Subclinical cardiovascular dysfunction in adults with type 2 diabetes: characterisation and lifestyle interventions

Thesis submitted for the degree of
Doctor of Philosophy
At the University of Leicester

Gaurav Singh Gulsin, BSc (Hons), MBChB (Hons), MRCP (UK), MRes

Department of Cardiovascular Sciences

University of Leicester

March 2020

Abstract: Subclinical cardiovascular dysfunction in adults with type 2 diabetes: characterisation and lifestyle interventions

Dr Gaurav Singh Gulsin

Background

People with type 2 diabetes (T2D) are at increased risk of heart failure. Various measures of subclinical cardiovascular dysfunction have been reported, but it is unclear how these relate to functional limitation or whether they are reversible with lifestyle interventions.

Objectives

To comprehensively describe cardiovascular function in a multi-ethnic, asymptomatic population with T2D, and determine whether this is improved by a low-energy meal replacement plan (MRP) diet or exercise.

Methods

A comparison of adults with and without T2D and no cardiovascular disease was undertaken. A subset undertook a prospective, randomised, open-label, blinded endpoint (PROBE) trial and were assigned a 12-week intervention of: 1) routine care; 2) supervised exercise or 3) MRP. Echocardiography, cardiopulmonary exercise testing and cardiovascular magnetic resonance (CMR) were performed at baseline and post-intervention. The primary outcome was change in left ventricular (LV) peak early diastolic strain rate (PEDSR), measured by CMR.

Results

At baseline, 247 adults with T2D and 78 controls were compared. Subjects with T2D had concentric LV remodelling, diastolic dysfunction, aortic stiffening, reduced myocardial perfusion, and markedly lower peak VO_2. Key clinical determinants of cardiovascular dysfunction were diabetes duration, body mass index (BMI), smoking history, and systolic blood pressure (BP). MPR and diastolic filling were independently associated with peak VO_2.

Seventy-six T2Ds completed the PROBE trial (30 routine care, 22 exercise, and 24 MRP). The MRP arm lost weight, improved BP, glycaemia, LV mass:volume, and aortic stiffness. The exercise arm had negligible weight loss but increased exercise capacity. PEDSR increased in the exercise arm versus routine care ($p=0.002$) but did not improve with the MRP compared to routine care.

Conclusions

Concentric LV remodelling, diastolic dysfunction, aortic stiffening, and reduced MPR are key components of subclinical cardiovascular dysfunction in T2D. Exercise training improved diastolic function and despite beneficial effects on weight, glycaemic control, concentric LV remodelling and aortic stiffness, an MRP did not improve diastolic function.

Declaration

I confirm that this thesis is my own work.

Data from subjects with (n=247) and without (n=78) type 2 diabetes (T2D) recruited to four separate studies (EXPEDITION, LYDIA, DIASTOLIC and PREDICT) are included in this thesis. The EXPEDITION study (n=20 T2Ds and n=10 controls) had completed enrolment and imaging prior to my commencement. Recruitment to the LYDIA study (n=60 T2Ds) was conducted by staff at the Leicester Diabetes Centre, however cardiovascular magnetic resonance (CMR) imaging was performed at Glenfield Hospital and I supervised a proportion of these scans. Of the 87 people with T2D and 36 healthy volunteers included in the DIASTOLIC study, I recruited 62 patients and 16 controls. I supervised the vast majority of all the DIASTOLIC study assessment visits (including clinical assessments, blood tests, cardiopulmonary exercise testing and CMR scanning) at baseline and follow-up, as well as 10 test-retest reproducibility visits. Data from 70 people with T2D and 32 controls recruited to the PREDICT study are included. For this study, I wrote the British Heart Foundation Clinical Research Training Fellowship application, the study protocol and all study documents, secured research ethics approval, recruited all the participants and supervised all the study assessment visits (again including clinical assessments, blood tests, exercise testing, CMR scanning, and reproducibility scans). In total, I recruited 180 of the included participants in this thesis and supervised at least 270 of the included study assessment visits. I did not administer the exercise or dietary interventions in the DIASTOLIC study. However, I did provide medical oversight of almost all trial participants for the duration of the study.

Following completion of visits for the DIASTOLIC and PREDICT studies, I shared the CMR images and results of blood tests with each and every study volunteer. I explained in lay terms the findings of these investigations. Where identified, any significant clinical abnormalities were discussed with participants. I provided a full written report summarising the results of each study visit to participants and their general practitioners, with recommendations for further management where applicable.

I performed all the CMR image analysis for this thesis and undertook all the statistical analyses. The only exception was the statistical analysis for the primary outcome measure of the DIASTOLIC study (which was undertaken by a Leicester Clinical Trials Unit Statistician in accordance with a statistical analysis plan, which I co-authored).

Most importantly, I had a tremendous amount of fun undertaking this work.

Acknowledgements

Primary supervisor: Professor Gerry P McCann. Thank you for the wonderful education, mentoring, and friendship. You have inspired me to exceed my own lofty ambitions. Long may this continue.

Secondary supervisor: Professor Melanie J Davies. Thank you for the constant support, guidance, and for providing World-leading expertise in type 2 diabetes.

Co-investigators: The work presented in this thesis is the product of substantial contributions from multiple co-investigators:

Miss Stavroula Argyridou (Research dietitian); Dr Lavanya Athithan (Clinical Research Fellow); Dr Emma Baldry (Research dietitian; Dr John D Biglands (Physicist); Dr Emer M Brady (Postdoctoral research fellow); Dr Joseph Henson (Postdoctoral research fellow); Mr Nishal B Jaicim (Medical statistician); Professor Peter Kellman (Physicist); Professor Kamlesh Khunti (Professor of Primary Care Diabetes and Vascular Health); Dr Anna-Marie Marsh (Chief Cardiac Physiologist); Mr John McAdam (Senior Cardiac Physiologist); Mrs Kelly S Park (Senior Research Radiographer); Mr Manjt S Sian (Senior Cardiac Physiologist); Dr Daniel J Swarbrick (Consultant Cardiologist); Mrs Joanne V Wormleighton (Lead Research Radiographer); Professor Tom Yates (Professor of Physical Activity, Sedentary Behaviour and Health).

Thank you all for your efforts.

Other colleagues: Dr Matthew PM Graham-Brown. Thank you for being a constant source of banter, and for always being two steps ahead. It is incredibly motivating trying to keep up. Thank you also to all the members of the Cardiac Imaging Research Group, who have made it a genuine pleasure to come to work every day.

Volunteers: This thesis is dedicated to the hundreds of enthusiastic volunteers who generously gave their time to participate in our research. I hope to honour your contributions by helping to improve heart health in people with diabetes.

Funders: thank you to the British Heart Foundation, who have supported this work through a Clinical Research Training Fellowship (G S Gulsin, FS/16/47/32190) and the National Institute for Health Research, who funded the DIASTOLIC study through a Career Development Fellowship (G P McCann, CDF-2014-07-45).

Family: my wife (Maddy) and dog (Oscar), who are a constant reminder that success can be enjoyed both at home and at work.

Academic outputs

Publications

Arising from this thesis

1. Gulsin GS, Athithan L, McCann GP. *Diabetic cardiomyopathy: prevalence, determinants and potential treatments.* **Therapeutic Advances in Endocrinology and Metabolism**. 2019 Mar 27;10:2042018819834869. DOI: 10.1177/2042018819834869.

2. Graham-Brown MPM, **Gulsin GS**, Parke K, Wormleighton J, Lai FY, Athithan L, Arnold JR, Burton JO, McCann GP, Singh A. *A comparison of the reproducibility of two cine-derived strain software programmes in disease states.* **European Journal of Radiology**. 2019 Apr;113:51-58. DOI: 10.1016/j.ejrad.2019.01.026

3. Gulsin GS, Brady EM, Swarbrick DJ, Athithan L, Henson J, Baldry E, McAdam J, Marsh A-M, Parke KS, Wormleighton JV, Levelt E, Yates T, Bodicoat DH, Khunti K, Davies MJ, McCann GP. *Rationale, design and study protocol of the randomised controlled trial: Diabetes Interventional Assessment of Slimming or Training tO Lessen Inconspicuous Cardiovascular Dysfunction (the DIASTOLIC study).* **BMJ Open**. 2019; 9:e023207. DOI: 10.1136/bmjopen-2018-023207.

4. Gulsin GS, Swarbrick DJ, Athithan L, Brady EM, Henson J, Baldry E, Argyridou S, Jaicim NB, Squire G, Walters S, Marsh A-M, McAdam J, Parke KS, Biglands JD, Yates TY, Khunti K, Davies MJ, McCann GP. *Effects of low-energy diet or exercise on cardiovascular function working-age adults with type 2 diabetes: a prospective, randomized, open-label, blinded endpoint trial.* **Diabetes Care**. 2020: Mar 27:dc200129. DOI: 10.2337/dc20-0129.

5. Gulsin GS, Henson J, Brady EM, Sargeant JA, Wilmot EG, Athithan L, Parke KS, Squire G, Htike Z, Marsh A-M, Biglands JD, Kellman P, Khunti K, Webb D, Davies MJ, Yates T, McCann GP. *Cardiovascular determinants of aerobic exercise capacity in adults with type 2 diabetes.* **Diabetes Care.** 2020: Jul 17:dc200706. DOI: 10.2337/dc20-0706.

Related to this thesis

1. Webb DR, Htike ZZ, Swarbrick DJ, Brady EM, Gray LJ, Biglands J, **Gulsin GS**, Henson J, Khunti K, McCann GP, Waller HL, Webb MA, Sargeant J, Yates T, Zaccardi F, Davies MJ. A randomised, open label, active comparator trial assessing the effects of 26 weeks of liraglutide or sitagliptin on cardiovascular function in young obese adults with type 2 diabetes. **Diabetes, Obesity and Metabolism**. 2020;10.1111/dom.14023. DOI:10.1111/dom.14023

2. Gulsin GS, Graham-Brown MPM, Davies MJ, McCann GP. *Emerging glucose-lowering therapies: a guide for cardiologists*. **Heart**. 2019 Sep 24. pii: heartjnl-2019-315758. DOI: 10.1136/heartjnl-2019-315758.

3. Gulsin GS, Kanagala P, Chan DCS, Cheng ASH, Athithan L, Graham-Brown MPM, Singh A, Yang J, Li Z, Khunti K, Davies MJ, Arnold JR, Squire IB, McCann GP. *Differential left ventricular and left atrial remodeling in heart failure with preserved ejection fraction patients with and without diabetes.* **Therapeutic Advances in Endocrinology and Metabolism**. 2019 Jul 5;10:2042018819861593. DOI: 10.1177/2042018819861593. eCollection 2019.

4. Gulsin GS, Swarbrick DJ, Hunt WH, Levelt E, Graham-Brown MP, Parke KS, Wormleighton JY, Lai FY, Yates T, Wilmot EG, Webb DR, Davies MJ, McCann GP. *Relation of Aortic Stiffness to Left Ventricular Remodeling in Younger Adults with Type 2 Diabetes.* **Diabetes**. 2018 Apr 16. pii: db180112. DOI: 10.2337/db18-0112.

5. Sargeant JA, Yates T, McCann GP, Lawson CA, Davies MJ, **Gulsin GS**, Henson J. *Physical activity and structured exercise in patients with type 2 diabetes mellitus and heart failure.* **Practical Diabetes**. 2018. 35(4): 131-138.

Manuscripts under review

1. Gulsin GS, Abdelaty AM, Graham-Brown MP, Marsh A-M, Sian MS, Xue H, Budgeon CA, Kellman P, Arnold JR, Deshpande A, Davies MJ, McCann GP. *Determinants and reproducibility of myocardial blood flow measurement in type 2 diabetes using quantitative MRI perfusion mapping,* Under review, **JCMR**.

International and National Presentations

Oral

1. *Differential effects of exercise versus low-energy diet on cardiovascular function in working-age adults with type 2 diabetes* (February 2020). Oral presentation at the Society for Cardiovascular Magnetic Resonance 23rd Annual Scientific Sessions, Orlando, USA.

2. *A Randomized Trial of Exercise Versus Low-Energy Meal Replacement Diet to Improve Cardiac Dysfunction in Working-Age Adults With Type 2 Diabetes* (November 2019). Oral presentation in the Early Career Investigator Competition at the American Heart Association Annual Scientific Sessions 2019, Philadelphia, USA.

3. *A Randomized Trial of Exercise Versus Low-Energy Meal Replacement Diet to Improve Cardiac Dysfunction in Working-Age Adults With Type 2 Diabetes* (December 2019). Oral presentation in the Early Career Investigator Competition at the British Society for Heart Failure Annual Autumn Meeting 2019, London, UK.

4. *Importance of non-obstructive coronary artery disease and blood pressure in determining stress myocardial blood flow in asymptomatic people with type 2 diabetes* (September 2019). Oral presentation at the British and Irish Hypertension Society Annual Scientific Meeting 2019, Edgbaston, UK.

Poster

1. Gulsin GS, Athithan L, Parke KS, Wormleighton JV, Singh A, Arnold JR, Xue H, Kellman P, Deshpande A, McCann GP. *Relationship between coronary artery calcium and hyperaemic myocardial blood flow in asymptomatic adults with type 2 diabetes* (May 2019). Presented at the EuroCMR 2019 Annual Meeting, Venice, Italy.

2. Gulsin GS, Hunt WH, Levelt E, Swarbrick DJ, Lai F, Yates T, Davies MJ and McCann GP. *Aortic stiffness is an independent predictor of concentric left ventricular hypertrophy in young adults with type 2 diabetes* (February 2018). Presented at the joint SCMR/EACVI meeting, CMR 2018, Barcelona, Spain.

Prizes

1. *British Society for Heart Failure 22nd Annual Autumn Meeting – Early Career Investigator Award – Winner* (Nov 2019). Awarded for the best early career researcher scientific research presentation.

2. *American Heart Association Council on Lifestyle and Cardiometabolic Health – Early Career Investigator Award – Winner* (Nov 2019). Awarded for the best early career researcher scientific research presentation at the American Heart Association Scientific Sessions 2019.

3. *British and Irish Hypertension Society Annual Scientific Meeting – Dr Robert Grayson Award - Winner* (Sep 2019). Awarded for the best research presentation relating to diseases of the aorta or blood vessels.

4. *University of Leicester Research Bytes – Postgraduate Student - Winner* (June 2019). For presenting doctoral research at the annual Research Bytes competition – a three-minute talk with one presentation slide, to a lay audience.

5. *Royal College of Physicians and Clinical Research Network awards – Trainee Award Winner* (Sept 2017). To recognize outstanding contributions of NHS consultants and trainees to the conduct of NIHR Clinical Research Network Portfolio studies.

Grants arising from this thesis

1. Remission of diabetes and improved diastolic function by combining Structured Exercise with meal replacement and food reintroduction - RESET. Medical Research Council UK-Canada Diabetes Research Team Grants 2019. **£606,147.**

2. Impact of exercise training in combination with dapagliflozin on physical function in adults with type 2 diabetes mellitus: a randomised controlled trial. AstraZeneca PLC. **£487,830.**

Table of contents

List of figures

List of tables

List of abbreviations

ACC=American College of Cardiology
AHA=American Heart Association
ASCVD=atherosclerotic cardiovascular disease
BMI=body mass index
BP=blood pressure
CoV=coefficient of variation
CT=computed tomography
CVD=cardiovascular disease
CVOT=cardiovascular outcomes trial
ECV=extracellular volume fraction
EDV=end-diastolic volume
EF=ejection fraction
ESV=end-systolic volume
GLP-1RA=glucagon-like peptide-1 receptor agonist
GLS=global longitudinal strain
HFpEF=heart failure with preserved ejection fraction
HFrEF=heart failure with reduced ejection fraction
LA=left atrium
LDL=low-density lipoprotein
LGE=late gadolinium enhancement
LV=left ventricle
MACE=major adverse cardiovascular events
MBF=myocardial blood flow
MPR=myocardial perfusion reserve
MRP=meal replacement plan
NICE=National Institute for Health and Care Excellence
NIHR=National Institute for Health Research
PEDSR=peak early diastolic strain rate
PPI=patient and public involvement
RER=respiratory exchange ratio
RV=right ventricle
SGLT2i=sodium glucose co-transporter-2 inhibitor
SV=stroke volume
TI=inversion time
TR=repetition time
T2D=type 2 diabetes

1 Introduction

1.1 The global burden of diabetes

Diabetes mellitus is a major global health concern. Recent estimates suggest that there are currently 451 million people with diabetes worldwide and this figure is projected to increase to 693 million by 2045(1). Importantly, almost half (49.7%) of people living with diabetes remain undiagnosed(1).

The vast majority (90%) of people with diabetes have type 2 diabetes (T2D), which is linked to increases in sedentary time and obesity and is largely preventable. Whereas T2D was once a rarity in young people, increasingly we are seeing the condition diagnosed in children, adolescents and adults under the age of 30 years(2,3). Globally there are now more obese than underweight people(4) and this dramatic rise in obesity and sedentary lifestyles, particularly in younger age groups, has resulted in up to 10-fold increase in the prevalence of T2D in younger adults(5).

1.2 Cardiovascular complications of T2D

The most deleterious consequence of developing T2D is a substantially elevated risk of cardiovascular disease (CVD). The risk of cardiovascular complications is 2-2.5 times greater in people with T2D compared to those without diabetes. In a meta-analysis combing data from 4,549,481 people with T2D, almost one third (32.7%) suffered from CVD and half of all deaths were attributable to CVD(6).

1.2.1 Atherosclerotic cardiovascular disease

Atherosclerotic cardiovascular diseases (ASCVDs, angina, myocardial infarction, stroke, and peripheral arterial disease) have typically been regarded as the predominant manifestations of CVD in T2D and are amongst the largest contributors to the direct and indirect healthcare costs associated with diabetes(7).

1.2.1.1 Epicardial coronary artery disease

Several studies have shown that, even when matched for age and CVD risk factors with non-diabetic people, the prevalence, extent and severity of coronary artery atherosclerosis is worse in people with T2D(8,9). Furthermore, the progression of coronary artery atherosclerosis is greater in people with diabetes(10), and total coronary plaque volume measured using computed tomography (CT) coronary angiography provides incremental predictive value over traditional risk factors for determination of coronary events(11). Male sex(10), duration of diabetes(12), higher total plaque volume(10) and extent of coronary artery calcification(13) are

important non-modifiable predictors of plaque progression in people with diabetes. More importantly however, in cross-sectional study of 224 asymptomatic people with T2D, body mass index (BMI, β=0.26, $p<0.001$) was the only modifiable clinical factor associated with coronary artery plaque volume measured by CT angiography(14).

1.2.1.2 Cerebrovascular disease

Cerebrovascular disease contributes to a two- to six-fold increased risk of stroke in people with T2D(15) and approximately one third of stroke patients have coexisting diabetes(16). Furthermore, acute hyperglycaemia and/or the presence of diabetes are associated with worse outcomes (mortality, neurological and functional recovery, hospital readmission and recurrence of stroke) following stroke(16,17). There is both cerebral microvascular and macrovascular disease evident in T2D. For example, in a mouse model of T2D, marked cerebral microvascular remodelling (with increased vessel tortuosity and neovascularisation) occurred after diabetes duration of only 5-6 weeks(18). Importantly, cerebrovascular remodelling was reversible with glycaemic control T2D(19).

1.2.2 Heart failure

Despite the long held notion that atherosclerosis is the predominant cause of CVD in diabetes, recent data from the United Kingdom National Diabetes Audit 2015-16, which includes data on over 2.7 million patients with diabetes, have shown that heart failure is the commonest cardiovascular complication of T2D and a major cause of premature mortality(20). Patients with diabetes have up to a 74% increased risk of developing heart failure, and diabetic patients with heart failure are four times more likely to die than those without heart failure(21).

Large, population-based studies have shown that the occurrence of heart failure in diabetes cannot be accounted for solely by the increased atherosclerotic risk(22-24) or the prevalence of other traditional risk factors, such as age, gender, hypertension, coronary artery disease, and dyslipidaemia, which are inherent in diabetic subjects. Even after adjustment for these factors, diabetes still confers a two-fold added risk for heart failure development(22-24). This has led to the identification of diabetes as an independent risk factor for heart failure and the recognition of the distinct clinical entity of "diabetic cardiomyopathy"(25), a term originally coined by Lundbaek in 1954(26).

Specifically in diabetes populations: increased age; increasing HbA1c, increased BMI, hypertension, coronary artery disease, longer duration of diabetes and the presence of microvascular complications are associated with heart failure development(27). The United Kingdom National Diabetes Audit 2015-16 showed that the association of heart failure with HbA1c level was relatively weak but considerably stronger for hypertension(28). Although females with T2D have relatively higher risk of cardiovascular complications than males(29), there are scarce data in relation to heart failure(27).

1.2.3 Age and cardiovascular complications of T2D

Although increasing age is associated with greater risk for development of CVD, the overall lifetime risk is highest in younger adults with T2D(30), who live with diabetes for longer durations. For example, recent findings from the Swedish National Diabetes Registry (comprising data from 318,083 people with T2D and almost 1.6 million controls) demonstrated the highest excess risk of cardiovascular disease occurred in those diagnosed with diabetes aged <40 years(30). This is especially true in the case of heart failure, where the risk of developing heart failure is four- to five-fold higher than matched controls (Figure 1.1)(30).

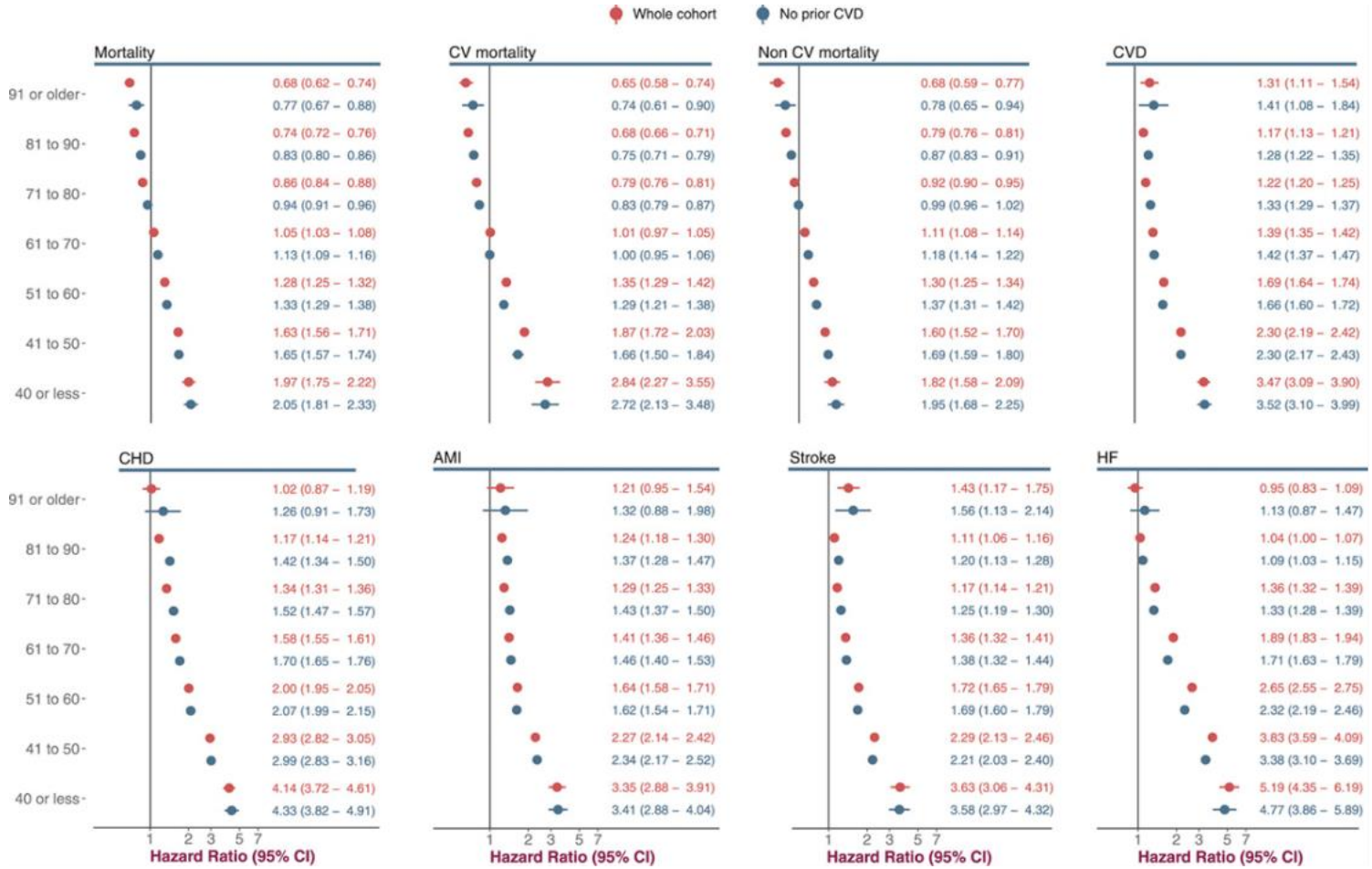


Figure 1.1 Adjusted hazard ratios (95% CI) for CVD in patients with T2D according to age at diagnosis, in comparison with matched controls in those without prior CVD (blue) and in the whole study cohort (red). Taken from(30). Abbreviations: AMI=acute myocardial infarction; CHD=coronary heart disease; CV=cardiovascular; CVD=cardiovascular disease; HF=heart failure.

1.3 Diabetic cardiomyopathy

Diabetic cardiomyopathy is defined as myocardial disease in patients with diabetes, not attributable to hypertension, coronary artery disease or other cardiac disease(25). Four stages of diabetic cardiomyopathy are described, and there is overlap with the heart failure classifications of both the American College of Cardiology (ACC)/American Heart Association (AHA) Stage and New York Heart Association Class (Table 1.1)(25). Patients in Stage 2 have annual mortality rates up to 20%(31) and are twice as likely to be hospitalised for heart failure than non-diabetics with heart failure with preserved ejection fraction (HFpEF)(32).

Table 1.1 Classification of diabetic cardiomyopathy, New York Heart Association Functional Class and American College of Cardiology/American Heart Association (ACC/AHA) HF stages. There is considerable overlap across the three classification schemes.

Diabetic cardiomyopathy stage	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**
	Diastolic HF with normal ejection fraction.	Symptomatic HF with combined systolic and diastolic dysfunction.	Symptomatic HF to which hypertension, microvascular disease and/or viral disease have contributed. No coronary artery disease.	Symptomatic HF, with contribution from multiple confounders including coronary artery disease.
NYHA Functional Class	**Class 1**	**Class 2**	**Class 3**	**Class 4**
	Asymptomatic, no limitation of physical activity.	Slight limitation during ordinary physical activity, with fatigue, palpitation, dyspnoea or angina.	Marked limitation, with symptoms occurring during minimal physical activity.	Symptoms present at rest. Unable to carry out any physical activity without discomfort.
ACC/AHA HF Stage	**Stage A**	**Stage B**	**Stage C**	**Stage D**
	At risk of HF, but no structural heart disease or symptoms.	Asymptomatic structural heart disease (e.g. previous MI, LV remodelling including LVH, systolic or diastolic dysfunction).	Symptomatic HF with structural heart disease.	Refractory HF requiring specialist interventions.

Abbreviations: ACC=American College of Cardiology; AHA=American Heart Association; HF=heart failure; LV=left ventricle; LVH=left ventricular hypertrophy; MI=myocardial infarction; NYHA=New York Heart Association.

1.3.1 Lessons from non-invasive imaging studies

Over the past two decades, the rapid evolution of advanced non-invasive cardiac imaging techniques has enabled detailed evaluation of heart structure and function *in vivo*. Application of these techniques to the study of diabetic cardiomyopathy has provided key insights to the relationship between T2D and undiagnosed heart failure.

1.3.2 Morphological changes in the diabetic heart

The occurrence of structural changes of the diabetic myocardium were first observed by Rubler in 1972(33). In four post-mortem specimens from diabetic patients free of hypertension, coronary artery disease, or valvular heart disease, Rubler described findings of left ventricle (LV) hypertrophy and diffuse myocardial fibrosis(33). There is now an abundance of data to support Rubler's initial findings and demonstrate that diabetes is associated with several alterations in LV geometry.

1.3.2.1 Alterations in LV mass and volumes

Increased LV mass is independently associated with diabetes in many (but not all) echocardiographic(34-36) and cardiovascular magnetic resonance imaging (CMR) studies(37-39)(Table 1.2). A 1% rise in HbA1c level was associated with a 3.0g (95% CI 1.5-4.6g) increase in LV mass in one report(35). Whilst these changes in LV mass are modest, increased LV mass is a recognised predictor of cardiovascular morbidity and mortality(40,41), and is likely to be a key contributor to heart failure development in T2D. When the increased LV mass is indexed for body surface area, however, the differences between diabetics and controls become inconsistent. These inconsistencies arise because adjustment of LV mass for body surface area inherently "permits" obese individuals to have higher LV mass(42). This is why indexing of LV mass to height is advocated(43,44).

Table 1.2 Key echocardiographic and cardiovascular magnetic resonance imaging studies evaluating the impact of diabetes on left ventricular mass.

Study	Subject group(s)	Inc/Exc criteria	Method(s) of assessment	Key findings
Galderisi 1991 (34)	**DM**: n=111, 62% M. **No-DM**: n=4,023, 42% M. Overall prevalence of HTN 16%.	**Inc**: Framingham cohort, sufficient echo windows. **Exc**: history of CVD.	Echocardiography.	↑LV mass/height in women with T2D vs. controls (100 vs. 82 g/m, p<0.001), but not in men. T2D independently associated with LV mass/height after adjustment for age, BMI, heart rate, hypertension and smoking.
Skali 2015 (35)	**DM**: n=1,322, 39% M, age 75y (72 - 69), A1c 6.4% (5.9 – 7.0), 91% HTN. **Pre-DM**: n=1,355, 34% M, age 75y (72 – 79), A1c 5.9% (5.7 – 6.0), 79% HTN. **No-DM**: 1,742, 39% M, age 75y (72 – 79), A1c 5.4% (5.2 - 5.5), 71% HTN.	**Inc**: Atherosclerosis Risk in the Community cohort. **Exc:** non-white subjects, history of CVD.	Echocardiography.	↑LV mass with progressive dysglycaemia (No-DM: 139±37 vs. Pre-DM: 140±40 vs. DM: 151±42g, p=0.003). After adjustment for age, sex, BMI, systolic BP, HTN, heart rate, only DM (but not Pre-DM) associated with ↑LV mass. ↑LV mass/height$^{2.7}$ (adjusted) in DM vs. No-DM 37.4 vs. 36.5 g/m$^{2.7}$, p<0.05.
De Marco 2011 (36)	**DM**: n=179, 42% M, age 32±6y, 65% HTN. **Pre-DM**: n=299, 53% M, age 29±8y, 25% HTN. **No-DM:** n=1,146, 40% M, age 25±7y, 11% HTN.	**Inc**: Strong Heart Study cohort, American Indians, age <40y. **Exc**: history of CVD.	Echocardiography.	↑LV mass with progressive dysglycaemia (No-DM: 145±37 vs. Pre-DM: 166±41 vs. DM: 169±43g, p<0.001), even after adjustment for age, sex, and systolic BP. ↑LV mass/height$^{2.7}$ with progressive dysglycaemia (No DM: 36±8 vs. Pre-DM: 39±9 vs. DM: 41±9g/m$^{2.7}$, p<0.001).
Bertoni 2006 (39)	**DM**: n=646, 53% M, 20% white, age 65±9y, 67% HTN. **Pre-DM**: n=1,334, 56% M, 34% white, age 63±10y, 48% HTN. **No-DM:** n=3,011, 43% M, 45% white, age 60±10y, 35% HTN.	**Inc:** MESA cohort. **Exc:** history of CVD.	Cardiac MRI.	LV mass ↑ across dysglycaemia categories in all ethnic groups except Chinese. Evidence of differential relationship of LV mass to glucose status depending on ethnicity (P for interaction =0.002). After adjustment for age, sex, height and weight, DM associated with ↑LV mass only in blacks and Hispanics.
Jensen 2019 (45)	**DM:** n=143, 57% M, 96% white, age 64±5y, 54% HTN. **No-DM:** n=3,841, 45% M, 96% white, age 61±8y, 18% HTN.	**Inc:** UK Biobank imaging sub-study cohort. **Exc:** known CVD, EF <50%.	Cardiac MRI.	NS difference in LV mass indexed to BSA between DM and No-DM groups (47.1±9.7 vs. 48.3±9.6g/m^2, p=0.16). ↑LV mass:volume in DM vs. No-DM (0.6±0.1 vs. 0.7±0.1g/mL, p<0.001).

Abbreviations: BSA=body surface area; BP=blood pressure; CVD=cardiovascular disease; DM=diabetes mellitus; EF=ejection fraction; HTN=hypertension; LV=left ventricle.

Other markers of LV remodelling are also apparent in diabetes; LV mass:volume(38,46), relative wall thickness(35,47-49), and septal thickness(49,50) are all increased in diabetes. In general, diabetes appears to be associated with reductions in the volumes of all four cardiac chambers. This was best demonstrated in a recent UK Biobank CMR Substudy, comparing 3,841 subjects without diabetes and 143 with diabetes, all of who were free of CVD(45). LV mass may be increased as a consequence of increased ventricular wall thickness or from chamber dilatation, i.e., the spectrum of LV hypertrophy ranges from concentric to eccentric hypertrophy. Whilst there is variation in both the degree and pattern of hypertrophy observed in patients with diabetes, concentric LV hypertrophy represents the main structural characteristic of diabetic cardiomyopathy. Concentric LV remodelling is associated with an increased risk of developing heart failure and other adverse cardiac events(51,52). However, LV geometry is also altered by gender(53), ethnicity(54), obesity(55) and hypertension(56), common confounders in diabetes, and adjusting for these confounders may in fact lessen the association of T2D with LV remodelling(57). The lack of standardisation in reported markers of LV remodelling makes comparisons between studies difficult(58) and limits knowledge of LV remodelling patterns in diabetes.

1.3.2.2 Left atrial volumes

Left atrial (LA) enlargement is increasingly being recognised for its association with adverse cardiovascular outcomes, including atrial fibrillation, stroke, all-cause cardiovascular mortality, and heart failure(59-61). Obesity has for many years been recognised to cause LA enlargement. In a large (n=1,212, mean age 48.5±13.7 years, 48% male) German cohort from the general population who underwent echocardiography at baseline and after 10 years, obesity was an independent determinant of LA enlargement(62). Despite the association between obesity and LA enlargement, surprisingly diabetes was not a predictor of LA enlargement in this cohort(62).

Conflicting data exist in studies describing the changes in LA structure associated with T2D. Some have reported smaller LA volumes(32) in subjects with T2D, whilst others have shown the opposite(63) (Table 1.3). In asymptomatic subjects, the UK Biobank CMR Substudy has shown that T2D is associated with smaller LA volumes(45). These findings extend to patients with T2D and HFpEF, a

disease that is typically associated with LA enlargement (and indeed LA enlargement is incorporated into guidelines for diagnosis of HFpEF)(64). In the RELAX HF Ancillary study, which comprehensively phenotyped HFpEF patients with (n=123) and without T2D (n=93) by CMR and echocardiography, smaller LA volumes were observed in people with T2D(32). We have recently performed a similar comparison of patients with and without T2D in our own cohort of prospectively recruited patients with HFpEF (total n=140, n=75 with T2D) who underwent CMR, echocardiography and plasma biomarker profiling. Despite being more obese, with higher LV filling pressures (E/e') than the patients without T2D, the diabetic HFpEF patients had smaller LA volumes. This finding was supported by lower NTproANP levels in the T2D group. It appears that diabetes has effects on the LA separate from the mechanisms in obesity that foster increased LA volumes. However, the mechanisms that drive smaller LA volumes in T2D are unclear, as are the prognostic implications of smaller LA volumes, both of which warrant further investigation.

Table 1.3 Key studies comparing left atrial volumes in asymptomatic people with and without diabetes.

Study	Subject group(s)	Inc/Exc criteria	Method(s) of assessment	Key findings
De Marco 2011 (36)	**DM**: n=179, 42% M, age 32±6y, 65% HTN. **Pre-DM**: n=299, 53% M, age 29±8y, 25% HTN. **No-DM:** n=1,146, 40% M, age 25±7y, 11% HTN.	**Inc**: Strong Heart Study cohort, American Indians, age <40y. **Exc**: history of CVD.	Echocardiography.	NS difference in LA diameter between groups after adjustment for age, sex, %body fat, and systolic BP.
Kadappu 2012 (63)	**DM:** n=73, age 43±11y, 59% HTN. **No-DM:** n=73, age 43±10, 0% HTN.	**Inc:** T2D, normal LV EF. **Exc:** evidence of CAD on ECG or echo, moderate or worse aortic or mitral valve disease.	Echocardiography.	↑LA vol. in DMs vs. No-DMs (79.8±29.3 vs. 38.2±9.9mL, p<0.001). ↑LA vol. indexed to BSA in DMs vs. No-DMs (38.2±9.9 vs. 20.5±4.8, p<0.001). DM associated with ↑LA vol. independent of HTN and diastolic function.
Skali 2015 (35)	**DM**: n=1,322, 39% M, age 75y (72 - 69), A1c 6.4% (5.9 – 7.0), 91% HTN. **Pre-DM**: n=1,355, 34% M, age 75y (72 – 79), A1c 5.9% (5.7 – 6.0), 79% HTN. **No-DM**: 1,742, 39% M, age 75y (72 – 79), A1c 5.4% (5.2 - 5.5), 71% HTN.	**Inc**: Atherosclerosis Risk in the Community cohort. **Exc:** non-white subjects, history of CVD.	Echocardiography.	After adjustment for age, sex, BMI, systolic BP, HTN, heart rate, progressive ↓LA volume across dysglycaemia categories (No-DM: 47.4 vs. Pre-DM: 46.2 vs. DM: 45.9mL, p=0.02). Same findings even when LA volume indexed to height$^{2.7}$ (No-DM: 12.2 vs. Pre-DM: 11.9 vs. DM: 11.8mL/m$^{2.7}$, p=0.006).
Jensen 2019 (45)	**DM:** n=143, 57% M, 96% white, age 64±5y, 54% HTN. **No-DM:** n=3,841, 45% M, 96% white, age 61±8y, 18% HTN.	**Inc:** UK Biobank imaging sub-study cohort. **Exc:** known CVD, EF <50%.	Cardiac MRI.	In multivariable analysis (with adjustment for age, sex, ethnicity, BMI, BP, physical activity), DM associated with ↓LA max. vol. indexed to BSA (β= -2.52, p=0.01).

Abbreviations: BMI=body mass index; BP=blood pressure; BSA=body surface area; CAD=coronary artery disease, CVD=cardiovascular disease; DM=diabetes mellitus; EF=ejection fraction; HTN=hypertension; LA=left atrium; LV=left ventricle.

1.3.3 Functional impairments in the diabetic heart

Cardiac dysfunction in diabetes is thought to lie on a continuum (Table 1.1) ranging from asymptomatic diastolic dysfunction through subclinical systolic dysfunction and then overt heart failure(25). The vast majority of these have HFpEF, which accounts for up to 83% of people with T2D and newly identified heart failure(65). For example, in two contemporary large-scale heart failure trials of the angiotensin-neprilysin inhibitor sacubitril-valsartan – PARAGON-HF (HFpEF patients, mean age 73y, LV EF ≥45%)(66) and PARADIGM-HF (heart failure with reduced ejection fraction, HFrEF, mean age 64y, LV EF ≤35%)(67) – prevalence of diabetes was 44% and 35%, respectively. Although there is therefore a high prevalence of diabetes in patients with both common forms of heart failure: HFrEF and HFpEF, it appears that people with T2D are particularly prone to developing HFpEF. Furthermore, a high proportion (almost one third) of patients with diabetes have asymptomatic LV dysfunction (in particular diastolic dysfunction)(65).

1.3.3.1 Diastolic dysfunction

It is often stated that diastolic dysfunction is the earliest functional change occurring in diabetic cardiomyopathy. Observational studies have found an increased frequency of diastolic dysfunction in T2D, predominantly using echocardiography, as cut-off values for diagnosing diastolic dysfunction by CMR are yet to be established. We have previously demonstrated evidence of subclinical diastolic impairment (using tagged CMR) even in young adults (mean age 32 years) with T2D compared to both obese and lean controls, despite their young age and relatively short duration of disease(38). There are, however, inconsistencies in the prevalence of diastolic dysfunction found in asymptomatic subjects with T2D (Table 1.4). Reported prevalence rates vary from 16 to 78%(63,68-71) and differ according to the technique used for diagnosis(68). Importantly, there is progressive deterioration of diastolic function with time in people with T2D. In a longitudinal study of 310 subjects with T2D and no overt CVD, prevalence of diastolic function increased from 49% to 67% (p=0.001) over three years' follow-up(72). The determinants of asymptomatic diastolic dysfunction in people with T2D are, however, unclear. Consistent associations of glycometabolic indices with diastolic function across studies have not been demonstrated, although HbA1c(69), systolic blood pressure (BP)(72), and microvascular complications of diabetes(72) have all

been linked with diastolic dysfunction in T2D. Despite controlling metabolic risk factors in T2D (e.g. elevated HbA1c, hypertension, raised BMI, dyslipidaemia, albuminuria), diastolic dysfunction persists in the absence of LV remodelling or systolic impairment(57).

Table 1.4 Summary of key studies reporting prevalence rates of diastolic dysfunction using echocardiography in asymptomatic people with type 2 diabetes.

Study	T2D group(s)	Inc/Exc criteria	Method(s) of assessment	Key findings
Boyer 2004 (68)	N=57, 47% M, age 48±8y, duration of T2D 5.3±4.4y.	**Inc**: T2D, age 31–59y. **Exc:** HTN, angina or dyspnoea, CAD or HF.	**Diastolic dysfunction defined as:** E/A <1 or >2, DT <150 or >220ms, relaxation time <60 or >100, e' <8cm/s.	Prevalence of diastolic dysfunction 75% No difference in age, sex, LV mass index, BMI, EF or T2D duration between patients with and without diastolic dysfunction.
Seyfeli 2008 (70)	N=57, 42% M, age 49±6y, duration of T2D 6.8±5.2y.	**Inc:** asymptomatic T2Ds. **Exc:** known CVD, abnormal ECG or echo.	Comparing conventional echocardiography with tissue Doppler. **Diastolic dysfunction defined as:** E/A <1 measured by conventional echo, and Em/Am <1 by tissue Doppler.	Prevalence of diastolic dysfunction 36% using conventional echocardiography and 74% using tissue Doppler echocardiography (p=0.001).
Yazici 2008 (69)	N=72, 50% M, age 49±10y, duration of T2D 5.1±4.2y.	**Inc:** T2D. **Exc:** CAD, HTN.	**Diastolic dysfunction defined as:** E/A <1 or E/A >1; e' <8cm/s and E/e' >10 and/or VE<45 cm/s and/or E/VE≥1.5.	Prevalence of diastolic dysfunction 68%.
Kadappu 2012 (63)	N=73, age 43±11y.	**Inc:** T2D, normal LV EF. **Exc:** evidence of CAD on ECG or echo, moderate or worse aortic or mitral valve disease.	**Diastolic function grading:** normal (DT=160 –240ms, E/A=0.9– 1.5, e' ≥10cm/s), impaired relaxation (DT=240ms, E/A <0.9, e' <10 cm/s), pseudonormal (DT=160–240 ms, E/A=0.9 –1.5, e' <8 cm/s) and restrictive (DT<160 ms, E/A >2.0, e' <5 cm/s)	Prevalence of diastolic dysfunction 78%: N=16 (22%) normal diastolic function. N=33 (46%) impaired relaxation. N=23 (32%) pseudonormal or restrictive filling.
Boonman de Winter 2012 (65)	N=581, 53% M, age 72±7y, duration of T2D 5.5y (3.0 – 15.2).	**Inc:** all T2Ds undergoing echocardiography.	**Diastolic function grading:** normal (0.75<E/A<1.5, 140<DT<320ms, S/D ≥1), impaired relaxation (grade 1, E/A ≤0.75, DT ≥180ms, S/D ≥1), pseudonormal filling (grade 2, 0.75<E/E<1.5, 140<DT<320ms, S/D <1), and restrictive filling (grade 3, E/A >1.5, DT <140ms, S/D <1).	Prevalence of asymptomatic diastolic dysfunction: n=151 (25.9%). Grade 1: n=149 (99%). Grade 2: n=2 (1%). Grade 4: n=0 (0%).
Faden 2013 (71)	N=386, 57% M, age 69±10y, duration of T2D 10±7y.	**Inc**: T2D, age >18y. **Exc:** angina or dyspnoea, history of CVD, ischaemia on stress ECG or echo.	**Diastolic function grading:** normal, mild (E/A ≤0.75, E/e' <10; moderate (E/A 0.75 – 1.5, DT>140ms, E/e' ≥10), severe (E/A >1.5, DT <140ms, E/e' ≥10).	Prevalence of isolated diastolic dysfunction 16%. N=51 (82%) mild diastolic dysfunction. N=6 (10%) moderate diastolic dysfunction. N=5 (8%) severe diastolic dysfunction.

