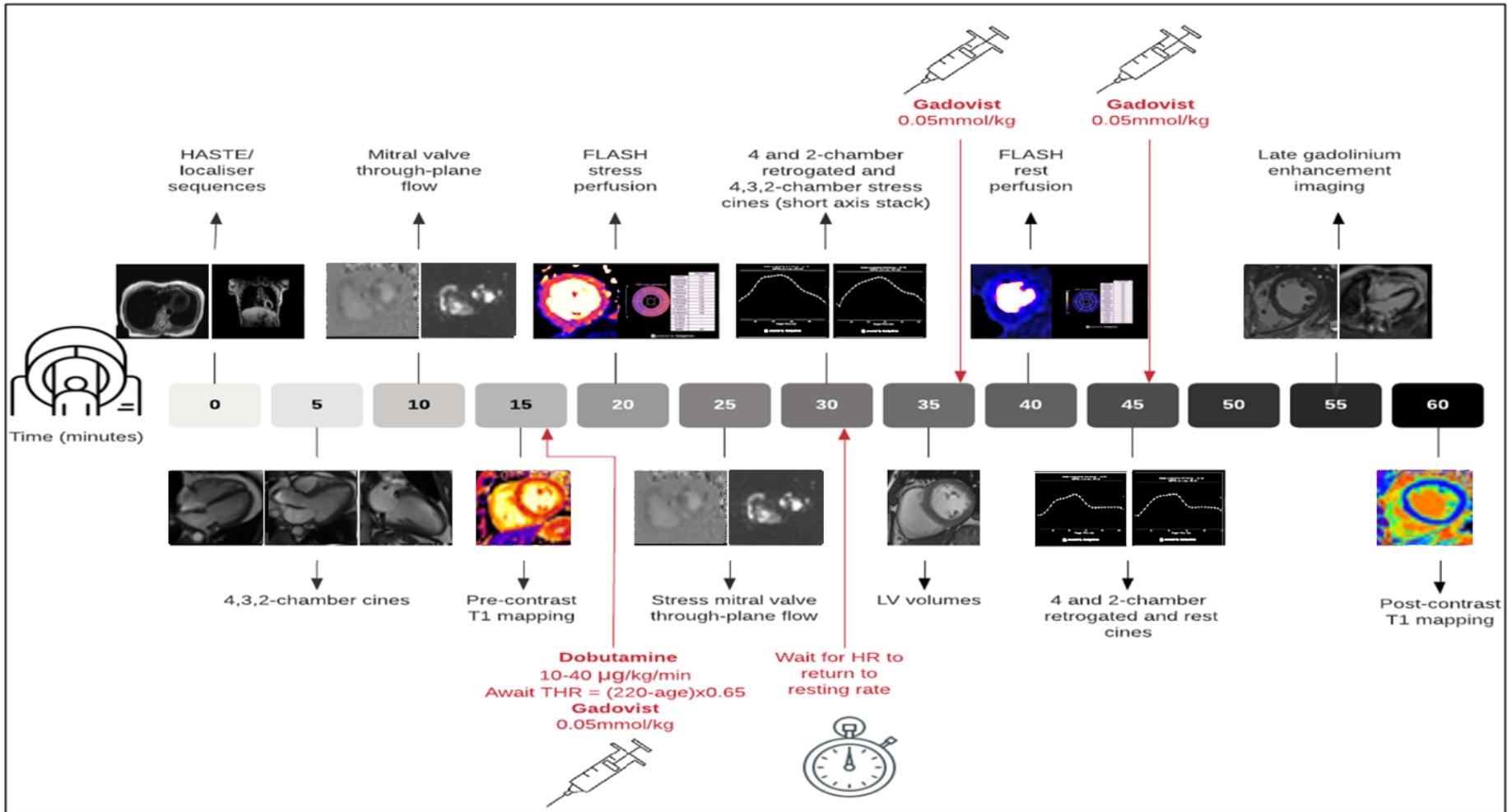
With the participant positioned supine on the scanner, free-breathing localiser and Half-fourier Single-Shot Turbo-spin Echo (HASTE) sequences were performed to identify the position and orientation of the heart within the thorax. Cine imaging was performed using true fast imaging with steady state precession (trueFISP), a balanced steady-state free precession (bSSFP) sequence in 4,3 and 2 chamber views using these typical parameters: spatial resolution 1.8 x 1.8 x 8mm, TR 37.8ms, TE 1.38ms, flip angle 42°, slice thickness 8 mm, parallel imaging factor 3, field of view adjusted for each patient and a phase reconstruction of 83.7%.

Native T1 mapping was acquired in three sections using a breath-held modified Look-Locker inversion recovery acquisition, as previously described (precontrast 5 seconds [3 seconds] 3 seconds and postcontrast 4 seconds [1 second] 3 seconds [1 second])

2 seconds schemes) (248). Postcontrast T1 mapping acquisition was performed 15 minutes after the last contrast injection using identical planning as the native T1 map. Perfusion imaging used a free-breathing, fast low-angle shot (FLASH) MR protocol with motion-corrected (MOCO) automated in-line perfusion mapping using the Gadgetron streaming software image reconstruction framework, as previously described (168). For stress perfusion imaging, dobutamine was infused at a rate of  $10\mu\text{g}/\text{kg}/\text{min}$  and up titrated at 2-minute intervals up to a maximum of  $40\mu\text{g}/\text{kg}/\text{min}$  to reach an individual target heart rate for the patient, calculated as  $(220-\text{age}) \times 0.65$ . A minimum 10-minute interval was kept between perfusion acquisitions to ensure equilibration of gadolinium kinetics and resolution of all hemodynamic effects of dobutamine. For each perfusion acquisition, an intravenous bolus of 0.05 mmol/kg of gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5 mL/s followed by a 20-mL saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer, Leverkusen, Germany). The blood pressure was monitored every two minutes using MasimoSET, WelchAllyn, Skaneateles Falls, NY, USA and continuous electrocardiograph monitoring was performed.

LGE imaging was performed using a phase-sensitive inversion recovery sequence in matching left ventricle (LV) short-axis planes and long-axis planes >8 minutes after contrast administration to exclude the presence of previous myocardial infarction or regional fibrosis.

Figure 6.2: MRI protocol



HASTE, Half-fourier Single-Shot Turbo-spin Echo; THR, target heart rate; FLASH, Fast Low Angle Shot; HR, heart rate

## **Quantitative analysis**

All CMR postprocessing analysis was performed offline and blinded to all participant details after completion of the study. The anonymization codes, which were generated using a random number generator, were unlocked only after all data analysis was completed. <sup>31</sup>P-MRS analysis was performed offline using Matlab version R2012a (Mathworks, Natick, Massachusetts) as previously described (273). Quantitative assessment of CMR data was performed offline using commercially available software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada)

Images for biventricular volumes and function were analyzed as previously described (182). The left atrial (LA) volume and LA ejection fraction were calculated using the biplane area-length method in the horizontal and vertical long axes, as previously described (249). Strain measurements were performed using cvi42 Tissue Tracking from balanced SSFP from the short-axis images and the horizontal long-axis and vertical long-axis views. The peak circumferential systolic strain and peak early diastolic strain rates and global longitudinal strain (GLS) were measured as previously described (250)

Myocardial perfusion image reconstruction and processing were implemented using the Gadgetron software framework as previously described (168). Rest/stress MBF was measured for each of the 16 segments using the American Heart Association (AHA) classification. MBF values for all remaining segments were averaged to provide a global value. Native T1 maps and extracellular volume (ECV) were analyzed using cvi42 software from a region of interest in the midwall of the septum using the native

precontrast and native postcontrast T1 times of myocardium, blood pool, and haematocrit, as previously described (250).

For LGE imaging analysis, areas of contrast enhancement were visually scored as absent or present. Hyperenhancement was considered present only if myocardial enhancement was confirmed on both short-axis and perpendicular long-axis locations.

### **Statistical analysis**

Statistical analysis was performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, New York) and GraphPad Prism Software (version 9.0.0). Categorical data were compared with the Pearson  $\chi^2$  test. Continuous variables are presented as mean [upper limit of 95% confidence interval- lower limit of 95% confidence interval] and they were checked for normality using the Shapiro–Wilk test. Comparison between two groups was performed by a Student's T-test. Bivariate correlations were performed using the Pearson correlation coefficient for parametric data or Spearman rank correlation for non-parametric data as appropriate. For these tests,  $p \leq 0.05$  was considered statistically significant.

### **EndoPAT**

EndoPAT™ (Peripheral Arterial Tone) 2000, Itamar Medical offers non-invasive assessment of endothelial function validated against the gold-standard (270). EndoPAT™ measures post-ischaemic vascular responsiveness after upper arm arterial occlusion through pulsatile volume changes in the fingertips. The system was used at 21-24°C, in line with recommendations (274). The participant's arms were supported at the level of the heart. Plethysmographic biosensors were placed on the

participant's right and left index fingers and inflated. A blood pressure cuff was placed on the participant's non-dominant arm. After a one-minute standby period, baseline data was recorded for five minutes. Following this, the brachial artery was occluded by inflating the cuff and data was recorded for a further 5 minutes. The cuff was then released with the restoration of blood flow producing reactive hyperaemia, with endothelial release of nitric oxide and endothelium-derived hyperpolarizing factor (274). The software then automatically analysed data obtained from both fingers to provide a post-occlusion to pre-occlusion ratio. The vasodilatory response was quantified as the reactive hyperaemia index, which is a measure of endothelial function, and has been validated against invasive methods of assessing microvascular dysfunction (270, 275).

### **Six-minute walk test**

The 6MWT is an inexpensive, widely available and well-tolerated method of assessing functional capacity and exercise tolerance. Even though maximal exercise tests, such as cardiopulmonary exercise testing (CPET), remain the gold-standard for functional assessments, the 6MWT is less resource-intensive and has been shown to have a moderate-to-strong correlation with CPET measures, such as peak aerobic capacity (peak  $\text{VO}_2$ ) (276). In patients with HF and reduced ejection fraction, decreased 6MWT performance has been associated with increased mortality, cardiovascular events and HF hospitalisations. Participants walked along a 30-metre corridor, attempting to cover the maximum possible distance in six minutes under medical supervision. At the end of six minutes, the distance the participants had walked was recorded in metres.

## **Study Outcomes**

### **Primary Outcome**

The primary study outcome was change in myocardial perfusion as measured by the MPR after treatment with Liraglutide or Pioglitazone.

### **Secondary Outcomes**

Secondary study outcomes after treatment with Liraglutide or Pioglitazone included percentage difference in myocardial PCr/ATP from rest to dobutamine stress, myocardial strain parameters, HOMA-IR, six-minute walk distance and peripheral endothelial function assessed by EndoPAT.