Abbreviations: CAD=coronary artery disease; CVD=cardiovascular disease; DT=deceleration time; EF=ejection fraction; Em/Am=ratio of early-to-late diastolic tissue velocities; HF=heart failure; HTN=hypertension; S/D=systolic-to-diastolic pulmonary venous flow ratio; T2D=type 2 diabetes; VE=early diastolic mitral annular velocity.

1.3.3.2 Systolic dysfunction

Despite the association of T2D with heart failure, few studies have shown that T2D causes a reduction in global LV ejection fraction (EF), which remains the most utilised measure of LV systolic performance. Using myocardial strain and strain rate measurement, subclinical impairments in systolic function with normal EF are now frequently reported in T2D. Some studies using Tissue Doppler imaging(48,73,74), speckle tracking echocardiography(35,75) and CMR(76,77) have shown that systolic LV global longitudinal strain (GLS) is lower in people with T2D than in non-diabetics (Table 1.5). These impairments in GLS worsen with time(78) and vary across the glycaemic spectrum (e.g. one study found GLS in controls was -18.5±2.3%, in subjects with pre-diabetes it was -18.1±2.5% versus -17.8±2.4% in those with T2D)(35). These subclinical abnormalities in contractility are widely considered to be a precursor to the onset of clinical heart failure in diabetes.

Table 1.5 Summary of key echocardiography and cardiac magnetic resonance imaging studies comparing global longitudinal strain in people with and without diabetes and normal left ventricular ejection fraction.

Study	Subject group(s)	Inc/Exc criteria	Method(s) of assessment	Key findings
Moir 2006 (48)	**DM:** n=22, 42% M, age 57±9y. **No-DM:** n=26, 62% M, age 54±11y.	**Inc:** T2D, normal resting echo. **Exc:** significant CAD on angiography, EF <55%, previous revascularisation.	Tissue Doppler echocardiography.	Peak systolic strain ↓ in DMs vs. No-DMs (-14.4±4.6 vs. -18.7±3.1%, p=0.006). In model containing age, BMI, LV mass index, pulmonary systolic velocity and lateral peak systolic velocity, DM independently associated with peak systolic strain.
Ng 2009 (75)	**DM:** n=47, 100% M, age 58±6y. **No-DM:** n=53, 100% M, 56±7y.	**Inc:** T2D, male, A1c <8.5%, BP <150/85mmHg. **Exc:** known CVD, positive dobutamine stress echo.	Speckle tracking echocardiography.	GLS ↓ in DMs vs. No-DMs (-18.3±2.2 vs. -19.9±1.9%, p<0.001). In model containing age, BMI, heart rate, and systolic BP, DM independently associated with GLS.
Skali 2015 (35)	**DM**: n=1,322, 39% M, age 75y (72 - 69), A1c 6.4% (5.9 – 7.0), 91% HTN. **Pre-DM**: n=1,355, 34% M, age 75y (72 – 79), A1c 5.9% (5.7 – 6.0), 79% HTN. **No-DM**: 1,742, 39% M, age 75y (72 – 79), A1c 5.4% (5.2 - 5.5), 71% HTN.	**Inc**: Atherosclerosis Risk in the Community cohort. **Exc:** non-white subjects, history of CVD.	Speckle tracking echocardiography.	Progressive worsening of GLS with dysglycaemia: (No-DM: -18.5±2.3 vs. Pre-DM: −18.1±2.5 vs. DM: -17.8±2.8%, p<0.001). Findings persisted even after adjustment for age, sex, ethnicity, BMI, systolic BP, heart rate and HTN.
Levelt 2015 (77)	**DM:** n=31, 58% M, age 55±9y, A1c 7.4±1.3%, T2D duration 7y (1 – 8). **No-DM:** n=17, 53% M, age 50±14y.	**Inc:** asymptomatic, T2D. **Exc:** history of CVD, uncontrolled HTN, insulin treated, significant CAD on CT coronary angiogram.	Tagged 3T CMR.	GLS ↓in DMs vs. No-DMs (-9.6±2.9 vs. -11.4±2.8%, p=0.049).
Liu 2018 (76)	**Newly diagnosed DM**: n=31, T2D duration <5y, 39% M, age 51±12y, A1c 7.2±1.4%. **Established DM**: n=40, T2D duration >5y, 73% M, age 57±10y, A1c 7.8±1.8%. **No-DM**: n=30, 57% M, age 53±9y, A1c 5.5±0.5.	**Inc:** T2D. **Exc**: evidence of CAD or HF (by echo, ECG or CT coronary angiogram), chest pain.	Feature tracking 3T CMR.	GLS ↓in established DMs vs. controls (-14.7±2.5 vs. -16.5±2.2%, p<0.017). NS difference in GLS between newly diagnosed DMs and No-DMs.

Abbreviations: BMI=body mass index, CAD=coronary artery disease; CMR=cardiovascular magnetic resonance imaging; CT=computed tomography; CVD=cardiovascular disease; DM=diabetes mellitus; EF= ejection fraction; HF=heart failure; HTN=hypertension; GLS=global longitudinal strain; LV=left ventricle; T2D=type 2 diabetes.

Longitudinal studies have found GLS to be an independent predictor of cardiovascular events and may provide incremental prognostic value in asymptomatic people with T2D(79,80). However, in the first of these studies the sample size was modest and there was significant risk of over-fitting of the multivariable regression model(79), and in the second study only echocardiographic parameters as predictors of outcomes were explored with no mention of clinical predictors(80). Importantly, the majority of cardiovascular events in these studies were atherosclerotic (myocardial infarction and stroke) and not heart failure-related events, and it remains unclear why reductions in GLS would predict atherothrombotic events independent of other clinical risk factors.

Lastly, in a single group intervention study of subjects with poorly controlled T2D free of prevalent CVD (n=105, age 54±10y, BMI 34±8kg/m^2, HbA1c 9.6% [8.7 – 11.4]) treated with 12 months intensive management of glycaemia (target HbA1c <7.0%), BP (target ≤130/80mmgH) and lipid levels (target LDL-cholesterol <2.0mmol/L), there was a progressive improvement in speckle tracking echocardiography-derived GLS in those patients who achieved the greatest reductions in HbA1c(81). In those patients (n=15) whose HbA1c rose despite the intervention, GLS actually worsened(81). These findings suggest that intensive glucose control may improve subclinical systolic dysfunction in T2D, although larger randomised trials have to demonstrate a benefit on heart failure outcomes with this approach (see below).

1.3.3.3 Combined systolic and diastolic dysfunction

The vast majority of studies that have examined both have shown that impaired systolic strain is associated with diastolic dysfunction(35,49,63,74,75,82-85). A small number have, however, reported reduced systolic strain without diastolic dysfunction(46,86,87). This could indicate that diastolic dysfunction is not the earliest functional change in the diabetic heart and is in fact preceded by impaired systolic strain. In most of these studies, diastolic function was determined by tissue Doppler velocities and not strain analyses, likely reducing the sensitivity of identifying diastolic impairments(86,87) Furthermore, it is acknowledged that different guidelines for grading diastolic dysfunction by echocardiography yield inconsistent results and may only be accurate for identifying the most severe cases(88). Assessment of diastolic strain rate may be a more sensitive measure of

early diastolic impairment(38). Only one CMR study(46) reported reduced LV systolic GLS (controls -11.4±2.8 vs. T2D -9.6±2.9, p=0.049) and preserved diastolic strain rate (controls 0.65±0.13 vs. 0.62±0.26s^{-1}, p=0.749), but these values are much lower than those seen in the prevailing echo and CMR literature where they are typically ~20% and 1.5-2.0s^{-1}, respectively, in controls.

1.3.4 Determinants of LV dysfunction in diabetic cardiomyopathy

The mechanisms contributing to the development of diabetic cardiomyopathy have been extensively explored in animal models(89). These include myocardial lipotoxicity and glucotoxicity, damage from advanced glycated end-products and reactive oxygen species, impaired calcium homeostasis, mitochondrial dysfunction, activation of the renin angiotensin aldosterone system, altered myocardial substrate utilisation, and cardiac autonomic neuropathy (Figure 1.2)(90).

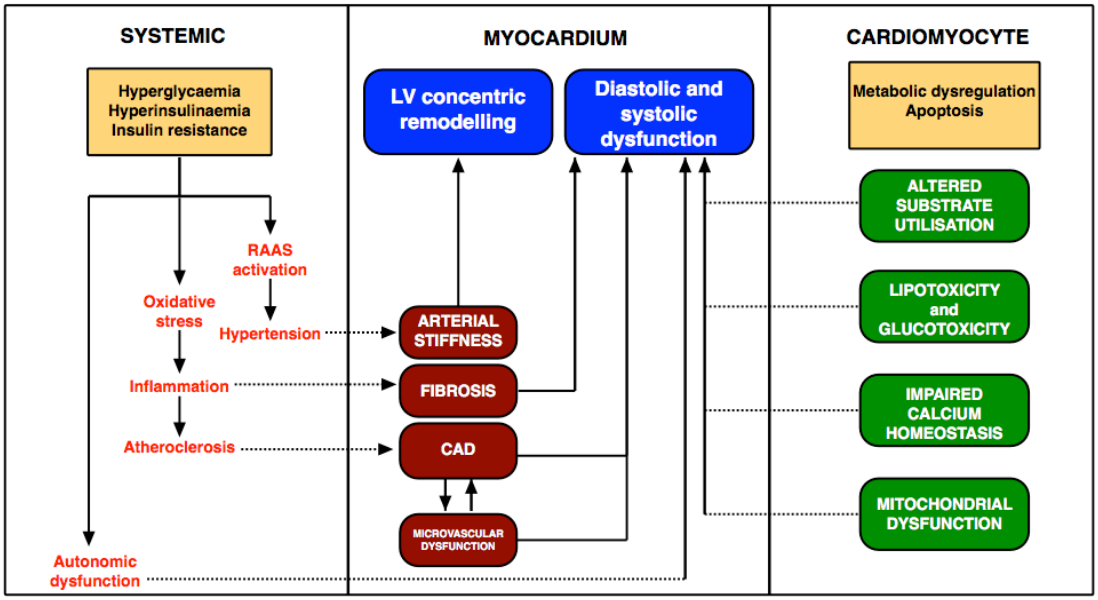


Figure 1.2 Local and systemic perturbations involved in the pathophysiology of diabetic cardiomyopathy. Abbreviations: RAAS=renin angiotensin aldosterone system; CAD=coronary artery disease; LV=left ventricle.

Several inverse correlations with diastolic dysfunction in T2D have been identified: HbA1c level(82), age(69), aortic stiffness(38), duration of diabetes(38), microvascular dysfunction(91) and myocardial triglyceride content(92-94). Similarly, multiple associations with LV systolic strain are reported; these include BMI(95), waist circumference(95), HbA1c(96), BP(85), gender(47), presence of microalbuminuria(47,97), LV relative wall thickness(47,97), coronary artery

disease(98) and, again, myocardial triglyceride(46). Importantly, these findings were made on the basis of multivariate analyses, which have been limited by small sample sizes (seldom greater than 100 subjects) and incomplete datasets with significant risk of overfitting the regression models(99).

1.3.4.1 Myocardial energy metabolism

Impaired myocardial substrate utilisation and altered energy metabolism has recently been described in T2D and is likely to contribute to the development of cardiac impairment (Figure 1.3). The normal heart derives 70% of its energy from free fatty acid metabolism and 30% from glucose metabolism(100). In T2D there is a shift toward increased free fatty acid utilisation by the myocardium in T2D due to increased free fatty acid availability. This is less energy-efficient, with lower adenosine triphosphate yield(101), and leads to metabolic inefficiency in the diabetic heart(102).

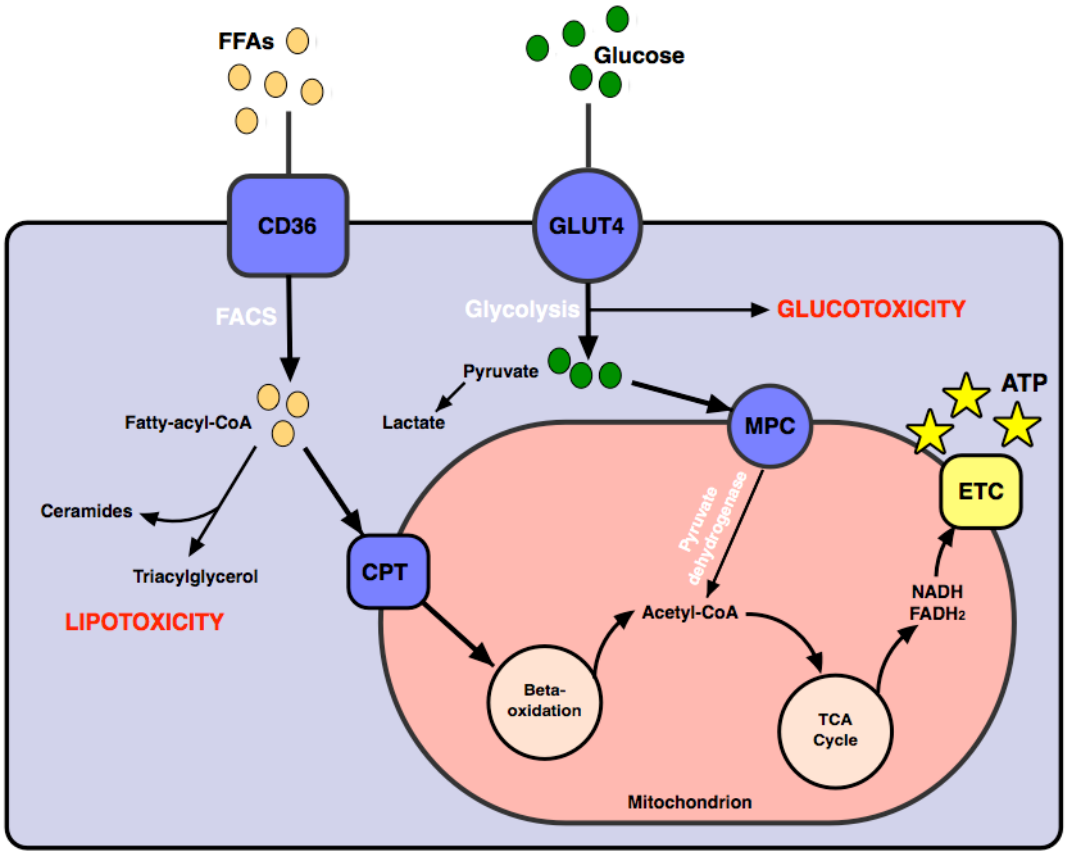


Figure 1.3 Myocyte energy metabolism and alterations that contribute to lipotoxicity and glucotoxicity. FFAs=free fatty acids; CPT=carnitine palmitoyltransferase; MPC=mitochondrial pyruvate carrier; TCA=tricarboxylic acid cycle; ETC=electron transport chain; ATP=adenosine triphosphate.

Myocardial energetics can now be assessed non-invasively by phosphorus magnetic resonance spectroscopy. This enables assessment of the myocardial PCr/ATP ratio, which is a sensitive index of the energetic state of the myocardium(103). Decreased myocardial PCr/ATP ratio has been demonstrated in T2D and suggests that myocardial energetic impairment is a key component in the pathophysiology of diabetic cardiomyopathy(77,104,105). Impairments in myocardial energetics have also been shown to worsen with exercise(77), are linked to reduced myocardial perfusion(106), and may reflect metabolic inflexibility in the diabetic heart. Importantly, decreased PCr/ATP ratio has been linked to contractile dysfunction and is a predictor of mortality, although this was in patients with dilated cardiomyopathy(107).

1.3.4.2 Myocardial steatosis

Metabolic dysregulation is central to the pathogenesis of diabetes. Insulin resistance results in decreased availability of glucose in the myocardium(108). There is an increased supply of free fatty acids, accumulation of intracellular lipids(109-111), and a shift towards fatty acid oxidation in the myocyte(112). Products of non-oxidative lipid metabolism include ceramide and diacylglycerol, which are toxic to cardiomyocytes and can induce myocardial dysfunction, apoptosis and fibrosis (Figure 1.3) (113). Excessive myocardial triglyceride accumulation (steatosis) was first demonstrated in mouse models of diabetes(114) and has emerged as a contributor to the development of diabetic cardiomyopathy.

Magnetic resonance spectroscopy now also allows the detection of myocardial steatosis *in vivo*, and several studies have confirmed elevated myocardial triglyceride content in T2D (Table 1.6)(46,92,94,115). Steatosis has been significantly correlated with concentric LV remodelling(116,117), diastolic strain rate on speckle tracking echocardiography(93) and on CMR(94), and was also an independent predictor of systolic strain in a small study with 66 patients(46). Myocardial steatosis precedes the development of overt diabetes, as it is also detectable in obesity and impaired glucose tolerance(118). However, all of the studies to date have involved small numbers.

Table 1.6 Summary of magnetic resonance spectroscopy studies evaluating myocardial triglyceride content in people with type 2 diabetes.

Study	Patients	Mean age (years)	M/F	Mean BMI (kg/m²)	Key outcomes
McGavock 2007 (119)	Lean controls (n=15) Obese (n=21) IGT (n=20) T2D (n=78)	35±3 36±12 49±9 47±10	7/8 10/11 5/15 37/41	23±2 32±5 31±6 34±7	Myocardial TG ↑ in IGT and T2D vs. controls (0.95±0.60 vs. 1.06±0.62 vs. 0.46±0.30 fat/water, p<0.05).
Rijzewijk 2008 (92)	Controls (n=28) T2D (n=38)	54±1 57±1	28/0 38/0	26.9±0.5 28.1±0.6	Myocardial TG ↑ in T2D vs. controls (0.96±0.07% vs. 0.65±0.05%, p<0.05).
Korosoglou 2012 (94)	Controls (n=16) T2D (n=42)	62±3 62±6	10/6 26/16	23.9±2.5 31.6±4.8	Myocardial TG in T2D 0.86±0.14%. Association between TG and mean diastolic strain rate (r=-0.71, p<0.001) and peak systolic strain rate (r=0.41, p=0.02).
Levelt 2015 (46)	Controls (n=20) T2D (n=46)	54±10 55±9	9/11 24/22	28.6±2.8 29.6±5.7	↑myocardial TG in T2D (1.13±0.78 vs. 0.64±0.52, p=0.017). Negative correlation between TG and systolic strain (R=-0.40, p=0.003).

Abbreviations: IGT=impaired glucose tolerance; TG=triglyceride; T2D=type 2 diabetes.

As myocardial steatosis has been shown to be modifiable(120,121), this provides the potential for novel therapies aimed at reducing concentric LV remodelling and improving cardiac function in diabetes. Successful reduction of myocardial steatosis has been observed with weight loss by a very low calorie diet(122), treatment with glucagon like peptide 1 receptor agonists (GLP-1RAs)(120), and mineralocorticoid receptor blockers(121), alongside improvements in concentric LV remodelling and diastolic function. However, larger studies are needed to confirm these observations.

1.3.4.3 Coronary microvascular dysfunction

Abnormalities of vascular function are well described in T2D. Impaired coronary microvascular function has been demonstrated in older(37,48,94), but not younger patients(38) with T2D. The mechanisms contributing to coronary microvascular dysfunction include: endothelial dysfunction, increased myocardial mass with reduced capillary density, myocardial fibrosis and reduced trans-myocardial perfusion gradient due to increased LV diastolic pressure(123). Animal models and *in vivo* human studies have shown that endothelial dysfunction is a common feature in the coronary microcirculation in T2D(124,125). Furthermore impaired myocardial perfusion reserve (MPR) has been associated with increased cardiovascular mortality(126). However, evidence of the association between microvascular dysfunction and diastolic impairment in T2D is conflicting (Table 1.7). Some CMR studies have failed to identify an association(38,94) whereas others have shown the opposite(76,127-129). In the most recent study, 193 patients with T2D who underwent both CMR for assessment of myocardial blood flow (MBF) and echocardiography for diastolic function, reduced MPR was associated with increasing E/e' even after adjustment for age, BP and LV mass(129). But none of these studies angiographically excluded epicardial coronary artery disease. The potential link between coronary microvascular dysfunction and diastolic impairment in T2D is, therefore, unclear. Moreover, microvascular dysfunction may co-exist with and potentiate the effects of epicardial coronary artery disease on cardiac dysfunction.

Table 1.7 Key studies evaluating the relationship between myocardial perfusion and diastolic function in T2D.

Study	Subject group(s)	Inc/Exc criteria	Method(s) of assessment	Key findings
Korosoglou 2011 (94)	**T2Ds**: n=42, 62% M, age 62±6y, A1c 7.4±0.8%. **Controls**: n=16, 63% M, age 62±3y, A1c 5.1±0.4%.	**Inc**: stable T2D. **Exc**: no clinical signs of HF or angina.	Stress CMR for perfusion, strain encoded CMR for diastolic function.	↓MPRI in T2Ds vs. controls (1.7±0.4 vs. 2.4±0.3, p<0.001). ↓diastolic strain rate in T2D vs. controls (46.9±10.9 vs. 79.6±5.5s^{-1}, p<0.001). NS association between MPRI and diastolic strain rate in T2Ds.
Khan 2014 (38)	**T2Ds**: n=20, 45% M, age 32±7y, A1c 7.1% (6.7 - 10.3)). **Lean controls**: n=10, 50% M, age 30±7y, A1c 5.6% (5.4 - 5.6). **Obese controls**: n=10, 40% M, age 31±6y, A1c 5.5% (5.3 - 5.9).	**Inc**: younger adults, age 18 - 39y.	Stress CMR for perfusion, tissue tagging for diastolic function.	NS difference in MPR between T2Ds and controls. ↓PEDSR in T2Ds vs. lean controls and obese controls (1.51±0.24 vs. 1.97±0.34 vs. 1.78±0.39s^{-1}, p<0.05). NS correlation between MPR and PEDSR.
Kawata 2015 (128)	**T2Ds**: n=67, 85% M, age 57±12y, A1c 7.8±1.5%. **Controls**: n=14, 64% M, age 56±10y, A1c 5.2±0.2%.	**Inc**: no symptoms of CAD, EF >50% **Exc**: CFR <2, +ve stress ECG.	Echo for diastolic function, CFR in distal LAD with colour Doppler and adenosine stress.	↓ CFR in T2Ds vs. controls (3.3±0.8 vs. 3.8±0.7, p=0.036). CFR (β=-0.24, p=0.043) independent determinant of E/e' in T2Ds.
Liu 2018 (76)	**New T2Ds**: n=31, duration <5y, 39% males, age 51±12y, A1c 7.2±1.4%. **Established T2Ds**: n=40, duration >5y, 73% males, age 57±10y, A1c 7.8±1.8%. **Controls**: n=30, 57% M, age 53±9y, A1c 5.5±0.5.	**Exc**: evidence of CAD or HF (by echo, ECG or CTA), chest pain.	Stress CMR for perfusion and feature tracking CMR for diastolic function.	Max signal intensity during stress ↓ in established T2Ds vs. new T2Ds vs. controls (p<0.017 for all). Radial, circumferential and longitudinal PEDSR all correlated with time to max signal intensity in T2Ds.
Sorensen 2019 (129)	**T2Ds**: n=193, 71% M, age 59±11y, A1c 7.9±3.5%. **Controls:** n=25, 68% M, age 57±11y, A1c 5.4±0.5%.	**Inc**: history of CAD. **Exc**: previous CABG, angina.	Stress CMR for perfusion, echo for diastolic function.	↑resting MBF, (0.81±0.19 vs. 0.63±012 mL/min/g, p<0.05), ↓ stress MBF (2.41±0.9 vs. 3.11±0.81 mL/min/g, p<0.05), and ↓ MPR (3.0±1.2 vs. 5.1±1.5, p<0.05) in T2Ds vs. controls. Multivariate analysis: age (β=0.19, p=0.001), hypertension (β=0.15, p=0.04), and MPR (β= -0.31, p=0.01) associated with increased E/e' in T2Ds.

Abbreviations: CAD=coronary artery disease; CFR=coronary flow reserve; CMR=cardiovascular magnetic resonance imaging; CTA=computed tomography angiography; EF=ejection fraction; MBF=myocardial blood flow; MPR=myocardial perfusion reserve; NS=no significant; PEDSR=peak early diastolic strain rate; T2D=type 2 diabetes.

1.3.4.4 Myocardial fibrosis

Given Rubler's observation of diffuse myocardial fibrosis in T2D, the development of LV hypertrophy in diabetic cardiomyopathy could be attributable to myocardial interstitial fibrosis(130), especially in the advanced stages of disease(33). The role of interstitial fibrosis in the pathogenesis of LV hypertrophy in stable/early diabetic cardiomyopathy is less clear, as abnormal myocyte hypertrophy rather than fibrosis appears to predominate in the early stages(130). Myocardial fibrosis is thought to be mediated by damage from advanced glycation end-products(131) and apoptosis caused by lipotoxicity(94). CMR techniques now allow the non-invasive detection of replacement myocardial fibrosis (late gadolinium enhancement, LGE) and an estimate of diffuse interstitial fibrosis using T_1 mapping and calculation of myocardial extracellular volume(132). Although not a consistent finding, several studies have shown that patients with diabetes have longer pre-contrast myocardial T_1(133) and shorter post-contrast myocardial T_1 times compared to controls, indicative of a higher burden of diffuse interstitial myocardial fibrosis(134) (Table 1.8). This is independently associated with myocardial systolic(134) and diastolic function(131,134). Furthermore, subjects with T2D have a higher extracellular volume fraction (ECV) than controls(135,136), although the differences found are small (1-2%)(136), and there is a large degree of overlap when control subjects are well matched to those with diabetes(46). Elevated ECV is associated with increased admissions for heart failure and mortality, but this was in patients already referred for a clinical CMR with inevitable selection bias(136).

Table 1.8 Key studies evaluating diffuse myocardial fibrosis by cardiovascular magnetic resonance imaging in diabetes.

Study	Subject group(s)	Inc/exc criteria	Method(s) of assessment	Key findings
Ng 2012 (134)	**T1D** (n=35) and **T2D** (n=15): 54% M, age 51±10y, A1c 8.0±1.4%. **Controls**: n=19, 63% M, age 45±15y.	**Exc**: history of CVD, LV EF <50%, focal LGE.	1.5T CMR, post-contrast T1, Look-Locker sequence.	↓ post-contrast T1 in diabetics vs. controls (425±72 vs. 504±34, p<0.001) Independent association between native T1 and GLS (β=-0.626, p<0.001) and septal e' (β=0.432, p<0.001).
Wong 2014 (136)	**T2D:** n=231, 65% M, age 61y (54 – 70). **Controls:** n=945, 58% M, age 53y (40 – 64).	**Inc:** subjects referred for clinical CMR, prev. MI included. **Exc:** T1D, amyloid, hypertrophic cardioymyopathy.	1.5T CMR, MOLLI sequence, average of base and mid-slices, focal areas of LGE excluded.	ECV ↑ in T2D vs. controls (30.2% (IQR 26.9 – 32.7) vs. 28.1% (IQR 25.9 – 31.0), p<0.001), even after adjustment for age, sex, ethnicity, smoking, hypertension, renal function, prev. MI. Over median FU 1.3y (IQR 0.8 – 1.9), ECV associated with combined endpoint of death or incident heart failure in T2D.
Storz 2018 (137)	**Pre-diabetes:** n=78, 65% M, age 58±9y, A1c 5.6±0.3%. **T2D**: n=47, 75% M, age 62±8y, A1c 6.7±1.4%. **Controls**: n=218, 50% M, age 54±9y, A1c 5.3±0.3%.	**Exc**: history of CVD.	3T CMR, MOLLI sequence, native T1 from mid short-axis slice.	NS difference in native T1 between pre-diabetes, T2D, or controls (1200±40 vs. 1202±46 vs. 1200±54ms, respectively, p=1.00). ↓ECV in T2D vs. controls (22.8±3.0 vs. 24.6±2.8%, p=0.003), but not after adjustment for age, sex, BP and BMI. NS difference in ECV between T2D and pre-diabetes.
Cao 2018 (133)	**T2D**: n=50, 56% M, age 55±7y, A1c 8.9±2.3%. **Controls**: n=32, 53% M, age 54±6y.	**Inc**: no history or symptoms of CVD, normal echocardiogram.	1.5T CMR, MOLLI sequence, global native T1 from base, mid and apical short axis slices.	↑Native T1 in T2D vs. controls (1027±30 vs. 1012±26ms, p=0.022). ↑ECV in T2D vs. controls (27.1±0.3 vs. 25.0±0.4%, p<0.001). A1c significantly associated with native T1 (β=4.735, p=0.008) and ECV (β=0.413, p=0.002) in multivariable analyses.

Abbreviations: CMR=cardiovascular magnetic resonance imaging; CVD=cardiovascular disease; ECV=extracellular volume fraction; EF=ejection fraction; LGE=late gadolinium enhancement; LV=left ventricle; M=male; MI=myocardial infarction; MOLLI=modified Look-Locker inversion recovery; NS=no significant; T1Ds=type 1 diabetes; T2D=type 2 diabetes.

1.3.4.5 Arterial stiffness

The aorta is a conduit for the delivery of blood to peripheral tissues. In addition, the elastic properties of the aorta act to dampen the sudden fluctuations in BP generated by blood ejected from the LV during each cardiac cycle. This transforms the pulsatile stroke volume into continuous blood flow through the peripheral arterial tree(138). This buffering capacity of the thoracic aortic is essential for maintaining normal LV structure and function. Aortic stiffening is an increase in the elastic resistance of the aorta to deformation and naturally occurs with ageing but is also accelerated by all the traditional cardiovascular risk factors(139,140). Increased aortic stiffness is a strong predictor of adverse cardiovascular events in several cohorts(141-143), including T2D(144,145). In a pilot study, our group has previously shown in young adults with T2D a modest but significant correlation exists between mean aortic distensibility and peak early diastolic strain rate (PEDSR, r=0.564, *P*=0.023)(38). This suggests that increased aortic stiffness is an early change in diabetes, which contributes to subclinical cardiac dysfunction in T2D, independent of age and BP.

Poorer blood glucose control is associated with accelerated aortic stiffness, particularly in younger adults with T2D(146). Reducing HbA1c levels may attenuate the progression of aortic stiffness and some studies have shown that aortic stiffness is modifiable by diabetes treatments(147-150).

Despite the recognition that aortic stiffening is a key determinant of LV dysfunction in several diseases, the precise mechanisms linking aortic stiffness with adverse cardiovascular outcomes are unclear. The most prominent hypothesis for the pathophysiological basis linking aortic stiffness with adverse cardiovascular events in T2D is the development of LV remodelling(151). Aortic stiffening disturbs the arterial-ventricular interaction, augmenting ventricular afterload and supplementing the development of LV hypertrophy. This results in increased LV filling pressures and impairment in the passive flow of blood from the left atrium to the LV in early diastole. Aortic stiffness therefore likely mediates the development of diabetic cardiomyopathy by stimulating LV hypertrophy. We have recently confirmed this in a cross-sectional study of 80 young adults with T2D, where we have demonstrated that aortic stiffness is an independent associated with concentric LV remodelling(152). Given that aortic stiffening is potentially reversible

with aggressive BP reduction(153), this may yield a potential therapeutic strategy for preventing and/or treating diabetic cardiomyopathy.

1.4 Preventing or reversing cardiovascular dysfunction in type 2 diabetes

1.4.1 Glycaemic control

The majority of previous large, multicentre randomised controlled trials such as UKPDS(154), ADVANCE(155), ACCORD(156) and VADT(157) have not demonstrated an improvement in macrovascular outcomes with tight blood glucose control. A meta-analysis of these four large trials, comprising over 27,000 patients assigned to more intensive versus less intensive blood glucose control showed only modest reduction in major adverse cardiovascular events (HR 0.91, 95% CI 0.84-0.99)(158). This was primarily driven by a reduction in myocardial infarction, with no overall benefit on all-cause mortality or cardiovascular death(158). Similarly, a meta-analysis of data from eight randomised trials comprising 37,229 people with T2D revealed no observed benefit of intensive glycaemic control on heart failure-related outcomes(159). Even with intensive glucose control, underlying epigenetic alterations that promote oxidative stress, myocardial inflammation and LV dysfunction persisted in a mouse model of diabetes. This may be a key mechanism driving the persistence of cardiac dysfunction in the context of tight glycaemic control, and targeting epigenetic networks has been proposed as a novel strategy to ameliorate LV dysfunction in T2D(160).

In 2008 the U.S. Food and Drug Administration, responding to concerns regarding the increased cardiovascular risk associated with the use of thiazoledinediones (specifically rosiglitazone)(161), mandated that all new glucose-lowering therapies for T2D be subjected to long-term cardiovascular outcomes trials (CVOTs) to demonstrate their safety(162). The European Medicines Agency later stipulated similar requirements(163). In the 11 years since these guidance were issued, 17 CVOTs of three classes of glucose-lowering medications (dipeptidyl peptidase 4 inhibitors, GLP-1RAs, and sodium glucose cotransporter-2 inhibitors, SGLT2i) have reported. All successfully demonstrated non-inferiority with respect to cardiovascular safety profiles compared to placebo. While the cardiovascular safety of dipeptidyl peptidase 4 inhibitors is well established, no overall benefit on major adverse cardiovascular events (MACE) is observed with this class of drugs. However, promising cardiovascular benefits were observed in several trials of GLP-

1RAs and SGLT2i, likely independent of their glucose-lowering effects. This has prompted growing optimism amongst clinicians regarding the potential for these agents to reduce the burden of cardiovascular disease in people with T2D. The use of GLP-1RAs and SGLT2i are now advocated as second-line agents in European and U.S. diabetes guidance for management of hyperglycaemia in people with T2D(164) and in joint ACC/AHA primary prevention of CVD guidelines(165). Furthermore, the most recent European Society of Cardiology guidelines on diabetes recommend these agents as first-line in high-risk groups(166), although there is debate surrounding the evidence base for this endorsement. Most importantly, however, is that lifestyle management such as encouraging dietary weight loss and increased physical activity, remain at the foundation of all guidelines on management of T2D.

1.4.2 Glucagon like peptide-1 receptor agonists

GLP-1RAs exert their effects by suppressing appetite, glucagon secretion, gastric emptying, and by stimulating the release of insulin(167). These actions lead to reductions in plasma glucose and weight loss (which is more pronounced in higher levels of obesity) (Figure 1.4). Several recent, but not all, CVOTs of GLP-1RAs have shown exciting results with improved glycaemic control as well as reductions in MACE in people with T2D(164)(Table 1.9). Notably, the benefits of GLP-1RAs appear to be on the on the prevention of atherosclerotic cardiovascular disease events (myocardial infarction and stroke), with no observed improvements on heart failure hospitalisations. The first of these trials to demonstrate cardiovascular benefit was the LEADER trial, in which people with T2D and high cardiovascular risk treated with liraglutide had lower rates of cardiovascular death compared to those treated with placebo(168). Subsequently, in high-risk T2D patients, cardiovascular event rates (death, non-fatal myocardial infarction and non-fatal stroke) have been found to be significantly lower with semaglutide(169), albiglutide(170) and dulaglutide(171). Three randomised trials of GLP-1RAs versus placebo, however, did not demonstrate cardiovascular benefit. The ELIXA trial of lixisenatide versus placebo achieved non-inferiority but not superiority for the primary composite endpoint of cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina. However, this was in patients within 180 days of an acute coronary event(172). In the EXSCEL trial, there was no overall cardiovascular risk benefit with exenatide, although this study included patients with or without a prior

history of CVD(173). Lastly, the recent PIONEER 6 trial of oral semaglutide (the first oral GLP-1RA) met the primary endpoint of non-inferiority versus placebo but did not achieve superiority, although rates of cardiovascular death were reduced. However, the follow-up duration in this trial was shorter (1.3 years) compared to the other GLP-1RA CVOTs(174).

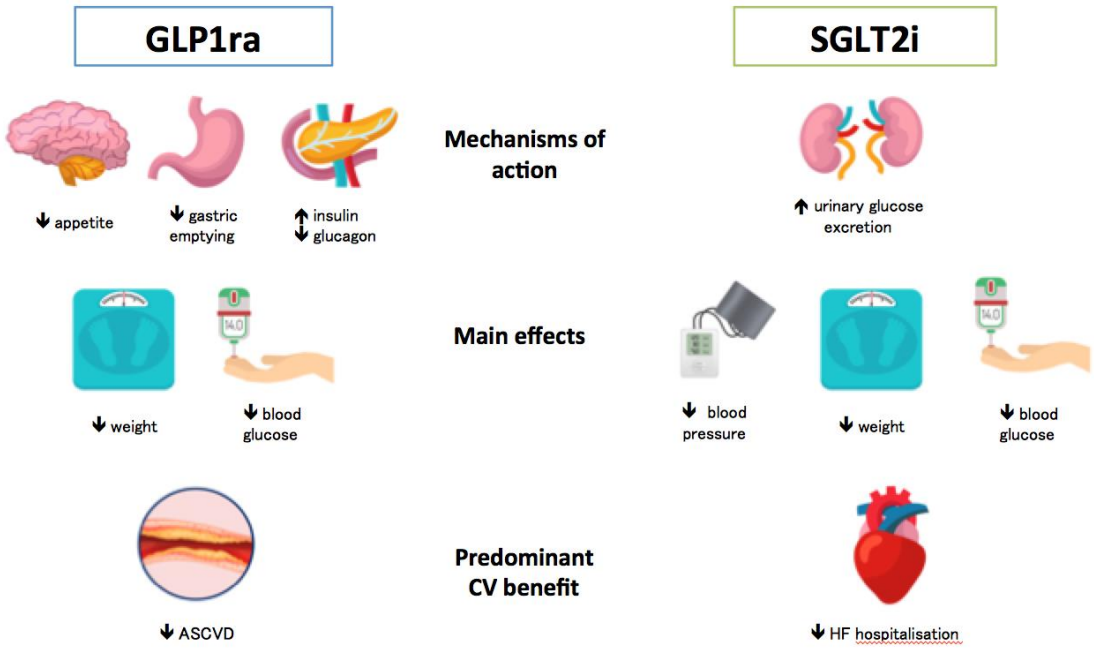


Figure 1.4 Mechanisms of action, main effects and cardiovascular benefits of glucagon-like peptide 1 receptor agonists and sodium glucose co-transporter 2 inhibitors. Abbreviations: ASCVD=atherosclerotic cardiovascular disease; GLPra=glucagon-like 1 receptor agonist; SGLT2i=sodium glucose co-transporter 2 inhibitor.

Table 1.9 Cardiovascular outcomes trials of glucagon like peptide 1 receptor agonists.

Study	Agent	Sample size (n)	Key inclusion criteria	Average age (y)	Follow up duration (y)	Key findings
LEADER (168)	Liraglutide	Total: 9340 Drug: 4668 Placebo: 4672	T2D and CVD, A1c ≥7.0%	64	3.8	MACE, HR 0.87 (95% CI 0.78-0.97); MI, HR 0.88 (95% CI 0.75-1.03); stroke, HR 0.89 (95% CI 0.72-1.11); CV death, HR 0.78 (95% CI 0.66-0.93); HF hospitalisation, HR 0.87 (95% CI 0.77-1.61).
SUSTAIN-6 (169)	Semaglutide	Total: 3297 Drug: 1648 Placebo: 1649	T2D and CVD, A1c ≥7.0%	65	2.1	MACE, HR 0.74 (95% CI 0.58-0.95); MI, HR 0.74 (95% CI 0.51-1.08); stroke, HR 0.61 (95% CI 0.38-0.99); CV death, HR 0.98 (95% CI 0.65-1.48); HF hospitalisation, HR 1.11 (95% CI 0.75-1.23).
EXSCEL (173)	Exenatide	Total: 14752 Drug: 7356 Placebo: 7396	T2D, 70% with CVD and 30% without, A1c 6.5-10%	62	3.2	MACE, HR 0.91 (95% CI 0.83-1.00); MI, HR 0.97 (95% CI 0.85-1.10); stroke, HR 0.85 (95% CI 0.70-1.03); CV death, HR 0.88 (95% CI 0.0.76-1.02); HF hospitalisation, HR 0.94 (95% CI 0.78-1.13).
HARMONY OUTCOMES (170)	Albiglutide	Total: 9463 Drug: 4731 Placebo: 4732	T2D and CVD, A1c >7%	64	1.5	MACE, HR 0.78 (95% CI 0.68-0.90); MI, HR 0.75 (95% CI 0.61-0.90); stroke, HR 0.86 (95% CI 0.66-1.14); CV death, HR 0.93 (95% CI 0.73-1.19).
ELIXA (172)	Lixisenatide	Total: 6068 Drug: 3034 Placebo: 3034	T2D, ACS ≤180 days, A1c 5.5-11%	60	2.1	MACE, HR 1.02 (95% CI 0.89-1.17); MI, HR 1.03 (95% CI 0.87-1.22); stroke, HR 1.12 (95% CI 0.79-1.58); CV death, HR 0.98 (95% CI 0.78-1.22); HF hospitalisation, HR 0.96 (95% CI 0.75-1.23).
PIONEER-6 (174)	Oral Semaglutide	Total: 3183 Drug: 1591 Placebo: 1592	T2D and CVD	66	1.3	MACE, HR 0.79 (95% CI 0.57-1.11); MI, HR 1.18 (95% CI 0.73-1.90); stroke, HR 0.74 (95% CI 0.35-1.57); CV death, HR 0.49 (95% CI 0.27-0.92); HF hospitalisation, HR 0.86 (95% CI 0.48-1.55).
REWIND (171)	Dulaglutide	Total: 9901 Drug: 4949 Placebo: 4952	T2D, with prior CVD or CV risk factors, A1c ≤9.5%	66	5.4	MACE, HR 0.88 (95% CI 0.79-0.99); MI, HR 0.96 (95% CI 0.0.79-1.15); stroke, HR 0.76 (95% CI 0.62-0.94); CV death, HR 0.91 (95% CI 0.78-1.06); HF hospitalisation, HR 0.93 (95% CI 0.77-1.12).

Abbreviations: ACS=acute coronary syndrome; CI=confidence interval; CVD=cardiovascular disease; HR=hazard ratio; MACE=major adverse cardiovascular events; T2D=type 2 diabetes.

The mechanisms by which GLP-1RAs lower rates of myocardial infarction and stroke in T2D are not fully understood. Studies examining the glucose-lowering effects of GLP-1RAs have shown that short-acting agents (exenatide and lixisenatide) decrease HbA1c to a lesser degree (-0.32% to -0.78%)(175,176) than longer-acting agents (liraglutide, albiglutide, dulaglutide)(-1.2% to -1.5%)(177,178). The BP lowering effects of GLP-1RAs are modest (mean systolic BP reduction between 1 and 5mmHg in one meta-analysis)(179), although the precise mechanisms by which this occurs are not yet known. Similarly trial data have shown favourable lipid lowering effects of GLP-1RAs, with reductions in total cholesterol, triglycerides and low-density lipoprotein cholesterol(180). Finally, GLP-1RAs result in weight loss (ranging between 0.4 and 5.1kg), although this varies between different agents and it should be noted that up to a third of patients do not lose weight(181). It is possible that the effects of GLP-1RAs on multiple ASCVD risk factors in combination leads to the observed benefits on cardiovascular events.