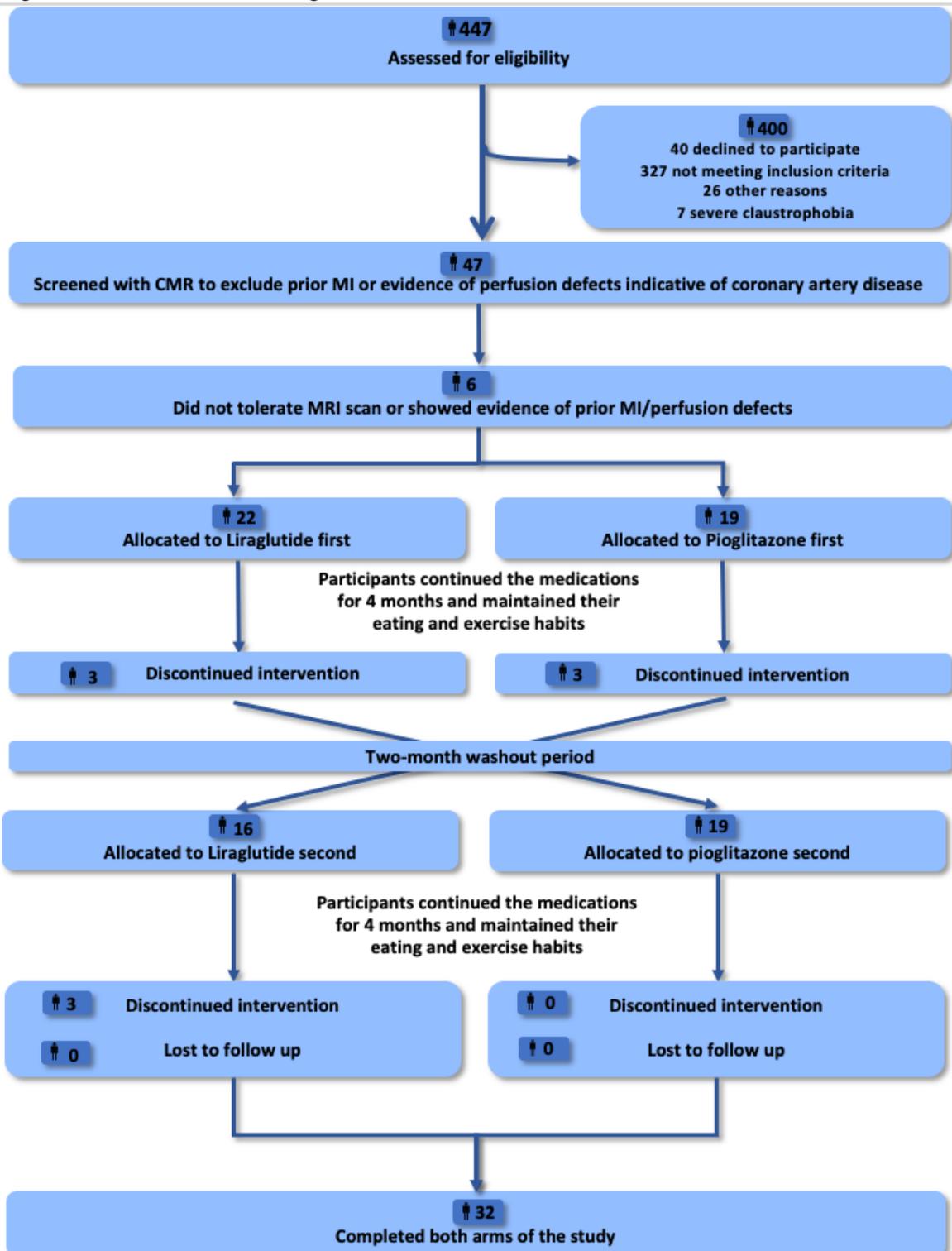
## **6.4 Results:**

### **Trial population**

A total of 447 patients were assessed for eligibility (figure 6.3). Out of these 400 were excluded (327 did not meet all the inclusion criteria, 40 declined to participate, 26 other reasons and 7 had severe claustrophobia) (figure 6.3). Out of the remaining 47 participants, six participants either did not tolerate the scan or showed evidence of prior MI or perfusion defects on the initial CMR. The remaining 41 participants were either randomized to the pioglitazone-first arm (n=19) or the liraglutide-first arm (n=22). In the pioglitazone-first arm three participants discontinued therapy due to the side effect of weight gain and mild ankle swelling. These symptoms resolved promptly with the discontinuation of the medication. In the liraglutide-first arm, three participants discontinued therapy due to gastrointestinal symptoms of nausea and fullness and therefore discontinued the medication. The remaining participants (pioglitazone-first,

n=16 and liraglutide-first, n=19) completed the first two study visits and the subsequent 8-week washout period successfully. A total of 19 participants were commenced on the pioglitazone-second arm and 16 participants were commenced on the liraglutide-second arm. In the pioglitazone-second arm, all the 19 participants completed 16-week treatment course whereas in liraglutide-second arm 13 participants completed the 16-week course of treatment (3 participants discontinued the treatment due to gastrointestinal side effects of nausea and fullness).

Figure 6.3: CONSORT diagram



### **Demographics, anthropometrics, and biochemical characteristics**

The demographics, anthropometrics, biochemical characteristics, and drug history are demonstrated in table 6.2. There were no significant differences in any of these parameters in between the groups that received pioglitazone-first or liraglutide-first. After treatment with pioglitazone, participants showed a significant increase in both weight (79.6kg [74.1–85.1] vs 81.4kg [75.9-86.9];  $p=0.0005$ ) and BMI (27.7kg/m<sup>2</sup> [26.0-29.5] vs 28.5kg/m<sup>2</sup> [26.8-30.3],  $p=0.0002$ ) (table 6.3) (Figure 6.4 A & B). Whereas treatment with Liraglutide resulted in a significant reduction in both the body weight (79.7kg [74.3-85.1] vs 78.0kg [72.4-83.5],  $p=0.0003$ ) and BMI (28.0kg/m<sup>2</sup> [26.3-29.8] vs 27.5kg/m<sup>2</sup> [25.8-29.3],  $p=0.0001$ ) (table 6.2). Neither drug had an effect on the systolic or diastolic blood pressure however, liraglutide did result in a significant increase in the resting heart rate (65bpm [62–68] vs 71bpm [68–74],  $p=0.0002$ ).

There was no significant difference in the blood glucose, HbA1c, NT-proBNP, troponin-I, HOMA-IR or triglyceride index between the participants who were commenced on pioglitazone-first or liraglutide-first (table 6.2). The improvement in glycaemic control was significantly higher with liraglutide compared to pioglitazone ( $p=0.03$ ) and only liraglutide led to significant reductions in fasting blood glucose (figure 6.4 C & D). Both medications resulted in a decline in the triglyceride levels and in the triglyceride index (table 6.3) (figure 6.4 E & F).

Liraglutide resulted in a significant improvement in the 6-minute walk test distance (479 [447-512] metres to 521 [481-561] metres;  $p=0.002$ ). Neither drug showed an improvement in the reactive hyperaemia index as measured by the EndoPAT test (table 6.3).

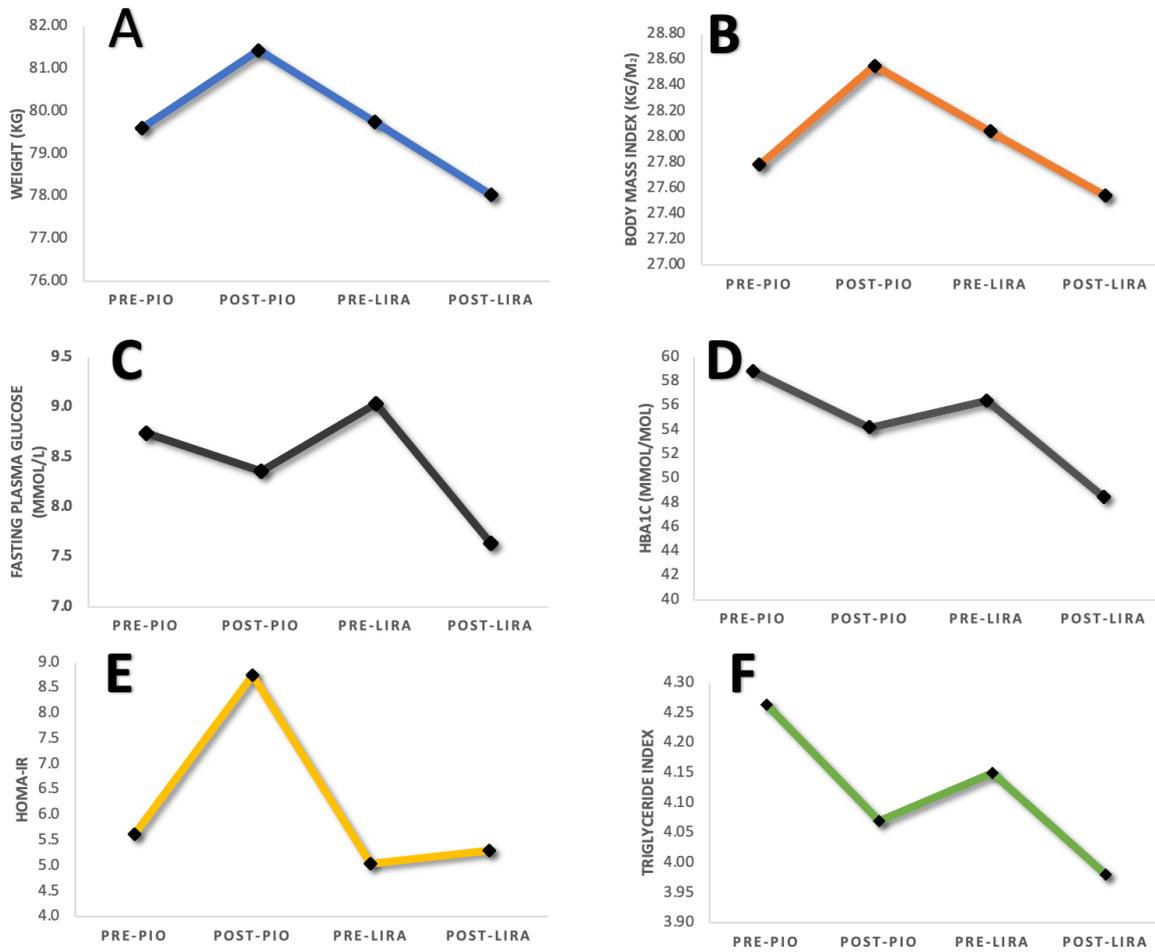


Figure 6.4: Changes in (A) weight, (B) body mass index, (C) fasting plasma glucose, (D) HbA1c, (E) HOMA-IR and (F) triglyceride index of study participants before and after treatment with pioglitazone or liraglutide (Pio- pioglitazone, Lira- liraglutide)

Table 6.2: Demographic, anthropometric and biochemical characteristics of the study population commenced on pioglitazone-first vs liraglutide-first showing means and 95% confidence intervals

	<b>Total study population (n=41)</b>	<b>Pioglitazone first (n=19)</b>	<b>Liraglutide first (n=22)</b>
Age (yrs)	63 [59-68]	64 [59-69]	63 [59-67]
Male (n,%)	66%	53%	66%
Ethnicity			
Caucasian	39 (98%)	18 (95%)	21 (100%)
South Asian	1 (3%)	1 (5%)	0 (0%)
Current or ex-smokers	15 (38%)	8 (42%)	7 (33%)
Duration of Diabetes (years)	10.8 [6.1-15.9]	12.2 [6.4-17.9]	9.5 [6.7-12.3]
Hypertension	20 (50%)	9 (48%)	11 (52%)
Hypercholesterolaemia	13 (33%)	6 (32%)	7 (33%)
Weight (kg)	79.6 [74.1-85.1]	74.9 [69.8-79.9]	83.1 [74.1-92.0]
Body mass index (kg/m <sup>2</sup> )	27.8 [26.1-29.5]	27.2 [25.2-29.1]	28.4 [25.6-30.6]
Waist circumference (cm)	99 [96-103]	98 [95-102]	98 [93-103]
Hip Circumference (cm)	101 [94-107]	102 [98-106]	103 [98-109]
Systolic Blood pressure (mmHg)	130 [124-136]	134 [127-140]	127 [118-135]
Diastolic blood pressure (mmHg)	77 [74-80]	78 [75-80]	81 [76-86]
Resting heart rate (bpm)	68 [65-72]	70 [63-76]	67 [62-70]
Fasting glucose (mmol/L)	8.7 [7.8-9.6]	9.3 [8.2-10.4]	8.2 [7.1-9.3]
Glycated haemoglobin (mmol/mol)	58.8 [54.1-63.4]	61.1 [56.2-65.9]	55.2 [52.4-57.9]
Insulin (pmol/L)	89 [58-119]	86 [40-133]	91 [61-121]
C-peptide (pmol/L)	839 [671-1008]	747 [507-987]	933 [690-1176]
Free fatty acids	0.50 [0.42-0.58]	0.52 [0.44-0.60]	0.45 [0.34-0.57]
D-3 hydroxy butyrate	0.18 [0.11-0.26]	0.13 [0.09-0.17]	0.27 [0.08-0.46]
Triglycerides (mmol/L)	2.49 [1.77-3.20]	2.56 [1.60-3.51]	1.87 [1.05-2.68]
NT-proBNP (pg/ml)	101 [58-132]	111 [39-183]	94 [63-126]
Troponin I	5.1 [2.9-7.7]	3.7 [3.1-4.5]	5.6 [3.2-7.9]
HOMA-IR	5.6 [3.7-7.4]	5.8 [2.9-8.7]	5.2 [3.2-8.3]
Triglyceride Index	4.3 [4.1-4.4]	4.4 [4.2-4.5]	4.1 [3.9-4.4]
6-minute walk test distance (metres)	491 [397-582]	484 [390-591]	493 [465-521]
Reactive hyperaemia index	0.68 [0.51-0.85]	0.70 [0.53-0.87]	0.64 [0.45-0.82]

Angiotensin Converting Enzyme- Inhibitor (ACE-I)	11 (28%)	4 (21%)	7 (33%)
Angiotensin Receptor Blocker	7 (18)	4 (21%)	3 (14%)
Beta blockers	4 (10%)	3 (16%)	1 (4%)
Statins	22 (55%)	11 (58%)	11 (52%)
Biguanide	24 (60%)	12 (63%)	12 (57%)
Sulphonylurea	7 (18%)	6 (32%)	1 (4%)
Thiazolidendiones	0 (0%)	0 (0%)	0 (0%)
Sodium-glucose co-transporter-2 inhibitors	5 (13%)	3 (16%)	2 (8%)
Gliptins	4 (10%)	3 (16%)	1 (4%)

All values are mean [95% confidence intervals]. There were no statistically significant differences between the group which received pioglitazone first and the group which received liraglutide first for any characteristic.