Trials examining the impact of GLP-1RAs on diastolic function have been contradictory. In a small (n=49) placebo-controlled randomised controlled trial in people with T2D and no prevalent CVD, six months treatment with liraglutide 1.8mg/day led to modest improvements in echocardiographic measures of LV diastolic filling and LV filling pressure(182). Conversely, in a second similar trial from the same group, treatment with liraglutide did not yield improvements in CMR measures of diastolic function, arterial stiffness or myocardial triglyceride in south Asians with T2D(183). Lastly, in our own prospective, randomised, open-label blinded endpoint (PROBE) trial of 6 months liraglutide 1.8mg/day versus the active comparator sitagliptin 100mg in young adults with T2D and obesity (n=76, age 44±6y, diabetes duration 4.4y, BMI 35.3±6.1kg/m^2), there were no significant between-group differences in CMR-measured LV PEDSR, LV mass, aortic distensibility or MPR(184). Given CVOTs have not demonstrated improvements in heart failure outcomes with GLP-1RAs, it is perhaps unsurprising that diastolic function may not be modifiable with these agents.

1.4.3 Sodium glucose co-transporter-2 inhibitors

SGLT2i prevent reabsorption of glucose in the proximal convoluted tubule promoting urinary glucose excretion and thereby lowering blood glucose levels. Secondary effects include weight loss, a modest diuretic effect and BP

reduction(185) (Figure 1.4). Four major CVOTs of SGLT2i have been completed (Table 1.10)(186-188). In the first two of these - the EMPA-REG OUTCOME(187) and CANVAS(188) studies - there was a relative risk reduction in MACE and hospitalisation for heart failure (~33% reduction) in patients with T2D with established or at high-risk of CVD. More recently, in the largest of the SGLT2i trials with the longest follow up duration - DECLARE-TIMI 58 study of the SGLT2i dapagliflozin versus placebo - reduced rates of hospitalization for heart failure were also observed in lower risk subjects with T2D(186). In a secondary analysis of patients from DECLARE TIMI 58 stratified according to LV EF at baseline (n=671 with HFrEF, n=1316 with HFpEF or unknown EF, and n=15173 with no history of heart failure), the greatest reductions in cardiovascular mortality and heart failure hospitalisations were observed in patients with reduced EF (HR 0.62, 95% CI 0.45-0.86)(189). This suggests that SGLT2i are of added benefit in patients with T2D and HFrEF. However, it is important that the results of these studies be viewed with a degree of caution. Heart failure risk reduction was not the primary endpoint in any of the studies and was based on investigator-reported heart failure events rather than objective measures (such as echocardiography or measurement of B-type natriuretic peptide levels). In the most recent large-scale trial of SGLT2i – the DAPA-HF trial – the effects of dapagliflozin in patients with HFrEF, both with and without diabetes, were compared to placebo(190). This trial assessed the effects of dapagliflozin in a well-described heart failure cohort: key inclusion criteria were a LV EF ≤40% and elevated N-terminal pro-B-type natriuretic peptide ≥600pg/mL, and patients were generally well treated with established heart failure drugs (although the proportion of patients on the angiotensin-neprilysin inhibitor sacubitril/valsartan was relatively low at around 11%). Overall, treatment with dapagliflozin was associated with a 26% relative risk reduction in the composite primary outcome measure of worsening heart failure or cardiovascular death (Table 1.10). This finding was consistent across patients with or without diabetes indicating a role for SGLT2i in all heart failure patients and not exclusively in those with diabetes. Trials are also underway to address the effects of empagliflozin in patients with heart failure and reduced EF (EMPEROR-Reduced, ClinicalTrials.gov Identifier: NCT03057977) and HFpEF (EMPEROR-Preserved, ClinicalTrials.gov Identifier: NCT03057951) in people both with and without T2D.

Mechanisms driving improved heart failure outcomes with SGLT2i are not fully understood. Echocardiographic indices of diastolic function appear to improve consistently in studies with SGLT2i, in both patients with and without heart failure(191-193). Only two CMR studies to date have assessed the impact of SGLT2i on cardiac structure in T2D. In one small (n=90) placebo-controlled randomised trial of six months treatment with empagliflozin in people with T2D and established coronary artery disease(194), there was a small but statistically significant improvement in LV mass index (-3.35g/m^2, 95% CI -5.91 to -0.81, p=0.01). In another smaller (n=25) randomised trial of six months treatment with canagliflozin, however, no improvement in LV mass was observed(195). Neither of these evaluated diastolic function. Other posited benefits of SGLT2i include reductions in arterial stiffness(196) and improvements in myocardial substrate utilisation(197), although convincing trial data do not yet exist. Overall it does appear that SGLT2i may improve diastolic function in people with T2D, which may be a key mechanism underlying the reductions in heart failure outcomes observed in large-scale CVOTs with these agents.

Table 1.10 Cardiovascular outcomes trials of sodium glucose co-transporter 2 inhibitors.

Study	Agent	Sample size (n)	Key inclusion criteria	Average age (y)	Follow up duration (y)	Key findings
EMPA-REG OUTCOME (187)	Empagliflozin	Total: 7020 Drug: 4687 Placebo: 2333	T2D and CVD, A1c 7-10%	63	3.1	MACE, HR 0.86 (95% CI 0.74-0.99); MI, HR 0.87 (95% CI 0.70-1.09); stroke, HR 1.18 (95% CI 0.89-1.56); CV death, HR 0.62 (95% CI 0.49-0.77); HF hospitalisation, HR 0.65 (95% CI 0.50-0.85).
CANVAS (188)	Canagliflozin	Total: 10142 Drug: 5795 Placebo: 4347	T2D and history of or high risk for CVD, A1c 7-10.5%	63	2.4	MACE, HR 0.86 (95% CI 0.75-0.97); MI, HR 0.89 (95% CI 0.73-1.09); stroke, HR 0.87 (95% CI 0.69-1.09); CV death, HR 0.87 (95% CI 0.72-1.06); HF hospitalisation, HR 0.67 (95% CI 0.52-0.87).
DECLARE TIMI 58 (186)	Dapagliflozin	Total: 17160 Drug: 8582 Placebo: 8578	T2D with and without history of CVD, A1c 6.5-12%	64	4.2	MACE, HR 0.93 (95% CI 0.84-1.03); MI, HR 0.89 (95% CI 0.77-1.01); stroke, HR 1.01 (95% CI 0.84-1.21); CV death, HR 0.98 (95% CI 0.82-1.17); HF hospitalisation, HR 0.73 (95% CI 0.61-0.88).
DAPA-HF (190)	Dapagliflozin	Total: 4,744 Drug: 2,373 Placebo: 2,371	Symptomatic HFrEF, with and without diabetes, LV EF ≤40%	66	1.5	Primary outcome (worsening HF or CV death) in all patients, HR 0.74 (95% CI 0.65 - 0.85); in diabetes, HR 0.75 (95% CI 0.63 – 0.90); no diabetes, HR 0.73 (95% CI 0.60 – 0.88).

Abbreviations: CI=confidence interval; CV=cardiovascular; CVD=cardiovascular disease; HF=heart failure; HFrEF=heart failure with reduced ejection fraction; HR=hazard ratio; LV EF=left ventricular ejection fraction; MACE=major adverse cardiovascular events; MI=myocardial infarction.

1.4.4 Blood pressure reduction

Patients with diabetes are twice as likely to suffer from hypertension than non-diabetics(198). Co-existence of these two conditions confers a greater risk of CVD, including coronary artery disease, LV hypertrophy, stroke and heart failure, compared with either diabetes or hypertension in isolation(199). Meta-analysis data convincingly demonstrate a reduction in overall mortality, cardiovascular death, myocardial infarction and stroke with BP reduction in patients with a baseline systolic BP ≥140mmHg, but no overall benefit on heart failure(200). Furthermore, the observed benefits of BP lowering on cardiovascular outcomes in patients with a baseline systolic BP <140mmHg are less clear(201), and there are inconsistencies in the recommended BP targets in these patients(202-204). Previous recommendations favoured an intensive approach to BP management in diabetes given the high cardiovascular risk profile of these patients. Indeed, in non-diabetic patients at high risk of CVD, intensive BP lowering (to a systolic BP below 120mmHg) dramatically lowers cardiovascular risk and all-cause mortality(205). It has therefore been suggested that tighter BP control be targeted in patients with T2D(206), although there are limited data to support this strategy as a means of overall cardiovascular risk reduction.

In the ACCORD BP study, which specifically addressed the issue of intensive BP lowering in T2D, there was no demonstrable survival benefit with intensive BP reduction (systolic BP<120mmHg) compared with a standard BP reduction target (<140mmHg) over a median follow-up period of 4.7 years in 4733 patients with T2D(207). The annual incidence of all-cause mortality was similar with either BP target (1.28 and 1.19%, respectively, HR 1.07; 95% CI 0.85-1.35, p=0.52). Intensive BP treatment was, in fact, harmful and led to increased incidence of syncope and hyperkalaemia(207). Even after longer term follow-up (median duration 8.8 years) of patients was carried out for 3,957 patients from the ACCORD study, there remained no reduction in the rate of a composite of fatal and non-fatal major cardiovascular events or mortality with intensive versus standard BP control(208). Similarly, patients with T2D and a history of coronary artery disease do not appear to benefit from an intensive BP lowering treatment strategy(209). Meta-analyses of studies evaluating the benefit of intensive BP control on cardiovascular risk in patients with diabetes do not convincingly support tight BP regulation for

cardiovascular risk reduction, and recent data suggest a U-shaped relationship between BP and cardiovascular outcomes in T2D, where systolic BP over 150mmHg or less than 110mmHg portends a poorer prognosis(210). Maintenance of BP within this range appears to be the most appropriate strategy in T2D. In view of these and other large prospective studies evaluating BP lowering targets in diabetes, current National Institute for Health and Care Excellence (NICE, NG28) recommendations are that BP be maintained below 140/80mmHg in uncomplicated T2D or below 130/80mmHg if there is a history of renal, eye or cerebrovascular disease(202).

1.4.5 Intensive risk factor management

The effect of intensive control of multiple modifiable risk factors in T2D was first explored in the Steno-2 trial(211). Patients with T2D (n=160, mean age 55.1 years) and microalbuminuria were randomly assigned to conventional versus intensified treatment, which included behavior modification and pharmacologic therapy with strict treatment goals (targeting glycaemia, dyslipidaemia, and microalbuminuria) along with secondary prevention of cardiovascular disease with aspirin. Those in the intensive therapy arm had a 53% lower risk of the composite primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, revascularization, and amputation after 7.8 years of follow up(211). However, heart failure outcomes were not assessed. Similarly, in a recent large (n=271,174 patients with T2D) cohort study, the observation that modifiable risk factors (dysglycaemia, hypertension, smoking, albuminuria and dyslipidaemia) being within target ranges was associated with a stepwise risk reduction in death, myocardial infarction and stroke over 5.7 years of follow up. When all five risk factors were found to be within their specified target ranges, people with T2D had little or no excess ASCVD risk compared to the general population, but the excess risk of heart failure persisted(212). These studies suggest that multifactorial risk factor management could be a strategy to lower risk of ASCVD in people with T2D, but not necessarily heart failure. Furthermore, multifactorial risk factor management strategies may not be pragmatic or realistic in the real world. In the Steno-2 trial intensive treatment required strict diet and physical activity modification as well as aggressive pharmacological therapy and close specialist outpatient follow-up(211) and in the study by Rawshani *et al.* only five per cent of the study population had all five risk factors within the designated target ranges(212).

1.4.6 Lifestyle interventions

1.4.6.1 Weight loss

Type 2 diabetes has long been regarded as a chronic condition capable of being ameliorated but not cured. There is usually a gradual decline in glycaemic control(213) and most patients ultimately require exogenous insulin therapy, often in combination with the newer classes of glucose-lowering therapies such as dipeptidyl peptidase-IV inhibitors, GLP-1RAs, and SGLT2i(214,215). However, proof that T2D is a reversible condition has been firmly established in patients undergoing bariatric surgery(216,217). The extent of weight loss is strongly linked to reversal of T2D, as is good glycaemic control before surgery. Insulin use, diabetes duration and high HbA1c levels reduce the chances of reversal(217). Importantly, bariatric surgery can achieve sustained weight loss (in up to a fifth of patients)(218), sustained remission of diabetes (in up to one third of patients)(219), and lower rates major adverse cardiovascular events (including heart failure) in people with T2D and obesity(220).

Like with bariatric surgery, the phenotypic characteristics of T2D (excess body weight, ectopic fat deposition, hypertension and insulin resistance) are largely normalised through low-energy diets via the meal replacement approach(221). In some instances, dramatic reductions in body weight (over 10kg) and BP (systolic BP falls >10mmHg) are observed after as little as six weeks(122,222). In one of the first studies using a meal replacement dietary intervention, normalisation of fasting blood glucose levels was achieved with an eight week very low energy diet, with 50% of subjects with even longstanding (>8 years duration) T2D attaining fasting glucose measurements in the non-diabetes range at the end of the study(223). This approach also led to a decrease in hepatic glucose production, and hepatic triglyceride content with substantial improvements in glucose disposal rates(224,225). In a larger (n=298) recent trial of a low-energy total diet replacement programme (825-853 kcal/day over 3-5 months) versus guideline-driven best practice, diabetes remission was achieved in almost half (46%) of those who received the diet (mean weight loss 10kg) compared to only 4% in the comparator group (mean weight loss 1kg)(226). Sustained remission of T2D at two years, closely linked to sustained weight loss, was achieved in over one third of people with the diet(227). Many of these improvements are seen within one week.

The majority of T2D individuals are also found to lose 50% of excess body weight within 8-12 weeks of very low energy diet(224,225). In a self–reported study of dietary induced weight loss, diabetes reversal was positively associated with the magnitude of weight loss and inversely with diabetes duration (being lowest in those with diabetes >8 years)(228). None of these reports, however, have assessed changes in cardiovascular function.

Several studies have explored the effects of dietary weight loss interventions on cardiac structure and function, mostly in people with obesity (Table 1.11). In obese subjects without T2D, sustained weight loss, either with diet or after surgery, has resulted in favourable reductions in CMR measured LV mass, volumes, arterial stiffness and diastolic function(229). Improved diastolic function following weight loss in obesity has been associated with improved energetics(230) and with reduced myocardial triglyceride content(231). In patients with insulin-treated T2D, a 471 kcal/day very low energy diet has also been shown to reduce myocardial steatosis (from 0.88±0.12% to 0.64±0.14%, p=0.02) in a small (n=12) single group study and was associated with improved diastolic filling on CMR(122). Interestingly, a recent brief report from the same group, suggests that in the first few days after commencing a very low energy diet there may actually be an increase in steatosis and reduced diastolic mitral filling(232). It has been posited that this transient worsening of cardiac function is the result of increased circulating free fatty acids that occurs with fat mobilisation during rapid weight loss, which in turn causes vascular injury and accumulation of myocardial triglycerides(233). However, it should be noted that there was a dramatic decrease in ventricular volumes, with a non-significant decrease in estimated filling pressure, which are likely to have affected the diastolic filling rate. Surprisingly weight regain up to 18 months after a very low calorie diet did not lead to regression of favourable LV remodelling and diastolic dysfunction, but was associated with a return of myocardial triglyceride levels back to baseline in a small (n=14) cohort of insulin-treated T2Ds(234). In none of these existing studies have subjects been randomised to diet programmes and the majority have been un-blinded single-group interventions with inevitable selection and potential ascertainment bias.

Table 1.11 Key studies evaluating the impact of dietary weight loss interventions on cardiac structure and function.

Study	Participant group(s)	Inc/Exc criteria	Weight loss intervention(s)	Method(s) of assessment	Key findings
Hammer 2008 (122)	**T2Ds:** n=12, age 48±3y, 58% M, BMI 35.6±1.2kg/m^2, A1c 7.9±0.4%.	**Inc:** T2D on insulin, obesity **Exc:** abnormal stress ECG.	Single group: 16-week very low calorie (450kCal/day) diet.	1.5T CMR and MRS.	Myocardial TG ↓0.88±0.12 to 0.64±0.14% (p=0.019), hepatic TG ↓21.2±4.2 to 3.0±0.9% (p<0.001). LV mass ↓118±7 to 99±6g (p<0.001) and E/A ratio ↑1.02±0.08 to 1.19±0.06 (p=0.019).
De las Fuentes 2009 (235)	**Obesity**: n=47, age 46±10y, 28% M, BMI 37±3kg/m^2.	**Exc:** T2D, use of weight loss or lipid lowering drug(s).	Low fat or low carbohydrate diet over 24 months.	Echocardiography at 3, 6, 12 and 24 months.	↑E' and ↓LV mass/Ht$^{2.7}$ at 3,6 and 12 months.
Syed 2009 (236)	**Obesity**: n=62, age 47±1y, 10% M, BMI 46.7±1.4kg/m^2.	**Inc:** patients attending weight loss clinic.	Single group: weight loss by diet, exercise, metabolic surgery, or combination.	Echocardiography after 10% weight loss or 6 months.	LV mass ↓256±12 to 228±12g (p=0.01), E/A ratio ↑1.30±0.05 to 1.32±0.06 (p=0.03).
Kosmala 2009 (237)	**Obesity +/- T2D:** n=124, age 41±13y, 24% M, BMI 36.4±5.7kg/m^2, 36% T2D.	**Exc:** history of CVD, abnormal stress echo.	Single group: 6 month diet (deficit 3,487-6,998kCal/week) and 150 mins/week unsupervised mod. intensity exercise.	Echocardiography	LV mass/Ht ↓49.8±12.2 to 44.8±9.6g/m$^{2.7}$, PSV ↑5.7±1.5 to 6.0±1.4cm/s (p=0.03), PEDV ↑6.5±2.1 to 8.0±2.5cm/s (p<0.001). Systolic strain ↑18.8±2.9 to 21.0±2.8% (p<0.001) and strain rate ↑1.51±0.28 to 1.65±0.34 (p<0.001). NS change in E/e' or E/A ratio.
Viljanen 2009 (222)	**Obesity:** n=33, age 44±8y, 30% M, BMI 33.7±0.7kg/m^2, A1c 5.6±0.1%.	**Exc:** history of CVD, hypertension, T2D.	Single group: 6-week very low calorie (645kCal/day) diet.	1.5T CMR (n=17) and MRS (n=8).	LV mass ↓109±7 to 101±6g (p=0.004), CO ↓8.3±0.4 to 6.8±0.3 L/min (p<0.001), myocardial TG ↓1.05 to 0.75% (p=0.076).
Jonker 2014 (234)	**T2Ds:** n=14, 57% age 53±2y, 57%M, BMI 35±1kg/m^2, A1c 8.4±0.3%.	**Inc:** on insulin, normal stress echo.	Single group: 16-week very low calorie (450kCal/day) diet.	1.5T CMR and MRS at baseline, 4 and 14 months.	LV mass/volume ↓0.67±0.03 to 0.59±0.03 to 0.56±0.03g/mL (p<0.05). E/A ratio ↑0.96±0.07 to 1.12±0.06 to 1.06±0.07. Myocardial TG ↓0.74 (0.41-1.10) to 0.45 (0.31-0.54) after 4 months, but ↑0.76 (0.65-1.32) by 14 months.

Abbreviations: CO=cardiac output; LV=left ventricle; PEDV=peak early diastolic velocity; PSV=peak systolic velocity; TG=triglyceride.

1.4.6.2 Exercise programmes

Large cohort studies have shown that increased aerobic exercise capacity is associated with significantly lower cardiovascular and overall mortality in men(238) and women(239) with diabetes. Peak exercise capacity (VO_2) is a recognised prognostic marker in subjects with cardiovascular disease(240) and in T2D(241). In a small study (n=19), exercise capacity was significantly reduced in subjects with T2D and diastolic dysfunction compared to those with normal diastolic function(242). This suggests that improvements in exercise capacity may yield improvements in cardiac dysfunction in T2D, at the very least in the early stages of diabetic cardiomyopathy. This is supported by a recent position paper of the European Association of Preventive Cardiology, which advocates the promotion of individualised exercised training programmes in people with T2D to improve both cardiovascular and metabolic function(243).

Exercise training has consistently been found to lower HbA1c by 0.6 to 0.7%, with greater effects seen with higher volumes of exercise(244,245). Importantly, these substantial benefits are seen even when exercise training does not result in weight loss(244,245). This is consistent with experimental studies that have elucidated key insulin-dependent and insulin-independent pathways linking physical activity to improved glucose regulation that do not act through adiposity(246,247). While the benefits of exercise training on glycaemic control are well established, the effects on diastolic function are less established, primarily due to insufficient data, differences in measurement, and poor study design. Several studies have been conducted in T2D assessing the effects of exercise training on diastolic function, but the results have been inconsistent (Table 1.12). In a small pilot study, three months of aerobic exercise training reversed diastolic dysfunction in almost half (45%) of individuals with T2D and grade 1 diastolic dysfunction(248), while another study found no overall effect(249). However in the latter study, a post-hoc analysis revealed that change in moderate–intensity physical activity was significantly associated with change in myocardial strain rate, although it is unclear whether this was systolic, diastolic or both(249). This mirrors the wider evidence where light-to-moderate aerobic exercise training has repeatedly been demonstrated to improve diastolic function across a number of groups(248,250).

Table 1.12 Key studies evaluating the impact of exercise interventions on cardiac structure and function in people with type 2 diabetes.

Study	Participant group(s)	Inc/Exc criteria	Exercise intervention(s)	Assessment method(s)	Key findings
Loimaala 2007 (251)	**Control:** n=24, age 53±6y, A1c 8.0±1.3%. **Intervention:** n=24, age 53±5y, A1c 8.2±2.1%.	**Inc:** T2D duration <3y. **Exc:** history of CVD, evidence of CAD on exercise ECG or RWMA on echo.	Randomised: 12-months, part-supervised, 2x/week moderate intensity aerobic exercise and 2x/week resistance training vs. standard care.	Echo	NS change in diastolic or systolic tissue velocities in either group.
Brassard 2007 (248)	**Intervention:** n=11, age 58±5y, A1c 5.8±1.3%. **Control:** n=12, age 57±6y, A1c 6.4±1.2%.	**Inc:** sedentary, oral hypoglycaemic or diet controlled T2D. **Exc:** history of CVD, hypertension.	Randomised: 3-month supervised aerobic exercise, cycle ergometer, moderate intensity, 3x/week vs. no intervention.	Echo	↑E/A 0.76±0.11 to 0.97±0.32, p<0.05, in exercise arm. Normalisation of diastolic dysfunction in 5/11 patients in exercise arm (p<0.001). NS change in LV mass, volumes or systolic function in either group.
Hordern 2009 (249)	**Intervention:** n=88, age 56±12, 53% M, A1c 7.5±1.6%. **Control:** n=88, age 55±8.5y, 57% M, A1c 7.6±1.3%.	**Exc:** known CVD, evidence of CAD on exercise echo.	Randomised: 1-month supervised gym, 11 months home-based with tel. follow-up, mod. intensity, 150mins/week, mixture of aerobic and resistance training vs. routine care.	Echo	↑e' both groups, NS difference between groups. ↑s' both groups, NS difference between groups. Post-hoc analysis: exercise group who did more MVPA ↑e'(p<0.01).
Jonker 2013 (252)	n=12, 58% M, A1c 6.7±0.3%.	**Exc:** microvascular T2D complications, hypertension, smoking, history of CVD.	Single group: 6-months, individualised training, 3-6.5h/week, mixture of resistance and aerobic.	1.5T CMR and MRS at baseline and 6 months.	NS change in diastolic or systolic function. NS change in myocardial TG. ↓hepatic TG 6.8±2.3 to 4.6±1.6% (p<0.01) and ↓pericardial fat volume 4.6±0.9 to 3.7±0.8ml (p=0.02).

Sacre 2014 (253)	**Control:** n=25, age 60±9y, 40% M, A1c 7.7±1.7%. **Intervention:** n=24, age 59±10y, 54% M, A1c 7.7±1.6%.	**Inc:** asymptomatic T2Ds, age ≥40y. **Exc:** known CVD, macro- or microvascular T2D complications, EF <50%.	Non-randomised: 6-month aerobic/resistance training, gym- and home-based, moderate intensity, 150mins/week, semi-supervised vs. standard care.	Echo	NS improvement in LV diastolic or systolic function with exercise.
Hollekim-Strand 2014 (254)	n=47, age 56±6y, 64% M, duration T2D 3.6±2.5y.	**Inc:** T2D <10y, no history CVD, e'<8cm/s.	Randomised: 12-weeks home-based MIE vs. supervised HIIT.	Echo	↑e' with HIIT vs. MIE at 12 weeks (+1.8±1.1cm/s, p<0.01 vs. +0.5±0.7/s, p=0.02). ↑E/A ratio with HIIT (0.93±0.21 to 1.07±0.26, p=0.03), but not MIE. ↑systolic strain/strain rate with HIIT vs. MIE at 12 weeks.
Cugusi 2015 (255)	n=18, age 52±9y, 100% M, A1c 8.1±0.9%.	**Inc:** LV EF ≥55%, no RWMAs on echo. **Exc:** heart valve disease, arrhythmia.	Single group: 12-week, supervised, aquatic-based sessions, 50mins, 3x/week.	Echo	↓E/e' 10.1±1.5 to 9.1±0.8 (p<0.005). NS change in LV volumes, EF, strain or strain rate.
Cassidy 2016 (256)	**Intervention:** n=12, age 61±9y, 83%M, A1c 7.0±1.0%. **Control:** n=11, age 59±9y, 73% M, A1c 7.0±0.5%.	**Exc:** overt CVD, take regular exercise, beta-blocker treatment.	Randomised: 3-month HIIT, cycle ergometer, unsupervised, 3x/week vs. standard care.	3T tagged CMR and MRS.	HIIT arm: ↑LV mass 104±17 to 116±20g (p=0.02), ↑EDV 118±30 to 126±30 (p=0.01), ↑stroke volume 76±16 to 87±19ml (p<0.001), ↑EF 65±8 to 70±6% (p=0.02) ↑early diastolic filling rate 241±84 to 299±89ml (p=0.01). NS change in PCr/ATP ratio.

Abbreviations: CAD=coronary artery disease; CVD=cardiovascular disease, e'=diastolic tissue velocity, EF=ejection fraction; HIIT=high intensity interval training; MIE=moderate intensity exercise; MRS=magnetic resonance spectroscopy; MVPA=moderate-to-vigorous physical activity; s'=systolic tissue velocity; RWMA=regional wall motion abnormality; TG=triglyceride.

The effectiveness of vigorous-intensity exercise or combined aerobic and resistance training is less well established, with at least one study showing the latter approach is not effective(257). Additional benefits of exercise training may include reductions in hepatic triglyceride content and pericardial fat volume(252), although these are even less well studied. Given this evidence base, it is important the efficacy of aerobic exercise training is investigated further.

1.4.6.3 Combined exercise and dietary interventions

Whether combined exercise and dietary interventions may improve cardiovascular function or prevent the development of heart failure in people with T2D is not known. In the randomised controlled Look AHEAD trial, the effects of intensive lifestyle intervention (which included the combination of a dietary weight loss and exercise programme) versus a diabetes support programme were evaluated in 5,145 overweight or obese (mean age 58.7 years, BMI 36 kg/m^2) people with T2D over a median follow-up duration of 9.6 years. Disappointingly, there was no difference in the rate of cardiovascular events in the intensive lifestyle intervention arm, despite a greater extent of weight loss, increased fitness and improved glycaemic control(258). However, the mean weight loss achieved in the intensive lifestyle intervention arm was only 6% by the end of the trial, and both study groups had intensive medical management of cardiovascular risk factors, which may have limited the treatment effect in the intervention arm.

Only one small (n=22) study has assessed the impact of a combined dietary and exercise intervention on cardiac structure and function in T2D(259). Patients (age 60 years, BMI 31 kg/m^2) were randomly assigned to a 12-week intervention of either: 1) Palaeolithic diet (based on fruits, vegetables, fish, eggs and lean meats), or 2) Palaeolithic diet and supervised aerobic and resistance exercise training sessions (one hour sessions, three times per week). Feature tracking CMR and MRS were performed pre- and post-intervention. In both arms there was a reduction in fat mass, markers of insulin resistance and glycaemic control, with additional preservation of lean mass noted in the combined diet and exercise group(260). Furthermore, improvements in concentric LV remodelling (13% reduction in LV mass:volume), myocardial steatosis (45% decrease in myocardial triglyceride content) and systolic strain (approximately 5% increase in GLS) only occurred with the diet and exercise intervention(259). These findings suggest an additive effect of

exercise over dietary restriction and weight loss for improving subclinical LV dysfunction in T2D. However, measures of diastolic function were not reported in this study, and the trial groups were not well matched at baseline. Further work is therefore needed to evaluate the impact of combined dietary and exercise interventions on in T2D.

1.5 Summary and study rationale

The prevalence of T2D has reached a pandemic scale. These patients are at a substantially elevated risk of developing CVD, with heart failure being a leading cause of morbidity and mortality. Even in the absence of traditional risk factors, diabetes still confers up to a two-fold increased risk of heart failure development. This has led to the identification that diabetes is an independent risk factor for heart failure and the recognition of the distinct clinical entity of diabetic cardiomyopathy. Typical features of diabetic cardiomyopathy, even before the development of symptoms, appear to include concentric LV remodelling, diastolic and systolic dysfunction (reduced diastolic strain rates and GLS), increased arterial stiffening, reduced myocardial perfusion, myocardial lipid accumulation and impaired myocardial energetics.

Despite a wealth of research interest, the prevalence and determinants of diabetic cardiomyopathy remain uncertain, particularly in a mixed ethnic population. This limited understanding of the pathophysiology of diabetic heart disease has also hindered the development of effective treatments. Tight blood glucose and BP control have not convincingly been shown to reduce macrovascular outcomes in T2D. There is, however, emerging evidence that T2D is reversible and that the metabolic abnormalities can be reversed with weight loss. Increased aerobic exercise capacity is associated with significantly lower cardiovascular and overall mortality in diabetes. Whether such lifestyle modifications as weight loss and exercise may ameliorate the structural and functional derangements of the diabetic heart has yet to be established.

1.6 Aims

The aims of this study are:

1. To compare the inter-study reproducibility of myocardial strain measurements (specifically PEDSR and GLS) derived from CMR cine

images using two different commercially available software packages: cmr42 Tissue tracking and Diogenes Feature Tracking.

2. To comprehensively describe the cardiovascular structural and functional perturbations associated with T2D in a multi-ethnic population using multiparametric CMR, echocardiography, and cardiopulmonary exercise testing (CPET).
3. To identify which clinical factors (e.g. BP, glycaemic control, lipid levels) are independently associated with markers of subclinical cardiovascular dysfunction in people with T2D.
4. To evaluate which markers of subclinical cardiovascular dysfunction are independently related to aerobic exercise capacity (peak VO_2), a strong surrogate marker of outcome in people with T2D.
5. To determine if subclinical cardiovascular dysfunction can be improved by either a low-energy meal replacement diet or a supervised aerobic exercise programme, compared to routine care.

1.7 Original hypotheses

The following hypotheses will be tested:

1. **H_0**: there will be no difference in the inter-study reproducibility of PEDSR measurements derived from CMR SSFP cine images using cmr42 Tissue Tracking compared to Diogenes Feature Tracking.

   H_a: in asymptomatic people with T2D, the inter-study reproducibility of cmr42 Tissue Tracking will be superior to Diogenes Feature tracking for measurement of PEDSR from CMR cine images.
2. **H_0**: When matched for age, sex and ethnicity, there will be no difference in cardiovascular structure or function between people with and without T2D, who have no prior history, signs or symptoms of CVD.

   H_a: In people with T2D and no history, signs or symptoms of CVD, there will already be evidence of subclinical cardiovascular dysfunction (concentric LV remodelling, diastolic and systolic dysfunction, aortic stiffening, and reduced myocardial perfusion) compared to age-, sex- and ethnicity-matched controls.

3. **H_0**: There will be no association between clinical indicators of metabolic dysfunction (e.g. BMI, BP, HbA1c, diabetes duration, and dyslipidaemia) and markers of subclinical cardiovascular dysfunction in adults with T2D.

   H_a: Clinical indicators of metabolic dysfunction, such as BMI, BP, diabetes duration, and lipid levels, will be independently associated with markers of subclinical cardiovascular dysfunction (e.g. LV mass:volume, GLS, PEDSR, aortic distensibility, and MPR) in adults with T2D.

4. **H_0**: In people with T2D, there will be no association between CMR markers of subclinical cardiovascular dysfunction and age- and sex-corrected peak VO_2.

   H_a: In people with T2D, CMR markers of subclinical cardiovascular dysfunction (e.g. LV mass:volume, GLS, PEDSR, aortic distensibility, and MPR) will be independently associated with age- and sex-corrected peak VO_2.

5. **H_0**: Neither a low-energy meal replacement plan (MRP) diet nor aerobic exercise training will cause improvements in subclinical cardiovascular dysfunction in people with T2D, when compared to standard care.

   H_a: A low-energy MRP diet or aerobic exercise training will both lead to favourable but differential improvements in subclinical cardiovascular dysfunction in people with T2D when compared to standard care.

2 General methods

2.1 Overall study design

This was a single-centre study, which comprised a baseline cross-sectional case-control analysis, followed by a three-arm, 12-week, PROBE trial of a low-energy MRP and an exercise programme compared with standard care in adults with T2D (the DIASTOLIC study, see below). Detailed methods of the DIASTOLIC study are presented in **Chapter 7**. Therefore, only those methods pertaining to baseline cross-sectional analyses will be described here.

2.2 Study population

For baseline cross-sectional analyses, participants with and without T2D recruited to four separate studies were included (Table 2.1)(38,261-263). Participants were identified from both primary and secondary care in Leicester, Leicestershire and Rutland using electronic databases and with support from the National Institute for Health Research (NIHR) East Midlands Local Clinical Research Network. At-risk groups, such as slimming clubs/weight watchers and the local South Asian Health Foundation group were also contacted within the local community. Details of each study's key inclusion and exclusion criteria are displayed in Table 2.1 and individuals with absolute contraindications to CMR were also excluded.

Table 2.1 Studies from which participants were included for baseline cross-sectional analyses.

Study title and acronym	Funding	Trial registry/REC reference	Key inclusion/Exclusion criteria	Synopsis
The Emerging Epidemic of Type 2 Diabetes in Young Adults – the EXPEDITION study(38)	Medical Research Council Interdisciplinary Bridging Award	ISRCTN: 60207691 REC: North Nottinghamshire, 09/H0407/9	**Inc:** stable T2D, age 18-39y. **Exc:** angina or limiting dyspnoea (>NYHA class II), history of CVD, arrhythmia, moderate or worse heart valve disease, eGFR <30mL/min/1.73m^2.	Case-control study to assess the cardiovascular, anthropometric and biochemical determinants of diastolic dysfunction in young adults with T2D using multiparametric CMR.
Effects of Liraglutide in Young Adults with Type 2 Diabetes – the LYDIA study(262)	Jointly funded by Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit and by Novo Nordisk.	ClinicalTrials.gov ref: NCT02043054 REC: West Midlands, 13/WM/0311	**Inc:** stable T2D, age 18-50y, BMI ≥30kg/m^2 (≥27 kg/m^2 if south Asian or BME), A1c 6.5-10%. **Exc:** eGFR <30mL/min/1.73m^2, active CVD including MI within past 6 months and/or heart failure (NYHA class III and IV), on GLP-1RA or DPPIVi.	Open-label, randomised, active-comparator trial investigating the cardiometabolic effects of Liraglutide (a GLP-1RA) compared to that of its clinically relevant comparator Sitagliptin (a DPPIVi) in young adults with T2D.
Diabetes Interventional Assessment of Slimming or Training to Lessen Inconspicuous Cardiovascular Dysfunction – the DIASTOLIC study(261)	National Institute for Health Research Career Development Fellowship (G. P. McCann)	ClinicalTrials.gov ref: NCT02590822 REC: West Midlands, 15/WM/0222	**Inc:** stable T2D, age 18-65y, BMI ≥30kg/m^2 (≥27 kg/m^2 if south Asian or BME), A1c 6.5-10%. **Exc:** T2D duration >12y, angina or limiting dyspnoea (>NYHA class II), history of CVD, arrhythmia, moderate or worse heart valve disease, eGFR <30mL/min/1.73m^2.	PROBE trial with nested case-control study: 1) to determine the cause of diastolic dysfunction, assessed by CMR, in young adults with T2D and 2) determine if diastolic dysfunction can be reversed by either a low energy meal replacement diet or an exercise programme.
Prevalence and Determinants of Subclinical Cardiovascular Dysfunction in Adults with Type 2 Diabetes – the PREDICT study(263)	British Heart Foundation Clinical Research Training Fellowship (G. S. Gulsin)	ClinicalTrials.gov ref: NCT03132129 REC: West Midlands, 17/WM/0192	**Inc:** age 18-75y. **Exc:** angina or limiting dyspnoea (>NYHA class II), history of CVD, arrhythmia, moderate or worse heart valve disease, eGFR <30mL/min/1.73m^2.	Cross-sectional study to identify the prevalence of subclinical cardiovascular dysfunction and identify the key aetiological factors in adults with T2D.

Abbreviations: BME=Black or other minority ethnicity; CMR=cardiovascular magnetic resonance imaging; DPPIVi=dipeptidyl peptidase-IV inhibitor; GLP-1RA=glucagon-like peptide-1 receptor agonist; ISRCTN=International Standard Randomised Controlled Trials Number; REC=research ethics committee; T2D=type 2 diabetes.

2.3 Ethical approval and consent to participate

Ethical approval for all included studies was granted by the National Research Ethics Service (Table 2.1). Each study was conducted in accordance with International Conference on Harmonisation-Good Clinical Practice guidelines and the Declaration of Helsinki. All participants provided written informed consent in advance of entering into either study. The study sponsor was University of Leicester.

2.4 Assessments

Eligibility of the potential participant was checked and written informed consent obtained. Participants subsequently underwent bio-anthropometric assessment, comprehensive CMR scanning, transthoracic echocardiography, and CPET, performed as outlined below.

2.4.1 Demographics and bio-anthropometrics

Baseline data including patients' demographics, medical history and anthropometric measures (height, weight, BMI, BP) were collected. A fasting blood sample was taken to check a range of measures including a biochemical profile for diabetes control (fasting glucose and HbA1c), liver and kidney function, and lipid profile. Insulin resistance was estimated using the homeostatic model assessment-insulin resistance method where applicable (HOMA-IR)(264).

2.4.2 CMR scanning

CMR scanning was performed using a standardised protocol (Figure 2.1) on Siemens scanners (Erlangen, Germany) at either 1.5T (Siemens Aera) or 3T (Siemens Skyra) with an 18-channel cardiac coil and retrospective electrocardiographic (ECG) gating. Patients were advised to abstain from caffeine-containing products at least 12 hours prior to CMR scanning.

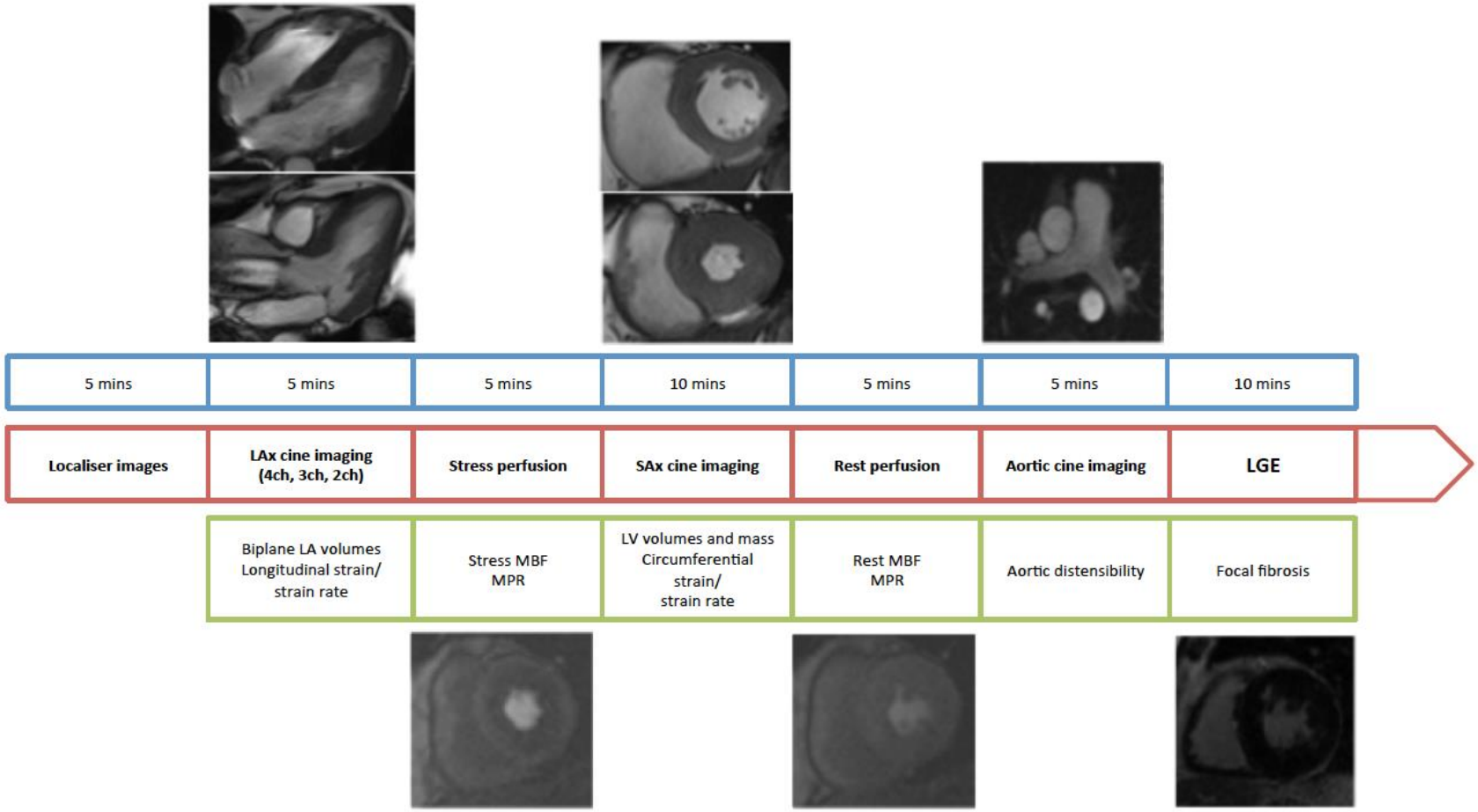


Figure 2.1 Cardiovascular magnetic resonance imaging protocol overview. Abbreviations: LA=left atrium; LAx=long axis; LGE=late gadolinium enhancement; LV=left ventricle; MBF=myocardial blood flow; MPR=myocardial perfusion reserve; SAx=short axis.

2.4.2.1 Cine imaging

After localisers, balanced steady-state free precession (SSFP) cine images were acquired in four-, three- and two-chamber views. After stress imaging (detailed below) a stack of short axis slices was obtained to provide coverage of the entire LV (Figure 2.2). Typical imaging parameters at 3T were: acquisition voxel size 1.66x1.33x8mm, temporal resolution 48ms, repetition time (TR) 3.44 and echo time (TE) 1.55, and at 1.5T: acquisition voxel size 1.90x1.52x8mm, temporal resolution 48ms, TR 2.76 and TE 1.15. All acquisitions were reconstructed to 30 phases.