Table 6.3: Demographic, anthropometric and biochemical characteristics of the study population showing means and 95% confidence intervals

	Pioglitazone baseline (n=35)	Pioglitazone treatment (n=35)	P value	Liraglutide baseline (n=32)	Liraglutide treatment (n=32)	P value	ANCOVA (Liraglutide vs Pioglitazone)
Age (yrs)	63 [52-66]			63 [52-66]			
Male (n,%)	62			62			
Weight (kg)	79.6 [74.1-85.09]	81.4 [75.9-86.9]	<b>0.0005</b>	79.7 [74.3-85.1]	78.0 [72.4-83.5]	<b>0.0003</b>	<b>&lt;0.001</b>
Body mass index (kg/m <sup>2</sup> )	27.7 [26.0-29.5]	28.5 [26.8-30.3]	<b>0.0002</b>	28.0 [26.3-29.8]	27.5 [25.8-29.3]	<b>0.0001</b>	<b>&lt;0.001</b>
Waist circumference (cm)	102 [99-105]	101 [97-104]	0.4	96 [90-103]	97 [93-102]	0.6	0.8
Hip Circumference (cm)	100 [94-107]	102 [98-105]	0.6	99 [92-105]	101 [98-105]	0.6	0.6
Systolic Blood pressure (mmHg)	130 [124-136]	128 [121-135]	0.3	127 [119-136]	128 [122-134]	0.7	0.8
Diastolic blood pressure (mmHg)	77 [74-80]	74 [71-76]	0.3	78 [74-81]	77 [74-80]	0.2	0.6
Resting heart rate (bpm)	68 [65-72]	66 [62-69]	0.2	65 [62-68]	71 [68-74]	<b>0.0002</b>	<b>0.001</b>
Fasting glucose (mmol/L)	8.7 [7.8-9.6]	8.4 [7.4-9.3]	0.4	9.0 [8.0-10.0]	7.6 [6.6-8.6]	<b>0.04</b>	0.2
Glycated haemoglobin (mmol/mol)	58.8 [54.1-63.4]	54.2 [50.5-57.8]	<b>0.03</b>	57.2 [53.3-61.2]	49.4 [45.7-53.0]	<b>0.005</b>	<b>0.03</b>
Insulin (pmol/L)	89 [58-119]	123 [56-190]	0.4	77 [58-97]	97 [68-126]	0.8	0.4
C-peptide (pmol/L)	840 [671-1008]	707 [561-854]	0.8	885 [714-1054]	879 [743-1014]	0.9	0.3
Free fatty acids	0.52 [0.44-0.60]	0.43 [0.37-0.50]	0.1	0.45 [0.38-0.52]	0.58 [0.48-0.67]	0.08	0.5

D-3 hydroxy butyrate	0.18 [0.11-0.26]	0.16 [0.12-0.20]	0.2	0.20 [0.09-0.30]	0.17 [0.11-0.23]	0.4	0.4
Triglycerides (mmol/L)	2.49 [1.77-3.20]	1.67 [1.25-2.08]	<b>0.01</b>	1.94 [1.41-2.48]	1.51 [1.23-1.80]	<b>0.04</b>	0.5
NT-proBNP (pg/ml)	139 [63-217]	143 [100-187]	0.9	104 [73-136]	95 [50-140]	0.5	0.2
Troponin I	7.4 [1.8-13.0]	10.5 [2-22]	0.3	9.7 [1.2-19.0]	9.4 [1.2-17.7]	0.7	0.6
HOMA-IR	5.2 [3.4-7.0]	6.8 [2.0-11.6]	0.5	4.0 [2.4-5.5]	3.8 [2.4-5.2]	0.9	0.6
Triglyceride Index	4.3 [4.1-4.4]	4.0 [3.9-4.2]	<b>0.002</b>	4.1 [4.0-4.3]	3.9 [3.8-4.1]	<b>0.01</b>	0.7
6-minute walk test (metres)	485 [437-532]	518 [486-549]	0.4	479 [447-512]	521 [481-561]	<b>0.002</b>	<b>0.008</b>
Reactive hyperaemia index	0.69 [0.58-0.81]	0.52 [0.40-0.64]	0.5	0.66 [0.55-0.77]	0.60 [0.44-0.77]	0.48	0.4

Values are mean [95% confidence intervals]. Values in bold signify a statistically significant difference with  $p < 0.05$ .

### CMR parameters

All CMR parameters comparing the study population commenced on pioglitazone-first and liraglutide-first at baseline are summarised in table 6.4. All CMR parameters comparing the effect of treatment with pioglitazone and liraglutide when compared to baseline are summarised in table 6.4

Table 6.4: CMR and  $^{31}\text{P}$ -MRS characteristics of the study population commenced on pioglitazone-first vs liraglutide-first showing means and 95% confidence intervals

	Pioglitazone first (n=19)	Liraglutide first (n=22)
LV end-diastolic volume (ml)	121 [105-139]	131 [118-144]
LV end diastolic volume index (ml/m <sup>2</sup> )	65 [58-72]	66 [60-73]
LV end systolic volume (ml)	50 [40-59]	53 [45-60]
LV end systolic volume index (ml/m <sup>2</sup> )	26 [22-31]	27 [23-31]
LV stroke volume (ml)	72 [63-80]	78 [72-85]
LV ejection fraction (%)	60 [57-63]	62 [58-63]
LV mass (g)	90 [80-101]	107 [95-120]
LV mass index (g/m <sup>2</sup> )	48 [44-53]	54 [50-59]
LV mass /LV end diastolic volume (mg/ml)	0.77 [0.69-0.85]	0.83 [0.75-0.91]
RV end-diastolic volume (ml)	123 [106-140]	141 [128-154]
RV end-diastolic volume index (ml/m <sup>2</sup> )	66 [58-73]	71 [65-78]
RV end systolic volume (ml)	53 [44-62]	64 [56-71]
RV stroke volume (ml)	68 [58-77]	77 [73-89]
RV ejection fraction (%)	58 [55-61]	55 [53-58]

Stress RPP (bpm*mmHg)	17448 [16535-18360]	15372 [13739-17004]
Rest RPP (bpm*mmHg)	8907 [8146-9668]	8667 [7876-9458]
Increase in RPP (%)	86%	84%
Rest GLS, (%)	17 [15-19]	16 [14-18]
Stress GLS, (%)	21 [17-25]	19 [16-22]
Rest E/A	1.08 [0.83-1.33]	1.10 [0.90-1.29]
Stress E/A	0.82 [0.65-0.99]	0.84 [0.69-0.99]
Rest LV EF (biplanar) (%)	63 [60-67]	64 [60-68]
Stress LV EF (biplanar) (%)	74 [71-78]	74 [71-78]
Rest myocardial blood flow (ml/g/min)	0.66 [0.59-0.73]	0.69 [0.60-0.78]
Stress myocardial blood flow (ml/g/min)	1.57 [1.34-1.80]	1.67 [1.36-1.98]
Myocardial perfusion reserve	2.44 [2.06-2.82]	2.43 [1.95-2.91]
Native T1 (ms)	1144 [1105-1183]	1150 [1120-1187]
Extracellular volume (ECV)	21 [20-22]	23 [20-26]
Epicardial adipose tissue (cm <sup>2</sup> )	113 [96-130]	118 [99-138]
Visceral adipose tissue (cm <sup>2</sup> )	405 [345-465]	453 [352-555]
Subcutaneous adipose tissue (cm <sup>2</sup> )	370 [278-461]	383 [292-472]
<sup>31</sup> P-Magnetic Resonance Spectroscopy		
Rest RPP (bpm*mmHg)	8945 [8082-9807]	8227 [7433-9019]
Stress RPP (bpm*mmHg)	17821 [16515-19127]	16716 [15215-18216]
Increase in RPP (%)	101%	108%
Rest PCr/ATP	1.49 [1.30-1.69]	1.49 [1.39-1.59]
Stress PCr/ATP	1.33 [1.21-1.45]	1.39 [1.29-1.49]

All values are mean [95% confidence intervals]. There were no statistically significant differences between the two groups for any characteristic.

There was no significant difference in any of the baseline CMR parameters between the study population treated with pioglitazone-first when compared to the population treated with liraglutide-first (table 6.4).

All CMR and <sup>31</sup>P-MRS parameters before and after treatment with pioglitazone and liraglutide are outlined in table 6.5.

Neither drug had a significant impact on the LV EDV or the LVEF, while there was a numeric increment in LV EDV in the pioglitazone arm. However, pioglitazone led to a significant increment in mean LV mass (98g [71–107] vs 105g [74-115]; p=0.004) (table 6.4), while no significant effect in myocardial mass or mass index were detected with liraglutide. Pioglitazone therapy also resulted in an increase in the RVEDV and RVESV (p=0.03 and p=0.0001 respectively). Liraglutide did not lead to a significant change in the parameters of RV size or function.

The native T1 and ECV remained unchanged after treatment with either drug.

Both pioglitazone and liraglutide did not affect the rest or stress global longitudinal strain or stress LVEF. However, Pioglitazone did lead to a significant improvement in the rest E/A measurement (1.08 [0.63–1.23] vs 1.34 [0.70-1.54];  $p=0.007$ ).

Liraglutide therapy alone resulted in a significant increase in the stress MBF (1.64ml/g/min [1.20-1.78] to 2.08ml/g/min [1.57-2.24];  $p=0.01$ ) and MPRI (2.42 [1.57-2.69] to 2.90 [1.83-3.18];  $p=0.01$ ) (figure 6.5 A & B).

Neither drug had a significant impact on the epicardial, visceral or subcutaneous adipose tissue distribution.

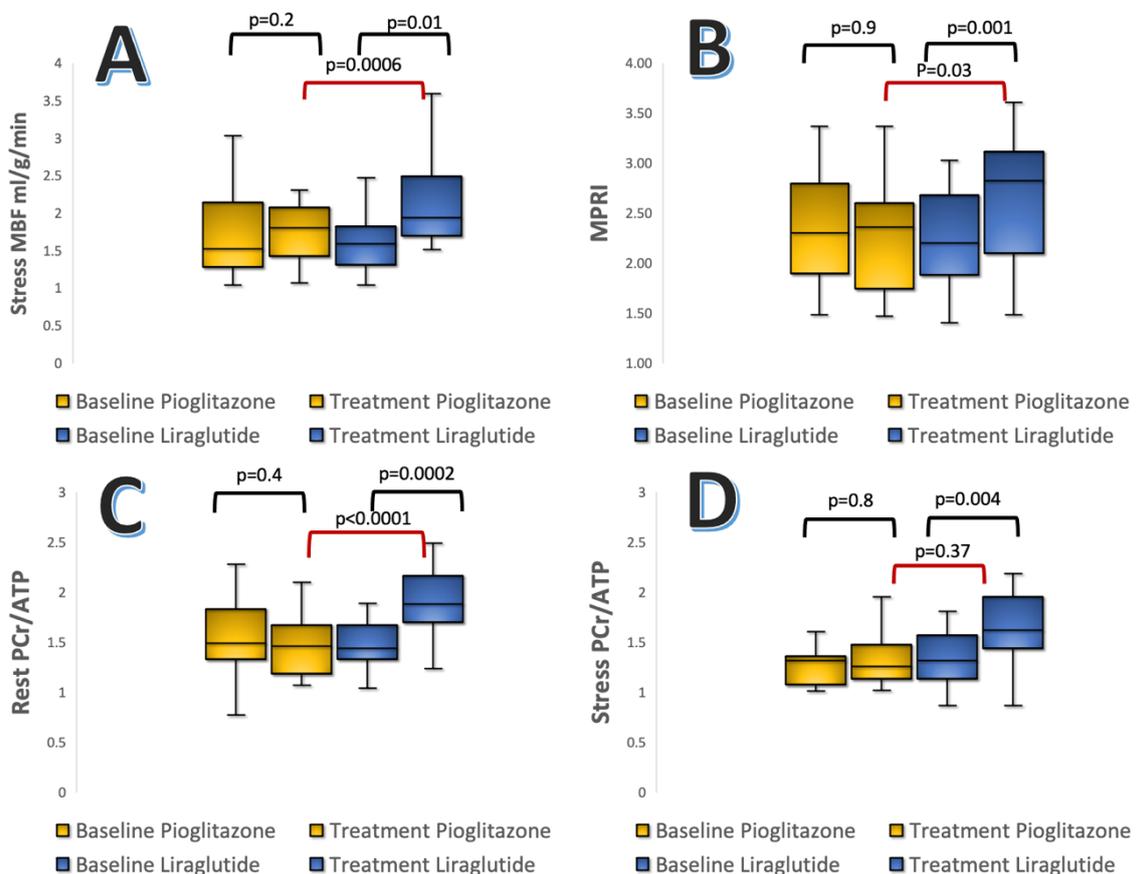


Figure 6.5: Changes in A) Stress myocardial blood flow (MBF), B) Myocardial Perfusion Reserve Index (MPRI), C) Rest PCr/ATP and D) Stress PCr/ATP in the two treatment arms of the study.

### <sup>31</sup>P-MRS parameters

The  $^{31}\text{P}$ -MRS parameters are summarised in table 6.5. In all cohorts the stress PCr/ATP was lower than the rest PCr/ATP. Liraglutide therapy alone resulted in a significant improvement of the rest PCr/ATP (1.49 [1.19-1.58] to 1.94 [1.52-2.08];  $p=0.004$ ) and stress PCr/ATP (1.35 [1.08-1.43] to 1.58 [1.19-1.71];  $p=0.004$ ) (figure 6.5 C & D)

*Table-6.5: CMR and  $^{31}\text{P}$ -MRS characteristics of the study population showing means and 95% confidence intervals*

	Pioglitazone baseline (n=35)	Pioglitazone treatment (n=35)	P value	Liraglutide baseline (n=32)	Liraglutide treatment (n=32)	P value	ANCOVA (Liraglutide versus Pioglitazone)
LV end-diastolic volume (ml)	129 [95-140]	144 [110-154]	0.06	125 [96-135]	120 [88-129]	0.2	<b>&lt;0.0001</b>
LV end diastolic volume index (ml/m <sup>2</sup> )	67 [52-72]	73 [60-78]	0.06	66 [52-72]	64 [49-69]	0.4	<b>&lt;0.001</b>
LV end systolic volume (ml)	52 [33-57]	59 [38-65]	<b>0.002</b>	53 [33-59]	50 [28-56]	0.2	<b>0.01</b>
LV end systolic volume index (ml/m <sup>2</sup> )	26 [18-29]	29 [20-32]	0.2	25 [17-30]	26 [17-30]	0.2	<b>0.02</b>
LV stroke volume (ml)	78 [59-84]	86 [68-92]	<b>0.007</b>	75 [59-80]	73 [55-78]	0.8	<b>&lt;0.0001</b>
LV ejection fraction (%)	61 [55-63]	61 [54-63]	0.3	60 [53-62]	61 [54-63]	0.3	0.6
LV mass (g)	98 [71-107]	105 [74-115]	<b>0.004</b>	101 [74-109]	97 [67-107]	0.16	<b>0.02</b>
LV mass index (g/m <sup>2</sup> )	51 [40-54]	53 [41-56]	<b>0.007</b>	53 [43-56]	51 [39-55]	0.2	<b>0.008</b>
LV mass /LV end diastolic volume (mg/ml)	0.77 [0.60-0.83]	0.73 [0.56-0.79]	<b>0.04</b>	0.80 [0.65-0.85]	0.81 [0.63-0.86]	0.9	0.09
RV end-diastolic volume (ml)	135 [99-146]	153 [116-165]	0.03	135 [104-145]	132 [97-143]	0.7	<b>&lt;0.001</b>
RV end-diastolic volume index (ml/m <sup>2</sup> )	70 [54-75]	78 [62-82]	0.001	70 [56-75]	68 [54-73]	0.8	<b>&lt;0.001</b>
RV end systolic volume (ml)	59 [40-65]	66 [45-73]	0.0001	62 [43-68]	60 [40-66]	0.8	<b>0.03</b>
RV ejection fraction (%)	57 [50-59]	57 [51-59]	0.7	55 [49-57]	56 [50-57]	0.7	0.4
Native T1 (ms)	1155 [1067-1183]	1173 [1096-1197]	0.2	1155 [1082-1178]	1165 [1071-1194]	0.6	0.1
Extracellular Volume (%)	22 [17-24]	22 [17-23]	0.3	21 [16-22]	22 [17-24]	0.05	0.07

Stress RPP (bpm*mmHg)	16410 [13235-17406]	15775 [12730-16729]	0.5	17312 [14567-18174]	16599 [12854-17775]	0.06	0.9
Rest RPP (bpm*mmHg)	8787 [7056-9331]	8386 [6752-8899]	0.4	8934 [7162-9490]	9081 [7522-9571]	0.9	0.2
Increase in RPP (%)	86%	101%		101%	96%		
Rest GLS, (%)	17 [14-18]	17 [14-18]	0.9	16 [12-17]	17 [14-18]	0.2	0.9
Stress GLS, (%)	21 [15-23]	20 [16-21]	0.6	19 [14-21]	19 [14-20]	0.4	0.1
Rest E/A	1.08 [0.63-1.23]	1.34 [0.70-1.54]	<b>0.007</b>	1.06 [0.58-1.22]	1.01 [0.62-1.13]	0.4	<b>0.004</b>
Stress E/A	0.81 [0.52-0.91]	0.95 [0.40-1.12]	0.08	0.90 [0.47-1.04]	0.88 [0.33-1.06]	0.7	0.4
Rest LV EF (biplanar) (%)	61 [55-64]	62 [55-64]	0.3	62 [53-65]	61 [53-63]	0.3	0.4
Stress LV EF (biplanar) (%)	75 [68-77]	74 [69-76]	0.8	73 [65-75]	71 [62-74]	0.2	0.1
Rest myocardial blood flow (ml/g/min)	0.68 [0.50-0.74]	0.73 [0.55-0.78]	0.15	0.69 [0.55-0.73]	0.68 [0.57-0.71]	0.4	0.08
Stress myocardial blood flow (ml/g/min)	1.72 [1.18-1.88]	1.3 [1.33-1.84]	0.2	1.64 [1.20-1.78]	2.08 [1.57-2.24]	<b>0.01</b>	<b>0.006</b>
Myocardial perfusion reserve	2.52 [1.75-2.76]	2.40 [1.78-2.59]	0.9	2.42 [1.57-2.69]	2.90 [1.83-3.18]	<b>0.01</b>	<b>0.03</b>
Epicardial adipose tissue (cm <sup>2</sup> )	117 [78-129]	121 [75-136]	0.5	120 [77-132]	119 [77-132]	0.2	0.8
Visceral adipose tissue (cm <sup>2</sup> )	437 [254-495]	419 [250-472]	0.1	431 [232-494]	401 [215-459]	0.8	0.2
Subcutaneous adipose tissue (cm <sup>2</sup> )	358 [159-420]	399 [184-466]	0.06	383 [182-446]	375 [155-444]	0.08	0.06

<sup>31</sup>P-Magnetic Resonance Spectroscopy

Rest RPP (bpm*mmHg)	8588 [6736-9168]	7969 [6358-8344]	0.06	8089 [6001-8747]	8707 [7053-9226]	0.2	0.09
Stress RPP (bpm*mmHg)	17231 [14059-18226]	16618 [13809-17509]	0.07	17614 [14409-18621]	16779 [13929-17673]	0.08	0.6
Increase in RPP (%)	101%	108%		112	98		
Rest PCr/ATP	1.54 [1.19-1.65]	1.47 [1.15-1.58]	0.4	1.49 [1.19-1.58]	1.94 [1.52-2.08]	<b>0.00002</b>	<b>&lt;0.001</b>
Stress PCr/ATP	1.31 [1.08-1.38]	1.35 [1.05-1.44]	0.8	1.35 [1.08-1.43]	1.58 [1.19-1.71]	<b>0.004</b>	<b>0.03</b>

LV- left ventricle, RV- right ventricle, RPP- rate pressure product, GLS- global longitudinal shortening, EF ejection fraction, PCr- Phosphocreatinine, ATP- adenosine triphosphate

## 6.5 Discussion:

In this randomised cross-over design trial we showed for the first time that four months treatment with GLP-1 receptor agonist liraglutide results in significant improvements

in myocardial stress perfusion and PCr/ATP ratio, while the insulin sensitiser pioglitazone shows no effect in modulation of these parameters. Pioglitazone results in significant increases in LV mass and an isolated improvement in rest diastolic function.

In a meta-analysis of the 7 trials (>56 000 patients in total) in patients with T2D, GLP-1 receptor agonist class of drugs were shown to be associated with significant reductions in major adverse cardiovascular events including cardiovascular death and myocardial infarction or stroke (by 12%), all-cause mortality (by 12%), outcomes related to kidney function (by 17%), and risk of hospitalization for heart failure (by 9%)(131, 277, 278). Reflecting these results, American Diabetes Association and European Society of Cardiology guidelines and consensus statements recommend this class of drugs as add-ons to lifestyle interventions with or without metformin in T2DM patients at high atherosclerotic cardiovascular disease risk.

This study shows that the myocardial energy metabolism and myocardial perfusion are both amenable to pharmacological intervention and that the improvements in energetics and perfusion indices may drive the cardiovascular beneficial effects of GLP1 receptor agonists.

### **Modulation of myocardial phosphocreatine to ATP ratio in type 2 diabetes**

Type 2 diabetes is a disorder of metabolic dysregulation. Decreased PCr/ATP ratio is a predictor of mortality(279), linked to contractile dysfunction(279, 280), and is a well-recognized complication of T2D(37, 267). Here we have shown for the first time that GLP-1 receptor agonist liraglutide improves both the rest and the stress PCr/ATP ratio after 4 months of treatment.

Energy starvation is detectable even in asymptomatic T2D patients preceding other abnormalities such as reductions in LVEF or increase in LV mass(40, 42, 61). These suggest that myocardial energy metabolism offers both early diagnostic and therapeutic opportunities to prevent or modulate diabetic HF.

Loss of flexibility in myocardial fuel selection(281), impaired mitochondrial function(282) and reduced myocardial blood supply(42, 283) are considered as key potential deleterious alterations in cardiac metabolism ultimately resulting in energy deficiency and impaired contractility in patients with T2D(284). In a recent study, significant improvements in myocardial function and energetics were detected despite no improvements in myocardial rest or stress blood flow, or myocardial perfusion reserve, ruling out blood flow changes among the beneficial CV effects of Empagliflozin (138)

While our study shows significant improvements in myocardial energetics by utilizing <sup>31</sup>P-MRS, comprehensive evaluation of cardiac metabolic alterations requires a multi-modal approach using both invasive and non-invasive testing. High resolution respirometry is the gold-standard technique for analyzing mitochondrial oxidative capacity and efficiency in human tissue samples(285-287), while coronary sinus sampling studies allow for measuring the transmural extraction of carbohydrates, fat, ketones, and amino acids *in vivo*(288). We have not performed these invasive tests in our study cohort, consequently we cannot speculate on the impact of liraglutide on myocardial metabolic flexibility in fuel uptake or mitochondrial oxidative capacity as the causes for the observed beneficial outcomes on myocardial energetics.