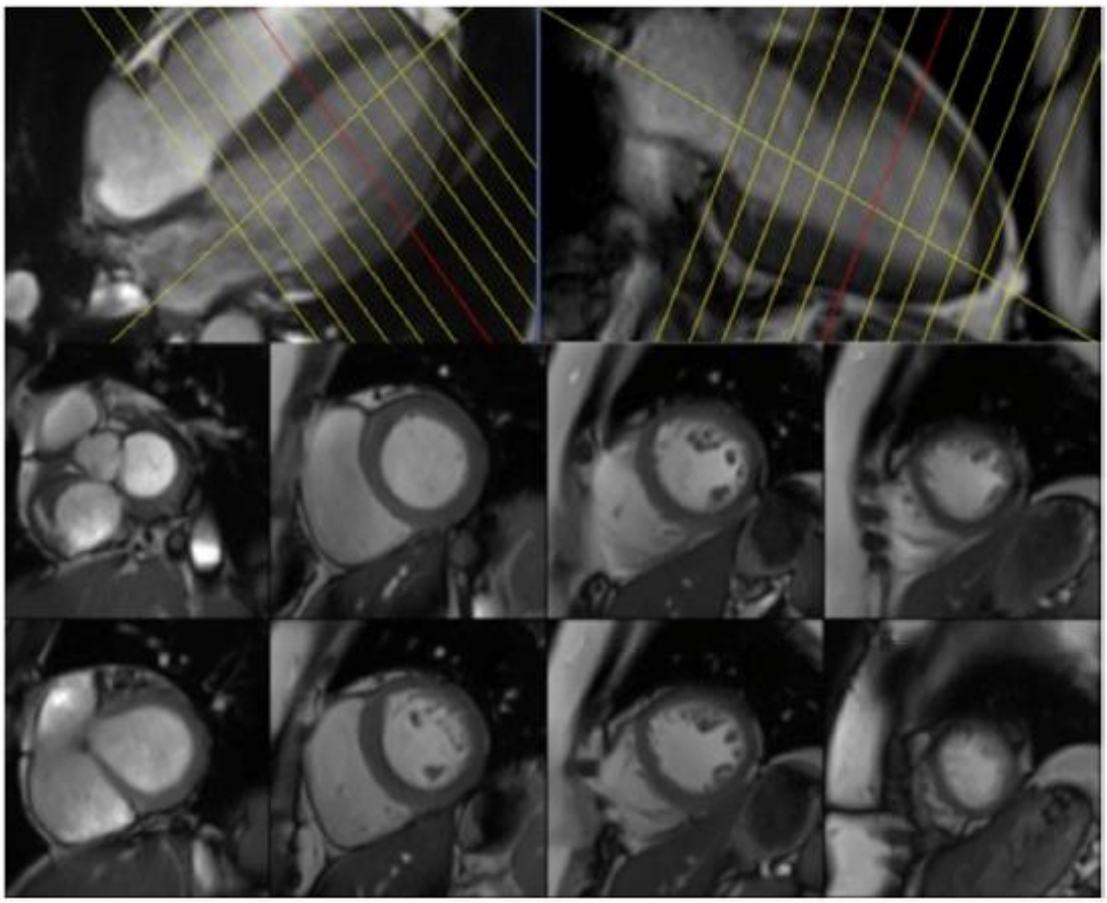


Figure 2.2 Example 4- and 2- chamber steady state free precession cine images, and planning lines for short axis stack. Selected short axis images are also displayed.

2.4.2.2 Perfusion imaging

Stress perfusion images were acquired after vasodilatory stress with adenosine (commencing at 140mcg/kg/min), infused for 3-5 minutes. Patients underwent pulse, BP and pulse oximetry monitoring at baseline and at one-minute intervals during adenosine infusion, with documentation of haemodynamic and symptomatic responses at the time of CMR scanning. A heart rate increase of ≥10 beats per minute

and/or systolic BP fall ≥10mmHg was regarded as an adequate stress response(265,266). Failure to achieve this at two minutes resulted in a dose increase to 170mcg/kg/min and, if required, to a maximum of 210mcg/kg/min. At peak stress, a gadolinium-based contrast agent was injected followed by a 20mL bolus of normal saline, at a rate of 5mL/s, and perfusion images were acquired at three short-axis slices (basal, mid and apical) using a saturation recovery gradient echo pulse sequence (at 1.5T), with signal intensity versus time curves converted concentration using a linear signal response to contrast agent with Fermi-constrained deconvolution(267), or using a dual sequence gradient echo method, with inline automated reconstruction and post-processing (Gadgetron software framework) for MBF quantification (at 3T)(268). Rest imaging was performed approximately 10 minutes after stress.

2.4.2.3 Aortic distensibility

For measurement of aortic distensibility, high temporal resolution SSFP aortic cine images were acquired in a plane perpendicular to the thoracic aorta at the level of the pulmonary artery bifurcation (Figure 2.3). Simultaneous BP was measured using an automatic oscillometric device. Typical imaging parameters were: approximate field of view 320x390mm, acquisition matrix 186x256mm, acquired temporal resolution <50ms, TR 3.1ms and TE 1.2ms, with a parallel imaging factor of 3.

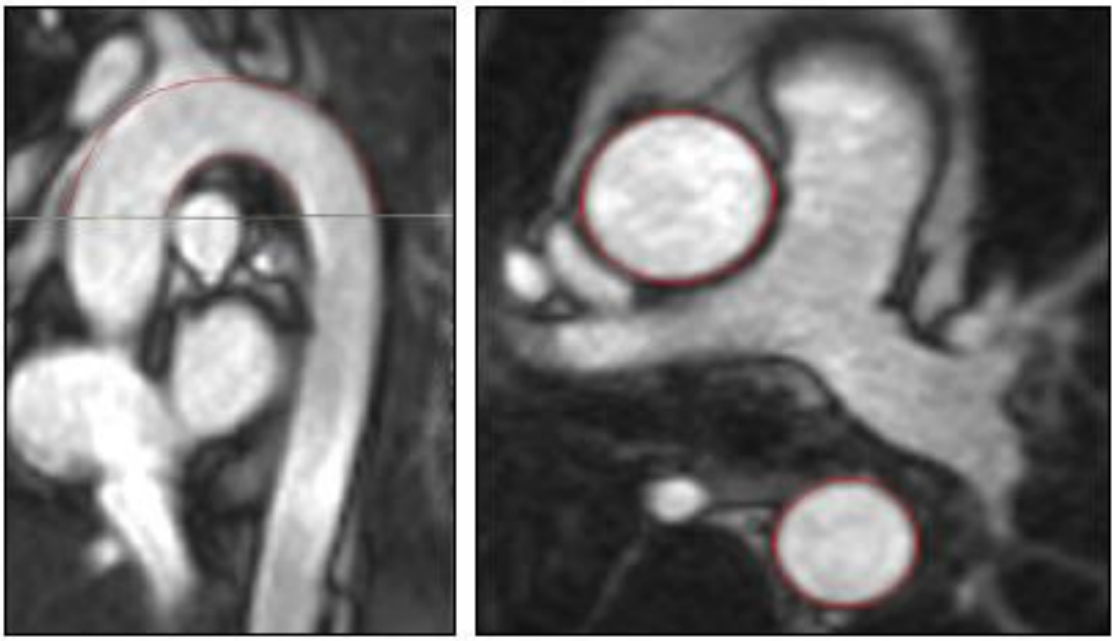


Figure 2.3 Steady state free precession aortic cine images. A sagittal oblique view is acquired, from which a transverse slice of the ascending and descending aorta is imaged, planned at the level of the pulmonary artery bifurcation.

2.4.2.4 Late gadolinium enhancement imaging

Late gadolinium enhancement images in three long axis views and a complete short axis stack were acquired 5-10 minutes after rest perfusion imaging. A Look Locker inversion time (TI) scout was performed on a long axis cine imaging slice position to determine the optimal TI for nulling unaffected myocardium. A segmented, phase-sensitive inversion recovery gradient echo sequence with a two beat trigger was used. The TR was set approximately 100 milliseconds less than the RR interval. The TI was progressively adjusted by 10ms approximately every 1-2 slices to ensure adequate nulling was maintained throughout image acquisition. In the event of an image showing doubtful enhancement, the acquisition was repeated with the phase encoding direction swapped.

2.4.3 CMR image analysis

Scans were anonymised and sent to a standalone workstation for blinded analysis as previously described(38), and all image analysis was undertaken by a single observer (GSG). Image quality was graded according to a four-point scale: 0=not analysable; 1=fair (does have artefact, e.g. breathing/mistriggering, but images still analysable); 2=very good (artefact present, but not in the region of interest); or 3=excellent.

2.4.3.1 LV volumes, mass and function

Left ventricular volumes, mass and function were calculated using commercially available software (cmr42 version 5.10, Circle Cardiovascular Imaging, Calgary, Canada). For quantification, LV epicardial and endocardial borders were contoured at end-diastole and end-systole, to allow calculation of LV end-diastolic volume (EDV), LV end-systolic volume (ESV), LV stroke volume (SV), LV EF and LV mass (Figure 2.4). Values were indexed to body surface area (denoted by the suffix "i"), or height$^{2.7}$.

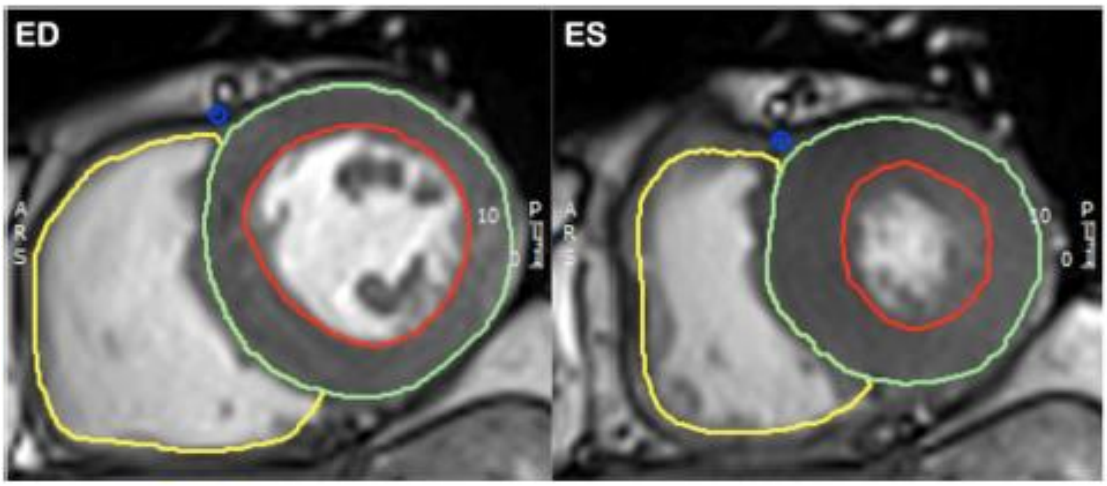


Figure 2.4 An example of left ventricle epicardial (green), endocardial (red), and right ventricular endocardial (yellow) contours at end-diastole (ED) and end-systole (ES) in a mid-ventricular slice position.

2.4.3.2 Myocardial strain

Systolic strain and PEDSR were quantified from cine images using cmr42 Tissue Tracking (version 5.10, Circle Cardiovascular Imaging, Calgary, Canada). Short and long axis SSFP cine images were loaded into the Tissue Tracking module, including the pre-existing endocardial and epicardial end-diastolic contours already defined for prior LV volumetric and function quantification. The superior and inferior right ventricular (RV) insertion points were additionally defined on short axis cine images, as well as the LV extent on long axis cines (Figure 2.5). The software automatically to defined strain measurements by tracking of myocardial features throughout the cardiac cycle. Based on the LV extent defined on long axis cine images, short axis basal and apical slices no longer containing myocardium due to through-plane motion during the cardiac cycle were automatically excluded.

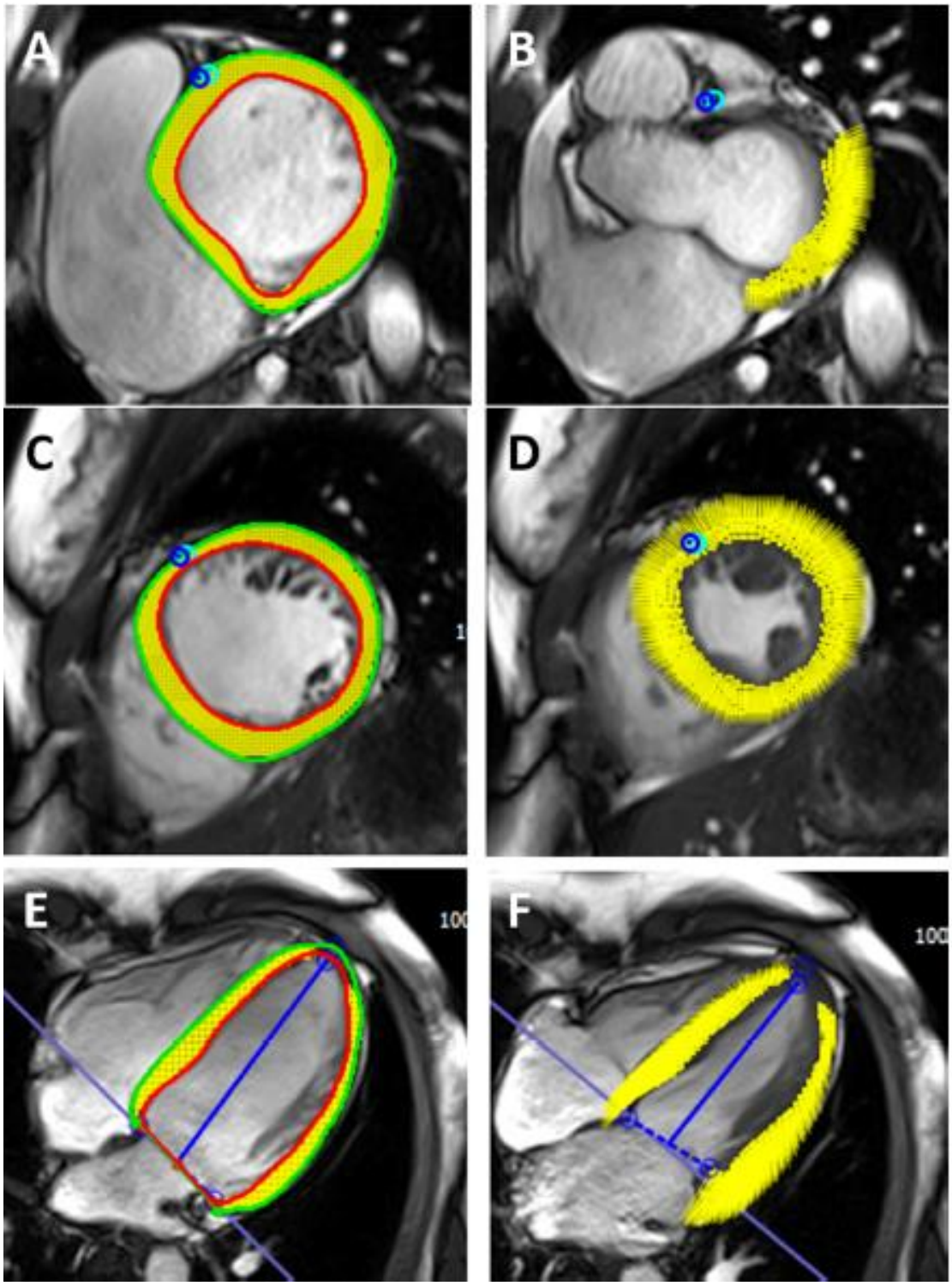


Figure 2.5 Typical myocardial strain images using cmr42 tissue tracking software for A: basal ventricular short axis slice in end-diastole to B: end-systole. C: mid ventricular short axis slice in end-diastole to D: end-systole. E: four chamber long axis image in end-diastole with definition of the LV extent (blue reference lines) to F: end-systole.

Inter-observer and intra-observer variability of myocardial strain measurements were calculated on at least 10 random datasets by two experienced observers (GSG and MGB) with a minimum interval of two weeks.

2.4.3.3 Aortic distensibility

Aortic distensibility was analysed using Java Image Manipulation version 6 (Xinapse Software, Essex, UK), from aortic SSFP cine images. Each sequence contained 30 phases, displaying pulsatile motion of the ascending and descending aorta throughout one cardiac cycle. Using a manual contouring tool, the endovascular-blood pool interface was defined at the ascending and descending aorta every six frames. Contours were then propagated, with manual checking to ensure accurate border definition of the aortic cross-sectional area. The software then calculated the cross-sectional areas of both the ascending and descending aorta at minimal and maximal distention. Aortic distensibility was subsequently calculated by dividing the change in cross sectional areas of both the ascending and descending aorta, by the simultaneously measured brachial pulse pressure per the formula:

$$\textbf{Aortic distensibility } (\text{mmHg}^{-1}\text{x}10^{-3}) = (A_{max} - A_{min})/A_{min} \text{ x pulse pressure}$$

Where A is the aortic cross-sectional area. Mean aortic distensibility was calculated as the average of ascending and descending aortic distensibility. The inter- and intra-observer variability of aortic distensibility measurements have previously been tested using 10 randomly selected datasets, with good or excellent reproducibility(152).

2.4.3.4 Myocardial perfusion

Stress and rest perfusion images were assessed qualitatively by two observers (GSG and GPM) for the presence of reversible perfusion defects as per Society for Cardiovascular Magnetic Resonance guidance(269). If present, perfusion defects were categorized into ischaemia likely due to epicardial coronary artery disease or microvascular dysfunction.

For perfusion imaging at 3T, MBF was calculated on a pixel-wise basis using motion-corrected signal intensity flow maps generated within the Gadgetron software framework(268) (Figure 2.6). Endo- and epicardial contours and RV insertion points were defined manually for each slice (base, mid and apex) on stress

and rest images. A 10% endocardial and epicardial offset was applied to minimise partial volume effects.

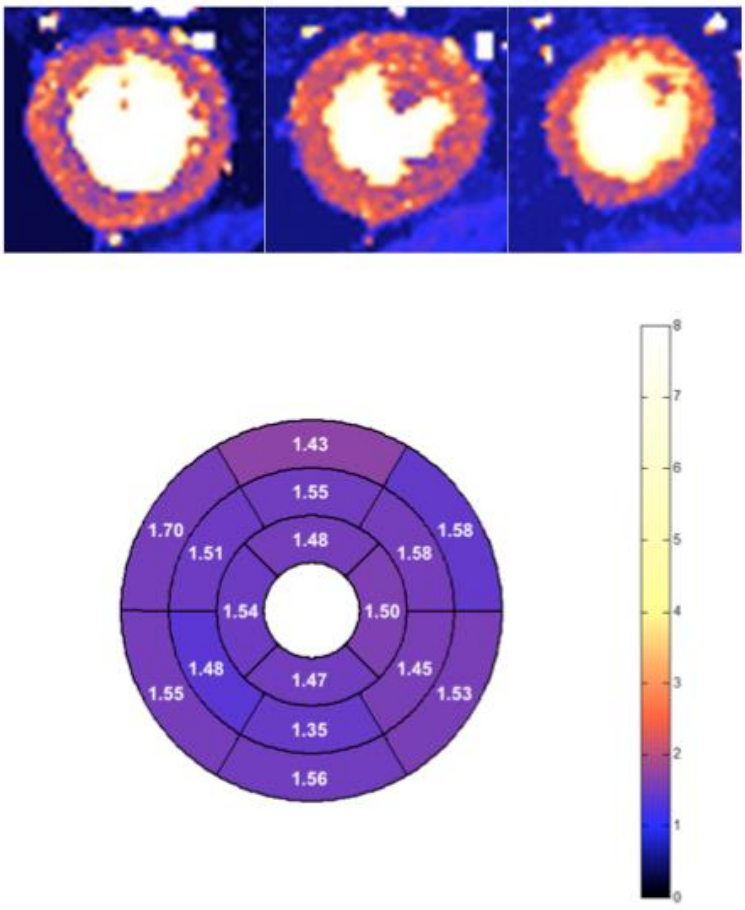


Figure 2.6 Cardiovascular magnetic resonance perfusion imaging at 3T. Representative example of automatically generated flow maps at base, mid, and apical slice positions (top), with resulting myocardial blood flow values (mL/g/min) in each of the 16 left ventricular segments (bottom). Images are from a subject with type 2 diabetes at peak stress.

For images acquired at 1.5T, the epicardium and endocardium were manually contoured at base, mid and apical short axis slice positions, on rest and stress perfusion images. A region of interest in the LV blood pool was also defined. Signal intensity versus time curves were subsequently generated for the myocardium and blood pool (Figure 2.7) and resulting outputs transferred to Dr John D. Biglands (University of Leeds) for absolute MBF quantification. The arterial input function (contrast baseline) was obtained from the basal slice. All pre-contrast signal estimates were taken from the stress study. To avoid remnant contrast agent

from the stress perfusion scan affecting the rest perfusion analysis the pre-contrast signal intensity was subtracted from the rest perfusion curves before analysis. Automated methods were used to subtract the pre-contrast baseline from the signal intensity versus time curves, correct for differences in contrast bolus arrival time and heart rate between the arterial input function and the myocardium, and to limit the data to the first pass of contrast through the myocardium(270).

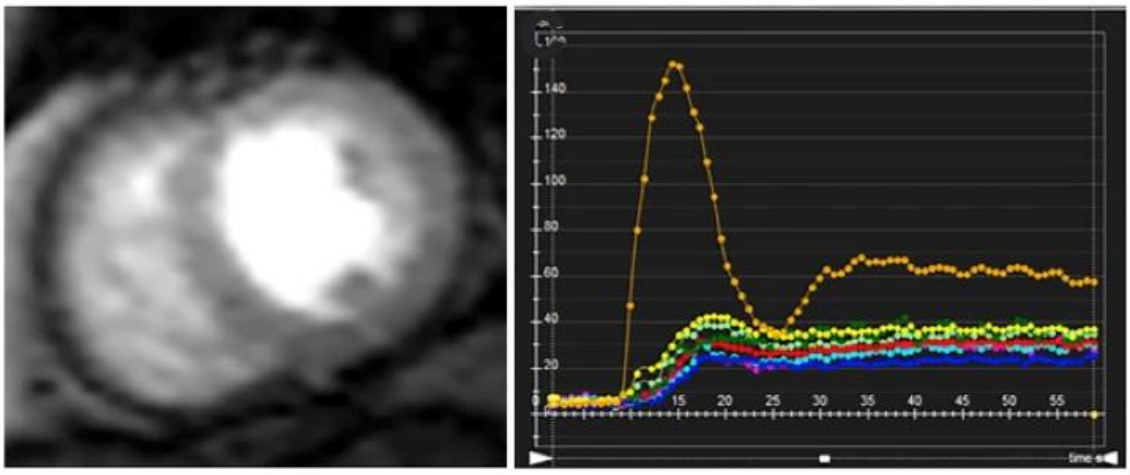


Figure 2.7 Cardiovascular magnetic resonance perfusion imaging at 1.5T. Representative example shown is a mid-ventricular slice imaged at peak stress in a subject with type 2 diabetes. Coloured lines represent signal intensity versus time curves in the blood pool (orange) and myocardial segments (coloured).

Stress and rest MBF at basal, mid, and apical slices were averaged to derive global stress and rest MBF, and determine MPR (calculated as global stress MBF/global rest MBF).

2.4.3.5 Late gadolinium enhancement images

Late gadolinium enhancement images were assessed by two observers (GSG and GPM) for focal fibrosis and categorized as present or absent. If present, fibrosis was further dichotomised into either infarct or non-infarct pattern fibrosis. Infarct was defined as area(s) of LGE hyper-enhancement present in orthogonal long- and short-axis images and involving at least the sub-endocardium in a coronary artery distribution(269).

2.4.4 Transthoracic echocardiography

Comprehensive transthoracic echocardiography was performed according to British Society of Echocardiography guidelines(271) by one of three accredited operators (A-MM, JM, and MSS) using an iE33 system with S5-1 transducer (Philips

Medical Systems, Best, The Netherlands). Each participant underwent assessment for: tissue Doppler indices of diastolic filling, exclusion of valvular abnormalities, assessment of LV size and function. Given the difficulties in grading diastolic dysfunction in mild and moderate disease(88), the primary focus on echocardiography was on E/e', a non-invasive surrogate of LV filling pressure(272).

2.4.5 Cardiopulmonary exercise testing

A symptom-limited incremental CPET was performed on a stationary electromagnetically braked cycle ergometer with expired gas analysis to determine peak VO_2(273). One-minute workload increments were based on patient age, sex, height and weight(273). Each test was physician supervised (predominantly GSG) with continuous ECG monitoring and BP recording at two-minute intervals. Indications for medical termination were as previously described(274). Subjects with ST-segment ECG changes indicative of myocardial ischaemia during exercise testing were excluded from subsequent analyses. Peak VO_2 was determined as the average of 30s using breath-by-breath data. Indications for medical termination were as previously described(274). A quality control cardiopulmonary exercise test is undertaken every six weeks using a biological control in our unit. The coefficient of variation for VO_2 (L/min) during steady state at workloads of 75, 100, 125 and 150 watts is consistently <8%.

2.5 Patient and public involvement

The University of Leicester and the Leicester NIHR Biomedical Research Centre have very active patient and public involvement (PPI) groups in both Diabetes and Cardiovascular Sciences. The study outline was presented to our PPI groups for feedback prior to designing the study protocol. In addition, PPI focus groups were held about the study design and conduct before the protocol was finalised. The PPI groups discussed the following:

1. Initially it was planned not to provide clinical reports of the results of imaging studies performed on study participants, as these investigations were not indicated on clinical grounds. Members of both the Diabetes and Cardiovascular Sciences PPI groups expressed that the results of study investigations should be made available to participants for two main reasons: i) they may reveal underlying pathology (cardiovascular and other)

amenable to intervention and from which a direct clinical benefit could be derived, and ii) as an incentive to participate in the research, likely increasing recruitment numbers. As a consequence of this discussion, clinical reports of all stress CMR scans were provided to participants and their General Practitioners. Participants were also invited to view their own imaging studies with the fellow (GSG).

2. The burden of the exercise and acceptability of the dietary intervention arms of the DIASTOLIC study on study participants to ensure appropriate support was available to help compliance with these interventions. The frequency of clinical reviews during trial interventions was determined after these discussions (**Chapter 7**).

Furthermore, the DIASTOLIC trial steering committee (see **Chapter 7**) had at least two lay members for study oversight.

2.6 Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS), version 25 (IBM Corp, Armonk, New York), performed by GSG, unless otherwise stated. Normality was assessed using histograms, the Shapiro-Wilk test and Q-Q plots. Continuous data are expressed as mean (± standard deviation), if normally distributed or median (25-75% interquartile range) if not. Multiple imputation was used to impute missing CMR and echocardiography data, where appropriate. A p value <0.05 was considered statistically significant, unless otherwise stated.

Patient and control groups were compared by independent *t*-tests or Mann-Whitney tests as appropriate. Categorical variables were compared using the Chi-squared test. Biochemical, CMR, echocardiography and CPET variable between-group comparisons were undertaken using a general linear univariate analysis of variance, with adjustments for variables age, sex and ethnic group.

Correlations were assessed using Pearson's or Spearman's correlation coefficients, where appropriate. Multivariable linear regression analysis using generalised linear modelling was performed to identify independent associations of specified dependent variables separately in patients with and without T2D.

2.6.1 Reproducibility and observer variability

Inter-study reproducibility of CMR measures was compared using paired t-tests, coefficient of variation (CoV), intraclass correlation coefficients (ICCs), and the Bland-Altman method(275). A CoV of <10% was considered excellent, <15% considered good, <20% average and >20% poor. Interpretation of ICCs were: <0.40 poor, 0.40-0.59 fair, 0.60-0.74 good and ≥0.75 excellent(276). Intra- and inter-observer reproducibility of CMR measures were compared using Bland-Altman plots and ICCs.

3 A comparison of the inter-study reproducibility of two cine-derived strain software programmes in people with type 2 diabetes

3.1 Abstract

3.1.1 Background

Myocardial systolic strain and early diastolic strain rates are increasingly recognised measures of subclinical cardiac dysfunction, but the inter-study reproducibility of myocardial strain measures derived from CMR SSFP cine images using different software packages is not known.

3.1.2 Objective

To compare the test-retest reproducibility of LV strain measures derived from two commercially available software packages in people with T2D.

3.1.3 Research design and methods

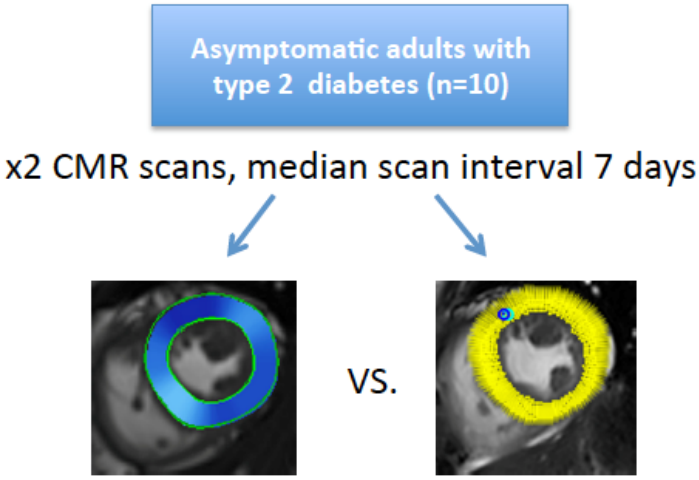
We prospectively enrolled 10 subjects with T2D and no prevalent cardiovascular disease. Participants underwent baseline and repeat CMR scanning at 1.5T with SSFP long- and short-axis cine imaging within two weeks. Inter-study reproducibility of GLS and PEDSR measured using cmr42 Tissue Tracking and Diogenes Feature Tracking were compared.

3.1.4 Results

The absolute values of GLS and PEDSR were consistently higher using Feature Tracking compared with Tissue Tracking (both $p<0.001$). The test-retest reproducibility of GLS was similar for Feature Tracking and Tissue Tracking (CoV 6.4 and 4.8%, respectively, $p>0.05$). However, the reproducibility of Feature Tracking-derived PEDSR was worse than that of Tissue Tracking (CoV 18 and 11.7%, respectively, $p=0.02$). Bland-Altman analysis revealed no systematic bias with either software package, with tighter limits of agreement for Tissue Tracking-derived PEDSR.

3.1.5 Conclusions

In asymptomatic people with T2D and normal ejection fraction, CMR cine-derived quantification of GLS is highly reproducible with both Feature Tracking and Tissue Tracking software. However, Tissue Tracking is superior to Feature Tracking for measurement of PEDSR, with excellent test-retest reproducibility.



	Scan 1	Scan 2	CoV (%)	Bias ± SD difference	BA limits of agreement
Feature Tracking					
GLS, %	-22.5±3.2	-23.4±3.9	6.4	0.85±2.9	-4.9, 6.6
PEDSR, s^{-1}	2.0±0.6	1.8±0.6	18	-0.23±0.7	-1.6, 1.1
Tissue Tracking					
GLS, %	-16.2±2.1	-16.3±3.2	4.8	-0.03±1.7	-3.3, 3.2
PEDSR, s^{-1}	0.84±0.14	1.0±0.1	11.7	-0.02±2.1	-0.4, 0.03

Figure 3.1 Inter-study reproducibility of cardiovascular magnetic resonance imaging cine-derived global longitudinal strain and peak early diastolic strain rate, comparing Feature Tracking with Tissue Tracking. Abbreviations: BA=Bland-Altman; CoV=coefficient of variation; GLS=global longitudinal strain; PEDSR=peak early diastolic strain rate.

3.2 Introduction

Echocardiography remains the most widely used modality for the assessment of myocardial systolic and diastolic function. In routine clinical practice, LV EF is the commonest descriptor of cardiac systolic function and is relied upon to direct clinical decisions in most cardiac conditions(277,278). The usual calculation of LV EF by two-dimensional echocardiography is based upon geometric assumptions that do not take into consideration the complex architecture of the myocardium in different planes and its value may therefore not express true contractility(279). Similarly, the commonest echocardiographic measure of diastolic function - the ratio of early mitral inflow to tissue velocity of the mitral annulus (E/e') – is only an indirect measure of LV filling pressure. Despite having the strongest correlation with invasively measured LV filling pressures of all echocardiographic measures of diastolic function, the correlation of E/e' with invasive filling pressures is modest at best(272).

Myocardial deformation analysis, by speckle tracking echocardiography and CMR provide a direct assessment of regional contraction and relaxation of the LV. Deformation of myocardial segments ("strain") is described in circumferential, radial and longitudinal planes, and represents a more integrated view of myocardial mechanics than EF(280-282). Importantly, LV systolic strain and early diastolic strain rates are increasingly recognised measures of subclinical cardiac dysfunction, providing incremental prognostic and earlier diagnostic information in disease states in multiple studies(283-287).

Myocardial strain can be assessed using CMR with several techniques including: myocardial tissue tagging; phase velocity mapping; displacement-encoding with stimulated echoes; and strain encoded images. All these techniques require acquisition of additional sequences and lengthy post-processing, limiting their clinical utility(288-290). Software developments now allow assessment of LV strain measure from routinely acquired SSFP cine images, shortening scan times and streamlining analysis(291). Strain derived from these methods have been shown to have good agreement with strain derived from speckle tracking echocardiography(292,293) and reasonable agreement compared to strain derived from CMR tagging methods(294-296). We therefore selected LV PEDSR, a CMR SSFP

cine image derived measure of the speed of myocardial relaxation, as the primary outcome measure in the DIASTOLIC study (**Chapter 7**).

A variety of software packages now allow quantification of myocardial strain indices from SSFP cine sequences, each of which automatically tracks image features at the cavity-tissue interface throughout the cardiac cycle in a similar manner to speckle tracking echocardiography(297). However, the comparable test-retest reproducibility of cine-derived strain techniques in people with T2D is not known, which will be an important consideration for selecting which technique is best-suited to analyse the primary outcome measure, PEDSR, in the DIASTOLIC trial.

The aim of this study was to compare the test-retest reproducibility of LV strain measures derived from two commercially available software packages in a subset of individuals from our asymptomatic cohort of people with T2D.

3.3 Research design and methods

A detailed description of the general study methods and analyses is provided in **Chapter 2**.

3.3.1 Study participants

Ten individuals with T2D prospectively enrolled to the DIASTOLIC study gave additional written informed consent to return for a repeat non-contrast CMR scan within 14 days of their initial CMR study.

3.3.2 CMR image acquisition

All CMR studies were conducted at 1.5T (Siemens Aera, Erlangen, Germany). SSFP end-expiratory breath-held cine images were acquired, with retrospective ECG triggering and an 18-channel cardiac coil. Images were acquired in four-, three- and two-chamber views, in addition to a short-axis stack covering the entire LV using the typical parameters described in **Chapter 2**.

3.3.3 Image analysis

All analysis was performed offline, blinded to participant and scan details. LV mass and volumetric analyses were performed as described in **Chapter 2** using cmr42 version 5.10 (Circle Cardiovascular Imaging, Calgary, Canada). Image quality of LV short and long axis cines was categorized as “excellent”: no artefact in any images; “good”: artefact present but not in the region of interest, “moderate”: artefact present in the region of interest but images still analysable; and “unanalysable”:

artefact renders the scan uninterpretable. LV GLS and circumferential PEDSR were calculated using Tissue Tracking and Feature Tracking software, as outlined below.

3.3.3.1 Tissue Tracking strain analysis, cmr42 (Circle Cardiovascular Imaging)

Short and long axis cine images were loaded into the cmr42 Tissue Tracking module. These included the original endocardial and epicardial end-diastolic contours, defined for prior volumetric and function quantification. The superior and inferior RV insertion points were additionally defined on the short axis cines and the LV extent on long axis cines, as described in **Chapter 2**. The software automatically tracked features throughout the cardiac cycle to define GLS and PEDSR. All Tissue Tracking strain analysis was carried out by a single, blinded observer (GSG).

3.3.3.2 Feature Tracking strain analysis, Diogenes (TomTec)

Short and long-axis cine images were loaded into the Diogenes Feature Tracking software package. Endo- and epicardial contours were drawn on a single end-diastolic phase and propagated automatically by the software to all phases (Figure 3.2), generating endocardial strain and strain rate curves. In cases of poor border tracking, manual adjustment was performed and the images re-propagated. No further post-processing was required and the software automatically generated GLS and PEDSR graphs. Details of the algorithm used by this software has been described previously(294). All Feature Tracking strain analysis was carried out by a single, blinded observer (GSG).

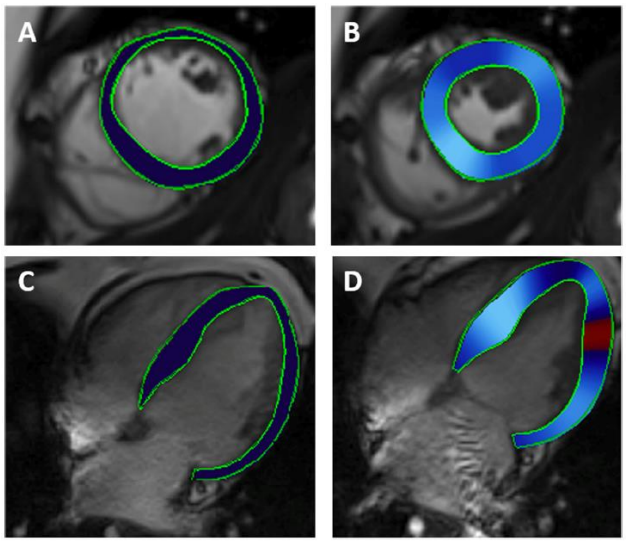


Figure 3.2 A representative image using Diogenes Feature Tracking software for A: mid-ventricular short-axis slice in end-diastole to B: end-systole. C: four-chamber long-axis in end-diastole to D: end-systole.

3.3.4 Specific statistical analysis

A general description of statistical methods is detailed in **Chapter 2**. Test-retest reproducibility was compared using paired t-tests, CoVs and the Bland-Altman method(275). For each subject and technique, the squared difference between test-retest results was used as an estimate of the within-subject variance for the method. This was then log-transformed and a paired t-test method was used to compare the test 1- and test 2-squared differences, as previously described(298). Statistical analysis was performed using SPSS version 25 (Chicago, IL, USA).

3.4 Results

Baseline demographic characteristics and LV mass of volumes data of included participants are displayed in Table 3.1, below. Median duration between baseline and repeat CMR studies was seven days. Image quality was rated as excellent (n=4) or good (n=16).

Table 3.1 Baseline demographic characteristics and left ventricular mass and volumes.

Type 2 diabetes (n=10)	
Demographics	
Age, years	49±8
Sex, n (%) male	5 (50)
Body mass index, kg/m^2	36.2±3.7
Systolic blood pressure, mmHg	147±20
Diastolic blood pressure, mmHg	89±7
Heart rate, beats/min	72±12
LV mass and volumes	
Ejection fraction, %	61.3±5.9
Mass, grams	111.7±24.2
Mass index, g/m^2	57.8±13.5
End diastolic volume, mL	164±33
Mass:volume, mL/m^2	0.69±0.10

Abbreviations: LV=left ventricle.

3.4.1 Comparison of absolute strain values acquired using Tissue Tracking and Feature Tracking

Mean GLS was consistently higher using Feature Tracking compared to Tissue Tracking (-22.5±3.2 vs. -16.2±2.1%, respectively for scan 1, $p<0.001$). Similarly, mean PEDSR was consistently higher using Feature Tracking compared to Tissue Tracking (2.0±0.6 vs. 0.84±0.14, respectively for scan 1, $p<0.001$).

3.4.2 Test-retest reproducibility of Tissue Tracking and Feature Tracking strain measurements

The inter-study reproducibility of Tissue Tracking and Feature Tracking derived GLS and PEDSR are shown in Table 3.2. Reproducibility of GLS was similar for both software packages ($p>0.05$). The reproducibility of PEDSR, however, was worse for Feature Tracking than for Tissue Tracking ($p=0.02$).

Table 3.2 Inter-study reproducibility of global longitudinal strain and peak early diastolic strain rate assessed by Feature Tracking and Tissue Tracking in 10 subjects with type 2 diabetes.

	Scan 1	Scan 2	CoV (%)	Bias ± SD difference	BA limits of agreement
Feature Tracking					
GLS, %	-22.5±3.2	-23.4±3.9	6.4	0.85±2.9	-4.9, 6.6
PEDSR, s^{-1}	2.0±0.6	1.8±0.6	18	-0.23±0.7	-1.6, 1.1
Tissue Tracking					
GLS, %	-16.2±2.1	-16.3±3.2	4.8	-0.03±1.7	-3.3, 3.2
PEDSR, s^{-1}	0.84±0.14	1.0±0.1	11.7	-0.02±2.1	-0.4, 0.03

Abbreviations: GLS=global longitudinal strain, PEDSR=peak early diastolic strain rate.

Bland-Altman analysis did not reveal any systematic bias for GLS or PEDSR (**Error! Reference source not found.**). The limits of agreement were tighter for Tissue Tracking PEDSR than Feature Tracking PEDSR.

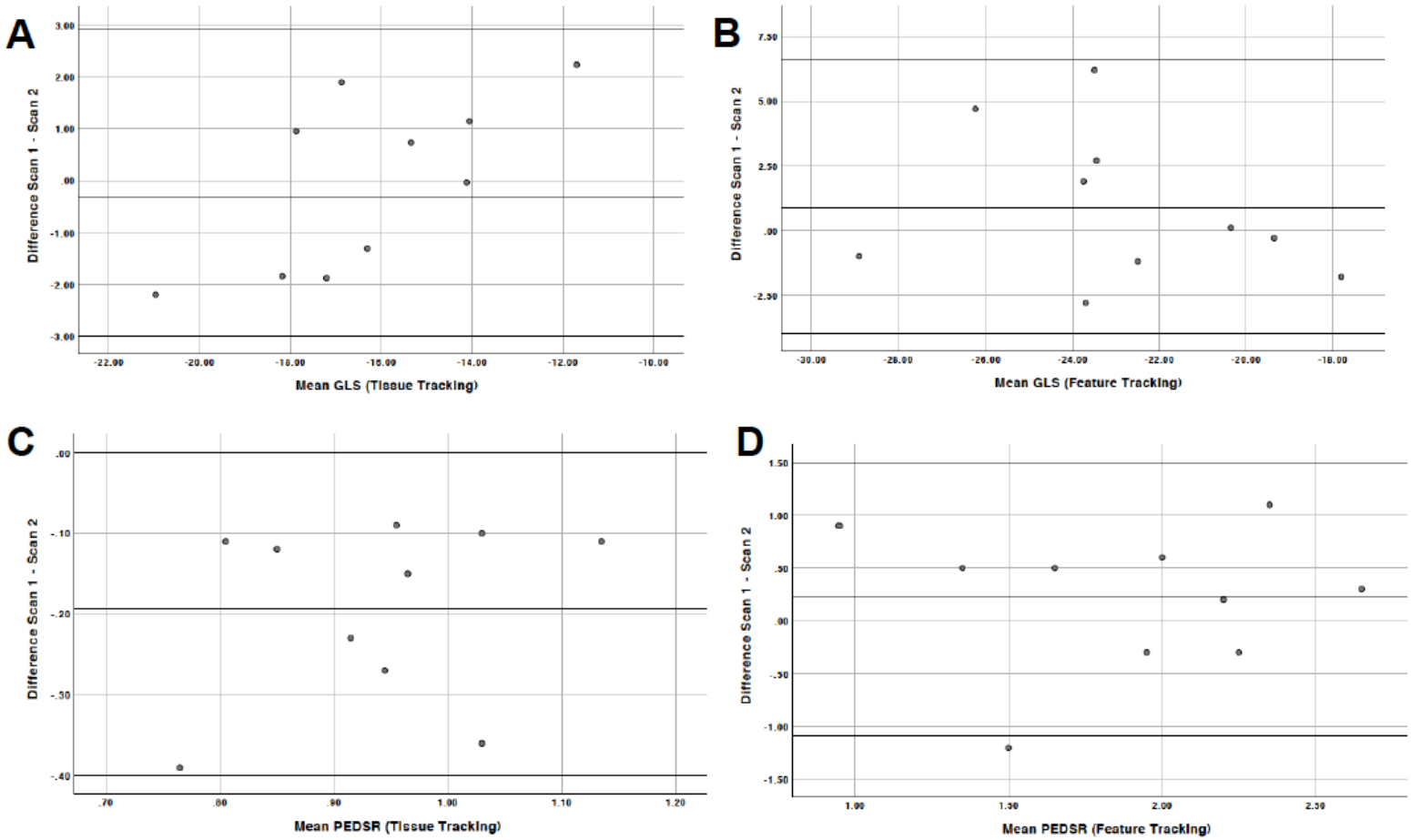


Figure 3.3 Bland-Altman analysis of inter-study reproducibility of Tissue Tracking (plots A and C) and Feature Tracking (plots B and D) derived left ventricular global longitudinal strain and peak early diastolic strain rate. Abbreviations: GLS=global longitudinal strain; PEDSR=peak early diastolic strain rate.