### **Modulation of myocardial perfusion**

In the absence of significant epicardial CAD, failure to increment myocardial blood flow (MBF) during acute increases in cardiac workload is indicative of coronary microvascular dysfunction (CMD). CMD has emerged as a candidate mechanism of heart disease in T2D(19, 91), preceding clinical HF manifestation (206, 207) and carrying important prognostic information(18, 208). Moreover, CMD has also been demonstrated in obese individuals without T2D(209, 210). As liraglutide treatment resulted in both the weight reduction and improvements in diabetes control, both these factors might have contributed to the improvements in stress myocardial blood flow as well as myocardial perfusion reserve. In this study we have not detected any significant correlations of weight change with the stress or rest perfusion indices, however, our study was not powered to detect these relationships; larger studies are needed to assess these.

Clinical studies examining the effects of GLP-1 receptor agonists on coronary vascular function have been scarce; but small scaled studies suggested enhanced endothelial function and increased perfusion by GLP-1 receptor agonists in patients with T2D(289). GLP-1 receptor agonist exenatide ameliorated both high glucose- and lipid-induced endothelial dysfunction, and stimulated endothelial AMP Kinase pathway activity, resulting in greater endothelial nitric oxide synthase activation and nitric oxide production(289) which might be relevant mechanism for liraglutide action.

### **Modulation of diastolic function**

Confirming the findings of a previous study by van der Meer et al, in our study pioglitazone treatment was associated with improvements in LV diastolic function in patients with well-controlled, uncomplicated T2D, but led to no change in myocardial PCr/ATP ratio or perfusion indices(290). In this study, the effects of liraglutide and

pioglitazone on BMI have been on opposite directions with significant elevations with pioglitazone and significant reductions with liraglutide. While we have not measured myocardial or hepatic triglyceride content changes in this study, a prior study showed pioglitazone had no effect on myocardial triglyceride content, but significantly lowered hepatic triglyceride content. Therefore, van der Meer and colleagues had concluded that the effects of pioglitazone on diastolic function were not related to myocardial metabolism. They had detected significant increment of LV EDV and LV stroke volume with pioglitazone and interpreted these changes as improved myocardial compliance which is also in keeping with diastolic function improvements we have also detected. In our study LV EDV changes showed a similar trend and significant improvements in LV stroke volume in line with the findings of van der Meer. Liraglutide arm did not show any significant changes in diastolic function or LV stroke volume. The increment in LV mass with pioglitazone is a product of change in overall cardiac size.

**Conclusions:**

In this randomised cross-over study we showed for the first time that four months treatment with GLP-1RA liraglutide results in significant improvements in myocardial perfusion and energetics, while the insulin sensitiser pioglitazone shows no effect in modulation of these parameters. Pioglitazone results in significant increases in LV mass and an isolated improvement in rest diastolic function.

## **Chapter 7**

### **General Conclusions**

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The work in this thesis was carried out to study disease mechanisms in asymptomatic patients with uncomplicated T2D who had no prior diagnosis of cardiovascular disease. Even in the absence of coronary artery disease, T2D is associated with an increased risk of HF and cardiovascular mortality. The two main candidate mechanisms that potentially underpin this dysfunction are impaired cardiac high energy phosphate metabolism and coronary microvascular dysfunction. This work combined advanced CMR and MRS techniques to further investigate these two mechanisms. Furthermore, potential pharmacological interventions in the form of PPAR- $\gamma$  agonist (Pioglitazone) and GLP-1RA (Liraglutide) were studied to examine improvement in myocardial perfusion and energetics.

There is a distinct lack of longitudinal studies utilizing CMR in T2D. This work has demonstrated that over a 6-year follow up period, even in the absence of overt clinical CAD, significant valvular disease, uncontrolled hypertension or change in BMI, patients with T2D showed significant reductions in cardiac size and biventricular systolic function over time. Plasma hs-cTnT measured at baseline was associated with change in LVEF over time highlighting a potential role in risk-stratifying patients with T2D and higher risk for developing HF. It was also shown that higher LV mass and concentric remodelling at baseline was associated with higher rates of MACE during the follow up period.

This work has also shown that not only is T2D associated with greater visceral adiposity in the presence of overweight/obesity, T2D participants with a normal body weight also demonstrated excess visceral adipose tissue deposition at similar levels to overweight/obese controls. Another important finding was the reduction in

myocardial stress perfusion and myocardial perfusion reserve index only in the overweight/obese T2D participants with no such reduction in the normal body weight T2D participants or the overweight/obese healthy volunteers.

The intricate relationship between myocardial perfusion, systolic and diastolic function, and myocardial energetics in both the healthy and diabetic human heart, not only at rest but with pharmacological (dobutamine) stress has been further demonstrated in this work. In response to dobutamine stress, patients with T2D, healthy volunteers and veteran athletes all showed a reduction in the myocardial energetics and an increase in global longitudinal shortening and LVEF. However, it was only the participants with T2D who demonstrated a blunted increase in the stress myocardial blood flow. This work further showed an association between myocardial blood flow and LVEF and also an association between energetics and diastolic parameters.

T2D is a chronic metabolic disorder characterised by dysregulated insulin secretion and/or resistance to insulin action. Both these abnormalities are potentially amenable to pharmacological treatment with PPAR- $\gamma$  agonist which target peripheral insulin sensitivity or GLP-1RA which promote insulin secretion. This work involved a randomised, phase-2, single centre, open-label, cross-over trial involving the two above mentioned drugs in a population of patients with T2D and no prior cardiovascular disease. Four-months of treatment with GLP-1RA resulted in significant improvement in myocardial energetics and perfusion. Pioglitazone also led to an isolated improvement in diastolic function at rest.

Science has progressed considerably in the understanding of disease mechanisms underlying T2D. This body of work provides further insight into the role of myocardial perfusion, ectopic adiposity and myocardial energetics in the diabetic heart. In addition, it also provides us with an understanding of the changes in perfusion and myocardial energy utilization during acute hemodynamic stress. At the same time this work sheds some light on the treatment modalities available to help improve outcomes in patients with T2D.

The answers provided by this body of work stimulate further questions. Longer and larger longitudinal studies are required to further characterize myocardial changes in T2D. In addition, studies examining diastolic parameters and the association with myocardial energy utilization would be of use to help identify patient with T2D prior to the onset of HF to potential change the course of their disease process. The benefits seen with the use of GLP-1RA in the study population warrant a larger Phase III trial to further target and individualise the treatment of T2D.

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# **Appendix**

Appendix 1: Ethics approval for chapters 3,4 and 5

**Health Research Authority****Yorkshire & The Humber - Leeds East Research Ethics Committee**NHSBT Newcastle Blood Donor Centre  
Holland Drive  
Newcastle upon Tyne  
NE2 4NQ

Telephone: 0207 104 8081

**Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval**

10 May 2018

Professor Sven Plein  
BHF Professor of Cardiology and Honorary Consultant Cardiologist  
University of Leeds  
LICAMM  
LIGHT building  
University of Leeds  
LS2 9JT

Dear Professor Plein

**Study title:**                    **Advanced Magnetic Resonance Imaging: Optimization of Image Acquisition and Analysis Methods (AMaRI)**  
**REC reference:**                **18/YH/0168**  
**IRAS project ID:**             **245109**

The Research Ethics Committee reviewed the above application at the meeting held on 01 May 2018. Thank you for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact [hra.studyregistration@nhs.net](mailto:hra.studyregistration@nhs.net) outlining the reasons for your request. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

### Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

### Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1. The Committee asked that a cold-calling approach is not used to recruit potential participants.
2. The Participant Information Sheet needs to be amended to specify the potential risk of bruising or irritation at the cannula site.

**You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.**

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

*Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).*

*Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at [www.hra.nhs.uk](http://www.hra.nhs.uk) or at <http://www.rdforum.nhs.uk>.*

*Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.*

*For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.*

*Sponsors are not required to notify the Committee of management permissions from host organisations.*

### Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact [hra.studyregistration@nhs.net](mailto:hra.studyregistration@nhs.net). The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

#### *NHS Sites*

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

#### **Summary of discussion at the meeting**

Senior Research Nurse Petra Bijsterveld was welcomed to the meeting.

- **Social or scientific value; scientific design and conduct of the study**

Participants received between one and four MRI scans. Members queried how the number of scans each individual participant received had been determined.

*Ms Bijsterveld advised that the study followed a similar design to another study which was already running. They wanted to carry out pilot work, looking at a condition in particular, involving some pharmacological interventions and stress. Not all participants would have contrast. The invitation letters were generic and the study was further explained to those who showed interest.*

*Participants may receive just one scan. The researchers may wish to look at something sequential, in this instance participants would be invited for further scans. She expected that it would be very rare for a participant to receive four scans.*

Members asked whether the study was pseudo-randomised.

*Ms Bijsterveld explained that the study was not randomised in the sense they would look for volunteers and describe the type of scan they may have. The scans participants received would depend on the information the researchers needed.*

The Committee was satisfied with the responses given.

- **Recruitment arrangements and access to health information, and fair participant selection**

The Committee noted that this was an interesting study, which was part of a larger portfolio of studies.

The Committee asked if there was a screening process for identifying abnormalities in the healthy volunteers, particularly health problems that the volunteers may be unaware of.

*Ms Bijsterveld explained that participants were sent a screening form to ask about known health issues. She expected that most of the volunteers would be medical students, who tended to be young, although some older members of staff may also volunteer.*

Members asked whether participants received a pre-emptive ECG.

*Ms Bijsterveld explained that participants received an ECG while they were being prepared for the MRI.*

The first approach to patient participants was potentially made by telephone call. Members queried whether this was a "cold call" and how the researchers planned to gain the contact details of potential participants.

*Ms Bijsterveld confirmed that cold calling was used. This method of recruitment had been included, subject to ethical approval, as their experience in previous studies had been that if a potential participant was sent a letter they may not understand the study. If contacted by telephone, the researcher could explain the study and ask if they wanted to receive further information in the post. Potential participants would be told that the study related to the condition or disease they were being investigated for.*

Members were satisfied with the responses given, apart from the cold calling aspect of recruitment. The Committee asked that the cold calling of potential participants was removed. Members were unsure where the contact details for the potential participants were obtained from and whether the researchers were able to access this information. The REC suggested potential participants were instead sent a letter or were approached in clinic.

- **Care and protection of research participants: respect for potential and enrolled participants' welfare and dignity**

The Committee requested clarification on how the data collected on the healthy volunteers was managed.

*Ms Bijsterveld advised that data was stored on a secure server. This may change with the introduction of GDPR, but the data would be on a secure drive.*

The Committee asked whether the medical records of healthy volunteers were accessed.

*Ms Bijsterveld advised they were not. Participants GPs were informed of any incidental findings and asked to investigate, so there was no need for the researchers to access any records.*

The Committee asked how the doses for Adenosine and Dobutamine had been determined.

*Ms Bijsterveld advised that the doses were used as standard in clinical practice.*

The Committee was satisfied with the responses given.

- **Informed consent process and the adequacy and completeness of participant information**

The format of the Participant Information Sheet was not user friendly and members were concerned about its accessibility to participants. Members suggested that the researchers asked the Public and Patient Involvement group to review the Information Sheet with regards to its format and specificity.

The Participant Information Sheet did not mention whether there was a risk of reaction at the cannula site. Members queried whether there was a risk of bruising or irritation and, if so, that this was described in the Information Sheet.

The Committee noted that other issues may be raised in correspondence.

Ms Bijsterveld was thanked for attending and left the meeting.

**Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.**

Please contact the REC Manager if you feel that the above summary is not an accurate reflection of the discussion at the meeting.