3.5 Discussion

This study compared the inter-study reproducibility of GLS and PEDSR derived from routinely acquired CMR SSFP cine images between two commercially available software packages in 10 subjects with T2D. We found the reproducibility of GLS derived from Feature Tracking and Tissue Tracking to be equivalent. For PEDSR, however, the reproducibility of Tissue Tracking was superior to that of Feature Tracking. Both GLS and PEDSR strain values were higher for Feature Tracking than Tissue Tracking.

Our findings suggest that PEDSR measurement using Tissue Tracking is the preferred method for assessment of diastolic function in our subjects with T2D. Furthermore, neither systolic nor diastolic strain measures are interchangeable between software vendors. This has important implications for the DIASTOLIC study, where PEDSR is the chosen primary endpoint pre- and post-intervention. Clearly the technique with the highest reproducibility will minimise the risk of measurement errors and we have therefore selected Tissue Tracking for this purpose.

That there were absolute differences in GLS and PEDSR between the two software packages is consistent with previous studies showing that strain values derived from Feature Tracking analysis tend to be higher than those derived from tissue tagging techniques(296). The wider implications for these findings are that they suggest a requirement for standardisation of cine-derived strain assessment across CMR platforms and software packages before these novel measures may be adopted in clinical practice. Furthermore, imaging units will need to define their own reference ranges for normal strain values to enable the differentiation between health and disease. For this reason, significant efforts have been made to establish normal strain values for different software vendors in echocardiographic studies(299). Indeed assessment of GLS with echocardiography is now a recommended part of multi-modality imaging evaluation of adult patients responses to cancer therapies(300).

Although there have been significant advances in the quantification of strain/strain rates with the advent of tracking techniques based on routinely acquired cine images, these issues will continue to hinder their real-world application and limit their diagnostic and prognostic value. In the DIASTOLIC trial

we have therefore opted to include a nested case-control study, to enable us to determine whether PEDSR is indeed abnormal in our cohort with T2D and support our assertion that this group has diastolic dysfunction.

3.5.1 Limitations

This study has several important limitations. We have not compared strain measures derived from SSFP cine images with those from myocardial tissue tagging, nor have we compared these with echocardiographic strain measures. However, we have purposely selected CMR cine-based strain assessment techniques to mitigate the requirement for additional acquisitions and to shorten post-processing times. Furthermore we have already shown that Tissue Tracking gives higher reproducibility than tagging in patients with aortic stenosis and in patients post myocardial infarction(301,302). As our cohort with T2D will also have obesity, we are conscious that a substantial proportion of them will have limited acoustic windows for echocardiographic evaluation of diastolic function, which is not a limitation of CMR. Lastly, we have not included inter-observer variability in these analyses, as we have demonstrated excellent inter-observer agreement for all strain measures in wider cohort of different disease states(303).

3.6 Conclusions

In asymptomatic people with T2D and normal EF, CMR cine-derived quantification of GLS is highly reproducible with both Feature Tracking and Tissue Tracking software. However, Tissue Tracking was superior to Feature Tracking for measurement of PEDSR, with excellent test-retest reproducibility. CMR Tissue Tracking is therefore the preferred method of assessment of diastolic function by CMR in the DIASTOLIC study; a trial of diet or exercises versus standard care in asymptomatic people with T2D.

4 A cross-sectional comparison of cardiovascular function in people with and without type 2 diabetes

4.1 Abstract

4.1.1 Background

Heart failure is a leading cause of morbidity and mortality in T2D. There is a high prevalence of asymptomatic cardiovascular dysfunction in adults with T2D, placing this group at impending risk of developing clinical heart failure.

4.1.2 Objective

To confirm and characterise the presence of subclinical cardiovascular alterations in our regional multi-ethnic population of adults with T2D.

4.1.3 Research design and methods

Cross-sectional study. We prospectively enrolled adults with T2D and no history, signs or symptoms of cardiovascular disease, representative of our regional population. Age-, sex-, and ethnicity-matched controls were recruited for comparison. Participants underwent bio-anthropometric profiling, echocardiography, CPET, and stress perfusion CMR imaging.

4.1.4 Results

Two hundred and forty seven adults with T2D (age 51.8±11.9 years, 55% males, HbA1c 7.4±1.1%, duration of diabetes 61 (32 – 120) months) and 78 matched controls were included. Subjects with T2D had increased concentric LV remodelling (LV mass:volume 0.84±0.14 vs. 0.76±0.11 g/mL, $p<0.001$), lower aortic distensibility (2.75 (1.74 – 4.03) vs. 4.92 (2.65 – 7.13) $mmHg^{-1}x10^{-3}$, $p<0.001$), impaired systolic (GLS -16.2±2.4 vs. −17.4±1.9%, $p<0.001$) and diastolic dysfunction (E/A ratio 0.84 (0.66 – 1.05) vs. 1.10 (0.83 – 1.23), $p=0.006$), and reduced MPR (2.60±1.24 vs. 3.54±1.15, $p<0.001$) compared with controls.

4.1.5 Conclusion

Asymptomatic adults with T2D already have evidence of subclinical cardiovascular dysfunction, with concentric LV remodelling, arterial stiffening, systolic and diastolic dysfunction, and reduced MPR, when compared with matched controls.

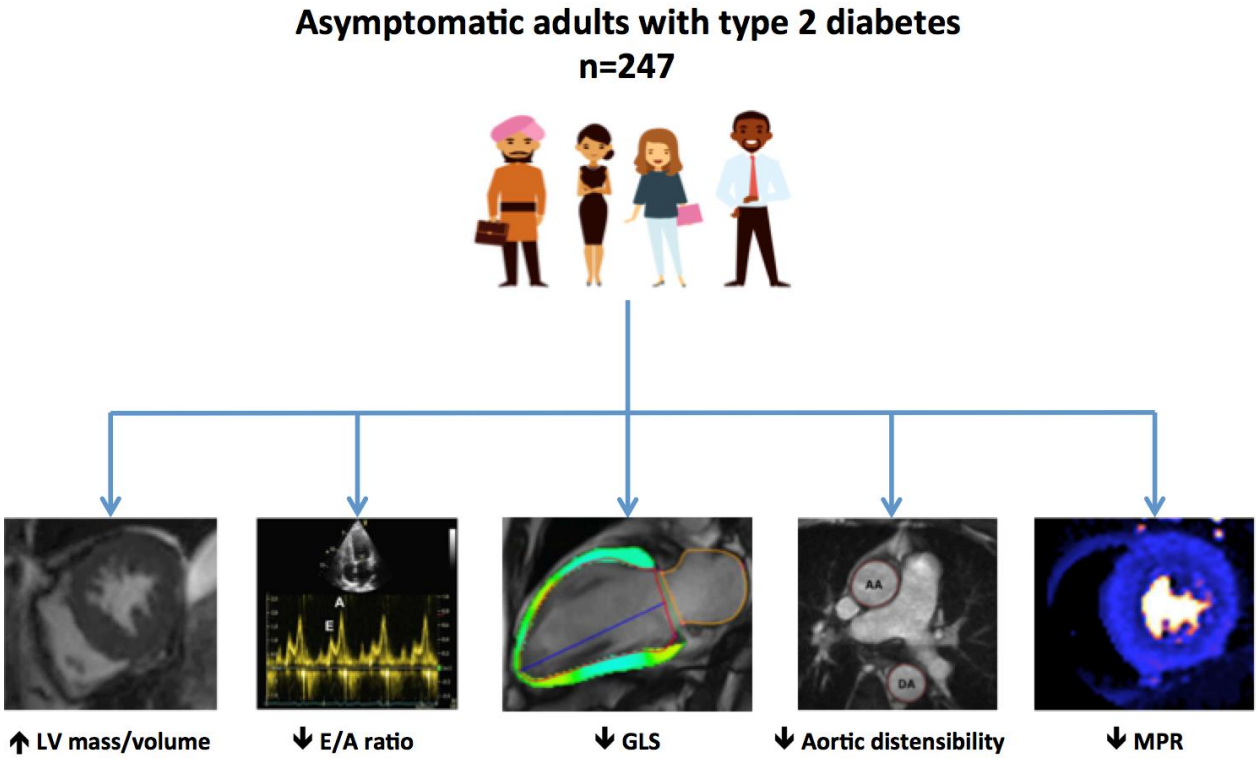


Figure 4.1 In a case-control comparison of 247 asymptomatic adults with type 2 diabetes and 78 matched controls, subjects with diabetes had evidence of concentric left ventricular remodelling, diastolic and systolic dysfunction, increased arterial stiffening and lower myocardial perfusion reserve. Abbreviations: GLS=global longitudinal strain, MPR=myocardial perfusion reserve.

4.2 Introduction

In asymptomatic individuals with T2D there is a high prevalence of LV systolic and diastolic dysfunction or cardiac remodelling(68,69). The AHA has classified such individuals as having stage B heart failure(304). Isolated abnormalities of LV diastolic dysfunction and reduced GLS are associated with incident heart failure in T2D, and their identification may permit earlier identification and treatment of patients most at risk(305). However, there are numerous inconsistencies in the prevalence of asymptomatic LV dysfunction and remodelling in subjects with T2D. Reported prevalence rates vary from 15 to 78% and differ according to the technique(s) used for diagnosis(68-70). Therefore, characterisation of subclinical cardiovascular dysfunction in our own, ethnically diverse, regional population is vitally important.

Cardiovascular magnetic resonance imaging is the gold standard imaging modality for assessment of cardiac volumes, mass and EF, aortic stiffness, and with the addition of stress perfusion imaging has the ability to provide accurate quantification of MBF. By combining CMR with echocardiography and CPET, comprehensive cardiovascular phenotyping of asymptomatic adults with T2D is possible.

The aim of this study was to identify the presence and nature of subclinical cardiovascular dysfunction associated with T2D in our regional population, using multiparametric CMR, echocardiography and CPET.

4.3 Research design and methods

Detailed descriptions of the study methods and analyses are provided in **Chapter 2**.

4.3.1 Study participants

This was a pooled analysis of individual baseline patient data from participants recruited to one of four studies, as described in **Chapter 2** and Table 2.1. Participants included in these analyses were aged 18 to 75 years, with no prior history, clinical signs or symptoms of cardiovascular disease and no contraindications to CMR imaging or CPET. Exclusion criteria were: type 1 diabetes, stage 4 or 5 chronic kidney disease (estimated glomerular filtration rate <30mL/min/1.73m^2), known macrovascular disease (including myocardial infarction, transient ischaemic attack, stroke, peripheral artery disease), presence of arrhythmia, history of heart failure, moderate or worse valvular heart disease, cardiovascular symptoms (such as angina or limiting dyspnoea during normal physical activity). Age-, sex- and ethnicity-matched controls without dysglycaemia and free of prevalent cardiovascular disease were recruited for comparison.

4.3.2 Assessments

All subjects had demographics, medical history, anthropometric measures, fasting blood tests, transthoracic echocardiography, CMR scanning and CPET performed in a single assessment visit. A detailed description of all study assessments is provided in **Chapter 2**.

4.3.3 Inter-study reproducibility of myocardial blood flow measurement using quantitative MRI perfusion mapping

Ten subjects with T2D underwent repeat CMR stress perfusion imaging at 3T within 1-3 weeks for assessment of inter-study reproducibility of MBF quantification by quantitative perfusion mapping. Intra- and inter-observer variability of MBF quantification were assessed by repeating the analyses in the same ten subjects with T2D after a minimum interval of one month by the same observer (GSG) and by a second observer (AMA).

4.3.4 Statistical analysis

All statistical analyses were performed as described in **Chapter 2**.

4.4 Results

The study profile is displayed in Figure 4.2 . At baseline 259 subjects with T2D and 85 controls were recruited. Twelve subjects with T2D were excluded after consent and reasons for ineligibility are shown in Figure 4.2 . A total of 247 subjects with T2D were therefore included in the analysis. Eighty-five healthy volunteers were enrolled for case-control comparison. Seven of these were subsequently excluded (three after blood sampling revealed a glycated haemoglobin level ≥6.0% and <6.5% indicating the presence of pre-diabetes, three who were unable to undergo CMR scanning due to claustrophobia, and one who developed arrhythmia during CPET). A total of 78 healthy volunteers were therefore included in case-control comparisons.

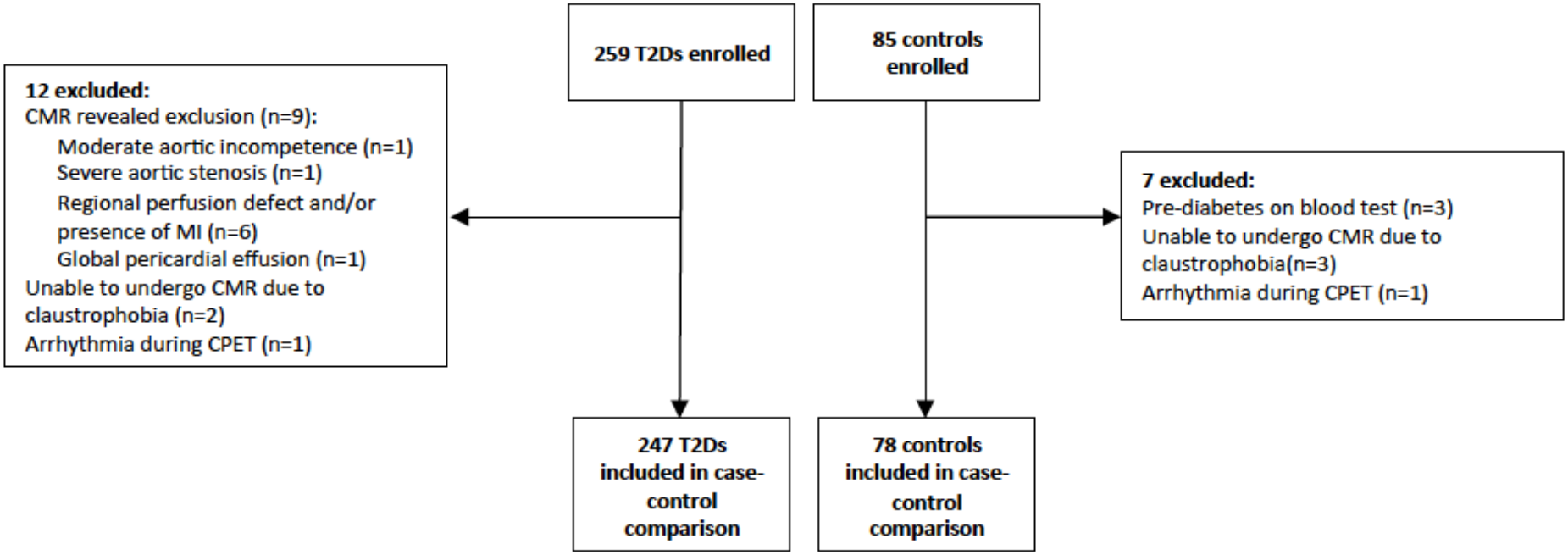


Figure 4.2 Study profile. Abbreviations: CMR=cardiovascular magnetic resonance imaging; CPET=cardiopulmonary exercise testing; MI=myocardial infarction; CPET=cardiopulmonary exercise testing.

4.4.1 Bio-anthropometric characteristics

The baseline demographic characteristics of subjects with T2D and controls are shown in Table 4.1. Mean age of participants with T2D was 51.8±11.9 years, mean BMI was 34.2±6.0 kg/m^2, median duration of diabetes was 61 (32 – 120) months, 45% were women, and 37% were from a black or minority ethnic group. The control group were similar for age, sex and ethnicity, but had lower overall body weight and BMI. Those with T2D had a higher proportion of individuals with a history of smoking, hypertension and dyslipidaemia compared with controls. Antihypertensive and lipid-lowering medication use was therefore higher in those with T2D compared to controls.

Fasting blood test results, adjusted for age, sex and ethnicity, are displayed in Table 4.2. Both groups had similar renal function. Subjects with T2D had higher overall glycated haemoglobin (7.4±1.1 vs. 5.4±0.3%, p<0.001) (57±12 vs. 36±3mmol/mol, p<0.001), lower total cholesterol and LDL cholesterol than controls.

Table 4.1 Demographic, clinical and bio-anthropometric characteristics of subjects with type 2 diabetes and controls

	T2Ds (n=247)	**CONTROLS (n=78)**	**P-value**
Demographics			
Age, years	51.8±11.9	51.5±12.3	0.898
Sex, n (%)			
Male	136 (55)	42 (54)	0.851
Female	111 (45)	36 (46)	
Ethnic origin, n (%)			
Caucasian	155 (63)	53 (68)	0.405
Black or other minority ethnicity	92 (37)	25 (32)	
Anthropometrics			
Height, cm	168±10	170±10	0.111
Weight, kg	96.9±19.1	72.0±13.6	**<0.001**
Body mass index, kg/m^2	34.2±6.0	24.8±3.1	**<0.001**
Systolic blood pressure, mmHg	138±16	129±18	**<0.001**
Diastolic blood pressure, mmHg	87±8	81±9	**<0.001**
Heart rate, beats/min	76±12	63±11	**<0.001**
Medical history			
Diabetes duration, months	61 (32 - 120)	N/A	N/A
Smoking history, n (%)			
Never smoked	140 (56)	50 (64)	**0.023**
Ex-smoker	68 (28)	25 (32)	
Current smoker	39 (16)	3 (4)	
Hypertension, n (%)	121 (49)	5 (6)	**<0.001**
Dyslipidaemia, n (%)	148 (60)	7 (9)	**<0.001**
Medications			
ACE inhibitor, n (%)	67 (27)	4 (5)	**<0.001**
ARB, n (%)	28 (11)	0 (0)	**0.002**
Beta blocker, n (%)	16 (6)	0 (0)	**0.024**
Calcium channel blocker, n (%)	50 (20)	1 (1)	**0.001**
Statin, n (%)	144 (58)	7 (9)	**<0.001**
Metformin, n (%)	214 (87)	N/A	N/A
Sulfonylurea, n (%)	50 (20)	N/A	N/A
DPP-IV inhibitor, n (%)	16 (6)	N/A	N/A
SGLT2 inhibitor, n (%)	36 (15)	N/A	N/A
GLP-1 receptor agonist, n (%)	17 (7)	N/A	N/A
Insulin, n (%)	20 (8)	N/A	N/A

Data are n (%), mean±SD, or median (IQR). Abbreviations: ACE=angiotensin converting enzyme; ARB=angiotensin receptor blocker; GFR=glomerular filtration rate; GLP-1=glucagon-like peptide-1; DPP-IV=dipeptidyl peptidase-IV; LDL=low-density lipoprotein; SGLT2=sodium glucose cotransporter-2.

Table 4.2 Fasting blood test results in subjects with type 2 diabetes and controls.

	T2Ds (n=247)	CONTROLS (n=78)	P-value
Fasting blood tests			
Urea, mmol/L	5.3±1.3	5.4±1.4	0.656
Creatinine, mmol/L	74±16	76±15	0.147
Estimated GFR, mL/min	84±10	83±9	0.811
Glucose, mmol/L	7.7 (6.7 - 9.5)	5.0 (4.8 - 5.3)	**<0.001**
HbA1c, %	7.4±1.1	5.4±0.3	**<0.001**
HbA1c, mmol/mol	57±12	36±3	**<0.001**
Total cholesterol, mmol/L	4.5±1.0	5.5±1.0	**<0.001**
Triglycerides, mmol/L	1.8 (1.2 - 2.6)	1.0 (0.7 - 1.4)	**<0.001**
LDL, mmol/L	2.4±0.8	3.2±0.9	**<0.001**
Haemoglobin, g/L	144±15	144±13	0.985

Data are mean±SD, or median (IQR). Abbreviations: LDL=low-density lipoprotein.

4.4.2 Cardiovascular structure, function, and fitness

Baseline CMR imaging, echocardiography and CPET data comparing T2Ds and controls with adjustment for age, sex and ethnicity are displayed in Table 4.3.

Table 4.3 Cardiovascular magnetic resonance, echocardiography and cardiopulmonary exercise testing data in subjects with type 2 diabetes and controls.

	T2D (n=247)	CONTROLS (n=78)	*P*-value
Cardiovascular magnetic resonance imaging			
LV EDV, mL	145±35	149±37	0.403
LV EDVi, mL/m^2	68±12	81±15	**<0.001**
LV ESV, mL	48±18	52±19	0.083
LV ESVi, mL/m^2	23±7	28±9	**<0.001**
LV EF, %	67±7	66±6	0.055
LV mass, g	119±27	112±31	**0.011**
LV MI, g/m$^{2.7}$	29±5	26±5	**<0.001**
LV mass:volume, g/mL	0.84±0.14	0.76±0.11	**<0.001**
LV global longitudinal strain, %	−16.2±2.4	−17.4±1.9	**<0.001**
LV PEDSR, s^{-1}	1.02±0.23	1.05±0.22	0.206
LA EDV, mL	31±13	30±13	0.794
LA EDVi, mL	14±5	18±6	**<0.001**
LA ESV, mL	70±23	73±24	0.438
LA ESVi, mL	31±9	43±12	**<0.001**
LA EF, %	55±11	56±13	0.278
Stress MBF, mL/g/min	3.11±1.26	3.14±1.22	0.645
Rest MBF, mL/g/min	1.17±0.53	0.92±0.39	**<0.001**
Myocardial perfusion reserve	2.60±1.24	3.54±1.15	**<0.001**
Aortic distensibility, mmHg^{-1}x10^{-3}	2.75 (1.74 - 4.03)	4.92 (2.65 - 7.13)	**<0.001**
LGE present, n (%)	35 (14)	12 (15)	0.740
Echocardiography			
E/A ratio	0.84 (0.66 - 1.05)	1.10 (0.83 - 1.23)	**0.006**
Average E/e'	7.1 (3.1 – 9.4)	7.1 (5.2 - 8.3)	0.478
Cardiopulmonary exercise testing			
Peak VO_2, mL/kg/min	18.0±6.6	27.8±9.0	**<0.001**
Peak VO_2, L/min	1.74±0.70	1.99±0.70	**0.002**

Data are mean±SD or median (IQR). Abbreviations: EDVi=end-diastolic volume indexed to body surface area; ESVi=end-systoic volume indexed to body surface area; EF=ejection fraction; LA= left atrium; LGE=late gadolinium enhancement; LV=left ventricle; MBF=myocardial blood flow; MPR=myocardial perfusion reserve; MI=mass indexed to height; PEDSR=peak early diastolic strain rate.

Patients with T2D had similar absolute LV volumes but smaller indexed LV volumes and higher LV mass, with increased concentric LV remodelling (LV mass:volume 0.84±0.14 vs. 0.76±0.11g/mL, p<0.001) compared to controls. Similarly, there was no difference in absolute LA volumes but indexed LA volumes were smaller in T2Ds versus controls.

Overall there was no significant difference in LV EF between groups, however LV GLS was lower in T2Ds versus controls (-16.2±2.4 vs. -17.4±1.9%, p<0.001). LA ejection fraction was similar in both groups (p=0.278). With regards

to diastolic function, there was no significant difference in LV PEDSR (1.02±0.23 vs. 1.05±0.22, p=0.206) or E/e' (7.1 (3.1 – 9.4) vs. 7.1 (5.2 – 8.3), p=0.438) between groups, but E/A ratio was significantly lower in T2Ds (0.84 (0.66 – 1.05) vs. 1.10 (0.83 – 1.23), p=0.006).

Aortic distensibility was significantly lower in those with diabetes compared with controls (2.75 (1.74 – 4.03) vs. 4.92 (2.65 – 7.13) $mmHg^{-1}x10^{-3}$, p<0.001). Stress and rest perfusion imaging was performed in 208 T2Ds and 77 controls. There was no significant difference in stress MBF between groups, but rest MBF was higher in the T2D group versus controls (1.17±0.53 vs. 0.92±0.39 mL/g/min, respectively, p<0.001). Overall MPR was therefore lower in subjects with T2D (2.60±1.24 vs. 3.54±1.15, respectively, p<0.001).

Overall prevalence of non-ischaemic LGE was low and there was no significant difference in the presence of LGE between T2Ds and controls (14 vs. 15%, p=0.740).

After adjustment for age, sex and ethnicity, both absolute and bodyweight corrected peak VO_2 were significantly lower in the T2Ds versus controls.

4.4.3 Inter-study, inter-observer and intra-observer reproducibility of MBF measurements

Test-retest, inter-observer and intra-observer reproducibility data are presented in Table 4.4. In the 10 subjects with T2D who underwent repeat CMR (median scan interval 8 (IQR 7 – 10) days), mean age was 65.3±7.2 years and 60% were males. There was excellent inter- and intra-observer agreement for all measures of MBF (ICCs 0.993 to 1.00). There were no significant differences in global stress MBF, rest MBF, or MPR measurements between baseline and repeat CMR scans. For all measures of MBF, agreement between baseline and repeat CMR scans was good or excellent (ICCs 0.806 to 0.912). Bland-Altman analysis did not reveal any systematic bias (Figure 4.3).

Table 4.4 Inter-study, inter-observer and intra-observer agreement of myocardial blood flow measurements using quantitative MRI perfusion mapping at 3T in ten subjects with type 2 diabetes.

	Intra-observer agreement (observer 1 vs. observer 1)			**Inter-observer agreement (observer 1 vs. observer 2)**			**Inter-study reproducibility**			
	Repeat observer 1	**ICC**	**95% CI**	**Observer 2**	**ICC**	**95% CI**	**Study 1**	**Study 2**	**ICC**	**95% CI**
Global stress MBF (mL/g/min)	1.68±0.51	1.00	0.999 to 1.000	1.67±0.52	0.999	0.994 to 1.00	1.69±0.52	2.03±0.85	0.831	0.318 to 0.958
Global rest MBF (mL/g/min)	0.62±0.10	0.999	0.994 to 1.000	0.62±0.10	0.993	0.969 to 0.998	0.62±0.10	0.67±0.17	0.885	0.489 to 0.974
Myocardial perfusion reserve	2.74±0.86	1.00	0.998 to 1.000	2.73±0.80	0.997	0.987 to 0.999	2.77±0.86	3.05±0.87	0.912	0.610 to 0.980

Abbreviations: MBF=myocardial blood flow; ICC=intraclass correlation coefficient.

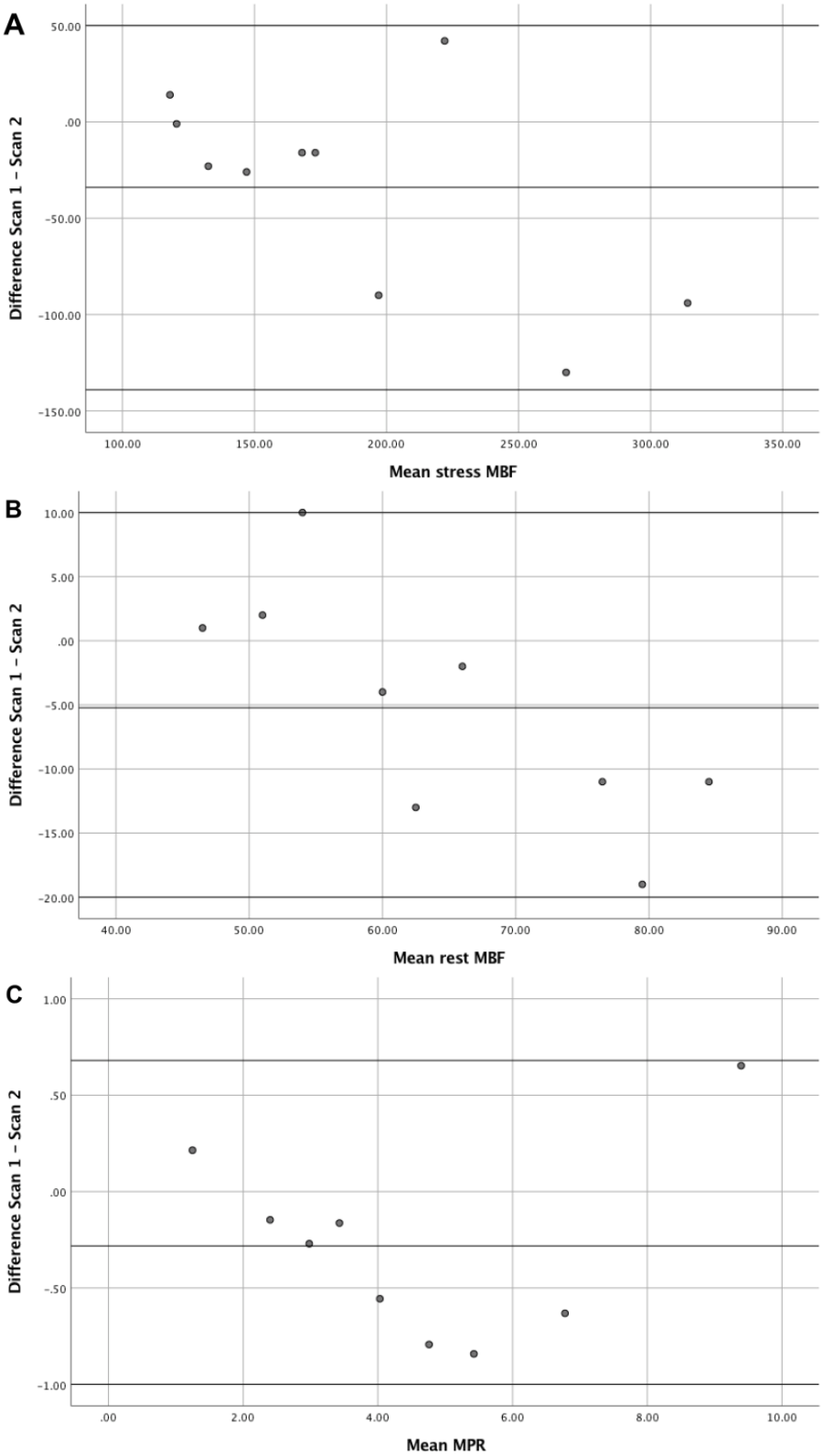


Figure 4.3 Bland-Altman plots for baseline and repeat myocardial blood flow (MPR) and myocardial perfusion reserve (MPR) measurements using quantitative cardiovascular magnetic resonance perfusion mapping at 3T. Plots shown are for A: stress MBF, B: rest MBF, and C: MPR.

4.5 Discussion

This is amongst the first studies to comprehensively describe subclinical cardiovascular dysfunction in adults with T2D using a combination of multiparametric CMR, echocardiography, and CPET. The major strengths of the study are the detailed cardiovascular phenotyping undertaken, the large sample size, the rigorous exclusion of those with established CVD, and the high proportion of both females and ethnic minorities, which make the results more generalizable to our regional population. Compared to well-matched controls, we have confirmed several markers LV dysfunction in asymptomatic people with T2D: concentric LV remodelling, subtle impairments of diastolic and systolic function, reduced MPR, increased aortic stiffness, and markedly lower aerobic exercise capacity (Figure 4.1). Furthermore, we demonstrated good to excellent inter-study, intra- and inter-observer reproducibility of CMR automated pixel-wise myocardial perfusion mapping, supporting the use of this technique for MBF quantification in subjects with T2D.

Despite having no signs, symptoms or history cardiovascular disease, our T2D cohort already have evidence of AHA stage B heart failure, confirming findings from existing literature. This group are therefore at risk of progression to overt clinical heart failure. Concentric LV remodelling(306), aortic stiffening(145), subclinical diastolic(307) and systolic dysfunction(308,309) have all been independently associated with poorer outcomes in T2D, and lower MPR is associated with impaired LV function in asymptomatic people(310) (although studies assessing clinical outcomes associated with lower MPR have been in patients referred for clinical perfusion imaging with inevitable selection bias). Furthermore, longitudinal studies have demonstrated the temporal progression of the early manifestations of diabetic cardiomyopathy(72,146,311), with the exception of MPR. Strategies that attenuate or reverse the progression of subclinical LV dysfunction could therefore be critical for lowering the risk of incident heart failure development in T2D.

We have recently shown in younger adults with T2D, that aortic distensibility is associated with concentric LV remodelling independent of age, sex, diabetes duration, and systolic BP(152). Targeting aortic stiffness specifically may by proxy reduce LV hypertrophy and could potentially improve survival in subjects with T2D.

In patients with hypertension, aortic stiffness may be improved by intensive BP reduction, regardless of the types of antihypertensive agents used(153). Alternatively, tight blood glucose control could lessen aortic stiffness and subsequent cardiac remodelling(149), especially given that aortic stiffening has been shown to worsen across the glycaemic spectrum(312). Similarly, emerging data suggest that SGLT2i may also ameliorate aortic stiffness, although whether these effects occur independently of BP and glucose-lowering effects is not known(313). Lastly, lifestyle interventions should be considered for reversal of aortic stiffness and cardiac remodelling. Dramatic weight loss, either with bariatric surgery or with dieting, can improve aortic stiffening both in T2D and obesity(229,314) and this may be particularly relevant with the recent success of low-energy diet treatments for T2D administered in primary care(315).

Subclinical reductions in LV diastolic and systolic dysfunction have been reported in numerous cross-sectional studies comparing asymptomatic adults with T2D to healthy volunteers(316). In the present study, the only indicator of diastolic dysfunction in subjects with T2D was an overall E/A ratio <1, with no difference in LV filling pressure (E/e') or PEDSR between patients and controls. At most this is suggestive of only mild (grade 1) diastolic dysfunction. However, subjects with T2D already had clear evidence of systolic dysfunction, with a lower GLS compared to controls. These findings question the notion that abnormalities in diastolic function, even when mild, occur in isolation and before the onset of subtle systolic compromise.

Given that poorer glycaemic control and BP are associated with a higher prevalence of diastolic and systolic dysfunction(317), aggressive reduction of blood glucose and BP levels may lead to improved cardiac function. However, trial data to support this assertion are limited. Optimisation of blood glucose levels (HbA1c ≤7.0%), resting BP (≤130/80 mmHg) and cholesterol (total cholesterol ≤4.0 mmol/L) led to improvements in GLS and early diastolic mitral annular velocities in a single-group study of 105 patients with poorly controlled T2D (age 54±10 years, duration of T2D 10±7 years, HbA1c 9.6% (8.7 – 11.4))(81). The largest improvements in systolic strain were observed in those who achieved the greatest reductions in HbA1c. Similarly, six-months treatment with the GLP-1RA liraglutide led to improvements in LV filling pressures in a small (n=23) echocardiography,

placebo-controlled randomised controlled trial(182). However, the fact that only modest reductions in major adverse cardiovascular events (primarily myocardial infarction) are observed with intensive glycaemic control(158) indicates that these short-term improvements in diastolic function do not translate to longer-term reductions in heart failure incidence.

There is a growing body of evidence to support beneficial effects of weight loss and exercise interventions on cardiovascular structure and function in people with T2D, with improvements in diastolic function, arterial stiffness and LV remodelling demonstrated in several small (predominantly single-group) studies(316). Furthermore, weight loss by bariatric surgery has been shown to reduce heart failure incidence(220), and increased physical activity levels are associated with lower risk of heart failure development(318). Clearly the impact of lifestyle interventions to encourage weight loss and improved fitness, which already underpin major guidelines for management of T2D(164,166), on subclinical cardiac dysfunction warrant further investigation, and this is a core objective of this thesis.

4.5.1 Limitations

The pooled cohort of subjects from four separate studies of cardiac structure and function in our unit is a limitation, owing to small differences in inclusion criteria. However, we adhered to pre-specified inclusion and exclusion criteria for these pooled analyses to limit heterogeneity of included subjects. Although all CMR scanning utilised a standardised imaging protocol, the impact of different MRI field strengths on measures of cardiovascular structure and function are not known and may introduce a source of variation in our imaging variables. This may have impacted absolute stress and rest MBF values but should not significantly affect MPR. Furthermore, similar proportions of patients and controls were studied with each method, which mitigates the risk that differences in MPR between groups occurred due to varying perfusion quantification techniques. Although we excluded significant epicardial coronary artery disease by LGE imaging, stress perfusion CMR(319), and exercise electrocardiography, invasive coronary angiography remains the gold-standard technique for assessment of coronary disease and individuals with significant diffuse three-vessel disease may not have been detected by CMR imaging alone. CMR measures of diffuse myocardial fibrosis (native T_1 relaxivity and ECV) were not included, as these were only measured in a small

subset of patients and are subject to variation when imaged at different MR field strengths(320), both of which limited their application in these analyses. We were also not able to measure myocardial triglyceride content in our participants, owing to technical issues with magnetic resonance spectroscopy on our wide bore 3T scanner, and we are currently not equipped to assess myocardial energetics, both of which are possible mechanisms underlying diabetic cardiomyopathy.

4.6 Conclusions

In an asymptomatic cohort of adults with T2D representative of our regional population, there is already evidence of subclinical cardiovascular dysfunction, with concentric LV remodelling, arterial stiffness, diastolic and systolic function, and lower MPR, when compared with age-, sex- and ethnicity-matched non-diabetic controls. Further studies are needed to determine whether these markers of cardiovascular dysfunction are related to limitations in exercise capacity and are amenable to weight loss or exercise interventions.

5 Clinical associations with subclinical cardiovascular dysfunction in adults with type 2 diabetes

5.1 Abstract

5.1.1 Background

Subclinical cardiovascular dysfunction is highly prevalent in adults with T2D, but the clinical determinants are incompletely understood.

5.1.2 Objective

To identify independent associations between key clinical characteristics and cardiovascular structural and functional alterations in asymptomatic adults with T2D.

5.1.3 Research design and methods

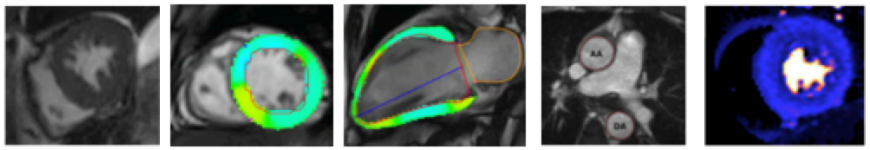
Cross-sectional study. We prospectively enrolled asymptomatic adults with T2D from a multi-ethnic population with no prevalent cardiovascular disease. Participants underwent bio-anthropometric profiling, echocardiography, and adenosine stress perfusion CMR imaging. Multivariable linear regression analysis was performed to identify independent associations between clinical and biochemical characteristics with measures of cardiovascular structure and function.

5.1.4 Results

In a series of multivariable linear regression models containing age, sex, ethnicity, smoking history, systolic BP, duration of diabetes, BMI, HbA1c, creatinine, and LDL-cholesterol, independent determinants of LV mass:volume were: age (β=0.023) and systolic BP (β=0.026); GLS were: never smoking (β= -1.175), systolic BP (β=0.32), and duration of diabetes (β=0.355); PEDSR were: age (β= -0.11), male sex (β= -0.111), never smoking (β=0.092), and BMI (β= -0.031); MPR were: age (β= -0.362) and male sex (β= 0.453); and aortic distensibility were: age (β= -0.630) and systolic BP (β= -0.349). HbA1c was not independently associated with any index of subclinical cardiovascular dysfunction.

5.1.5 Conclusions

In asymptomatic adults with T2D, the major determinants of subclinical cardiovascular dysfunction are increasing age, duration of T2D, systolic BP, BMI, and smoking history.



	LV mass:volume	Peak early diastolic strain rate	Global longitudinal strain	Aortic distensibility	Myocardial perfusion reserve
Age	↑	↓	–	↓	↓
Male sex	–	↓	–	–	↑
White ethnicity	–	–	–	–	–
Never smoked	–	↑	↑	–	–
Diabetes duration	–	–	↓	–	–
Systolic blood pressure	↑	–	↓	↓	–
Body mass index	–	↓	–	–	–
HbA1c	–	–	–	–	–
Creatinine	–	–	–	–	–
LDL cholesterol	–	–	–	–	–

Figure 5.1 Multivariable associations between key clinical characteristics and indices of cardiovascular structure and function in adults with type 2 diabetes. Increasing age, male sex, smoking history, duration of diabetes, systolic blood pressure and body mass index were independent determinants of subclinical cardiovascular dysfunction. Abbreviations: LDL=low-density lipoprotein; LV=left ventricle.

5.2 Introduction

As outlined in the **Chapter 1**, the clinical factors contributing to early cardiovascular dysfunction in people with T2D are poorly understood. Previous imaging studies have been hindered by variations in methods of assessing cardiac structure and function, as well as small sample sizes (seldom greater than 100 subjects) and incomplete datasets with significant risk of overfitting the regression models.

Cardiovascular magnetic resonance is the gold standard imaging modality for assessment of cardiac volumes, mass and EF, aortic stiffness, and with the addition of stress perfusion imaging has the ability to provide accurate quantification of MBF. By combining CMR with echocardiography, comprehensive cardiovascular phenotyping of asymptomatic adults with T2D is possible. Using these techniques, we have already described evidence of subclinical cardiovascular dysfunction in our multi-ethnic population with T2D, with concentric LV remodelling, arterial stiffening, abnormalities in diastolic and systolic function, and lower MPR, when compared with age-, sex- and ethnicity-matched non-diabetic controls. These perturbations are increasingly recognised as pathognomic features of subclinical cardiovascular dysfunction in people with T2D and are likely precursors to the onset of clinical heart failure.

The aim of this study was to identify independent associations between clinical characteristics and key cardiovascular perturbations in a multi-ethnic cohort of asymptomatic adults with T2D.

5.3 Research design and methods

A detailed description of the study methods, including participant recruitment, and assessments, and general statistical analysis, is provided in **Chapter 2**. Only subjects with T2D were included in these analyses, to enable specific clinical associations with measures of cardiovascular structure and function in our patient cohort to be explored. The same inclusion and exclusion criteria used in **Chapter 4** were applied.

5.3.1 Specific statistical analysis

Correlations between clinical characteristics (age, diabetes duration, body weight, BMI, BP, heart rate, biochemistry) and CMR and echocardiographic measures of cardiovascular structure and function (LV mass:volume, systolic and diastolic function, MPR, and aortic distensibility) were assessed using Pearson correlation

coefficient in subjects with T2D. Generalised linear modelling was performed to identify independent associations of clinical characteristics with measures of cardiovascular structure and function (specifically LV mass:volume, GLS, PEDSR, MPR, aortic distensibility, E/A ratio, and average E/e'). A separate model, with each CMR and echocardiographic measure as the dependent variable, tested individually against a combination of key clinical characteristics presumed to be associated with diabetic cardiomyopathy(316) was performed. Clinical variables included in each model were age, sex, ethnicity, smoking history, systolic BP, duration of diabetes, BMI, HbA1c, serum creatinine and LDL cholesterol. Continuous predictor variables were standardised prior to inclusion in the regression models to permit more direct comparisons of the magnitude of their effects on the dependent variable. Regression coefficients (β) are presented as point estimate and 95% confidence intervals. Statistical analysis was performed using SPSS version 25.0 (Statistical Package for Social Sciences, Chicago, IL). A p value <0.05 was considered statistically significant.

5.4 Results

This analysis included the same 247 subjects with T2D as detailed in **Chapter 4**. The study profile, including details of excluded participants, is displayed Figure 4.2 .

5.4.1 Bio-anthropometric characteristics

Detailed bio-anthropometric characteristics of included subjects with T2D are presented in **Chapter 4**, Table 4.1. Mean age of participants was 51.8±11.9 years, mean BMI was 34.2±6.0 kg/m^2, median duration of diabetes was 61 (32 – 120) months, 45% were women, and 37% were from a black or minority ethnic group.

5.4.2 Cardiovascular structure and function

Cardiovascular magnetic resonance imaging and echocardiography data for included subjects with T2D are presented in **Chapter 4**, Table 4.3.

5.4.3 Correlations of clinical characteristics with cardiovascular structure and function

Correlations of key CMR and echocardiographic measures of cardiovascular structure and function with patient clinical characteristics are displayed in Table 5.1.

Table 5.1 Correlations between clinical characteristics and key measures of cardiovascular structure and function in subjects with type 2 diabetes

	E/A	E/e'	LV mass:vol.	LV GLS	LV PEDSR	Stress MBF	Rest MBF	MPR	Aortic distensibility
	r	r	r	r	r	r	r	r	r
Age	0.086	0.502**	0.261**	0.025	−0.369**	−0.678**	−0.509**	−0.227**	−0.344**
T2D duration	0.015	0.353**	0.162*	0.119	−0.161*	−0.355**	−0.294**	−0.127	−0.216**
Weight	−0.08	−0.214**	−0.117	−0.022	−0.159*	0.256**	0.161*	0.102	0.086
BMI	−0.098	−0.134*	−0.102	−0.146*	−0.001	0.261**	0.348**	−0.109	0.112
Systolic BP	−0.011	0.325**	0.243**	0.129*	−0.191**	−0.113	−0.080	−0.105	−0.255**
Diastolic BP	−0.034	−0.009	0.187**	0.209**	−0.068	0.085	0.102	−0.019	−0.087
Creatinine	0.104	0.26**	0.177**	0.168**	−0.206**	−0.242**	−0.368	0.104	−0.106
Glucose	−0.14	−0.154*	0.032	0.103	−0.041	0.084	0.009	0.095	−0.003
HbA1c	−0.018	−0.155*	−0.002	0.146*	−0.012	0.124	0.078	0.094	0.036
LDL	−0.074	−0.096	−0.075	-0.07	0.004	0.201**	0.109	0.052	0.008

Data shown are correlations coefficients (r). *=p-value <0.05. **=p-value <0.01. Abbreviations: BMI=body mass index; BP=blood pressure; GLS=global longitudinal strain; LDL=low-density lipoprotein; MBF=myocardial blood flow; MPR=myocardial perfusion reserve; PEDSR=peak early diastolic strain rate.

5.4.3.1 Left ventricular mass:volume

Age (r= 0.261), duration of T2D (r= 0.162, Figure 5.2), systolic (r=0.243) and diastolic BP (r=0.187), and serum creatinine (r=0.177) correlated significantly with LV mass:volume.

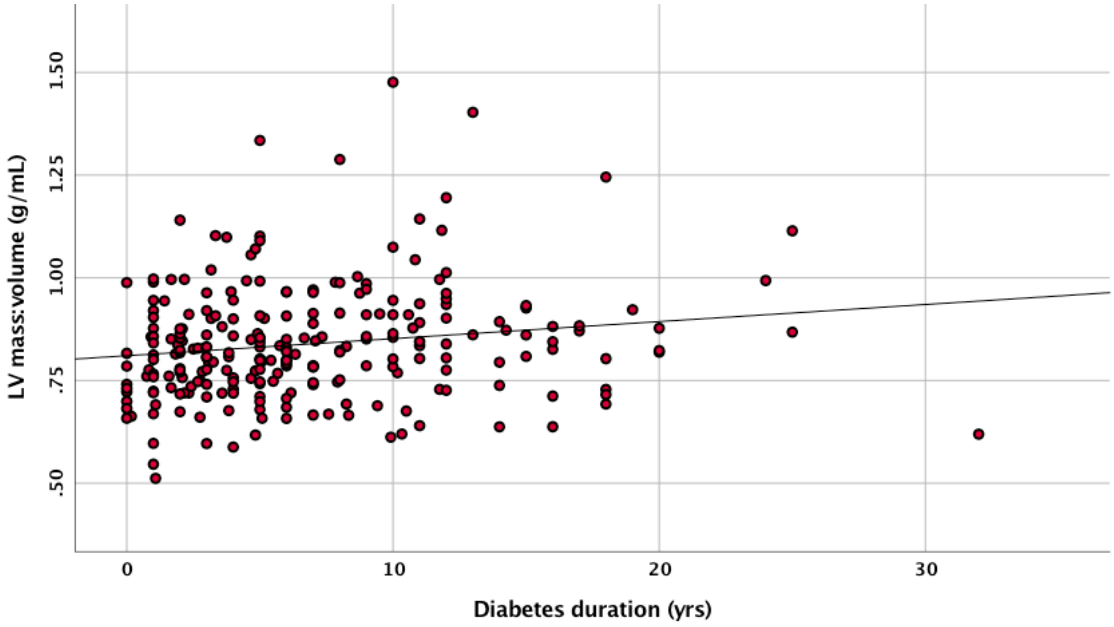


Figure 5.2 Scatterplot displaying the correlation between left ventricular (LV) mass:volume and diabetes duration (r= 0.162, p=0.011).

5.4.3.2 Left ventricular systolic function

Body mass index (r= -0.146), systolic (r=0.129) and diastolic BP (r=0.209), serum creatinine (r=0.168), and HbA1c (r=0.146) (Figure 5.3 3) correlated significantly with LV GLS.

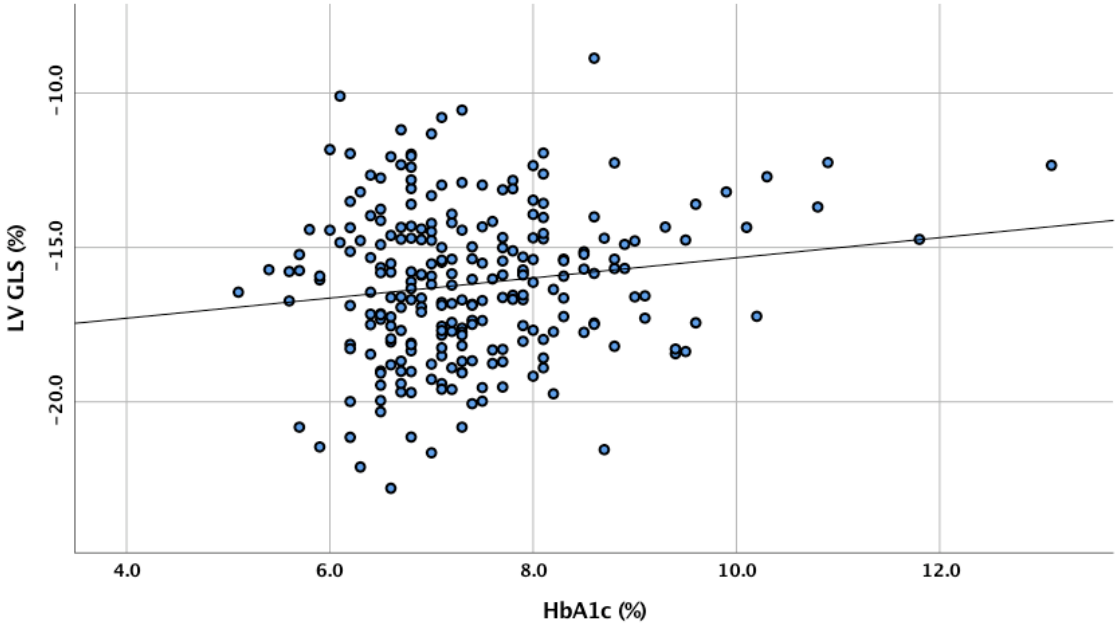


Figure 5.3 Scatterplot displaying the correlation between left ventricular global longitudinal strain (LV GLS) and HbA1c (r=0.146, p=0.022).

5.4.3.3 Left ventricular diastolic function

No significant correlations were observed between clinical characteristics and E/A ratio. Age (r=0.502), duration of T2D (r=0.353), body weight (r= -0.214) and BMI (r= -0.134), systolic BP (r=0.325), serum creatinine (r=0.26), fasting glucose (r= -0.154) and HbA1c (r= -0.155) all correlated with echocardiographic estimates of LV filling pressure (E/e'). Age (r= -0.369), duration of T2D (r= -0.161), body weight (r= -0.159), systolic BP (r= - 0.191) (Figure 5.4 4), and serum creatinine (r= -0.206) had significant correlations with CMR-measured LV PEDSR.

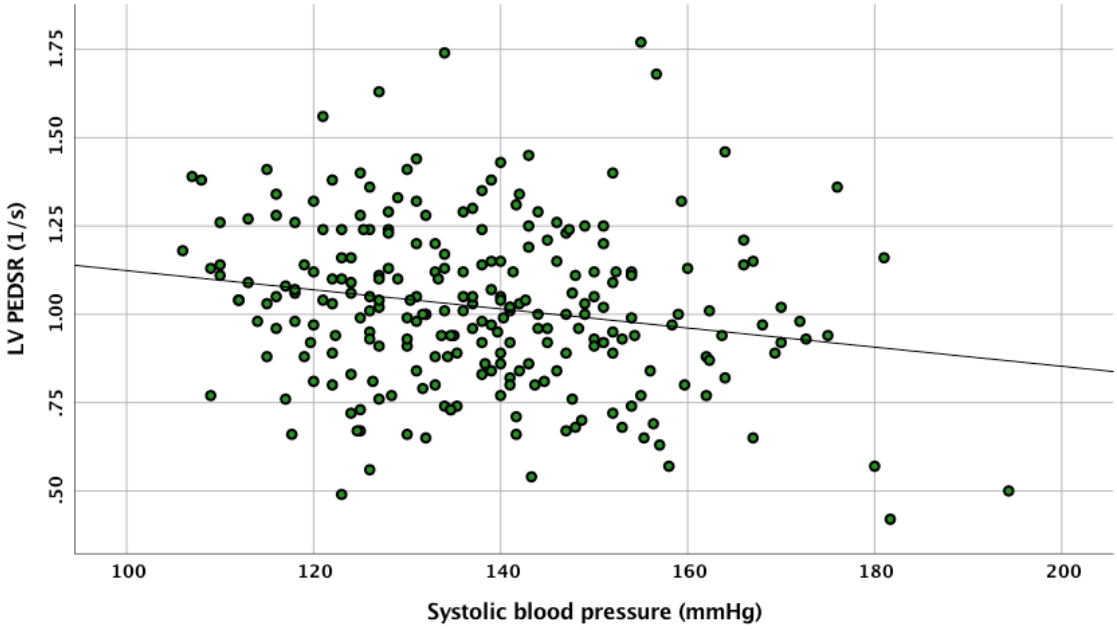


Figure 5.4 Scatterplot displaying the correlation between left ventricular peak early diastolic strain rate (LV PEDSR) and systolic blood pressure (r= -0.191, p=0.003).

5.4.3.4 Myocardial perfusion

Age (r= -0.678), duration of T2D (r= -0.355), body weight (r= 0.256) and BMI (r=0.261), serum creatinine (r= -0.242), and LDL cholesterol (r= 0.201) were significantly correlated with stress MBF. Similarly, age (r= -0.509), duration of T2D (r= -0.294), body weight (r=0.161) and BMI (r=0.348), correlated significantly with rest MBF. Only age was significantly associated with MPR, with which it had an inverse correlation (r= -0.227) (Figure 5.5 5). There was a non-significant towards reduced MPR with increasing diabetes duration (r= -0.127, p=0.054).

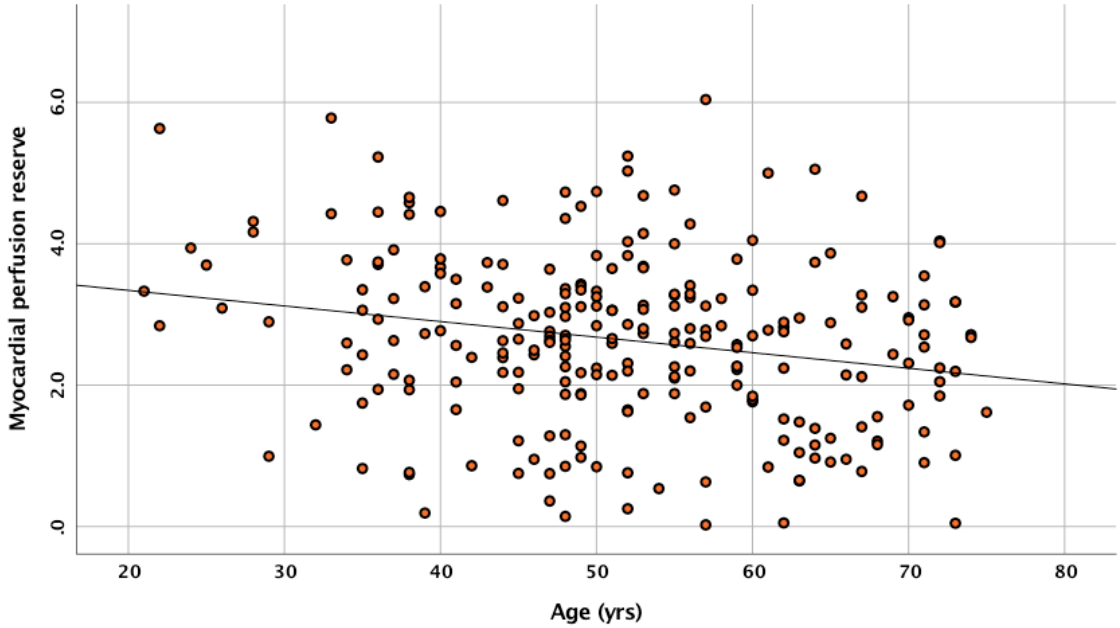


Figure 5.5 Scatterplot displaying the correlation between age and myocardial perfusion reserve (r= -0.227, p<0.001).

5.4.3.5 Aortic distensibility

Age (r= -0.344), duration of T2D (r= -0.216) and systolic BP (r= -0.255) (Figure 5.6) were the only significant correlations with aortic distensibility, each of which had an inverse association.

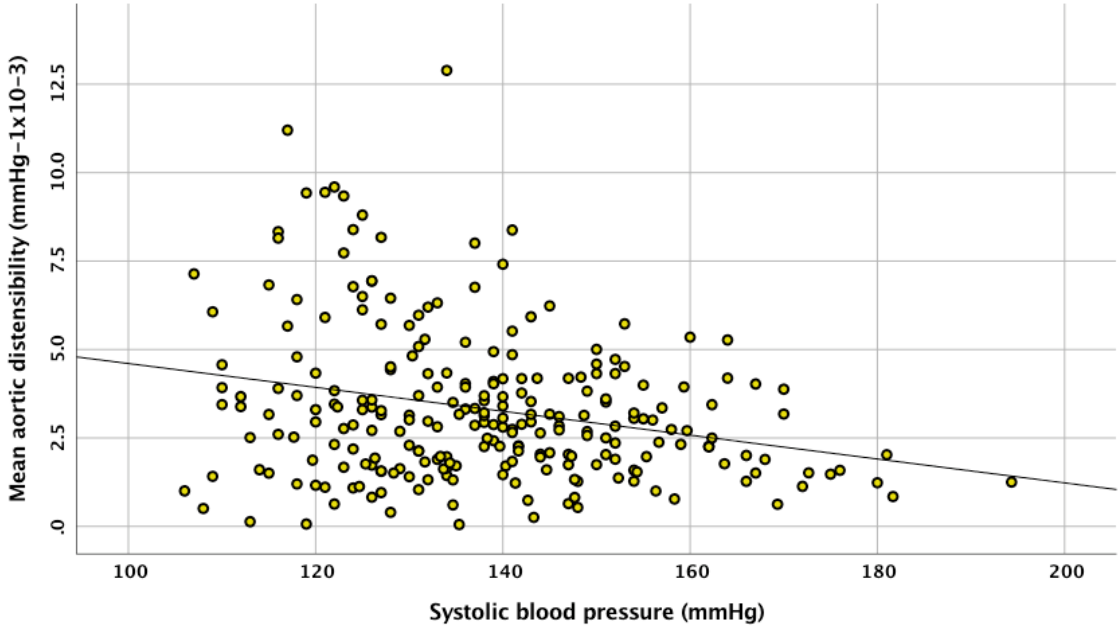


Figure 5.6 Scatterplot displaying the correlation between aortic distensibility and systolic blood pressure (r= -0.255, p<0.001).

5.4.4 Multivariable associations of clinical characteristics with cardiovascular structure and function

5.4.4.1 Left ventricular mass:volume

Multivariable associations of key clinical parameters with LV mass:volume are displayed in Table 5.2, below. Only age and systolic BP were independently associated with LV mass:volume ratio.

Table 5.2 Multivariable associations of clinical variables with left ventricular mass:volume in adults with type 2 diabetes.

	Dependent variable: LV mass:volume		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	0.023	0 to 0.047	**0.047**
Male sex	0.009	−0.032 to 0.050	0.66
White ethnicity	−0.014	−0.053 to 0.024	0.471
Never smoked	−0.029	−0.080 to 0.022	0.262
Systolic blood pressure (per 1SD increase)	0.026	0.008 to 0.045	**0.006**
T2D duration (per 1SD increase)	0.006	−0.015 to 0.026	0.605
Body mass index (per 1SD increase)	−0.001	−0.020 to 0.018	0.946
HbA1c (per 1SD increase)	0.005	−0.013 to 0.023	0.604
Creatinine (per 1SD increase)	0.011	−0.010 to 0.032	0.320
LDL cholesterol (per 1SD increase)	−0.001	−0.020 to 0.017	0.911

5.4.4.2 Global longitudinal strain

Multivariable associations of key clinical parameters with CMR-measured GLS are displayed in Table 5.3, below. Smoking history, systolic BP, and duration of T2D were independently associated with GLS.

Table 5.3 Multivariable associations of clinical variables with left ventricular global longitudinal strain in adults with type 2 diabetes.

	Dependent variable: global longitudinal strain		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	−0.229	−0.614 to 0.157	0.245
Male sex	0.491	−0.187 to 1.169	0.156
White ethnicity	−0.020	−0.664 to 0.623	0.951
Never smoked	−1.175	−2.022 to −0.328	**0.007**
Systolic blood pressure (per 1SD increase)	0.320	0.009 to 0.632	**0.044**
T2D duration (per 1SD increase)	0.355	0.006 to 0.704	**0.046**
Body mass index (per 1SD increase)	−0.305	−0.622 to 0.012	0.059
HbA1c (per 1SD increase)	0.240	−0.059 to 0.540	0.116
Creatinine (per 1SD increase)	0.104	−0.249 to 0.457	0.564
LDL cholesterol (per 1SD increase)	−0.110	−0.419 to 0.199	0.485

5.4.4.3 Peak early diastolic strain rate

Multivariable associations of clinical parameters with CMR-measured PEDSR are presented in Table 5.4, below. Age, sex, smoking history and BMI were independently associated with PEDSR.

Table 5.4 Multivariable associations of clinical variables with peak early diastolic strain rate in adults with type 2 diabetes.

	Dependent variable: peak early diastolic strain rate		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	−0.110	−0.143 to −0.077	**<0.001**
Male sex	−0.111	−0.169 to −0.053	**<0.001**
White ethnicity	−0.053	−0.108 to 0.002	0.059
Never smoked	0.092	0.020 to 0.165	**0.012**
Systolic blood pressure (per 1SD increase)	−0.007	−0.033 to 0.020	0.618
T2D duration (per 1SD increase)	0.001	−0.028 to 0.031	0.936
Body mass index (per 1SD increase)	−0.031	−0.058 to −0.004	**0.024**
HbA1c (per 1SD increase)	−0.017	−0.042 to 0.009	0.197
Creatinine (per 1SD increase)	<0.001	−0.030 to 0.030	0.992
LDL cholesterol (per 1SD increase)	−0.022	−0.048 to 0.005	0.107

5.4.4.4 Myocardial perfusion reserve

Multivariable associations of clinical parameters with MPR are presented in Table 5.5, below. Age and sex were the only independent associations with MPR. There was a non-significant trend towards an inverse association between BMI and MPR.

Table 5.5 Multivariable associations of clinical variables with myocardial perfusion reserve in adults with type 2 diabetes.

	Dependent variable: myocardial perfusion reserve		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	−0.362	−0.564 to −0.160	**<0.001**
Male sex	0.453	0.102 to 0.805	**0.012**
White ethnicity	0.113	−0.219 to 0.445	0.504
Never smoked	0.190	−0.241 to 0.622	0.388
Systolic blood pressure (per 1SD increase)	−0.109	−0.268 to 0.050	0.177
T2D duration (per 1SD increase)	−0.020	−0.201 to 0.160	0.825
Body mass index (per 1SD increase)	−0.165	−0.331 to 0.001	0.052
HbA1c (per 1SD increase)	0.046	−0.117 to 0.208	0.582
Creatinine (per 1SD increase)	0.138	−0.049 to 0.326	0.148
LDL cholesterol (per 1SD increase)	0.039	−0.139 to 0.217	0.662

5.4.4.5 Aortic distensibility

Multivariable associations between key clinical parameters and aortic distensibility are displayed in Table 5.6, below. The only variables independently associated with aortic distensibility were age and systolic BP.

Table 5.6 Multivariable associations of clinical variables with aortic distensibility in adults with type 2 diabetes.

	Dependent variable: aortic distensibility		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	−0.630	−1.058 to −0.203	**0.005**
Male sex	−0.082	−0.757 to 0.594	0.812
White ethnicity	−0.148	−0.751 to 0.455	0.629
Never smoked	0.372	−0.463 to 1.207	0.380
Systolic blood pressure (per 1SD increase)	−0.349	−0.637 to −0.061	**0.018**
T2D duration (per 1SD increase)	−0.156	−0.481 to 0.169	0.345
Body mass index (per 1SD increase)	0.095	−0.254 to 0.445	0.585
HbA1c (per 1SD increase)	0.002	−0.278 to 0.283	0.986
Creatinine (per 1SD increase)	0.099	−0.249 to 0.447	0.574
LDL cholesterol (per 1SD increase)	−0.155	−0.441 to 0.131	0.288

5.4.4.6 Echocardiographic diastolic function

Multivariable associations of key clinical parameters with echocardiographic indices of diastolic function are presented in Table 5.7 and Table 5.8, below. Serum creatinine was the only clinical variable independently associated with E/A ratio. Age, sex, systolic BP, duration of T2D, and serum creatinine were all independently associated with average E/e'.

Table 5.7 Multivariable associations of clinical variables with E/A ratio in adults with type 2 diabetes.

	Dependent variable: E/A ratio		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	−0.033	−0.095 to 0.029	0.292
Male sex	−0.089	−0.213 to 0.035	0.155
White ethnicity	−0.053	−0.160 to 0.055	0.336
Never smoked	0.041	−0.144 to 0.226	0.648
Systolic blood pressure (per 1SD increase)	0.021	−0.040 to 0.082	0.490
T2D duration (per 1SD increase)	−0.003	−0.057 to 0.051	0.915
Body mass index (per 1SD increase)	−0.009	−0.063 to 0.044	0.726
HbA1c (per 1SD increase)	−0.008	−0.068 to 0.053	0.796
Creatinine (per 1SD increase)	0.083	0.026 to 0.140	**0.005**
LDL cholesterol (per 1SD increase)	−0.006	0.082 to 0.145	0.859

Table 5.8 Multivariable associations of clinical variables with average E/e' in adults with type 2 diabetes.

	Dependent variable: average E/e'		
	β	**95% CI**	**P-value**
Age (per 1SD increase)	1.274	0.709 to 1.839	**<0.001**
Male sex	−1.292	−2.283 to −0.300	**0.011**
White ethnicity	−0.791	−1.765 to 0.184	0.112
Never smoked	0.372	−0.890 to 1.634	0.563
Systolic blood pressure (per 1SD increase)	0.735	0.287 to 1.184	**0.001**
T2D duration (per 1SD increase)	0.518	0.005 to 1.030	**0.048**
Body mass index (per 1SD increase)	0.024	−0.469 to 0.516	0.924
HbA1c (per 1SD increase)	−0.307	−0.753 to 0.140	0.178
Creatinine (per 1SD increase)	0.707	0.172 to 1.242	**0.010**
LDL cholesterol (per 1SD increase)	0.074	−0.390 to 0.538	0.755

5.5 Discussion

This is the first study to explore clinical determinants of early diabetic cardiomyopathy in a large, multi-ethnic cohort of asymptomatic adults with T2D who were comprehensively phenotyped with both multiparametric CMR and echocardiography. The major clinical determinants of cardiovascular structure and

function in our T2D cohort were increasing age, duration of T2D, systolic BP, BMI, and smoking history.

Interestingly, HbA1c was not associated with any measure of cardiovascular function in our multivariable analyses, although there was a modest univariate correlation between HbA1c and GLS. The lack of association between glycaemic control and measures of diastolic and systolic dysfunction are perhaps not surprising given the lack of evidence to suggest that improved glycaemic control lowers incident heart failure in people with T2D(159). Our findings contradict others, where both diastolic(82) and systolic function(82,96) have been found to worsen across the glycaemic spectrum. For example, in a study examining patients with pre-diabetes (n=44) and T2D (n=48), echocardiographic estimates of LV end-diastolic filling pressure correlated modestly with HbA1c (r=0.358 and r=0.447 in patients with pre-diabetes and T2D, respectively), but the small study sample size did not permit multivariable analyses(82). Similarly, in a small study (n=68, mean age 60±10 years) comparing GLS measured by speckle tracking echocardiography in people with well-controlled (HbA1c 6.1±0.5%) versus poorly controlled (HbA1c 9.1±1.4%) T2D, patients with poorly controlled diabetes had worse GLS (-17.7±2.6 vs. -16.2±2.4, respectively, p=0.039)(96). In a multivariable model containing age, duration of diabetes, systolic BP, LDL cholesterol, LV mass index, E/e', HbA1c was independently associated with GLS (β= -0.274, p=0.0224)(96). However, the multivariable model contained both groups of patients with and without poorly controlled T2D, which is likely to have confounded the results, and the number of variables included in the model is likely to have caused overfitting.

Despite no multivariable association with HbA1c, longer duration of T2D was associated with worsening GLS and increasing LV diastolic filling pressure (E/e') independent of age and other clinical parameters. These observations suggest that the longer diabetes duration drives progressive cardiac dysfunction regardless of glucose control, and are supported by longitudinal data that have shown marked deterioration of diastolic function occurs over as little as three years(72). It is possible that early interventions to reverse T2D and prevent subsequent years of dysglycaemia may attenuate the progression of cardiac dysfunction. Indeed it has already been discussed in **Chapter 1** that those individuals diagnosed with T2D at

younger ages have the highest lifetime risk of developing heart failure, even with excellent blood glucose and cardiovascular risk factor control(30).

Contrary to our findings with glucose control, increasing systolic BP was independently associated with more concentric LV remodelling, worse GLS and E/e', and lower aortic distensibility. The associations between T2D, BP, arterial stiffening and LV hypertrophy are well described(139,140), and these relationships are confirmed in our own cohort. Furthermore, hypertension has been shown to cause reductions in GLS (especially in patients with long-standing disease)(321), is associated with a higher prevalence of diastolic dysfunction in asymptomatic individuals(322), and is a recognised risk factor for HFpEF(323). Co-existence of T2D and hypertension is known to confer a greater risk of CVD, including coronary artery disease and heart failure, compared with either diabetes or hypertension in isolation(199). Intensive BP reduction, however, does not appear to lower the risk of incident heart failure (although overall mortality, cardiovascular death, myocardial infarction and stroke rates do improve with tighter BP control)(200). Like with T2D duration, perhaps earlier, aggressive BP reduction in younger adults with T2D at highest risk may be required to halt progression of subclinical cardiac dysfunction and prevent heart failure development. Similarly, a history of never having smoked was strongly associated with higher PEDSR and better GLS, supporting a role for smoking cessation to prevent or reduce both diastolic and systolic dysfunction in people with T2D, amongst other cardiovascular benefits.

The other marker of metabolic disease examined in this study – BMI – had an inverse correlation with LV diastolic filling and an inverse association with PEDSR on multivariable analysis. Obesity and overweight are known determinants of diastolic dysfunction(324) and risk factors for HFpEF(325). Very low-calorie diets have been found to improve diastolic function in people with obesity (**Chapter 1**)(233), suggesting that weight loss could be a key target for reversing diastolic dysfunction in T2D, which is the focus of **Chapter 7**. Our data also suggest that increasing BMI leads to hyperdynamic LV function (given the observed association of BMI with "better", more negative GLS). It is recognised that obesity is associated with increased sympathetic activity, which may result in hyperdynamic LV function(326). This may also explain the positive correlations noted between BMI and rest and stress MBF, although in multivariable analyses there was actually a

trend towards an inverse association between BMI and MPR. Whether weight loss interventions lead to subsequent reversal of hyperdynamic GLS and improvements in MPR and diastolic function is yet to be established.

Lastly, we found in our T2D patients that only increasing age and female sex were associated with lower MPR. Although microvascular dysfunction is thought to be a key determinant of diabetic cardiomyopathy, none of our modifiable clinical risk factors were significantly associated with MPR in multivariable analysis. Two previous studies have reported correlations between fasting glucose(327,328) and HbA1c (328), but both these studies were limited by small sample sizes (n=25 and n=23)(327,328) preventing multivariable analyses. The metabolic determinants of microvascular dysfunction and the relationship between MPR and cardiac systolic and diastolic dysfunction in T2D, therefore, warrant further investigation.

5.5.1 Limitations

Major strengths and limitations are as described in **Chapter 4**. Our large sample size permitted rigorous multivariable modelling of several key clinical variables thought be associated with measures of cardiac dysfunction in T2D. The inclusion of a diverse ethnic mixture of patients enabled an assessment of the impact of ethnicity on cardiovascular structure and function. However, we were not able to assess the impact of microvascular complications of T2D (such as retinopathy or microalbuminuria) on cardiac dysfunction, as these assessments were not performed in our cohort. It is also acknowledged that HbA1c represents glycaemic control over the past three months and may not be a true reflection of overall glucose control (where glycaemic variability is more representative). Of course, these analyses describe associations between clinical and cardiovascular parameters, but do not specifically indicate causation. No measures of diffuse myocardial fibrosis, myocardial triglyceride content or substrate utilisation were included, and as such the determinants of these pathophysiological mechanisms in diabetic cardiomyopathy have not been explored. The lack of univariate correlation or multivariate association between any of the selected clinical characteristics and E/A ratio in our T2D cohort was likely confounded by the complex pattern of changes in E/A ratio that occur with progressive diastolic dysfunction: an initial reduction, followed by pseudonormalisation and then increase with severely reduced LV compliance. These non-linear changes were therefore unlikely to be

detected by linear correlations or regression modelling. Grading of diastolic dysfunction has not as yet been undertaken in our study cohort, though it is not anticipated that a large proportion have grade 2 diastolic dysfunction.

5.6 Conclusion

In a large, asymptomatic, multi-ethnic cohort of adults with T2D, the major clinical determinants of cardiovascular dysfunction in our T2D cohort were increasing age, duration of T2D, systolic BP, BMI, and smoking history. HbA1c was not associated with early heart failure. Whether early interventions to treat modifiable risk factors (such as weight loss, BP reduction, reversal of T2D and smoking cessation) improve subclinical cardiovascular dysfunction is not known.

6 Cardiovascular determinants of aerobic exercise capacity in adults with type 2 diabetes

6.1 Abstract

6.1.1 Background

Aerobic exercise capacity is strongly related to cardiovascular outcomes, but the determinants of peak VO_2 in people with T2D, a group at high risk of heart failure, are unknown.

6.1.2 Objective

To assess the relationship between subclinical cardiovascular dysfunction and aerobic exercise capacity in adults with T2D.

6.1.3 Research design and methods

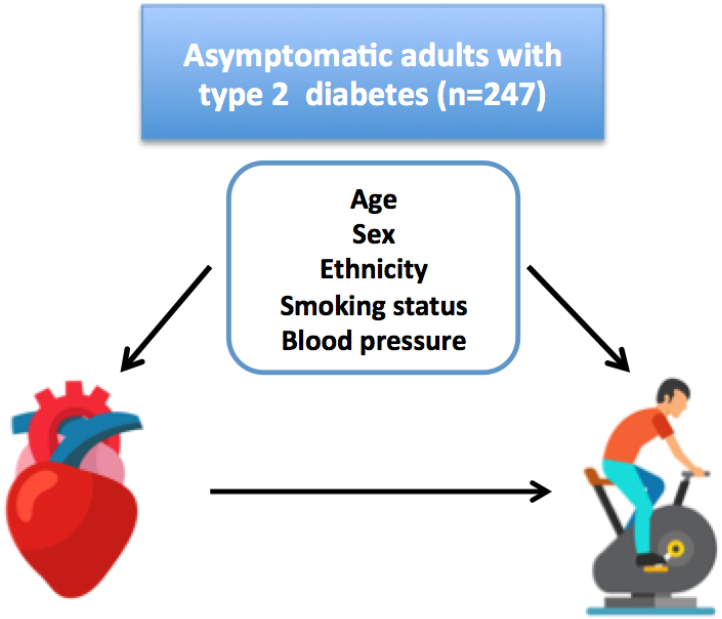
Cross-sectional study. We prospectively enrolled multi-ethnic adults with and without T2D, and no history, signs or symptoms of cardiovascular disease. Participants underwent bio-anthropometric profiling, echocardiography, CPET and adenosine stress perfusion CMR imaging. Multivariable linear regression analysis was undertaken to identify independent associations between measures of cardiovascular structure and function and peak VO_2.

6.1.4 Results

Two hundred and forty seven adults with T2D (age 51.8±11.9 years, 55% males, HbA1c 7.4±1.1%, 37% black or South Asian ethnicity, duration of diabetes 61 (32 – 120) months) and 78 controls were included. In a multivariable linear regression model containing age, sex, ethnicity, smoking status and systolic BP; only MPR (β=0.822, p=0.006) and E/e' (β= -0.388, p<0.001) were independently associated with peak VO_2 in subjects with T2D (Figure 6.1).

6.1.5 Conclusions

In a multi-ethnic cohort of asymptomatic people with T2D, MPR and LV diastolic filling are key determinants of aerobic exercise capacity, independent of age, sex, ethnicity, smoking status, or BP.



	β	95% CI	P-value
Age	−0.104	−0.172 to −0.036	**0.003**
Male sex	2.345	0.909 to 3.781	**0.001**
White ethnicity	1.415	−0.041 to 2.871	0.057
Never smoked	2.034	0.193 to 3.874	**0.030**
Systolic blood pressure	−0.017	−0.062 to 0.027	0.443
LV ejection fraction	−0.041	−0.150 to 0.067	0.453
LV global longitudinal strain	0.214	−0.072 to 0.499	0.142
Myocardial perfusion reserve	0.822	0.235 to 1.409	**0.006**
Average E/e'	-0.388	−0.595 to −0.180	**<0.001**

Figure 6.1 Association between cardiovascular structure, function, and aerobic exercise capacity in adults with type 2 diabetes. E/e' and MPR were associated with peak VO_2, independent of age, sex, ethnicity, smoking status and systolic blood pressure. Abbreviations: GLS=global longitudinal strain; MPR=myocardial perfusion reserve.

6.2 Introduction

Individuals with T2D are recognised to have limitations in aerobic exercise capacity, even in the absence of underlying CVD(329,330). Peak VO_2 is the gold standard method of assessing maximal aerobic capacity(331) and reduced peak VO_2 is a strong risk factor for the development of CVD(332), including heart failure(333), both in the general population and in people with T2D. However, the relationship between cardiovascular function and aerobic exercise capacity in asymptomatic people with T2D is not fully understood.

Cardiovascular magnetic resonance imaging is the gold standard imaging modality for assessment of cardiac volumes, mass and ejection fraction, and with the addition of stress perfusion imaging has the ability to provide accurate quantification of MBF. No studies to date have used this technique to assess the associations of cardiovascular structure and function with aerobic exercise capacity in people with T2D.

The aim of this study was to evaluate whether, in asymptomatic adults with T2D, markers of subclinical cardiovascular dysfunction are independently related to peak VO_2.

6.3 Research design and methods

A detailed description of the study methods, including participant recruitment, and assessments, and general statistical analysis, is provided in **Chapter 2**. The same inclusion and exclusion criteria used in **Chapter 4** were applied and the same cohort of participants with T2D and controls were included in the present analyses.

6.3.1 Specific Statistical analysis

Correlations with peak VO_2 were assessed using Pearson correlation coefficient separately in participants with T2D and controls. Generalised linear modelling was performed to identify independent associations of aerobic exercise capacity separately in participants with and without T2D. The dependent variable was peak VO_2 corrected for body weight. Only patients who achieved a respiratory exchange ratio (RER) ≥1 on CPET were included in correlation and regression analyses (total n=23 T2Ds excluded), to mitigate the confounding effects of tests where reaching of peak VO_2 was highly unlikely. A base model was adjusted for age, sex, ethnicity, smoking status, and systolic BP, factors that are recognised for their associations

with aerobic exercise capacity(334). CMR and echocardiographic variables that significantly correlated with peak VO_2, separately in cases and controls, were first analysed individually in the base model. Those CMR or echocardiographic variables found to be individually associated with peak VO_2 in the base model were then further selected and simultaneously entered into the base model to provide an assessment of whether these were associated with peak VO_2 independently of one another. A correlation matrix of included factors was undertaken to assess for potential multicollinearity; variables correlated at r >0.5 or < -0.5 were not included in the same regression model. Regression coefficients (β) are presented as point estimate and 95% confidence intervals. Statistical analysis was performed using SPSS version 25.0 (Statistical Package for Social Sciences, Chicago, IL). A p value <0.05 was considered statistically significant.

6.4 5.4 Results

The present analyses included the same cohort of participants as detailed in **Chapter 3**, with the additional exclusion of those individuals who did not achieve a peak RER ≥1 on CPET (**Error! Reference source not found.**2). A total of 224 T2Ds and 78 controls were therefore included in these analyses. Mean age of included participants with T2D was 52±12 years, 57% were male, 38% were of black or south Asian ethnicity, mean HbA1c was 7.4±1.1%, and median duration of diabetes was 60 (33 – 120) months.

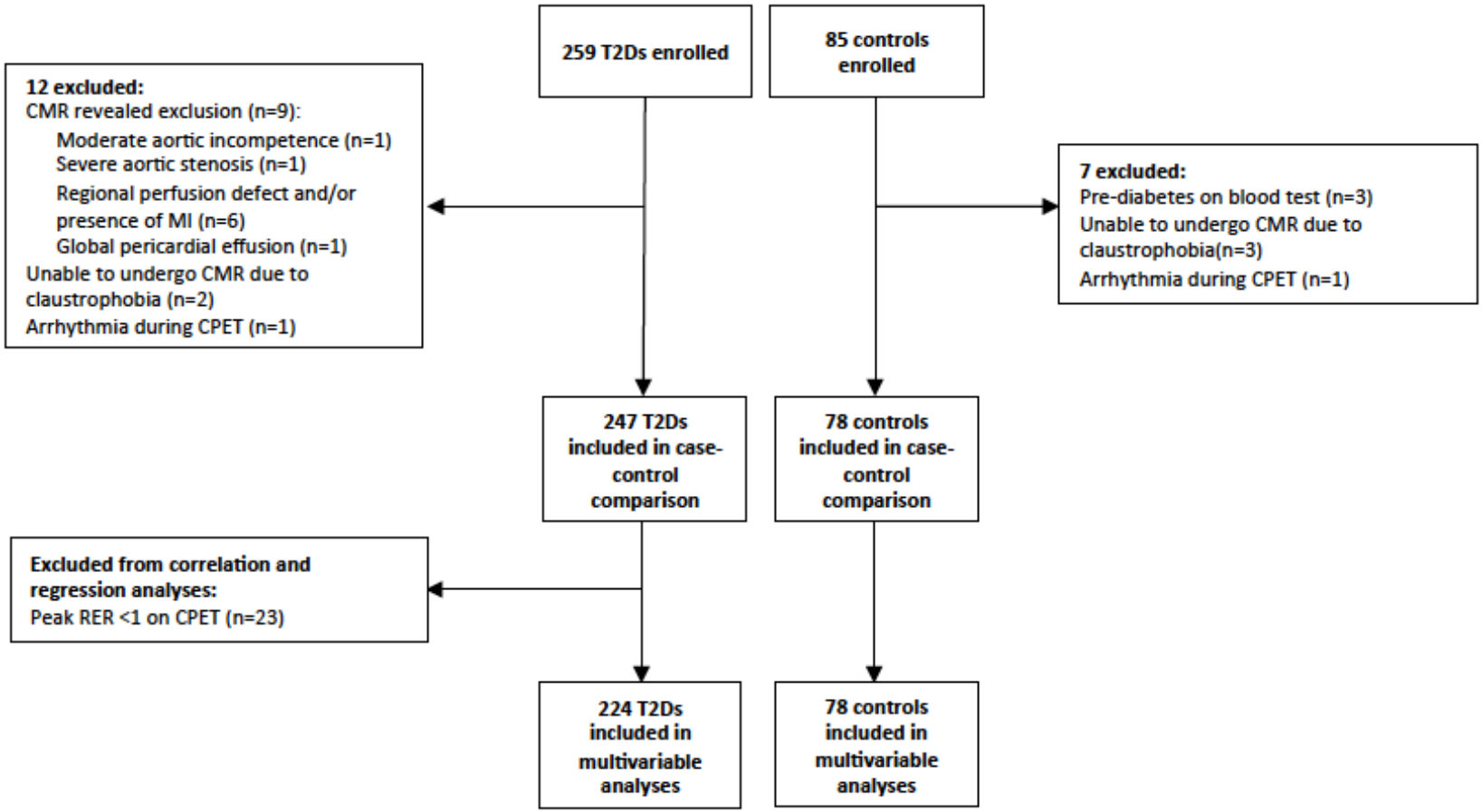


Figure 6.2 Study profile. Abbreviations: CMR=cardiovascular magnetic resonance; CPET=cardiopulmonary exercise testing; MI=myocardial infarction; RER=respiratory exchange ratio; T2D=type 2 diabetes.

6.4.1 Cardiovascular structure, function and fitness

Baseline case-control comparisons of CMR, echocardiography and CPET data are as presented in **Chapter 4**. In summary, subjects with T2D had increased concentric LV remodelling (LV mass:volume 0.84±0.14 vs. 0.76±0.11 g/mL, p<0.001), lower aortic distensibility (2.75 (1.74 – 4.03) vs. 4.92 (2.65 – 7.13) $mmHg^{-1}x10^{-3}$, p<0.001), evidence of systolic (GLS -16.2±2.4 vs. −17.4±1.9%, p<0.001) and diastolic dysfunction (E/A ratio 0.84 (0.66 – 1.05) vs. 1.10 (0.83 – 1.23), p=0.006), and reduced MPR (2.60±1.24 vs. 3.54±1.15, p<0.001) compared with controls.

After adjustment for age, sex and ethnicity, both absolute and body-weight corrected peak VO_2 were significantly lower in the T2Ds versus controls (18.0±6.6 vs. 27.8±9.0mL/g/min, p<0.001)(Table 4.3).

6.4.2 Correlations with aerobic exercise capacity

Correlations of participant characteristics and CMR measures of cardiac structure and function, with peak VO_2 in subjects with and without T2D are displayed in Table 6.1.

In subjects with T2D, significant correlations were observed between peak VO_2 and age, T2D duration, systolic BP, absolute and indexed LV volumes, LV EF, LV mass, LV GLS, average E/e' (Figure 6.3 3) and MPR (Figure 6.4 4). In controls, significant correlations were observed between peak VO_2 and absolute and indexed LV volumes, LV EF, LV mass, absolute and indexed LA volumes, LV PEDSR, E/e', MPR, aortic distensibility.

Table 6.1 Correlations between bio-anthropometrics, CMR measures of cardiac structure and function, and peak VO_2.

	T2Ds (n=224)		Controls (n=78)	
	Correlation coefficient (r)	**P-value**	**Correlation coefficient (r)**	**P-value**
Age	−0.346	**<0.001**	−0.652	**<0.001**
T2D duration	−0.246	**<0.001**	N/A	N/A
Systolic blood pressure	−0.142	**0.036**	−0.122	0.289
Diastolic blood pressure	−0.018	0.813	−0.219	0.054
HbA1c	0.065	0.338	−0.035	0.762
E/A ratio	-0.076	0.361	0.246	0.052
Average E/e’	-0.393	**<0.001**	-0.524	**<0.001**
LV EDV	0.164	**0.015**	0.414	**<0.001**
LV EDVi	0.204	**0.002**	0.571	**<0.001**
LV ESV	0.231	**0.001**	0.418	**<0.001**
LV ESVi	0.244	**<0.001**	0.497	**<0.001**
LV ejection fraction	−0.191	**0.004**	−0.299	**0.008**
LV mass	0.226	**0.001**	0.31	**0.005**
LV mass/volume	0.074	0.277	−0.098	0.392
LA EDV	0.008	0.907	0.267	**0.018**
LA EDVi	−0.010	0.886	0.283	**0.012**
LA ESV	−0.038	0.58	0.309	**0.006**
LA ESVi	−0.066	0.33	0.31	**0.006**
LA ejection fraction	−0.069	0.306	0.049	0.67
LV global longitudinal strain	0.158	**0.019**	0.176	0.123
LV peak early diastolic strain rate	0.093	0.188	0.232	**0.041**
Myocardial perfusion reserve	0.301	**<0.001**	0.304	**0.007**
Aortic distensibility	−0.089	0.223	0.46	**<0.001**

Abbreviations: BP=blood pressure; EDV=end-diastolic volume; EF=ejection fraction; ESV=end-systolic volume; LV=left ventricle.