**Approved documents**

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants [AMaRI recruitment email]	1.0	27 March 2018
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Confirmation of Liability]		21 September 2017
IRAS Application Form [IRAS_Form_06042018]		06 April 2018
Laboratory Manual [Laboratory manual]	1.0	01 March 2018
Letter from funder [BHF Programme Grant]		24 May 2016
Letter from sponsor [confirmation of sponsorship]		27 March 2018
Letters of invitation to participant [AMaRI invitation letter]	1.0	27 March 2018
Other [AMaRI PIS and consent (volunteers)]	1.0	27 March 2018
Participant information sheet (PIS) [AMaRI PIS and consent (patients)]	1.0	27 March 2018
Research protocol or project proposal [AMaRI Protocol]	1.0	27 March 2018
Summary CV for Chief Investigator (CI) [CV]		01 November 2017

**Membership of the Committee**

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

#### **User Feedback**

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: <http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

#### **HRA Training**

We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

<b>18/YH/0168</b>	<b>Please quote this number on all correspondence</b>
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With the Committee's best wishes for the success of this project.

Yours sincerely

pp



**Dr Rhona Bratt**  
**Chair**

E-mail: [nrescommittee.yorkandhumber-leedseast@nhs.net](mailto:nrescommittee.yorkandhumber-leedseast@nhs.net)

*Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments*

*"After ethical review – guidance for researchers" [SL-AR2 for other studies]*

*Copy to: NHS Research Ethics Officer, University of Leeds  
Ms Anne Gowing, Leeds Teaching Hospitals NHS Trust, Research & Development*

**Yorkshire & The Humber - Leeds East Research Ethics Committee**

**Attendance at Committee meeting on 01 May 2018**

**Committee Members:**

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Miss Jennifer Blaikie	Senior Research Ethics Administrator	Yes	
Dr Rhona Bratt	Retired Multimedia Project Manager	Yes	
Professor Kenneth Brodlie	Retired Professor of Visualization	Yes	
Dr Alexandros Chatziagorakis	Consultant Psychiatrist	No	
Dr Deborah Jane Fox	Senior Lecturer in Nursing	Yes	
Mr Aidan Hindley	Tissue Bank Coordinator, GIFT Research Tissue Bank (Cadaveric)	Yes	
Mrs Ann Kay	Retired Special Needs Coordinator	Yes	
Dr Nicky Kime	Senior Research Fellow	No	
Dr Nicolas Orsi	Senior Research Fellow	Yes	
Dr Robert Phillips	Senior Clinical Academic	Yes	
Dr Andrew Pollard	Consultant Anaesthetist	No	
Mr Satti Saggi	Research Project Manager	Yes	
Dr Anna Schuberth	Specialty Registrar in Palliative Medicine	No	
Dr Nana Theodorou	Research & Innovation Coordinator	Yes	
Mr Tom Wilson	Consultant ENT Surgeon	Yes	
Miss Kate Woodrow	Associate Director of Pharmacy – Patient Care	Yes	

**Also in attendance:**

<i>Name</i>	<i>Position (or reason for attending)</i>
Ms Katy Cassidy	REC Manager

## Appendix 2: Ethics approval for Chapter 6



**West Midlands - Black Country Research Ethics Committee**

The Old Chapel  
Royal Standard Place  
Nottingham  
NG1 6FS

**Please note:** This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

16 January 2020

Dr Eylem Levelt  
LICAMM  
University of Leeds  
Leeds  
LS2 9JT

Dear Dr Levelt

<b>Study title:</b>	<b>Targeting beta-cell failure in lean patients with type 2 diabetes</b>
<b>REC reference:</b>	<b>19/WM/0365</b>
<b>Protocol number:</b>	<b>N/A</b>
<b>IRAS project ID:</b>	<b>265208</b>

Thank you for your letter, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

**Conditions of the favourable opinion**

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or NHS management permission (in Scotland) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

#### Registration of Clinical Trials

It is a condition of the REC favourable opinion that **all clinical trials are registered** on a publicly accessible database. For this purpose, 'clinical trials' are defined as the first four project categories in IRAS project filter question 2. Registration is a legal requirement for clinical trials of investigational medicinal products (CTIMPs), except for phase I trials in healthy volunteers (these must still register as a condition of the REC favourable opinion).

Registration should take place as early as possible and within six weeks of recruiting the first research participant at the latest. Failure to register is a breach of these approval conditions, unless a deferral has been agreed by or on behalf of the Research Ethics Committee ( see here for more information on requesting a deferral:

<https://www.hra.nhs.uk/planning-and-improving-research/research-planning/research-registration-research-project-identifiers/>

As set out in the UK Policy Framework, research sponsors are responsible for making information about research publicly available before it starts e.g. by registering the research project on a publicly accessible register. Further guidance on registration is available at: <https://www.hra.nhs.uk/planning-and-improving-research/research-planning/transparency-responsibilities/>

You should notify the REC of the registration details. We will audit these as part of the annual progress reporting process.

**It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**

#### **After ethical review: Reporting requirements**

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study, including early termination of the study
- Final report

The latest guidance on these topics can be found at <https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/>.

### Ethical review of research sites

#### NHS/HSC sites

The favourable opinion applies to all NHS/HSC sites listed in the application subject to confirmation of Capacity and Capability (in England, Northern Ireland and Wales) or management permission (in Scotland) being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

#### Non-NHS/HSC sites

I am pleased to confirm that the favourable opinion applies to any non-NHS/HSC sites listed in the application, subject to site management permission being obtained prior to the start of the study at the site.

### Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants	1.0	25 June 2019
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		26 September 2019
GP/consultant information sheets or letters	2.1	23 October 2019
IRAS Application Form [IRAS_Form_11112019]		11 November 2019
Laboratory Manual	1.1	27 November 2018
Letter from funder		08 January 2019
Letter from sponsor		08 November 2019
Letters of invitation to participant	1.0	07 May 2019
Other [Insurance Certificate]		
Other [MHRA Notification CTA Not Required]		12 September 2019
Other [Academic Supervisor CV]		30 November 2017
Other [Participant Diary- Liraglutide]	1.1	02 January 2020
Other [Participant Diary- Pioglitazone]	1.1	02 January 2020
Other [Cover Letter Response to REC ]	N/A	03 January 2020
Participant information sheet (PIS)	2.2	02 January 2020
Participant information sheet (PIS) [Participant Information Sheet]	2.2	02 January 2020
Research protocol or project proposal	2.1	23 October 2019
Summary CV for Chief Investigator (CI)		10 September 2018
Summary CV for student		

### Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**User Feedback**

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:

<http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

**HRA Learning**

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at:

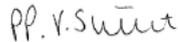
<https://www.hra.nhs.uk/planning-and-improving-research/learning/>

<b>19/WM/0365</b>
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<b>Please quote this number on all correspondence</b>
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With the Committee's best wishes for the success of this project.

Yours sincerely



**Dr Hilary Paniagua  
Chair**

Email: nrescommittee.westmidlands-blackcountry@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Jean Uniacke

## Appendix 3: Patient information sheet for Chapters 3,4 and 5

**PARTICIPANT INFORMATION SHEET - PATIENTS**  
Version 2.0 –16 Sept 2020  
**AMaRI**

Advanced Magnetic Resonance Imaging: Optimization of Image Acquisition and Analysis Methods

**Chief Investigator: Professor Sven Plein**

Dear Patient,

You are invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

**Purpose of the study**

Magnetic Resonance Imaging (MRI) is a test which produces detailed pictures of your internal organs by putting you within a strong magnetic field. MRI allows us to detect abnormalities in many organs in the human body with a very high sensitivity. Importantly, MRI is a safe test and does not use any harmful radiation. It is therefore an increasingly used test in many areas of medicine with over 100,000 MRI scans performed in the NHS every year.

In Leeds, we have an ongoing research programme that aims to continuously improve the way we acquire MRI pictures. This is mostly achieved by making scans shorter, increasing the detail in the image or finding out new information from within the acquired images. These developments are first tested in phantoms (bottles filled with a special liquid) and later need confirmation in volunteers and then in patients.

**Why have I been chosen?**

This study is looking at up to 300 people like you, who may have a range of conditions that are of interest to our research into improving imaging. We are also asking 400 healthy volunteers to participate in the study.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care that you receive from the NHS. If there is a possibility that you might be pregnant, you should not take part in the study. Our research team will be happy to discuss any other questions that you may have concerning your suitability for the study, before you decide whether to take part.

**What will happen to me if I take part?**

Most patients will have a single MRI scan. A small group of participants in this study will be asked to undergo up to four MRI scans to allow comparisons between different ways of obtaining MRI pictures. It is entirely up to you how many scans you wish to volunteer for, and you will remain free to withdraw from the study at any time. All scans will be performed at the Leeds General Infirmary, and will be performed on separate days.

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The MRI scan will take approximately 60 to 90 minutes to complete. You lie in a short 'tunnel', which holds a large magnet. Short bursts of radio waves from the MRI scanner allow images to be created. You will hear periodical loud "banging" noises while we are acquiring the images, so we protect your ears with headphones through which you can listen to the radio or one of your own CDs. We will remain in communication with you throughout the scan.

For most scans we will insert one or two cannulae (small plastic tubes) into veins in your arm. It is likely that we will inject a contrast dye during the scan. Usually people are not aware of the contrast dye injection. At one point we may also inject a medication (Adenosine, or occasionally Dobutamine) into a vein in your arm, which is a drug to increase the blood flow to your heart. This can cause a brief feeling of warmth, breathlessness or chest discomfort. However all of these feelings, if they occur, usually settle within one or two minutes of the medication being stopped. A doctor will stay in the room with you whilst you are having the medication. In some cases instead of using adenosine we may immerse your hands or feet in cold water for up to 2 minutes to achieve the same increased blood flow to the heart muscle, or we may ask you to use a cycle ergometer, a bicycle which can be used whilst lying down in the scanner.

If we wish to obtain specific images of your heart arteries we will wrap a belt around your abdomen to help improve the quality of the pictures. This is not painful and is a recognized method of doing this type of scan. You may be given a nitrate (GTN) spray under the tongue which helps us to obtaining good images. If your heart beat is quite fast we would give you a beta blocker tablet to reduce your heart rate. Again, these methods are widely used in other centres worldwide and are used in normal clinical work too.

As this study is about improving our scan protocols on an ongoing basis for a period of four years the information we give you has to describe all the different techniques we wish to use in the study overall, but not all the techniques described above will be used during your scan(s). Before you sign the consent form we will discuss with you the specific scanning protocol that we are going to use.

We may ask you for a blood sample (5 to 10 mls. or 1 to 2 teaspoons), which would be taken whilst we insert the cannula in your arm for the contrast, so there are no extra needles involved. Knowing your haematocrit (the volume percentage of red blood cells in the blood) helps us to create specific images which are applicable to clinical practice. We may also test your blood glucose, lipid fatty acid and ketone levels. With your permission we may store serum samples and analyse them at the end of the study for markers of heart function. We may ask you to come for the scan in a fasted state, or offer to scan you following a meal which we will provide you with, so that we can assess the influence of fed or fasted state on the heart scan assessments.

We may ask you to have an ECG, this is a heart tracing to measure the electrical impulses within the heart. It involves having 10 stickers applied to your chest for 5 minutes.

In the unlikely event of any abnormality we will, with your permission, inform your GP.

### Risks and discomforts

Magnetic Resonance Imaging (MRI) is safe and no x-rays or radiation are used for this scan. There are no known risks from this technique. Some people may experience claustrophobia. Our MRI staff will do all that they can to make you feel comfortable during the scan, and will be monitoring you via a video camera and an audio link. If we are unable to make you feel comfortable in the scanner, we will not go ahead with scanning. You may experience minor bruising or irritation at the site where we place the cannula in your arm. The contrast medication which we use is very safe but, as with any injection, reactions may occur. These

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include a warm sensation at the injection site, nausea or vomiting and transient skin rash. These effects usually only last for a few minutes. People with a history of allergy are more likely to suffer a more severe reaction, but this is rare (less than 1 in 3000). The department is equipped to cope with allergic reactions if they happen. Adenosine, the medication we use to increase the blood flow to the heart, can cause flushing, breathlessness and chest discomfort. However, all of these feelings usually subside within one or two minutes or even more quickly if the medication is stopped. Immersing your hands or feet in cold water is unpleasant, but the effects wear off very quickly. Nitrates and a beta blocker can cause temporary light headedness. For this reason if these drugs are used you will be kept under observation until the effects have worn off.