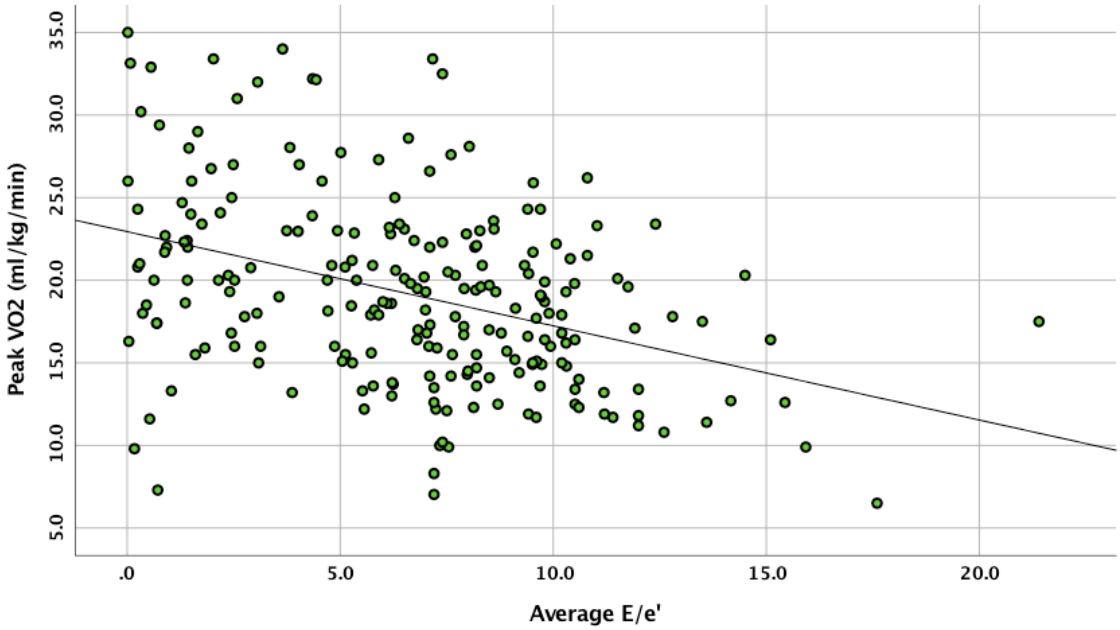


Figure 6.3 Scatterplot displaying the correlation between peak VO_2 and average E/e' in subjects with type 2 diabetes (r= -0.393, p<0.001).

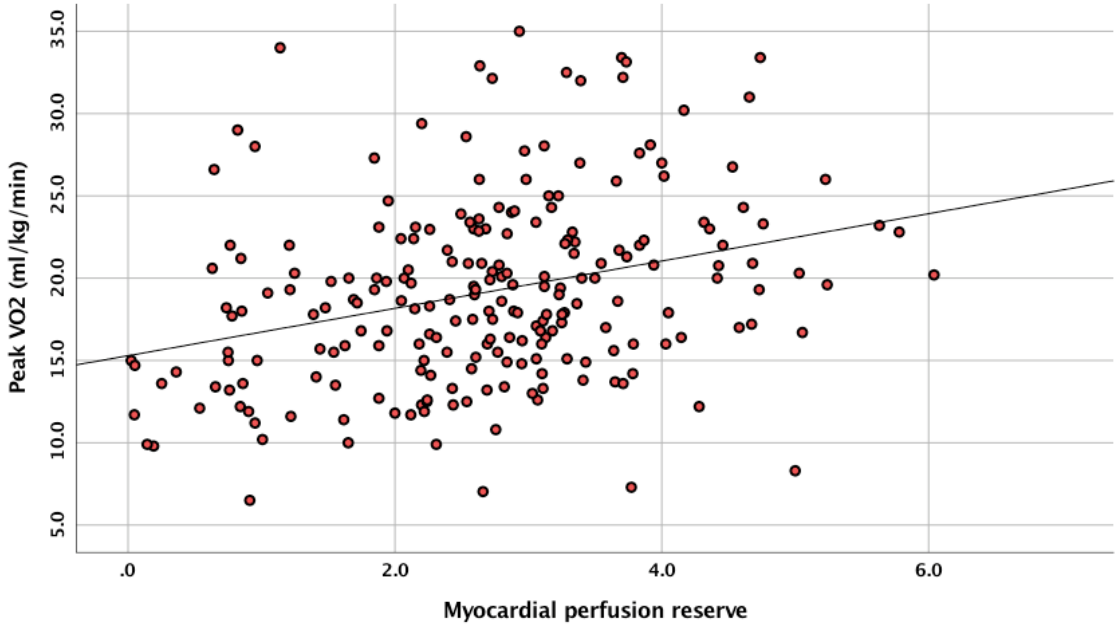


Figure 6.4 Scatterplot displaying the correlation between peak VO_2 and myocardial perfusion reserve in subjects with type 2 diabetes (r= 0.301, p<0.001).

6.4.3 Multivariable associations with aerobic exercise capacity

6.4.3.1 Participant characteristics

Multivariable associations between participant characteristics and peak VO_2 in subjects with and without T2D are displayed in Table 6.2. In both groups with and without T2D, variables significantly associated with peak VO_2 were age, sex, and ethnicity. Smoking status and resting systolic BP were not significantly associated with peak VO_2 in either T2Ds or controls.

Table 6.2 Associations of bio-anthropometric characteristics with peak VO_2 in people with type 2 diabetes and controls.

	T2Ds (n=224)			**Controls (n=78)**		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Age	−0.195	−0.260 to −0.130	**<0.001**	−0.448	−0.578 to −0.318	**<0.001**
Male sex	3.537	2.147 to 4.928	**<0.001**	3.31	0.347 to 6.273	**0.029**
White ethnicity	1.878	0.422 to 3.334	**0.011**	4.915	1.638 to 8.193	**0.003**
Never smoked	1.406	−0.563 to 3.375	0.161	−5.920	−13.308 to 1.468	0.116
Systolic blood pressure	−0.035	−0.082 to −0.012	0.144	0.04	−0.047 to 0.127	0.369

Abbreviations: CI=confidence interval; T2D=type 2 diabetes.

6.4.3.2 CMR and echocardiographic measures of cardiovascular structure and function

Associations of CMR measures of cardiovascular structure and function with peak VO_2, tested individually against the base model of bio-anthropometric characteristics, in participants with T2D and controls are shown in Table 6.3. In patients with T2D, LV EF (β= -0.108, p=0.037), LV GLS (β=0.265, p=0.046), MPR (β=0.798, p=0.005), and E/e' (β= -0.385, p<0.001) had significant individual associations with peak VO_2. In controls, only LV EDV (β=0.082, p<0.001), LV EF (β= -0.297, p=0.012) and LV mass (β=0.129, p<0.001) were significantly associated with peak VO_2.

Table 6.3 Individual associations of cardiac MRI measures of cardiovascular structure and function with peak VO_2, added to a base model containing age, sex, ethnic group, smoking status and systolic blood pressure.

	T2Ds (n=224)			**Controls (n=78)**		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Average E/e'	-0.385	-0.594 to -0.176	**<0.001**	-0.607	1.410 to 0.196	0.138
LV end-diastolic volume	−0.010	−0.034 to 0.014	0.429	0.082	0.036 to 0.129	**<0.001**
LV ejection fraction	−0.108	−0.209 to −0.007	**0.037**	−0.297	−0.529 to −0.064	**0.012**
LV mass	0.023	−0.010 to 0.057	0.175	0.129	0.072 to 0.185	**<0.001**
LV global longitudinal strain	0.265	0.004 to 0.526	**0.046**	0.525	−0.256 to 1.307	0.188
Myocardial perfusion reserve	0.798	−0.235 to 1.360	**0.005**	0.78	−0.531 to 2.092	0.243
Mean aortic distensibility	N/A	N/A	N/A	−0.307	−0.051 to 0.124	0.466
Indexed LA end-systolic volume	N/A	N/A	N/A	0.092	−0.019 to 0.203	0.104
LV peak early diastolic strain rate	N/A	N/A	N/A	1.062	−6.628 to 8.753	0.787

Abbreviations: CI=confidence interval; LA=left atrium; LV=left ventricle; N/A=not applicable due to no significant correlation demonstrated.

Multivariable associations between CMR measures of cardiovascular structure and function with significant individual associations with peak VO_2, simultaneously added to the base model of bio-anthropometric characteristics, are shown in Table 6.4. In subjects with T2D, only E/e' (β= -0.388, p<0.001) and MPR (β=0.822, p=0.006) were significantly associated with peak VO_2 independent of age, sex, ethnicity, smoking status and systolic BP (Figure 6.1). Addition of HbA1c to the model did not significantly affect these associations (Table 6.5). In controls, only LV mass was significantly associated with peak VO_2 (β=0.116, p=0.012).

Table 6.4 Multivariable associations between cardiac MRI measures of cardiovascular structure and function with peak VO_2 in people with type 2 diabetes and controls.

T2Ds (n=224)				Controls (n=79)			
Variable	**β**	**95% CI**	**P-value**	**Variable**	**β**	**95% CI**	**P-value**
Age	−0.104	−0.172 to −0.036	**0.003**	Age	−0.446	−0.563 to −0.329	**<0.001**
Male sex	2.345	0.909 to 3.781	**0.001**	Male sex	−0.461	−3.596 to 2.675	0.773
White ethnicity	1.415	−0.041 to 2.871	0.057	White ethnicity	2.929	−0.220 to 6.078	0.068
Never smoked	2.034	0.193 to 3.874	**0.030**	Never smoked	−5.636	−12.185 to 0.914	0.092
Systolic blood pressure	−0.017	−0.062 to 0.027	0.443	Systolic blood pressure	−0.037	−0.125 to 0.052	0.417
LV ejection fraction	−0.041	−0.150 to 0.067	0.453	LV EDV	<0.001	−0.072 to 0.072	0.998
LV GLS	0.214	−0.072 to 0.499	0.142	LV ejection fraction	−0.143	−0.375 to 0.089	0.227
Myocardial perfusion reserve	0.822	0.235 to 1.409	**0.006**	LV mass	0.116	0.026 to 0.206	**0.012**
Average E/e’	-0.388	−0.595 to −0.180	**<0.001**				

Abbreviations: CI=confidence interval; EDV=end-diastolic volume; GLS=global longitudinal strain; LV=left ventricle.

Table 6.5 Multivariable associations between cardiac MRI measures of cardiovascular structure and function with peak VO_2 in people with type 2 diabetes, including HbA1c.

	T2Ds (n=224)		
	β	**95% CI**	**P-value**
Age	-0.110	-0.179 to -0.041	**0.002**
Male sex	2.352	0.898 to 3.806	**0.002**
White ethnicity	1.425	-0.030 to 2.880	0.055
Smoking history (ref: never smoked)	2.041	0.197 to 3.885	**0.030**
Systolic blood pressure	-0.018	-0.063 to 0.027	0.427
HbA1c	-0.189	-0.782 to 0.403	0.531
LV ejection fraction	-0.043	-0.153 to 0.066	0.436
LV global longitudinal strain	0.266	-0.061 to 0.514	0.123
Myocardial perfusion reserve	0.808	0.217 to 1.399	**0.008**
Average E/e'	-0.383	-0.592 to -0.174	**<0.001**

Abbreviations: CI=confidence interval; LV=left ventricle.

6.5 Discussion

This is the first study to comprehensively describe the associations of aerobic exercise capacity, a strong surrogate marker of outcomes, with cardiovascular structure and function in asymptomatic people with T2D. The major strengths of the study are the detailed phenotyping with both CMR and echocardiography, the large sample size, the rigorous exclusion of those with established CVD or low RER, the high proportion of both females and ethnic minorities which make the results more generalizable and the use of CPET for exercise capacity. Of the markers of subclinical cardiovascular dysfunction in people with T2D we identified in **Chapter 4**, only E/e', and MPR were independently associated with peak VO_2 in subjects with T2D. By contrast, only LV mass was associated with peak VO_2 in controls.

People with T2D are at two-fold increased risk of developing heart failure, independent of traditional risk factors, and a distinct diabetic cardiomyopathy is now well described(316). Even though we carefully screened and excluded individuals with prior CVD and who reported exercise limitation due to symptoms of dyspnoea or angina, exercise capacity in the T2D group was markedly lower than controls. This suggests that our T2D cohort already have asymptomatic stage B heart failure and are at incipient risk of developing clinical symptoms. Key features that typify diabetic cardiomyopathy – diastolic dysfunction and reduced MPR – were present in our subjects with T2D and were intimately linked with reduced exercise capacity.

To our knowledge one other (n=170) study published over 15 years ago has assessed the cardiac determinants of exercise capacity in people with T2D, but only using echocardiography(335). In a model containing age, male, sex, BMI and HbA1c, the only independent cardiac determinant of exercise capacity was basal early diastolic velocity. However, no measures of myocardial perfusion or aortic stiffening were performed. Exercise capacity was measured during treadmill stress testing performed for assessment of coronary artery disease and was estimated in metabolic equivalents. We assessed aerobic exercise capacity by CPET with expired gas analysis for peak VO_2, which is the gold standard measure of cardiorespiratory fitness. Furthermore, we assessed cardiovascular structure and function by multiparametric CMR, the gold standard method that enabled comprehensive evaluation of cardiac geometry, EF, myocardial perfusion and aortic distensibility in

a single examination without the limitation of poor acoustic windows and operator dependency as in echocardiography.

Concentric LV remodelling, with smaller LV volumes and higher LV mass, is a consistent finding in studies of cardiac alterations in people with T2D(316). Whereas endurance training is associated with enlargement of LV chamber dimensions, physical inactivity causes the opposite(336). However, we found no association between concentric LV remodelling and peak VO_2 in our T2D subjects, suggesting that concentric remodelling occurs as the result of other cardiovascular perturbations in T2D and may not therefore be a central mechanism causing heart failure. This finding is consistent with the multi-ethnic study of atherosclerosis where LV mass:volume was not independently associated with a higher risk of heart failure development(52).

No previous studies have explored the relationship between MPR and exercise capacity in people with T2D. The findings of this study indicate that impaired myocardial perfusion is a central mechanism leading to reduced exercise capacity in people with T2D and is highly likely to play a key mechanistic role in the development and progression of diabetic cardiomyopathy. Our group has previously shown that MPR is independently associated with peak VO_2 in patients with both severe symptomatic(123) and asymptomatic moderate-severe aortic stenosis(337). This consistent finding is also physiologically plausible, as myocardial perfusion must progressively increase during incremental exercise to ensure sufficient myocardial oxygenation, driven by increased heart rate and BP.

Abnormalities of vascular function are well described in T2D, and impaired MPR has been associated with increased cardiovascular mortality(126). A striking finding in our cohort is that, even after excluding subjects with reversible perfusion defects, previous myocardial infarction on CMR, and myocardial ischaemia on exercise ECG, subjects with T2D had lower overall MPR than controls. Subclinical alterations in myocardial perfusion may therefore be linked to early heart failure. However, studies evaluating the relationship between myocardial perfusion and diastolic function have to date yielded inconsistent findings(94,129), possibly due to different selection criteria. Nevertheless, it is possible that targeting even subclinical impairments in myocardial perfusion may lower the risk of incident heart failure development in people with T2D.

Lastly, despite having a normal LV EF, our T2D cohort had reduced GLS compared with controls, suggesting that they have evidence of subclinical LV systolic dysfunction that correlated with exercise capacity. Surprisingly this was not associated with peak VO_2 in multivariable analyses, nor was our CMR measure of diastolic function, PEDSR. By contrast, although E/e' (an estimate of LV filling pressure) did not differ between cases and controls, it was independently associated with peak VO_2 only in subjects with T2D. This may be because diastolic function, which is typically regarded to precede systolic dysfunction in diabetic cardiomyopathy, is an early determinant of exercise limitation whereas worsening systolic dysfunction may predominate in symptomatic patients. Whether worsening of both systolic and diastolic function along the course of diabetic cardiomyopathy leads to greater impact on exercise capacity and subsequent heart failure development remains to be established.

6.5.1 Limitations

Limitations are as described in **Chapters 4** and **5**, and include the pooled cohort of baseline CPET and CMR data from participants of studies in our unit, with minor differences in recruitment criteria. However, we used pre-specified inclusion and exclusion criteria for the present analyses to unify the study cohort, and all imaging was performed with standardised protocols and analysis techniques. We excluded significant coronary artery disease by exercise electrocardiography, LGE and adenosine stress perfusion CMR imaging, which has demonstrated excellent sensitivity and specificity for obstructive epicardial coronary artery disease(319). However, we acknowledge that invasive angiography remains the gold standard modality for assessment of coronary artery disease, and subjects with diffuse, three-vessel coronary artery disease may not have regional perfusion defects detectable by CMR. Different perfusion acquisition and analysis methods were used between the different pooled studies, which may have introduced systematic differences in MPR values(338). However, each sub-study had its own T2D cases and controls, which were analysed with a common method, so differences in MPR between groups were not affected by analysis method.

6.6 Conclusions

E/e' and MPR are key determinants of aerobic exercise capacity in people with T2D, independent of age, sex, ethnicity, smoking status, BP, or glycaemic control, and may

drive the progression of stage B heart failure. Further studies are needed to determine whether strategies to reverse subclinical cardiovascular abnormalities in cardiovascular function lead to improvements in exercise capacity and prevent heart failure development in T2D.

7 Effects of low-energy diet or exercise on cardiovascular function in younger adults with type 2 diabetes

7.1 Abstract

7.1.1 Background

Asymptomatic adults with T2D are at increased of developing heart failure, which has limited effective treatment options.

7.1.2 Objectives

To confirm the presence of subclinical cardiovascular dysfunction in younger adults with T2D and determine if this is improved by a low-energy MRP diet or exercise training.

7.1.3 Research design and methods

Prospective, randomised, open-label, blinded endpoint trial with nested case-control study. Asymptomatic adults with T2D were randomized 1:1:1 to a 12-week intervention of: 1) routine care; 2) supervised aerobic exercise training or 3) a low-energy (≈810kcal/day) MRP. Participants underwent echocardiography, cardiopulmonary exercise testing and CMR at baseline and 12-weeks. The primary outcome was change in LV PEDSR, measured by CMR. Healthy volunteers were enrolled for baseline case-control comparison.

7.1.4 Results

Eighty-seven T2Ds (age 51±7y, HbA1c 7.3±1.1%) and 36 matched controls were included. At baseline, T2Ds had evidence of diastolic dysfunction (PEDSR 1.01±0.19 vs. 1.10±0.16s^{-1}, p=0.02) compared with controls. Seventy-six participants completed the trial (30 routine care, 22 exercise, and 24 MRP). The MRP arm lost 13kg weight, improved BP, glycaemia, LV mass:volume and aortic stiffness. The exercise arm had negligible weight loss but increased exercise capacity. PEDSR increased in the exercise arm versus routine care (β=0.132, p=0.002), but did not improve with the MRP (β=0.016, p=0.731).

7.1.5 Conclusions

In asymptomatic younger adults with T2D, exercise training improved diastolic function. Despite beneficial effects on weight, glycaemic control, concentric LV remodelling and aortic stiffness, a low-energy MRP did not improve diastolic function.

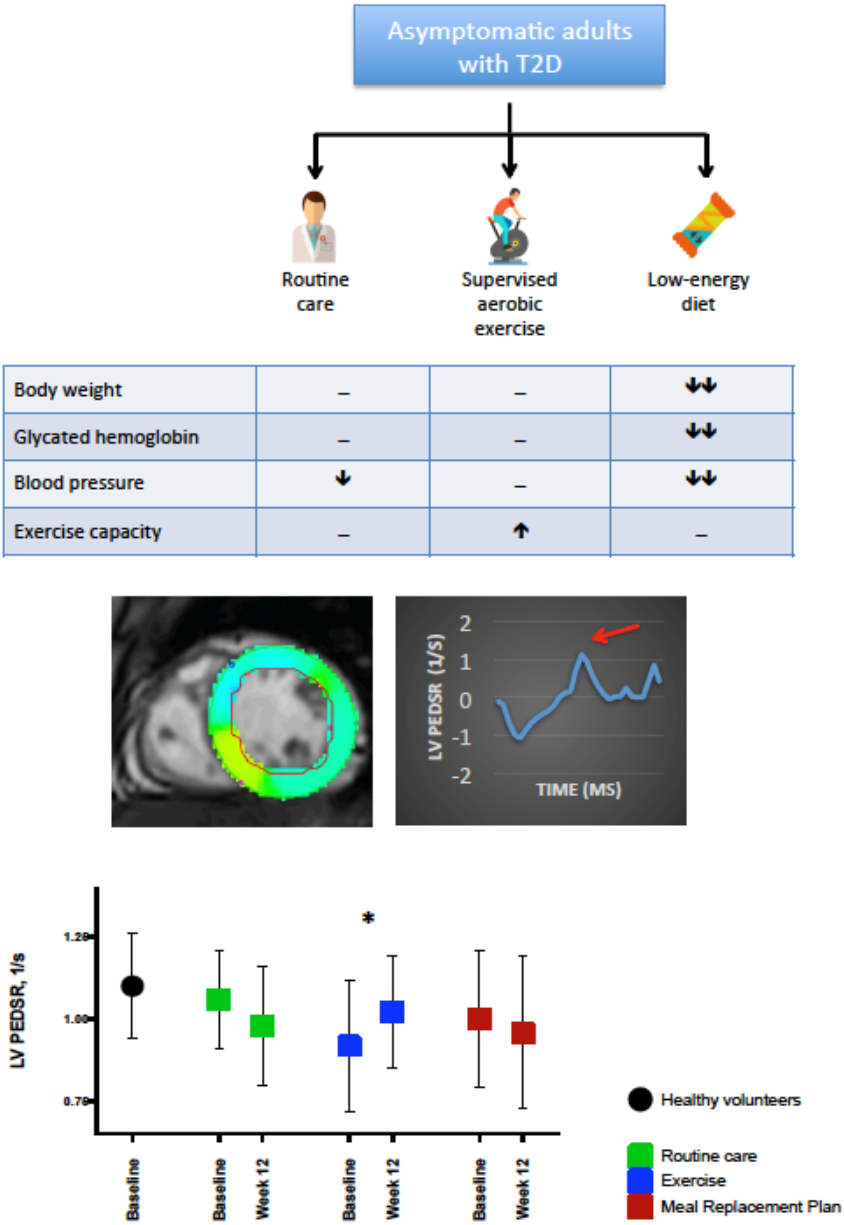


Figure 7.1 Prospective, randomized, open-label, blinded-endpoint trial of routine care versus a supervised aerobic exercise programme versus a low-energy (≈810 Kcal/day) meal replacement plan. With routine care, there was a modest reduction in blood pressure. Exercise training led to improvements in exercise capacity. The dietary intervention led to dramatic reductions in body weight, glycaemia and blood pressure. The primary outcome measure was cardiac MRI-derived left ventricular peak early diastolic strain rate (LV PEDSR, red arrow, a measure of the speed of myocardial relaxation). At baseline, LV PEDSR was significantly lower in people with type 2 diabetes compared with controls (p=0.02). After the 12-week interventions, PEDSR increased in the exercise arm versus routine care (β=0.132, 97.5% CI 0.038 to 0.225, p=0.002), but did not improve with the diet (β=0.016, 97.5% CI -0.075 to 0.106, p=0.731).

7.2 Introduction

The likelihood of developing CVD is markedly increased in younger adults with T2D, who have the highest lifetime risk(30). For example, recent findings from the Swedish National Diabetes Registry (comprising data from 318,083 people with T2D and almost 1.6 million controls) demonstrated the highest excess risk of CVD occurred in those diagnosed with diabetes aged <40 years(30).

Whilst the development of ASCVD is well recognised in T2D, the risk of heart failure has received less attention. However, the United Kingdom National Diabetes Audit 2015-16, which includes data on over 2.7 million people with diabetes, found that heart failure is the commonest cardiovascular complication and a major cause of premature mortality(20). This is especially the case in younger adults with T2D, where the risk of heart failure development is four- to five-fold higher than matched controls(30). Importantly, undiagnosed heart failure is highly prevalent in people with T2D(65). We have previously demonstrated evidence of subclinical diastolic impairment in young adults (mean age 32 years) with T2D compared to both obese and lean controls, despite their young age and relatively short duration of disease(38). Indeed, isolated diastolic dysfunction is widely regarded as the earliest manifestation of cardiac impairment in people with T2D. This is a known precursor of symptomatic heart failure(305), with the vast majority developing HFpEF, a condition with no effective treatments(65). It is therefore imperative to find strategies that prevent heart failure in T2D, specifically targeting younger adults before symptoms develop and when cardiac dysfunction is likely to be reversible.

Although the risk of ASCVD can be mitigated by strict risk factor management in T2D, this has little to no effect on the excess risk of heart failure development(212). Therefore, the development of effective therapies to prevent and treat heart failure in people with T2D represents an important unmet need in this population.

It is now recognised that reversal of T2D can be achieved with weight loss, accomplished either by bariatric surgery(217) or via a low-energy MRP diet(226). Exercise training also leads to modest but sustained improvements in glycaemic control, improvements in insulin resistance, and improved cardiovascular fitness, even in the absence of accompanying weight loss(244,339). Whether weight loss or exercise training can improve subclinical cardiac dysfunction in people with T2D

remains to be established. There have been no randomised controlled trials assessing cardiac function with MRP and the results of trials in exercise training have been inconsistent(340).

The aims of this study were: (1) to confirm the presence and nature of subclinical cardiovascular dysfunction in younger adults with T2D, and (2) to determine if diastolic function can be improved by either a low-energy MRP or a supervised aerobic exercise programme, compared to routine care.

7.3 Methods

A detailed description of the general study methods is provided in **Chapter 2**.

7.3.1 Study design and participants

The rationale and study design and conduct, including details of participant recruitment and planned analyses, have been published previously(261). In brief, this was a single-centre PROBE trial with a nested case-control study, at the NIHR Leicester Biomedical Research Centre. Ethical approval was granted by the National Research Ethics Service (Research Ethics Committee West Midlands – Coventry, reference: 15/WM/0222). The trial is registered at https://clinicaltrials.gov (unique identifier: NCT02590822).

Participants with T2D were identified from both primary and secondary care in Leicestershire, UK, using electronic databases and with support from the NIHR East Midlands Local Clinical Research Network. A list of the complete study inclusion and exclusion criteria is detailed in Table 7.1**.** Healthy volunteers free of T2D, obesity, hypertension, or prevalent cardiovascular disease were recruited for baseline case-control comparison. All participants provided written informed consent in advance of entering the study.

Table 7.1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Capacity to provide informed consent before any trial-related activities	Diabetes duration >12 years
Established T2D (≥3months)	Currently taking more than three glucose lowering therapies
HbA1c ≤ 9% if on triple therapy or ≤ 10% on diet & exercise or monotherapy or dual therapy	Weight-loss of >5kg in the preceding 6 months
Current glucose lowering therapy either mono, dual or triple of any combination of metformin, sulphonylurea, DPP-IV inhibitor, GLP-1 therapy or an SGLT2i +/- diet and exercise	Stage 4 or 5 chronic kidney disease (eGFR< 30ml/min/1.73m^2)
Poorly managed diet-controlled diabetes (with HbA1c $\geq$ 6.5%, not currently taking any glucose lowering therapy, meeting BMI inclusion range)	Current therapy with Insulin, thiazolidinediones, steroids or atypical antipsychotic medication
Body mass index >30Kg/m^2 or >27 Kg/m^2 (South Asian)	Untreated thyroid disease
Diagnosis of T2D before the age of 60 years of age	Known macrovascular disease including coronary artery disease, stroke/TIA or peripheral vascular disease
Age ≥18 and ≤ 65 years	Presence of arrhythmia (including atrial fibrillation, atrial flutter, or 2nd or 3rd degree atrioventricular block)
	Known heart failure or other clinically relevant heart disease
	Inability to exercise or undertake MRP
	Absolute contraindication to CMR
	Cardiovascular symptoms (angina, limiting dyspnoea during normal physical activity)
	Inflammatory condition e.g. Connective tissue disorder, Rheumatoid arthritis

Abbreviations: T2D=type 2 diabetes; DPP-IV=dipeptidyl peptidase-IV inhibitor; SGLT2i=sodium glucose co-transporter 2 inhibitor; GLP-1=glucagon-like peptide; CMR=cardiovascular magnetic resonance imaging; MRP=meal replacement plan.

7.3.2 Assessment visits

Participants with T2D underwent two main study assessment visits, at baseline and 12 weeks. An overview of participant flow through the study is presented in Figure 7.2. Control subjects underwent the same assessments, but at baseline only. Each participant was assigned a unique identification number upon recruitment. Participant details were held separately on an independent database provided by the Leicester Clinical Trials Unit and only accessible by members of the study team to arrange follow-up visits. Anonymised source data were entered separately by members of the research team at site into in independent web-based database created by the Leicester Clinical Trials Unit. To encourage compliance with the trial research outcomes, participants were offered £50 compensation for each assessment visit completed.

A detailed description of all study assessments is provided in **Chapter 2**, which are therefore only outlined in brief below.

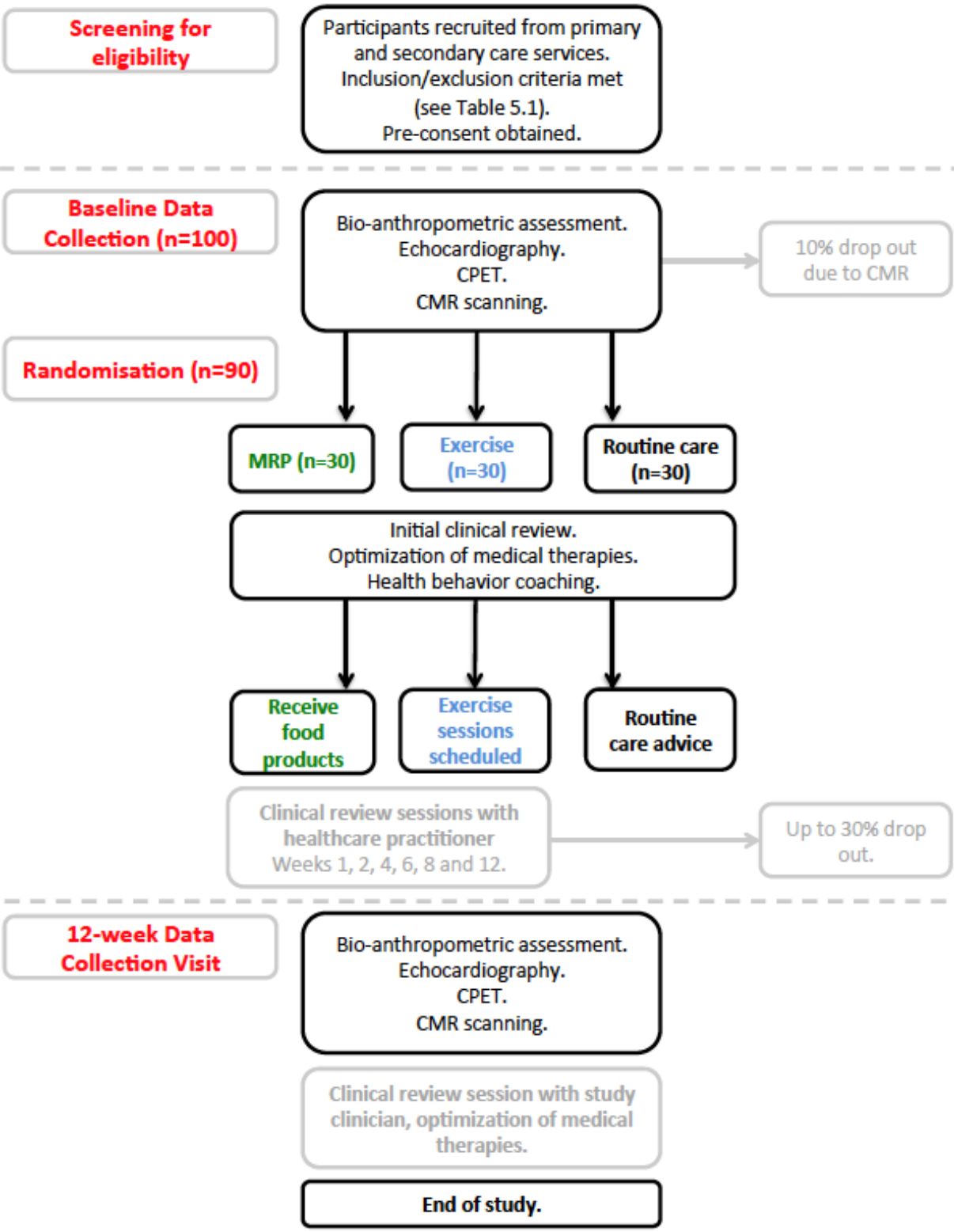


Figure 7.2 Study overview. Abbreviations: CMR=cardiovascular magnetic resonance imaging, CPET=cardiopulmonary exercise testing, MRP=meal replacement plan.

Demographics, medical history and anthropometric measures were collected at the assessment visits. A fasting blood sample was collected to obtain a biochemical profile for diabetes control, liver and kidney function, lipid profile, and adiposity (leptin and adiponectin), fasting insulin and C-peptide, and B-type natriuretic peptide. Insulin resistance was estimated using HOMA-IR(264). Participants in the MRP arm with a fasting glucose of <7.0mmol/L or HbA1c <6.5% without taking any hypoglycaemic agent post-intervention were considered to have remission of T2D(341).

7.3.2.1 Cardiovascular magnetic resonance imaging

All CMR scanning was performed on a 1.5T field strength scanner (Siemens Aera, Erlangen, Germany) using a standardised protocol (Figure 2.1). In summary, after localisers, SSFP cine images were acquired in four-, three- and two-chamber views. Perfusion images were then acquired after vasodilatory stress with adenosine (140µg/kg/min, infused intravenously for three minutes). At peak stress, a gadolinium-based contrast agent (Gadoterate meglumine, Dotarem, Guerbet LLC, France) was injected (0.04mmol/kg), followed by a 20mL bolus of normal saline, at a rate of 5mL/s, and perfusion images were acquired using a saturation recovery gradient echo pulse sequence for three slices (basal, mid and apical). Rest imaging was performed approximately 10 minutes after stress with a further 0·04mmol/kg contrast. In between rest and stress imaging, a stack of short-axis slices was obtained using cine images to obtain coverage of the entire LV.

CMR images were analysed offline blinded to all patient details and treatment group, by a single experienced observer (GSG), as described in **Chapter 2**. For the primary outcome measure, myocardial strain measurement was performed using cmr42 Tissue Tracking. Circumferential strain rate curves were generated to calculate PEDSR (Figure 7.1) from balanced SSFP short axis cine images.

7.3.2.2 Transthoracic echocardiography

Transthoracic echocardiography was performed as described in **Chapter 2**.

7.3.2.3 Cardiopulmonary exercise testing

A symptom-limited incremental CPET was performed as described in **Chapter 2.**

7.3.3 Randomization and blinding

Subjects with T2D were randomised at the end of the baseline visit in a 1:1:1 ratio, using an independent online computerised randomization system incorporating concealed allocation (Sealed Envelope®) to one of three arms (see below). Randomisation was stratified by sex, given different LV remodelling processes in males and females(342), and baseline glucose-lowering therapy (any GLP-1RA, dipeptidyl peptidase-IV inhibitor or SGLT2i versus none of these agents). The nature of the trial interventions prevented blinding of allocation.

7.3.4 Trial interventions

Recruited participants were randomised to one of three groups; 1. Routine care as per NICE guidance(343) including lifestyle advice, or 2. A low-energy MRP diet, or 3. supervised aerobic exercise training.

7.3.5 Routine care

The routine care group were provided with standard lifestyle advice according to NICE guidance(202), delivered in a single health coaching interview at week 0. This lifestyle advice was reinforced at the week 12 assessment.

7.3.6 Meal replacement plan diet

The MRP group received a low-energy MRP containing an average of ≈810 kcal/day (30% protein, 50% carbohydrate, 20% fat) (Cambridge Weight Plan®). This diet plan complies with all current guidance and government legislation (European Food Safety Authority) for macro- and micro nutrient content and quality(344). Participants randomised to the MRP group were asked to discontinue all glucose-lowering therapies following randomisation to avoid hypoglycaemia. Antihypertensive drugs were stopped on the day of low-energy diet commencement, as a safety measure because BP can fall substantially (mean drop in systolic BP >10mmHg) on the diet(122). Any alterations to medication were made at the discretion of the study clinician(s) (predominantly GSG). The clinical team included specialists in CVD and diabetes medicine to oversee alterations in antihypertensive and glucose lowering therapy. BP and glucose were monitored throughout the study at the clinical review sessions (see below).

The MRP was undertaken alongside health behaviour coaching and relapse prevention through weekly contact, where possible, with a qualified dietician or equivalent (EB or SA). Participants were asked to maintain their usual daily

activities and not initiate any additional physical activity for the duration of the study. Those participants in the MRP arm who did not achieve a loss of >2% body weight at week 1 and 4% at week 3 were considered non-compliant and excluded from the study. Investigator discretion was used when considering non-compliance in the event of borderline values. The diet was stopped, and a maintenance diet re-introduced once 50% excess body weight had been lost, or by 12 weeks, whichever came first. An individualised maintenance diet was then agreed with the participant, based on their estimated total energy expenditure at that point.

7.3.7 Supervised exercise training

The exercise group attended supervised sessions at the Leicester Diabetes Centre. The exercise program was undertaken thrice weekly, progressing to 50-minutes per session of moderate intensity aerobic exercise, in line with prevailing guidelines (150 minutes per week). Each exercise session consisted of a warm-up, stimulus and cool-down phase. Static stretching was undertaken at the end of each session. The stimulus phase included walking and/or lower extremity cycling. The duration of exercise in each training mode was increased gradually to achieve the 50-minute target. An initial assessment of cardiorespiratory fitness was performed, and exercise intensity titrated using the value from the baseline peak VO_2 assessment. Objective (heart rate monitoring) and subjective (Borg Rate of Perceived Exertion scale, graded from 6 to 20) measures were used to measure responses to the exercise sessions with exercise intensity adjusted progressively to take account of increasing fitness levels throughout the intervention period as follows:

Week 1&2: 15 mins at ~50% peak VO2
Week 3: 25 mins at ~60% peak VO2
Week 4: 30 mins at ~60% peak VO2
Week 5: 35 mins at ~60% peak VO2
Week 6: 40 mins at ~60% peak VO2
Week 7: 45 mins at ~60% peak VO2
Weeks 8-12: 50 mins at ~60% peak VO2

Compliance was assessed by attendance at the supervised sessions. Patients in the exercise arm who attended less than two-thirds of the supervised sessions in

the first four weeks and then throughout the remainder of the study period were excluded. Investigator discretion was used in cases where compliance was borderline.

Participants were asked to maintain their usual dietary intake during the exercise intervention. Participants in the exercise arm had their medication reviewed by the study clinician at baseline. Those with a HbA1c ≤8% who were taking a sulphonylurea had the dose reduced by 50% 72 hours prior to the first exercise session. Their regimes were continued to be down-titrated starting with the sulphonylurea and then moving, where applicable, onto their current SGLT2i or dipeptidyl peptidase-IV inhibitor or GLP-1RA therapies according to glycaemic control. The latter therapies were titrated down last given their relative low risk of hypoglycaemic events. For those participants with a HbA1c ≤8% who were not taking a sulphonylurea the study clinician down-titrated glucose-lowering therapy on an individual basis, taking into account the class and combination of therapies prescribed. All other medication was maintained unless the study clinician deemed alterations are necessary for a patient's best interest. Alterations in medication were supervised by clinicians specialised in diabetes medicine.

7.3.8 Clinical reviews

All participants had an initial clinical review post-randomisation at the time of their health coaching. In order to ensure their continued safety, each participant in the MRP or exercise arms were invited for frequent clinical reviews. They attended six reviews after randomisation in total (weeks 1, 2, 4, 6, 8, and 12), as well as receiving a telephone consultation at weeks 3 and 10. At these clinical reviews their BP, fasting glucose (weeks 1, 2 and 4 for MRP arm), and weight were measured. This was to inform; a) any additional alterations to medication that needed to be made and b) compliance to the MRP and exercise programmes as appropriate.

7.3.8.1 Week 12 final data collection visit

All measures and tests undertaken at the baseline visit were repeated at the 12-week final data collection visit. Participants underwent one further clinical review (see above) following the 12-week data collection visit, which was the final visit and the end of the study. Medications were reviewed and any medications re-introduced were tailored to the individual based on previous and current HbA1c and BP. This

review was undertaken by an experienced study clinician (GSG) and the outcome communicated to the patient's usual diabetes physician and General Practitioner.

7.3.9 Outcomes

The primary outcome measure was change in PEDSR, measured by CMR, from baseline to 12 weeks, in the two intervention arms (MRP and exercise) compared to routine care. PEDSR was selected as the primary outcome measure for the following reasons: we have previously demonstrated it is a sensitive measure for detecting subclinical diastolic dysfunction in younger adults with T2D(38), it has very good inter-study reproducibility in this cohort (**Chapter 3**)(303), is not reliant on the quality of acoustic windows that may limit echocardiographic measures of diastolic function in patients with obesity and is less load-dependent than measures of diastolic filling.

Key secondary outcomes were change in echocardiographic measures of diastolic function (E/A ratio and E/e'). Additional secondary outcomes were CMR measures of cardiac structure and function (LV mass and volumes, GLS) rest and stress MBF, aortic stiffness (distensibility) and peak VO_2(261).

7.3.10 Power calculation

The trial sample size calculation was determined according to published pilot data from our group(38). To detect a between-group difference in PEDSR of $0.2s^{-1}$ post-interventions, at least 21 participants with T2D completing each of the three trial arms were needed to provide 80% power, at alpha=0.025 (to allow for two primary comparisons, i.e. MRP vs. routine care and exercise vs. routine care). Assuming a maximum drop-out rate of 30%, we therefore targeted recruitment of 30 patients per group at baseline.

7.3.11 Specific Statistical analyses

Normality was assessed using histograms, the Shapiro-Wilk test and Q-Q plots. Continuous data are expressed as mean (± standard deviation), if normally distributed or median (25-75% interquartile range) if not. At baseline, patients and control groups were compared by independent *t*-tests or Mann-Whitney tests as appropriate. Categorical variables were compared using the Chi-squared test or Fisher's exact test as appropriate. For the primary and key secondary outcomes of the PROBE trial, statistical analysis was done by an independent trial statistician (NBJ) at the Leicester Clinical Trial Unit, according to a pre-specified statistical

analysis plan co-written by the study investigators (including GSG). For the analysis of the primary outcome, each intervention was compared with the routine care arm (i.e. MRP diet versus routine care, and exercise versus routine care) using linear regression adjusted for stratification factors (sex and baseline glucose lowering therapy) and baseline PEDSR. The treatment effect was presented as a point estimate, confidence interval and p-value. A Holm-Bonferroni correction was applied to these analyses to maintain an overall Family-Wise Error Rate of 5%. If the treatment effect for the models for the two primary comparisons had $p \geq 0.025$ then they were both declared non-significant. If at least one model had $p < 0.025$ then the threshold for significance was $p < 0.025$ for the smaller p-value and $p < 0{\cdot}05$ for the larger p-value. Changes in the key secondary outcomes (LV volumes, mass, systolic function, and echocardiographic diastolic function) were not formally assessed statistically: given the large number of additional secondary outcomes, formal statistical testing was not undertaken to reduce the risk of type II statistical errors. Differences from baseline to follow-up are presented with 95% confidence intervals.