### **Benefits to you**

This study does not form part of your normal clinical care and is done solely for research purposes. Your participation may however benefit future patients.

### **Expenses**

We will provide reasonable travel expenses should this be necessary for you to attend the MRI scan. We are also happy to arrange transport to the hospital and return you home if needs be.

### **Will my taking part be kept confidential?**

All information, which is collected about you during the course of the research will be kept strictly confidential. This information will be securely stored at the Cardiac MRI Unit at Leeds General Infirmary on paper and electronically, under the provisions of the 2018 Data Protection Act. The data collected will be coded and your personal details will be kept separately. If we keep any of your serum samples these will be stored in -80°C freezers in a secure environment, in University of Leeds or Leeds Teaching Hospitals NHS Trust Research laboratories. Stored serum samples will be anonymized and identified only by sample IDs. You will not be identified in any publication that may result from this research.

We will inform your General Practitioner (GP) in the event of an unexpected abnormality being found.

With your permission, your data may also provide a resource for future studies. If any information from this study is used to develop new research, data protection regulations will be observed and strict confidentiality maintained. Your anonymized data and/or images may be sent to institutions in the UK, the European Economic Area or outside the EEA. Ethical approval will be obtained for any future studies involving your data. You will not be identified in the results of any future studies.

The University of Leeds is the sponsor for this study based in the United Kingdom. We will be using information from you and/or your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The University of Leeds and the Leeds Teaching Hospitals NHS Trust (on behalf of the University of Leeds), will keep identifiable information about you for the purpose of the study for a maximum of 15 years after the study has finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information at [http://www.leeds.ac.uk/secretariat/data\\_protection.html](http://www.leeds.ac.uk/secretariat/data_protection.html)

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The University of Leeds will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from the University of Leeds and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Leeds Teaching Hospitals NHS Trust will pass these details to the University of Leeds along with the information collected from you and your medical records. The only people in the University of Leeds who will have access to information that identifies you will be people who need to contact you to organize the research or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number and contact details.

#### **What will happen to the results of the research study?**

When the study is complete the results will be published in a medical journal, but no individual participants will be identified. If you would like a copy of the published results, please ask your doctor.

#### **Indemnity/Compensation**

If you are harmed as a direct result of taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds to a legal action. Regardless of this, if you have any cause to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

#### **The research organisation**

This is a research project of the Department of Biomedical Imaging Science at the Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM).

#### **For further information please contact:**

Research Nurses  
CMR Clinical Research Group  
X47, Sunshine Corridor  
Leeds General Infirmary  
Leeds  
LS1 3EX  
T 0113 392 5481 or 392 5504  
cmrresearch@leeds.ac.uk

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## Appendix 4: Patient information sheet for Chapter 6

**PARTICIPANT INFORMATION SHEET**Short title: **Lean-DM**Study Title: **Targeting beta-cell failure in lean patients with type 2 diabetes**

Version 4.0, 21 Sep 2020

For all participants

**Chief Investigator:** Dr Eylem Levelt

Dear Participant,

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information.

**Part 1****Why are we doing this study?**

The majority of people with type 2 diabetes (T2D) are overweight, and while weight gain is a major contributor to diabetes, a minority of patients with T2D are not overweight or obese. The reasons why lean or normal body weight individuals develop T2D (lean-T2D) are not yet understood. T2D occurs when the body does not produce enough insulin, or becomes less sensitive to its effects. Insulin acts like a key to allow sugar into cells and if someone is overweight that key works less well. Recent research suggests that T2D in lean people should be considered a different disease from the diabetes associated with obesity and the main problem in lean-T2D patients may be a reduced capacity of insulin secretion. However, some researchers argue that many seemingly thin people carry more fat than muscle, making them trim on the outside, but fat on the inside, and they are in fact not truly lean. This implies that just like overweight diabetics, lean diabetics also have high resistance to insulin. The main aim of our research is to better understand the main driver of T2D in lean individuals, as this will determine how best to treat these individuals.

**What do we already know as a result of research carried out in this area?**

Many patients with T2D are not overweight as measured by body mass index (BMI). Lean-T2D patients have disproportionately reduced capacity of insulin secretion and less resistance to insulin action, but have a similar or even higher risk of cardiovascular disease as their overweight counterparts. Although BMI can be misleading, using MR scans the amount of internal excess fat in the liver and around the heart were shown to be less in lean-T2D patients compared to obese/overweight patients, and

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associated with this, they had lower blood insulin levels. As insulin improves the heart's blood supply, we have shown that the blood supply of the heart was more significantly impaired in lean-T2D patients and that this was associated with higher reductions in the energy levels of the heart during exercise compared to obese/overweight patients.

There are many different types of drugs for treating T2D. Liraglutide improves insulin secretion capacity of the pancreas. Pioglitazone reduces resistance to insulin action. We will compare the actions of these diabetes drugs on the blood supply and the heart's energy levels in lean-T2D and obese-T2D patients. This will allow us to determine the ideal treatment strategies for improving cardiovascular health in lean-T2D patients, and better understand the role of impaired insulin secretory capacity, insulin resistance and excess fat deposition specifically in this group.

#### **Why have I been chosen?**

This study is looking at people with either lean-T2D or obese-T2D. You have been chosen because you fit the criteria for either of these conditions, and do not have any heart problems.

#### **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care that you receive from the NHS. If there is a possibility that you might be pregnant, you should not take part in the study. Our research team will be happy to discuss any other questions that you may have concerning your suitability for the study, before you decide whether to take part.

#### **What will I have to do?**

The research will involve the participants to:

- 1) consent to taking part in the study by signing a form;
- 2) undergo the research procedures;
- 3) attend the research appointments;
- 4) take medication as instructed;
- 5) inform the investigators about any health problems; and
- 6) use contraceptive protection during the course of the study (if applicable).

Besides the inconvenience of hospital visits following an overnight fasting, all the investigations in this study are safe; both study medications are well-established and will only be prescribed if your blood tests confirm that an additional medication is indicated. Blood tests will be performed before enrolment, during treatment, and at the end of each treatment period to ensure your safety. With this information sheet we are giving you the contact details of study investigators to share any concerns you may have.

#### **What will happen to me if I take part?**

In this study, we will recruit lean and overweight participants with T2D and no history of heart disease to compare the effects of two established diabetes medications, Liraglutide and Pioglitazone, which improve diabetes control in different ways. Using Magnetic Resonance Imaging (MRI), we will assess the heart's energy status, fat levels, pumping function, restrictions of heart muscle blood flow, and fat deposition in the liver and around the pancreas in response to these medications. We will ask you to perform an exercise test (the distance a patient can walk within 6 minutes, also known as the 6 Minute

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Walk Test). We will take blood tests to evaluate diabetes control, insulin levels and resistance to insulin action. After these tests, we will add Liraglutide or Pioglitazone to your diabetes treatment. You will be given Liraglutide or Pioglitazone for 2 treatment periods of 16 weeks each. Treatment periods will be separated by 8 weeks in which you will take no study medication (washout period). You will be asked to attend 4 research visits over the course of roughly 10 months. You will be given the option to have these assessments over more visits than the 4 times in total should you wish for shorter research scan visits.

Pioglitazone will be started at the smallest dose (15 mg once daily) initially and the dose will be increased to 30mg once daily after 2 weeks and finally the dose will be titrated to target of 45 mg once daily after 2 weeks, if glucose levels permit this stepwise dose increase. This is to ensure you receive the medication at the most effective dose while ensuring your glucose levels are not too low. Blood assessments will be performed at our research centre by the study team.

Liraglutide will be started at the smallest dose (0.6mg once a day) initially and the dose will be increased to 1.2mg once a day after 2 weeks, if glucose levels permit this stepwise dose increase. This is to ensure you receive the medication at the most effective dose while ensuring your glucose levels are not too low. Blood assessments will be performed at our research centre by the study team.

Here is a summary of the study timeline and visits:

#### Visit 1

You will be asked to come to the Advanced Imaging Centre (AIC) research facility at the Leeds General Infirmary where you will meet the research team, discuss the study and consent to the study. This visit will take approximately 3 hours. You will be asked to attend the AIC after an overnight fasting (about 8 to 10 hours of no food intake). You will need to continue taking your medication(s). If you have any concerns or questions, please contact us. The researcher will explain the study and answer any questions you may have. If you decide to volunteer, we will ask you to sign a copy of the consent form. We will carry out the following tests:

1. Blood and urine tests
2. Electrocardiogram (ECG)
3. Height and weight, blood pressure and heart rate measurements, physical exam and vital signs
4. MRI scans
5. EndoPAT testing
6. 6 minute walk test

These tests are explained in more detail below.

At this visit, you will be randomised by a computer to determine which drug you will receive first (either Liraglutide or Pioglitazone). You will be given this drug along with a diary to keep track of when you take it. We will instruct you on how to take the drug and how to use your diary. You will know which drug you are taking. Before the drug is dispensed, female of participants of childbearing potential will be administered a urine pregnancy test.

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#### Visit 2 (approximately 4 months after Visit 1)

At this visit, we will carry out the same tests as in Visit 1. In addition, we will review with you your current medication list and your clinical status. We will also review your diary.

#### Visit 3 (approximately 6 months after Visit 1)

We will carry out the same tests as in Visit 1. We will give you the second drug (either Liraglutide or Pioglitazone, whichever drug you were not given at Visit 2), and remind you to track when you take it in your diary.

#### Visit 4 (approximately 10 months after Visit 1)

This will be your last visit for the study. We will carry out the same tests as in Visit 1. We will review your current medication list and your clinical status. We will also review and collect your diary.

#### **Blood and Urine Tests**

We will insert a small, sterile plastic tube into the arm, usually at the front of the elbow, using a small needle; we will collect blood samples from this cannula for metabolic assessments. The maximum amount taken will be about 20 ml, equivalent of 1 tablespoon. A small amount of urine sample will be collected and this will be for assessing the presence of a protein leak from the kidneys.

With your permission, we will collect plasma to store for up to 3 years for future research.

#### **Electrocardiogram (ECG)**

We will take an electrocardiogram (ECG) of your heart for approximately 5 minutes. This involves lying on the examination couch and having 10 adhesive patches placed on you, 6 on your chest and one on each limb. These are then attached to a machine that records the electrical activity of your heart.

#### **MRI scans**

Magnetic Resonance Imaging (MRI) is a test which produces detailed pictures of your internal organs by putting you within a strong magnetic field. With Cardiac MR we are able to detect several important abnormalities that are caused by heart disease, for example the scarring of the heart from heart attacks and the restrictions of blood flow to the heart muscle that lead to angina, or energy and fat levels in the heart. Also, MRI produces pictures of the heart with much greater detail than with other types of heart scans. Importantly, MRI is also a safer test than most other heart scans, because it does not expose patients to any harmful radiation and pictures of the heart can be taken "from the outside". Because of all of these qualities, MRI has become one of the most important tests in patients who suffer with different types of heart disease. We have been doing MRI scans of the heart in Leeds since 1995. We are continuously carrying out research into improving the images and thereby improving patient care.

In this study we are looking at the structure and function of the hearts of patients with type 2 diabetes. We know that type 2 diabetes can change the structure and function of heart muscle over time, and this research uses cardiac MRI to look at these changes in detail.

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The MR scan will be performed at the Leeds General Infirmary and will take approximately 120 minutes to complete. You lie in a short 'tunnel', which holds a large magnet. Short bursts of magnetic fields and radio waves from the MRI scanner allow images to be created. You will hear periodical loud "banging" noises while we are acquiring the images of your heart, though we do protect your ears with headphones. You can listen to the radio or watch a movie during these scans. We will remain in communication with you throughout the scan. Two types of MR scans will be performed:



***MR Spectroscopy:*** this scan measures fat in the heart and the liver and determines the energy levels of your heart at rest and dobutamine infusion (a medication that mimics exercise). This medication mimics the level of exercise similar to daily activities such as walking up stairs.

***MRI:*** Following the spectroscopy scans you will have an MRI scan to assess cardiac function. This is the traditional MR scan which is used routinely in clinical practice for assessing the structure and function of your heart. You will need to lie very still on your back as movement can blur the images. We will ask you to breathe in and out and hold your breath for several seconds for some of the scans. You will be given medication (called dobutamine) through a cannula in your arm. It is used routinely in MR scans in hospitals. The dobutamine stimulates the heart and will make you feel like you are exercising. This allows us to measure the blood supply to your heart. You will then be given a contrast dye (called gadolinium) through a cannula in your arm. The dye makes the images of your heart and blood flow more visible and is used routinely in MR scans in hospitals. This dye will also allow for assessment of any scarring in the heart.

Before the scan, we will take blood samples to be analysed as part of the research. It should be possible to take this at the same time as the cannula is put in your arm before the MRI scan, meaning no extra needles will be involved. With your permission, we will store some of these samples and analyze them at the end of the study for markers of heart function; some other samples such as diabetes control tests will be analysed immediately.

#### **EndoPAT Testing**

The endothelium is a thin lining inside of the heart and blood vessels. Endothelial cells release hormones and gases that control vessel relaxation and contraction as well as enzymes that control blood clotting, immune function and platelet (a colorless substance in the blood) adhesion. Clinical studies have demonstrated an association between diabetes and impairment of endothelium function leading to relaxation impairment. There are a number of methods to assess endothelium function. The EndoPAT 2000 is a new device that is used to assess endothelial vessel relaxation function in a rapid and non-invasive fashion. The Endo-PAT consists of two thimble-sized sensors that are placed over the index fingers to detect irregularities in blood flow. These changes are a sign of damage to the lining of the blood vessels.

The EndoPAT device is being used in research centres in more than 40 countries. If you are interested to learn about the results of your EndoPAT test we can provide this to you at your next scheduled visit.

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**The Endo-Pat 2000: Two thimble-sized sensors are placed over the index fingers to detect irregularities in blood flow.**

#### **6 minute walk test**

The 6 minute walk test is a widely accepted measure of exercise capacity. You will be instructed to walk along a 30-meter corridor at a pace you feel most comfortable with and cover the maximum distance in 6 minutes under the supervision of study investigators with medical training and with experience in conducting the test. The investigators will tell you how much time had elapsed every 2 minutes. At the end of 6 minutes, you will be asked to stop, and the distance walked will be measured in meters. You can stop and rest any time you want.

#### **Liraglutide and Pioglitazone**

Liraglutide and Pioglitazone are well-established diabetes drugs. Liraglutide is injected under the skin (subcutaneous injection) once a day. You will be trained how to inject yourself by the study team at the Leeds General Infirmary. If you are unable or unwilling to do the injection yourself, a member of the study team will do the injection for you. Pioglitazone is a pill that you take orally once a day.

Both drugs are considered safe; however they each do have side effects. Liraglutide is known to potentially cause nausea, vomiting, diarrhoea, constipation, headache, injection site erythema and diabetic ketoacidosis. Pioglitazone's side effects include bone fracture, increased risk of infection, numbness, visual impairment and increased weight. There have been very rare reports of liver dysfunction and bladder cancer. Your liver function will be checked by blood tests and the presence of blood in your urine will be evaluated before you begin taking Pioglitazone.

#### **Contraceptive protection**

If you are female and have childbearing potential, you must use a medically-approved, highly effective birth control method while on the study to prevent pregnancy. Acceptable methods of contraception include:

1. Barrier-type devices (e.g., female condom, diaphragm, and contraceptive sponge) used only in combination with a spermicide;
2. Intrauterine devices (IUDs);
3. Oral contraceptive agents started at least 90 days before enrolling in the study;
4. Depo-Provera (medroxyprogesterone acetate) shot;

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5. Levonorgestrel implants;
6. Naturally or surgically sterile (amenorrhic for at least 1 year and no record of childbirth for naturally sterile persons); or
7. Male partner is sterile and is the only sexual partner.

True or periodic abstinence, the rhythm method or contraception by the male partner only are not acceptable methods. If you are male with a female partner of childbearing potential, you do not have to use any birth control methods.

#### Expenses

We will reimburse travel and parking expenses for study visits (receipts are required). We will offer you light lunch and refreshments for each of the research visits. We are also happy to arrange transport to the hospital and return you home if needs be.

#### What are the possible disadvantages and risks of taking part?

MR scanning is used routinely in clinical practice to acquire images of various body parts. MR scans are safe, non-invasive and do not involve any ionizing radiation (x-rays). Some people find the space limitation in the scanner uncomfortable, but you will be given a chance to see the scanner to make sure that you are comfortable in it before the study starts. The whole time that you are in the scanner you will be given a buzzer that you will be able to use at any time if you wish to stop the study. The radiographer will go through a list of possible risks with you before you go into the scanner.

MR scans are painless but involve the use of a strong magnetic field, so if you have any of the following, you would not be suitable for a scan, and would not be able to take part in this study:

permanent pacemaker or defibrillator	metal clips in blood vessels of the brain
injury to the eye involving fragments of metal	shrapnel injuries
other metal or electronic implants	

If you are a woman who is pregnant, breast-feeding or who may become pregnant during the study period, you will not be able to take part.

If you feel claustrophobic, you can request that the scan be stopped. Unfortunately, if the scan is stopped for this reason, because the health information this study investigates can only be obtained through MR scans, you will be unable to continue to participate in the study.

The scan requires a gadolinium based contrast injection. This is the safest contrast agent used in medical practice with a very low risk of serious side effects (1 in 10 000). Because there was a historical concern that poor kidney function may in rare cases increase side effects with gadolinium, any subject with end-stage kidney disease will not be able to participate in this study.

In the unlikely event of us seeing any abnormalities on your MR scan, a member of our research team will discuss the implications with you and, with your permission, your GP may be notified. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research purposes only.

The department is equipped to cope with allergic reactions if they happen. Dobutamine, the medication we use to increase the blood flow to the heart, can cause flushing, breathlessness and

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chest discomfort. However, all of these feelings usually subside within one or two minutes or even more quickly when the medication is stopped.

Some people find having a drip in their arm uncomfortable and there can be bruising at the site of needle entry. Our staff are highly trained in drip insertion and we will make sure you are as comfortable as possible.

The 6 minute walk test may be tiring for you.

Liraglutide and Pioglitazone are safe, well-established drugs; however they do have side effects. These are explained in the previous "Liraglutide and Pioglitazone" section.

**What are the possible benefits of taking part?**

There are no direct benefits to you if you take part in this study. This study does not form part of your normal clinical care and is done solely for research purposes. It may be interesting to see moving images of your own heart and learn more about your liver function, and body organ fat content.

**What happens when the research study stops?**

You will officially end participation in the study when you have completed visit 4. Copies of any publications connected to this study will be available on request from Dr Eylem Levelt ([E.levelt@leeds.ac.uk](mailto:E.levelt@leeds.ac.uk)). Study drugs will be provided only for the duration of the study. We are unable to continue your prescriptions for Liraglutide or Pioglitazone after your participation in the study is completed. We will make recommendations to your GP; however it will be up to your GP to decide what to prescribe to you.

**What if there is a problem?**

The University has arrangements in place to provide for negligent harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which you are provided. If you wish to complain about the way you have been dealt with during the study or any possible harm you might have suffered, please contact Dr Levelt or the Leeds Teaching Hospitals NHS Trust Patient Advice and Liaison Service (PALS). Contact details can be found in Part 2.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence.

***If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decisions.***

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## Part 2

### What will happen if I don't want to carry on with the study?

You are free to withdraw at any time, without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. Any stored blood or urine samples that can still be identified as yours will be destroyed if you wish.

### Will my taking part be kept confidential?

All information collected about you during the course of the study will be kept strictly confidential. This information will be securely stored, electronically on the Leeds General Infirmary secure server, and on paper, under the provisions of the 2018 Data Protection Act. The data collected will be coded and your personal details will be kept separately. You will not be identified in any publication that may result from this research. We will retain your personal details for 15 years after the end of the study according to local archiving policy. At the end of these 15 years, your personal details will be destroyed. If we keep any of your plasma samples, these will be stored in -80°C freezers in an access-controlled, secure environment, in the Leeds Teaching Hospitals NHS Trust Research laboratories and University of Leeds laboratories. Only authorised staff have access to these laboratories. Plasma from blood samples will be stored for up to 3 years for future research after the study has ended. Urine samples will be destroyed after testing is completed. We will discuss your individual results with you after your tests.

With your permission, we will inform your GP of your participation in the study. If any unexpected abnormality or condition were found, we would inform your GP with your permission.

With your permission, your anonymised data may also provide a resource for future studies. If any information from this study is used to develop new research, data protection regulations will be observed and strict confidentiality maintained. Any information about you which leaves the hospital will have your name and address removed so that you cannot be identified. Your data and/ or images may be sent to institutions in the UK, the European Economic Area (EEA) or outside the EEA. Ethical approval will be obtained for any future studies involving your data. If you withdraw consent from further study follow-up, or if you were to become incapacitated, any data collected about you up to that point will remain on file and will be included in the final study analysis.

The University of Leeds is the sponsor for this study based in the United Kingdom. We will be using information from you and/or your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The University of Leeds will keep and store identifiable information about you for 15 years after the study has finished. This information will also be held by Leeds Teaching Hospitals NHS Trust. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out more about how we use your information by contacting the University Data Protection Officer at [DPO@leeds.ac.uk](mailto:DPO@leeds.ac.uk). You can also find the University of Leeds' Privacy Notice for Research Participants here: <https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/02/Research-Privacy->

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[Notice.pdf](https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/09/HRA-transparency-wording.pdf). You can also see the Health Research Authority's Patient Leaflet here: <https://dataprotection.leeds.ac.uk/wp-content/uploads/sites/48/2019/09/HRA-transparency-wording.pdf>

Leeds Teaching Hospitals NHS Trust and the University of Leeds will use your name, NHS number, and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from the University of Leeds and regulatory organizations may look at your medical and research records to check the accuracy of the research study. The Leeds Teaching Hospitals NHS Trust will pass these details to the University of Leeds along with the information collected from you and/or your medical records. The only people in the University of Leeds who will have access to information that identifies you will be people who need to contact you to confirm information or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

#### **What will happen to the results of the research study?**

When the study is complete the results will be published in a medical journal, but no individual patients will be identified. If you would like a copy of the published results, please ask your doctor.

#### **Abnormal & Incidental Findings**

It is unlikely that the scan will detect clinically significant cardiac abnormalities. However, should this occur, with your permission your GP will be informed in writing and they will be able to arrange further investigation and treatment if necessary. It is possible the finding of significant cardiac abnormalities could have an impact on your insurance. You may not be informed of subtle abnormalities that are not deemed to be of clinical relevance.

#### **Indemnity/Compensation**

The University has arrangements in place to provide for negligent harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which you are provided. If you are harmed as a direct result of taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds to a legal action. Regardless of this, if you have any cause to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

#### **The research organisation**

This is a research project of the Cardiac MRI department of the Leeds General Infirmary and the University of Leeds. This study is funded by Diabetes UK.

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#### Who has reviewed the study?

The study has been reviewed and funded by Diabetes UK. It is also approved both by a nationally approved Research Ethics Committee and your hospital's Research and Innovation Office. More details can be provided, on request, by your study doctor.

#### What if there are any problems?

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should Contact Dr Eylem Levelt on 0113 3438023 (or email [E.levelt@leeds.ac.uk](mailto:E.levelt@leeds.ac.uk)). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. The Leeds Teaching Hospitals NHS Trust Patient Advice and Liaison Service can be contacted on 0113 2066261 or [patientexperience.leedsth@nhs.net](mailto:patientexperience.leedsth@nhs.net).

#### Will I be informed of the results of the study?

On completion of the study, you will be given written feedback with the results and if you would like any further information we would be happy to discuss this with you.

#### Questions

If you have any further questions about the study, or would like to be included in this research, please call Dr Eylem Levelt on 0113 3438023 or write to Dr Levelt at the Cardiology Department, Jubilee Wing, Leeds General Infirmary, Great George Street, Leeds, LS1 3EX. You can also contact the Research Nurses here:

Research Nurses  
CMR Department  
Clarendon Wing, LGI, Leeds, LS1 3EX  
Email: [cmrresearch@leeds.ac.uk](mailto:cmrresearch@leeds.ac.uk)  
Tel: 0113 392 5481 or 0113 392 5224

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