7.3.12 Trial oversight and governance

The study was managed by the Leicester Clinical Trials Unit. A trial steering committee, comprising an independent chair and a group of experts, provided trial oversight. The independent chair of the trial steering committee was Professor Roy Taylor, Professor of Medicine and Metabolism and director of the Magnetic Resonance Centre, Newcastle University.

Data completeness and quality were reviewed by the Leicester Clinical Trials Unit. The quality of 10 randomly selected baseline CMR datasets was assessed by an independent laboratory (Prof Sven Plein, Leeds, UK) and all images were graded as excellent or good.

Given the open design of the study it was determined that a data monitoring and ethics committee was not required. All serious adverse events are reported to the sponsor according to current guidelines and the trial steering committee.

7.3.13 Sponsor and funding

The study sponsor was the University of Leicester. The study funder (NIHR) provided financial support but had no role in the study design (other than the external review process), data collection, data analysis, data interpretation or in the writing of the of the report. Cambridge Weight Plan® provided the dietary

supplements free of charge but were not involved in the conduct of the study, analysis or interpretation of the data, or writing of the report.

7.4 Results

The trial profile displayed in Figure 7.3 . Between November 2015 and May 2018, 260 patients were screened, of which 93 consented and enrolled. Three were found to be ineligible after consent and 90 subjects were randomized: 30 to routine care, 31 to the supervised exercise programme and 29 to the MRP diet. Three of these participants (two in the exercise arm and one in the MRP arm) were found to be ineligible after laboratory test results became available and did not undertake the intervention. A total of 76 patients with T2D completed the trial (30 in the routine care arm, 22 in the exercise arm and 24 in the MRP arm). Reasons for discontinuation are shown in Figure 7.3 . Thirty-nine healthy volunteers were enrolled for baseline case-control comparison. Three of these were subsequently excluded (one due to the presence of obesity and two who were unable to undergo CMR scanning due to claustrophobia). A total of 36 healthy volunteers were therefore included in case-control comparisons.

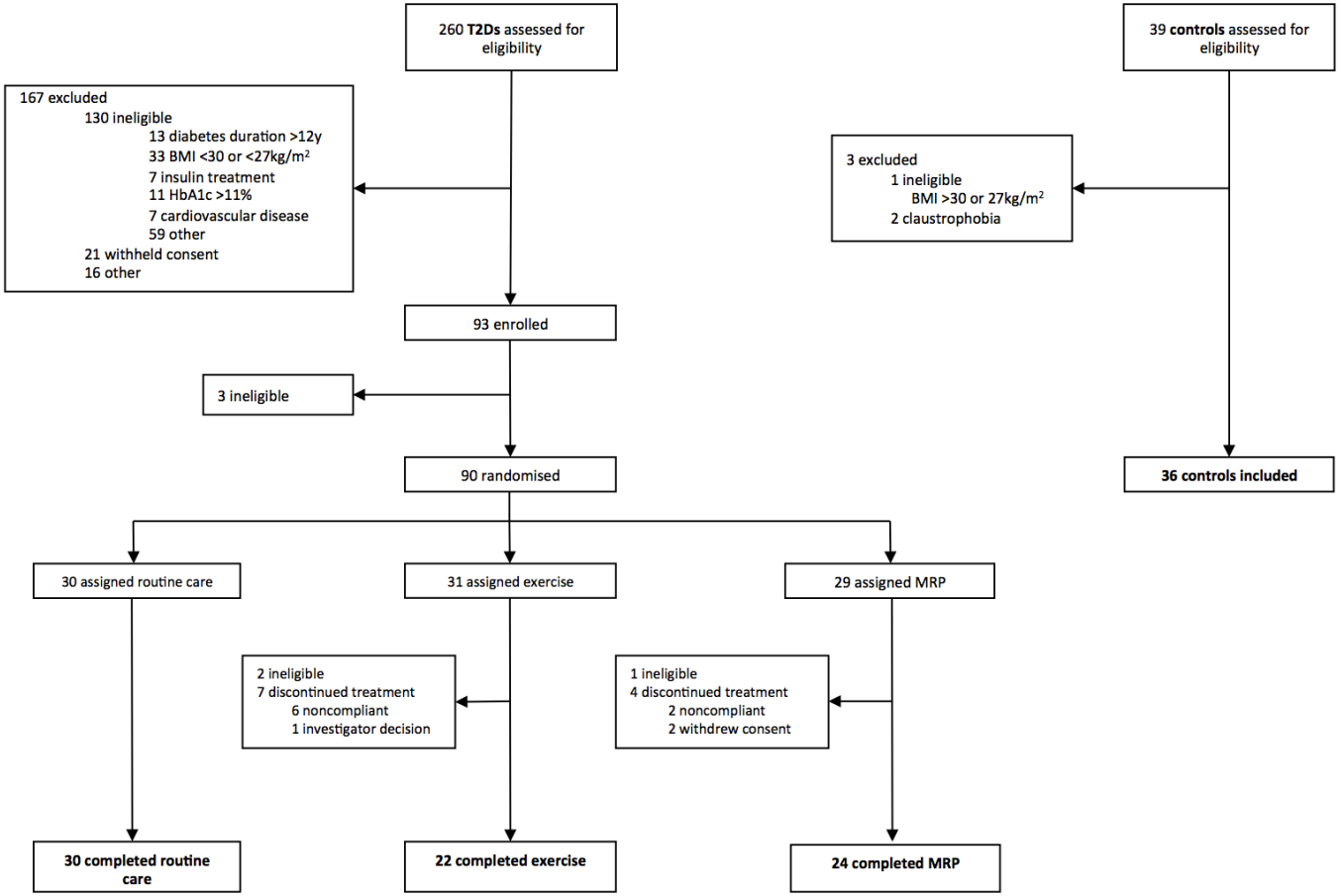


Figure 7.3 Trial profile. Abbreviations: BMI=body mass index; MRP=meal replacement plan; T2D=type 2 diabetes.

7.4.1 Baseline characteristics

The baseline demographic characteristics of subjects with T2D and controls are shown in Table 7.2. Mean age of participants with T2D was 50.5±6.5 years, mean BMI was 36.6±5.5 kg/m^2, mean duration of diabetes was 5.4±3.2 years, 41% were women, and 37% were from a black or minority ethnic group. The control group were similar for age, sex and ethnicity, but had lower overall body weight and BMI. Among those with T2D, 43% had a history of smoking and 52% had a history of hypertension. None of the control subjects had a history of hypertension or dyslipidaemia. Antihypertensive and lipid-lowering medication use was therefore higher in those with T2D compared to controls.

Table 7.2 Baseline characteristics of subjects with type 2 diabetes and controls.

	T2D (n=87)	**CONTROLS (n=36)**	***P*-value**
DEMOGRAPHICS			
Age, years	50.5±6.5	48.6±6.2	0.15
Sex, n (%)			
Male	51 (59)	19 (53)	0.552
Female	36 (41)	17 (47)	
Ethnic origin, n (%)			
Caucasian	55 (63)	25 (69)	0.51
Black or other minority ethnicity	32 (37)	11 (31)	
ANTHROPOMETRICS			
Height, cm	168±10	169±9	0.54
Weight, kg	103.3±16.7	70.4±10.8	**<0.001**
Body mass index, kg/m^2	36.6±5.5	24.5±2.4	**<0.001**
Systolic blood pressure, mmHg	140±15	121±13	**<0.001**
Diastolic blood pressure, mmHg	87±8	76±7	**<0.001**
Heart rate, beats/min	74±10	62±10	**<0.001**
MEDICAL HISTORY			
Diabetes duration, months	56 (32 – 94)	N/A	N/A
Smoking history, n (%)	39 (45)	9 (25)	**<0.001**
Hypertension, n (%)	44 (51)	0 (0)	**<0.001**
Dyslipidaemia, n (%)	56 (64)	0 (0)	**<0.001**
MEDICATIONS			
ACE inhibitor, n (%)	28 (32)	0 (0)	**<0.001**
ARB, n (%)	11 (13)	0 (0)	**0.025**
Beta blocker, n (%)	6 (7)	0 (0)	0.106
Calcium channel blocker, n (%)	19 (22)	0 (0)	**0.002**
Statin, n (%)	58 (67)	0 (0)	**<0.001**
Metformin, n (%)	82 (94)	N/A	N/A
Sulfonylurea, n (%)	13 (15)	N/A	N/A
DPP-IV inhibitor, n (%)	17 (20)	N/A	N/A
SGLT2 inhibitor, n (%)	10 (11)	N/A	N/A
GLP-1 receptor agonist, n (%)	10 (11)	N/A	N/A

Data are n (%), mean±SD, or median (IQR). Abbreviations: ACE=angiotensin converting enzyme; ARB=angiotensin receptor blocker; GLP-1=glucagon-like peptide-1; DPP-IV=dipeptidyl peptidase-IV; SGLT2=sodium glucose cotransporter-2.

Table 7.3 Fasting blood test results for subjects with type 2 diabetes and controls.

	T2D (n=87)	CONTROLS (n=36)	*P*-value
FASTING BLOOD TESTS			
Urea, mmol/L	5.4±1.2	5.2±1.4	0.59
Creatinine, mmol/L	76±15	79±12	0.332
Estimated GFR, mL/min	90 (80 - 90)	85 (78 - 90)	0.122
Glucose, mmol/L	7.6 (6.6 - 10.1)	5.0 (4.6 - 5.2)	**<0.001**
HbA1c, %	7.3±1.0	5.4±0.2	**<0.001**
HbA1c, mmol/mol	56±11	35±3	**<0.001**
Total cholesterol, mmol/L	4.6±1.0	5.7±0.8	**<0.001**
Triglycerides, mmol/L	1.88 (1.18 - 2.74)	0.98 (0.72 - 1.54)	**<0.001**
HDL, mmol/L	1.2 (1.0 - 1.4)	1.7 (1.6 - 2.0)	**<0.001**
LDL, mmol/L	2.4±0.8	3.3±0.8	**<0.001**
Cholesterol:HDL	3.9±0.9	3.3±0.8	**<0.001**
Haemoglobin, g/L	144±16	141±14	0.279
Adiponectin, ng/L	3460 (2550 - 5652)	9514 (4820 - 14589)	**<0.001**
Leptin, pg/L	18911 (9821 - 34115)	4811 (2400 - 9818)	**<0.001**
C-peptide, ng/L	2591 (1865 - 3371)	969 (743 - 1199)	**<0.001**
Insulin, miu/L	26.5 (18.8 - 35.8)	7.4 (4.9 - 10.4)	**<0.001**
HOMA-IR	9.2 (6.2 - 13.5)	1.6 (1.1 - 2.5)	**<0.001**
B-natriuretic peptide, ng/L	10.6 (4.5 - 17.9)	16.0 (8.7 - 22.6)	**0.048**

Data are n (%), mean±SD, or median (IQR). Abbreviations: HDL=high-density lipoprotein; HOMA-IR=homeostatic model assessment of insulin resistance; LDL=low-density lipoprotein.

Fasting blood test results are displayed in Table 7.3. Both T2Ds and healthy controls had similar renal function. Subjects with T2D had higher overall glycated haemoglobin (7.3±1.0 vs. 5.4±0.2%, p<0.001), lower total cholesterol (4.6±1.0 vs. 5.7±0.8mmol/L, p<0.001) and LDL cholesterol (2.4±0.8 vs. 3.3±0.8mmol/L, p<0.001) than controls, respectively. Adiponectin levels were significantly lower and leptin levels significantly higher (both p<0.001) in T2Ds versus controls. Fasting C-peptide and insulin levels were significantly higher (both p<0.001) in T2Ds compared with controls. Similarly, overall HOMA-IR was higher in T2Ds versus controls (9.2 [6.2 – 13.5] vs. 1.6 [1.1 – 2.5], respectively, p<0.001). B-natriuretic peptide levels were significantly lower in the T2D group compared to controls (10.6 [4.5 – 17.9] vs. 16.0 [8.7 – 22.6] ng/L, respectively, p=0.048).

7.4.2 Cardiovascular differences between people with type 2 diabetes and controls

Baseline CMR imaging, CPET, and echocardiography data comparing T2Ds and controls are displayed in Table 7.4. LV PEDSR was significantly lower in T2Ds

compared to controls (1.01±0.19 vs. 1.10±0.16 s^{-1}, p=0.02). Subjects with T2D also had smaller indexed LV volumes, higher LV EF, and higher LV mass than controls. In those with T2D, there was increased concentric LV remodelling (LV mass:volume 0.82±0.12 vs. 0.71±0.10 g/mL, p<0.001) and lower mean aortic distensibility (4.16±2.05 vs. 6.56±2.02 $mmHg^{-1}x10^{-3}$, p<0.001) than controls. There were no significant differences in indexed LV mass or GLS between groups. Stress MBF was lower, resting MBF higher, and MPR lower in T2Ds compared with controls.

Complete echocardiographic transmitral flow velocities were measurable in 84 subjects with T2D and all 36 controls. Mean E/A ratio was significantly lower in the T2D group compared with controls (0.95±0.21 vs. 1.21±0.25, p<0.001). Mitral annular velocities were measurable in 78 individuals with T2D and all 36 controls. Overall, average E/e' was higher in those with T2D compared to the control group (8.1 [6.2 – 9.6] vs. 6.2 [5.0 – 7.8], p<0.001).

All measures of cardiorespiratory fitness (maximum workload achieved, absolute and bodyweight corrected peak oxygen uptake) were lower in the T2Ds versus controls.

Table 7.4 Baseline CMR, cardiopulmonary exercise testing and echocardiographic data in subjects with T2D versus controls.

	T2D (n=87)	CONTROLS (n=36)	P-value
Cardiovascular magnetic resonance imaging			
LV EDVi, mL/m^2	67±10	83±19	**<0.001**
LV ESVi, mL/m^2	22±6	29±9	**<0.001**
LV EF, %	68±7	65±5	**0.016**
LV mass, g	121±25	107±32	**0.011**
LV mass index, g/m^2	55±9	58±14	**0.133**
LV mass:volume, g/mL	0.82±0.12	0.71±0.10	**<0.001**
LV GLS, %	−16.9±2.6	−17.6±1.5	0.179
LV PEDSR, s^{-1}	1.01±0.19	1.10±0.16	**0.02**
Stress MBF, mL/g/min	3.44±0.76	3.78±0.82	**0.037**
Rest MBF, mL/g/min	1.28±0.47	1.00±0.27	**0.002**
Myocardial perfusion reserve	3.02±0.98	3.98±1.01	**<0.001**
Aortic distensibility, mmHg^{-1}x10^{-3}	4.16±2.05	6.56±2.02	**<0.001**
Echocardiography			
E-wave, m/s	0.66±0.13	0.67±0.13	0.893
A-wave, m/s	0.72±0.16	0.56±0.11	**<0.001**
E/A ratio	0.95±0.21	1.21±0.25	**<0.001**
Septal e', cm/s	6.6±1.5	8.0±1.5	**<0.001**
Lateral e', cm/s	10.1±2.5	13.3±3.1	**<0.001**
Average e', cm/s	8.4±1.7	10.7±2.1	**<0.001**
Average E/e'	8.1 (6.2 - 9.6)	6.2 (5.0 - 7.8)	**<0.001**
Cardiopulmonary exercise testing			
Max workload achieved, W	125±47	173±67	**<0.001**
Peak VO_2, mL/kg/min	16.6±4.1	27.5±8.2	**<0.001**
Peak VO_2, L/min	1.70±0.46	1.96±0.73	**0.019**

Data are mean±SD or median (IQR). Abbreviations: LV=left ventricle; EDVi=end-diastolic volume indexed to body surface area; ESVi=end-systolic volume indexed to body surface area; EF=ejection fraction; GLS=global longitudinal strain; PEDSR=peak early diastolic strain rate; MBF=myocardial blood flow.

7.4.3 Prospective, randomised, open-label, blinded-endpoint trial

The baseline demographic characteristics and prescribed diabetes and anti-hypertensive medications of participants stratified by treatment arm in the trial are shown in Table 7.5. The three groups were well balanced.

Table 7.5 Baseline characteristics of prospective, randomised, open-label, blinded-endpoint trial participants stratified by treatment arm.

	Routine care (n=30)	**Exercise (n=31)**	**MRP (n=29)**
Age, years	50·7±6·4	50·5±7·2	49·7±6·3
Sex			
Male	18 (60%)	18 (58%)	17 (59%)
Female	12 (40%)	13 (42%)	12 (41%)
Ethnic origin			
Caucasian	18 (60%)	22 (71%)	16 (55%)
Black or other minority ethnicity	12 (40%)	9 (29%)	13 (45%)
Weight, Kg	102.6±14.9	102.8±18.3	104.6±16.9
Body mass index, Kg/m^2	36.7±4.8	36.0±5.8	37.2±6.1
Systolic BP, mmHg	138±13	138±18	144±18
Diastolic BP, mmHg	85±7	88±10	90±9
Medications			
RAAS inhibitor	15 (50%)	11 (35%)	15 (52%)
Other antihypertensive(s)	9 (30%)	8 (26%)	11 (38%)
Statin	21 (70%)	20 (65%)	19 (66%)
Metformin	30 (100%)	29 (94%)	25 (86%)
Sulphonylurea	6 (20%)	6 (19%)	2 (7%)
GLP-1 agonist	3 (10%)	4 (13%)	3 (10%)
DPP-IV inhibitor	7 (23%)	6 (19%)	5 (17%)
SGLT-2 inhibitor	4 (13%)	3 (10%)	4 (14%)
Fasting blood tests			
Glucose, mmol/L	8.2±2.3	8.9±2.4	8.1±2.5
HbA1c, %	7.3±0.9	7.6±1.3	7.2±1.1
HbA1c, mmol/mol	56±10	59±14	55±12
Cardiovascular magnetic resonance imaging			
LV EDVi, mL/m^2	65±10	68±10	69±11
LV ESVi, mL/m^2	21±5	23±7	21±7
LV EF, %	68±5	67±7	70±7
LV mass, g	116±22	122±25	126±27
LV mass:volume, g/mL	0.82±0.11	0.83±0.14	0.83±0.13
LV PEDSR, s^{-1}	1.06±0.15	0.93±0.18	1.03±0.22
LV GLS, %	-17.4±2.2	-16.8±2.7	-16.5±2.7
Myocardial perfusion reserve	2.8±0.9	3.3±1.0	2.9±1.0
Aortic distensibility, $mmHg^{-1}x10^{-3}$	4.23±1.91	4.30±2.27	3.90±1.90
Echocardiography			
E/A ratio	0.99±0.21	0.93±0.20	0.93±0.20
Average E/e'	8.05 (6.48 – 9.81)	7.84 (6.96 – 10.44)	9.79 (6.78 – 10.36)
Cardiopulmonary exercise testing			
Max workload achieved, W	123±42	121±43	129±54
Peak VO_2, mL/kg/min	16.7±3.7	16.7±4.1	16.3±4.3

Data are mean±SD or median (IQR). Abbreviations: LV=left ventricle; EDVi=end-diastolic volume indexed to body surface area; ESVi=end-systolic volume indexed to body surface area; EF=ejection fraction; GLS=global longitudinal strain; PEDSR=peak early diastolic strain rate.

7.4.3.1 Changes in bio-anthropometric measures, physical activity and cardiorespiratory fitness indices with interventions

Changes from baseline to 12 weeks in anthropometric measures, biochemical parameters, and cardiorespiratory fitness in subjects who completed the study are shown in Table 7.6.

In the routine care arm, body weight remained stable and there was no significant change in BMI. Markers of insulin resistance and glycaemic control remained similar from baseline to 12 weeks. Mean systolic BP dropped by 7 mmHg, driven by a guideline-directed increase in the doses of existing prescribed antihypertensive medications. Cardiopulmonary fitness did not change by the end of the trial period.

In the exercise arm there were small reductions in body weight (median weight loss 1.6 kg) and BMI (median reduction 0.8 kg/m^2). There was no significant change in glycaemic control, insulin resistance or BP. Although there was no significant change in peak oxygen uptake by week 12, subjects' total exercise duration and maximum workload achieved did increase (by 1.2 mins and 22 Watts, respectively).

In the MRP arm after 12 weeks, median weight loss was 13.6 kg, BMI fell by 4.8 kg/m^2, and mean systolic BP dropped by 13mmHg, despite a reduction in the number and/or dose of anti-hypertensive medications taken. Median HbA1c decreased by 0.75% (7.5 mmol/mol), with 20 (83%) participants achieving T2D remission. There was a non-significant trend for adiponectin to increase and median leptin decreased by 9,873 pg/mL, median HOMA-IR decreased by 6 units, and median B-type natriuretic peptide increased by 3.5 ng/L. There was a small increase in peak oxygen uptake when corrected for body weight (1.9 mL/kg/min), but not in absolute peak oxygen uptake. Other measures of cardiorespiratory fitness did not change by the end of the trial period.

Table 7.6 Bio-anthropometric measures at baseline, 12 weeks and change from baseline to 12 weeks in the three trial arms.

	Routine care (n=30)			Exercise (n=22)			MRP (n=24)		
	Baseline	**Week 12**	**Median change (95% CI)**	**Baseline**	**Week 12**	**Median change (95% CI)**	**Baseline**	**Week 12**	**Median change (95% CI)**
Anthropometrics									
Weight (kg)	102·6 (14·9)	100·4 (14·5)	-1.05 (-3.16, -0.01)	99·2 (16·3)	97·8 (16·6)	-1.55 (-2.51, -0.48)	106·7 (16·2)	93·0 (15·0)	-13.55 (-15.53, -11.90)
Body mass index (kg/m2)	35·0 (33·0 - 40·7)	34·5 (32·0 - 41·0)	-0.25 (-1.00, 0.00)	33·0 (31·8 - 35·0)	33·0 (31·0 - 34·7)	-0.75 (-1.00, -0.09)	35·2 (33·5 - 40·3)	30·3 (28·1 - 35·5)	-4.75 (-5.17, -4.00)
Systolic BP (mmHg)	137·8 (12·7)	130·8 (14·4)	-7.07 (-10.60, -3.54)*	135·5 (16·9)	133·0 (14·3)	-2.45 (-8.94, 4.03)*	145·9 (15·9)	132·9 (18·0)	-13.00 (-21.60, -4.40)*
Diastolic BP (mmHg)	85·3 (7·3)	83·5 (10·2)	-1.83 (-4.65, 0.99)*	87·2 (8·2)	86·7 (8·5)	-0.55 (-4.08, 2.98)*	91·1 (7·4)	86·5 (9·1)	-4.67 (-9.50, 0.17)*
Heart rate (bpm)	76·3 (7·5)	73·3 (9·6)	-3.03 (-6.06, -0.01)*	75·0 (12·7)	73·4 (9·6)	-1.55 (-4.94, 1.85)*	73·1 (8·6)	67·8 (9·8)	-5.29 (-8.55, -2.03)*
Fasting bloods									
HbA1c (%)	7·3 (0·9)	7·2 (1·1)	0.00 (-0.59, 0.10)	7·4 (1·1)	7·3 (1·1)	-0.10 (-0.31, 0.20)	7·2 (1·1)	6·2 (0·7)	-0.75 (-1.23, -0.40)
HOMA-IR	7·8 (4·7 - 9·3)	6·6 (3·3 - 11·7)	-0.81 (-2.07, 2.03)	9·8 (6·6 - 14·3)	6·5 (4·5 - 13·8)	-2.91 (-4.98, 0.39)	10·3 (8·0 - 13·6)	4·3 (3·0 - 6·0)	-5.98 (-9.48, -3.44)
Adiponectin (ng/mL)	4121·3 (3090·1 - 7550·2)	4006·9 (2417·5 - 6865·8)	17.81 (-795.87, 515.42)	3043·2 (2435·4 - 4169·0)	2767·5 (2186·9 - 3495·9)	-354.54 (-815.60, 256.48)	3714·4 (2546·3 - 4681·7)	4764·1 (3158·4 - 6159·5)	774.33 (-98.58, 2,784.91)
Leptin (pg/mL)	19606·6 (9617·2 - 34115·0)	18112·9 (8544·5 - 27105·2)	-2,035.80 (-4,300.81, -559.33)	16831·1 (11403·0 - 23753·9)	12691·9 (10098·3 - 21983·9)	-526.05 (-2,736.59, 2,248.05)	19294·6 (9808·7 - 51040·7)	6413·1 (3337·4 - 20558·8)	-9,873.31 (-13,360.80, -5,803.63)
BNP (ng/L)	9·4 (4·4 - 15·7)	8·3 (4·8 – 14·4)	0.00 (-1.43, 2.89)	7·4 (2·7 - 18·0)	7·5 (3·5 - 16·4)	1.05 (-3.91, 4.26)	10·8 (5·0 - 15·1)	13·6 (5·0 - 24·3)	3.45 (0.73, 8.61)
Cardiopulmonary exercise testing									
Peak VO_2 (mL/Kg/min)	16·7 (3·7)	16·2 (4·1)	-0.54 (-1.55, 0.47)*	17·2 (4·5)	18·2 (4·9)	0.97 (-0.46, 2.40)*	16·4 (4·5)	18·3 (5·5)	1.93 (0.64, 3.23)*
Peak VO2 (L/min)	1·72 (0·48)	1·63 (0·51)	-0.09 (-0.18, 0.01)*	1·67 (0·50)	1·73 (0·52)	0.06 (-0.08, 0.20)*	1·72 (0·45)	1·67 (0·46)	-0.05 (-0.16, 0.06)*
Exercise duration (mins:secs)	11·3 (2·2)	11·1 (2·2)	-0.15 (-0.60, 0.25)	10·6 (2·3)	11·8 (3·1)	1.20 (0.17, 2.07)	11·4 (2·2)	11·2 (1·7)	-0.37 (-0.62, 0.47)
Maximum workload (W)	123·0 (41·9)	122·0 (47·3)	-2.50 (-8.74, 3.00)	123·2 (47·1)	141·3 (54·9)	22.00 (5.81, 32.00)	132·5 (56·4)	132·3 (50·1)	-3.50 (-9.34, 10.01)

Data are n (%), median (IQR) or mean (SD), and median change (95% CI). *Data are mean change (95% CI). BP=blood pressure. RAAS=renin angiotensin aldosterone system. GLP-1=glucagon-like peptide-1. DPP-IV=dipeptidyl peptidase-IV. SGLT-2=sodium glucose cotransporter-2. HOMA-IR=homeostatic model assessment of insulin resistance. BNP=brain natriuretic peptide.

7.4.3.2 Primary and key secondary outcomes

Changes in the primary endpoint from baseline to 12 weeks are displayed in Figure 7.4 . For the primary outcome measure, participants in the supervised exercise programme arm demonstrated a significant improvement in PEDSR compared to those in the routine care arm of the trial (β=0.132, 97.5% CI 0.038 to 0.225, p=0.002). No improvement in PEDSR was observed in participants in the MRP arm versus those in the routine care arm of the trial (β=0.016, 97.5% CI -0.075 to 0.106, p=0.731).

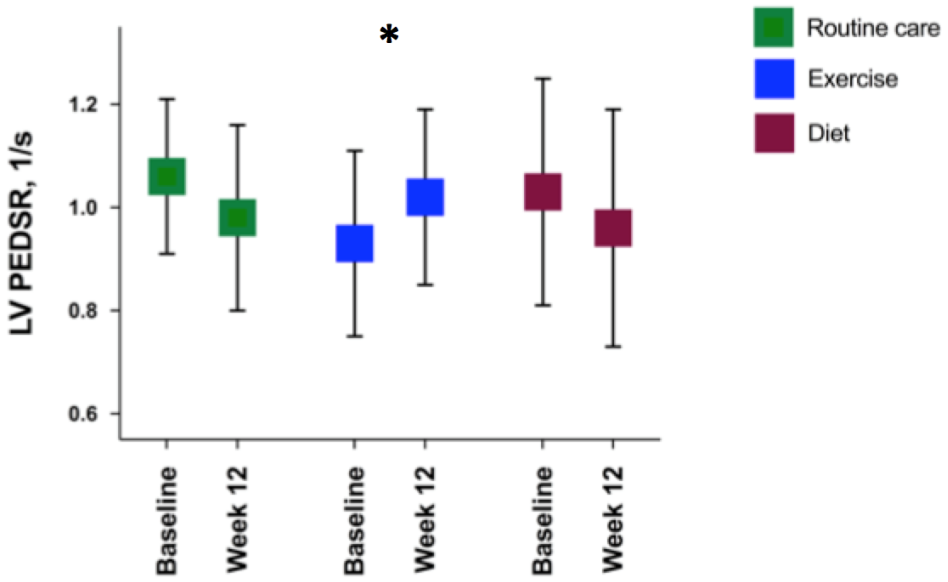


Figure 7.4 Box plots displaying change in left ventricular peak early diastolic strain rate (LV PEDSR) from baseline to 12 weeks in the three trial arms. *denotes statistical significance.

Average E/e' and early diastolic to late filling ratio (E/A) ratio could be obtained in 63 (83%) and 70 (92%) of participants who completed the trial, respectively. E/A ratio and non-invasive assessment of filling pressure (E/e') tended to improve in both intervention arms, but these changes were not significant compared to the routine care arm: average E/e' in the exercise arm of the trial versus the routine care arm at 12 weeks: β= -0.459, 95% CI -1.452 to 0.534, p=0.355, and E/A ratio: β=0.028, 95% CI -0.086 to 0.142, p=0.621. Similarly, there was no difference in average E/e' in the MRP arm versus routine care at 12 weeks (β=-

0.060, 95% CI -1.099 to 0.978, p=0.907), or E/A ratio (β=0.036, 95% CI -0.090 to 0.161, p=0.568).

Key secondary cardiac imaging endpoints at baseline and 12 weeks in the three trial arms are displayed in Table 7.7. In the routine care arm and the exercise arms there were negligible changes in most cardiac parameters. In the MRP arm there was a trend towards a reduction in LV mass (mean reduction 5.6 grams) and indexed LV end diastolic volume increased by 5 mL/m^2, with a corresponding significant reduction in concentric LV remodelling (mean LV mass:volume change -0.03 g/mL, 95% CI -0.06 to -0.01). Aortic distensibility increased by 0.90 $mmHg^{-1}x10^{-3}$ (95% CI 0.38 to 1.41). With regards to systolic function there was a significant lowering of ejection fraction (-4.54%, 95% CI -0.27 to -2.37) in the MRP arm. There were no significant changes in MPR in any group.

Table 7.7 Cardiovascular magnetic resonance imaging and echocardiography data at baseline and 12 weeks in the three trial arms.

	Routine care (n=30)			Exercise (n=22)			MRP (n=24)		
	Baseline	**Week 12**	**Mean change (95% CI)**	**Baseline**	**Week 12**	**Mean change (95% CI)**	**Baseline**	**Week 12**	**Mean change (95% CI)**
Cardiovascular magnetic resonance imaging									
LV PEDSR (s^{-1})	1·06 (0·15)	0·98 (0·18)	-0.07 (-0.13, -0.02)	0·92 (0·20)	1·02 (0·17)*	0.10 (0.04, 0.16)	1·00 (0·21)	0·96 (0·23)	-0.05 (-0.13, 0.03)
LV global longitudinal strain (%)	-17·4 (2·2)	-16·8 (1·8)	0.63 (-0.16, 1.41)	-16·3 (2·9)	-16·1 (2·5)	0.23 (-0.79, 1.25)	-16·6 (2·9)	-16·0 (1·8)	0.61 (-0.51, 1.72)
LV mass (g)	116·1 (22·8)	117·0 (24·2)	0.90 (-2.93 to 4.73)	123·1 (21·9)	122·0 (20·9)	-1.15 (-6.73, 4.44)	131·2 (26·9)	125·6 (27·0)	-5.56 (-11.53 to 0.40)
LV mass indexed to BSA (g/m^2)	53·0 (7·4)	53·9 (8·0)	0.93 (-0.92, 2.79)	57·0 (7·5)	56·9 (7·9)	-0.10 (-2.61, 2.41)	58·3 (10·1)	59·8 (10·7)	1.56 (-1.05 to 4.17)
LV mass/volume	0·82 (0·77 - 0·86)	0·83 (0·77 - 0·92)	0.02 (-0.01, 0.05)	0·86 (0·75 - 0·91)	0·85 (0·76 - 0·88)	-0.02 (-0.06, 0.02)	0·80 (0·74 - 0·91)	0·79 (0·72 - 0·87)	-0.03 (-0.06 to -0.01)
LV EDV (mL)	133·9 (125·3 - 148·8)	128·7 (117·4 - 149·6)	-0.37 (-4.75, 4.00)	147·2 (129·3 - 161·3)	145·1 (132·0 - 159·7)	2.45 (-4.36, 9.27)	172·5 (131·7 - 180·7)	172·3 (116·3 - 188·9)	-0.15 (-5.90 to 5.60)
LV EDVi (mL/m^2)	63·3 (58·1 - 67·8)	61·5 (56·3 - 69·0)	0.46 (-1.79, 2.72)	67·4 (62·0 - 70·6)	66·1 (62·9 - 72·4)	1.50 (-1.53, 4.52)	71·6 (59·9 - 78·4)	77·9 (64·3 - 87·5)	4.97 (2.22 to 7.73)
LV EF (%)	67·6 (5·4)	66·2 (5·3)	-1.42 (-3.76, 0.92)	66·8 (7·9)	66·0 (6·2)	-0.79 (-3.78, 2.20)	69·8 (7·4)	65·2 (6·1)	-4.54 (-6.89 to -2.18)
Myocardial perfusion reserve	2·7 (0·8)	3·2 (1·1)	0.49 (-0.03, 1.00)	3·3 (0·9)	3·4 (1·2)	0.10 (-0.54, 0.75)	3·0 (1·1)	3·2 (0·9)	0.18 (-0.44, 0.79)
Aortic distensibility ($mmHg^{-1}x10^{-3}$)	3.7 (2.9 – 5.5)	4·8 (3·0 – 5·8)	0.51 (-0.20, 1.21)	3.3 (2·7 – 5·7)	3·8 (2·9 - 4·6)	0.55 (-0.87, 1.97)	3·2 (2·3 – 4·3)	4·2 (3·1 – 6·1)	0.90 (0.38, 1.41)
Echocardiography									
Average E/e'	8·0 (6·5 – 9·7)	8·6 (7·1 – 9·3)	0.18 (-0.49, 0.85)	8·8 (7·0 – 10·6)	8·1 (6·8 – 9·3)	-0.70 (-1.78, 0.39)	10·1 (7·5 - 11·0)	8·5 (7·6 – 9·7)	-0.67 (-1.83, 0.48)
Average E/A	1·00 (0·21)	1·01 (0·25)	0.01 (-0.06, 0.09)	0·94 (0·19)	1·00 (0·21)	0.06 (-0.03, 0.15)	0·92 (0·20)	0·99 (0·23)	0.07 (-0.03, 0.17)

Data are mean (SD) or median (IQR), and mean (95% confidence interval). Abbreviations: MRP=meal replacement plan; LV=left ventricle; PEDSR=peak early diastolic strain rate; BSA=body surface; EDV=end diastolic volume; EDVi=end diastolic volume indexed to body surface area; ESV=end systolic volume; ESVi=end systolic volume indexed to body surface area; EF=ejection fraction.

7.5 Discussion

This is the first randomised controlled trial to compare the effects on cardiac structure and function of a low-energy diet versus an aerobic exercise programme or routine care in working-age adults with T2D. Compared with controls, individuals with T2D had reduced diastolic function, increased concentric LV remodelling, reduced myocardial perfusion, and increased aortic stiffening, consistent with asymptomatic stage B heart failure(345). A 12-week supervised aerobic exercise training programme led to favourable improvements in diastolic function in the absence of any major effects on LV remodelling, perfusion or aortic stiffening. Despite beneficial effects observed on glycometabolic profile, BP, aortic stiffness and concentric LV remodelling, a low-energy MRP diet did not lead to improved diastolic function.

Our younger T2D cohort may already have stage B heart failure(345), with clear evidence of reduced diastolic function by both CMR and echocardiographic measures. Diastolic dysfunction and concentric LV remodelling are typically the earliest manifestations of diabetic cardiomyopathy, and precursors to the onset of clinical heart failure(316). Our results suggest that supervised aerobic exercise training may improve the earliest functional consequence of T2D on the myocardium. Subjects with T2D had markedly lower aerobic exercise capacity compared to controls at baseline, and beneficial effects on diastolic function were observed even when only accompanied by small improvements in fitness.

Large cohort studies have shown that increased aerobic exercise capacity is associated with significantly lower cardiovascular and overall mortality in men(238) and women(239) with diabetes. Furthermore, diastolic function has been associated with exercise capacity in people with T2D, independent of age, sex, BMI and HbA1c(335), which suggests that improvements in exercise capacity may yield improvements in cardiac dysfunction in T2D. Several studies have assessed the effects of various exercise interventions on diastolic function in people with T2D, predominantly using echocardiography(340). The results of these studies have been inconsistent, likely due to differences in study populations, modes and duration of exercise intervention, and various measures of diastolic function being employed. In general, shorter durations (≈3 months) of exercise training appear to improve echocardiographic indices of diastolic function and LV filling pressures in

randomised studies of exercise versus standard care, regardless of the type of exercise intervention(248,254,256). Trials of longer durations of exercise interventions (6 - 12 months), however, have not always yielded improvements in diastolic function(249,251). This may reflect difficulties in compliance with longer-term exercise programmes. Although not significant statistically, there were trends for improvement in echocardiographic markers of diastolic function, which may have been compounded by relatively poor image quality in this obese cohort where 20% had inadequate windows for complete assessment.

The mechanism of benefit of aerobic exercise on diastolic function in our cohort is unclear. We did not observe significant improvements in myocardial perfusion or cardiac remodelling with exercise. Even short durations of exercise training have been found to improve endothelial function and promote angiogenesis(346), though it may be these effects were not sufficient to manifest as demonstrable improvements in myocardial perfusion in our study. It is also posited that exercise interventions cause improvements in myocyte calcium handling, mitochondrial function, inflammation and energy metabolism(106,340,347), which are linked to impaired cardiac contraction and relaxation(348). We were not able to assess these parameters in the current study, but given the lack of improvement in cardiac energetics following 12-weeks of high intensity interval training in a previous study in people with T2D(256), it seems this mechanism is unlikely to explain the benefit observed in diastolic function.

The ability to achieve remission of T2D by weight loss with administration of low-energy MRP diet was convincingly demonstrated in the DiRECT trial(226). However, improvements in cardiac function after weight loss in T2D have not been studied in a randomised controlled trial setting previously. Administration of a low-energy MRP diet in our patients led to dramatic improvements in body weight, BP and resting heart rate, fasting triglycerides, HbA1c, and markers of insulin resistance, mirroring the findings of the DiRECT trial(226). We also observed similar rates of remission of T2D to those in the DiRECT trial. However, PEDSR did not significantly change after MRP. This lack of improvement may have been influenced by a slightly higher baseline PEDSR in the MRP arm versus the exercise arm, although the analysis of the primary outcome measure was adjusted for baseline PEDSR. There were small, statistically non-significant reduction in E/e' and

increase in E/A ratio with the MRP that could suggest a trend towards improved diastolic function, but a much larger sample size would be required to assess this reliably.

We also observed a reduction in LV EF with a small rise in B-type natriuretic peptide levels. It is recognised that obesity is associated with increased sympathetic activity, which may result in hyperdynamic LV function(326). This is supported by our finding that LV EF was higher at baseline in those with T2D compared to healthy weight controls. The observed reduction in ejection fraction in the MRP arm may, therefore, reflect normalisation of hyperdynamic LV function with weight loss. Furthermore, obesity and T2D are both known to lower brain natriuretic peptide levels (which may explain why baseline levels were lower in T2Ds than controls) and its increase in the MRP arm of the trial is also likely to be a consequence of the weight loss rather than worsening of diastolic function(349,350).

Our interpretation is that diastolic dysfunction may be irreversible with improvements in glycometabolic derangements alone. Supporting evidence includes data from interventional trials that have not shown reductions in heart failure risk with strict glycaemic control(159). Although diastolic function did not improve, we did observe modest changes in cardiac remodelling and aortic distensibility in the MRP arm of the trial. Given that LV hypertrophy and smaller LV volumes are typically seen in diabetic cardiomyopathy (confirmed in our case-control analysis) and are associated with poorer cardiovascular outcomes(51,52), these changes may indicate favourable long-term effects of the dietary restriction or weight loss on the structural manifestations of heart failure in T2D. Furthermore, we have previously shown that aortic stiffening is an independent determinant of concentric LV remodelling(152) and the observed increase in aortic distensibility with MRP suggests that weight loss may ameliorate vascular stiffness in T2D and could promote reverse cardiac remodelling. It is possible that the best approach for improving stage B heart failure in people with T2D is a combination of exercise and dietary restriction to achieve weight loss, given the different effects of these interventions on diastolic function and cardiac remodelling in our study. Further trials are needed to assess the cardiovascular effects of combined exercise with dietary restriction and weight loss in people with T2D and for longer durations.

7.5.1 Strengths and limitations

Key strengths of our trial were the randomized design, the comprehensive cardiometabolic phenotyping undertaken, the use of CMR for cardiac outcomes, the blinded image analyses, and the robust independent statistical analyses undertaken by the Clinical Trials Unit. Although the younger, working-age population in this study have the highest lifetime risk of heart failure, they are under-represented in large-scale cardiovascular outcomes trials of T2D, and no studies have demonstrated effective therapies to prevent or treat heart failure in this group.

Our trial also has some important limitations, including the small sample size, unblinded design, and short duration of follow-up. Although the sample size is modest, the trial was powered in accordance with our pilot data(38), and the excellent reproducibility of our primary outcome measure of CMR-derived PEDSR in this very T2D cohort (**Chapter 3**)(303) allowed us to achieve the necessary statistical power as dictated by our power calculation. However, as PEDSR is a relatively novel measure of diastolic function on CMR, no prognostic data exist regarding subclinical alterations in PEDSR. The exclusion criteria were set to maximise the probability of remission of T2D with the MRP, and therefore the results are not generalizable to the entire population with T2D. The effects of sustained weight loss on cardiac structure and function were not assessed, nor the possibility that de-training could lead to worsening of diastolic function in those who undertook the supervised exercise arm of the trial. Although we achieved the necessary statistical power for our trial, the relatively high rate of non-compliance (19%) with the supervised exercise intervention may hinder its real-world application.

7.5.2 Conclusions

In younger adults with T2D and obesity without prevalent cardiovascular disease, there is already evidence of subclinical diastolic dysfunction, concentric LV remodelling and aortic stiffening. A 12-week supervised aerobic exercise training programme led to improvements in LV diastolic function without major effects on cardiac remodelling, weight loss, blood pressure, or glycaemic control. Conversely, a low-energy MRP diet led to improvements in glycometabolic profiles, concentric LV remodelling and aortic stiffness, but did not improve measures of diastolic function.

8 Conclusions and recommendations for future research

8.1 A comparison of the inter-study reproducibility of two cine-derived strain software programmes in people with type 2 diabetes (Chapter 3)

The purpose of this chapter was to compare the test-retest reproducibility of LV strain measures derived from two commercially available software packages (cmr42 Tissue Tracking and Diogenes Feature Tracking) in a subset of individuals from our asymptomatic cohort of people with T2D. This would guide which software vendor would be preferred for the primary outcome measure, PEDSR, of our PROBE trial. We found that the reproducibility of Tissue Tracking was superior to that of Feature Tracking. Furthermore, we found that both GLS and PEDSR strain values were higher for Feature Tracking than Tissue Tracking, confirming that different software packages for strain measurement cannot be used interchangeably.

8.1.1 Original hypothesis

H_0: there will be no difference in the inter-study reproducibility of PEDSR measurements derived from CMR SSFP cine images using cmr42 Tissue Tracking compared to Diogenes Feature Tracking: **rejected**.

8.1.2 Implications and recommendations for future research

Measurement of PEDSR using Tissue Tracking was the preferred method for assessment of diastolic function in our subjects with T2D. This was especially important for our PROBE trial, where a high degree of reproducibility was essential to mitigate the risk of obtaining a false-negative result for our primary outcome measure, PEDSR.

The fact that there were absolute differences in GLS and PEDSR between the two software packages, suggests a requirement for standardisation of cine-derived strain assessment across CMR platforms and software packages before these novel measures may be adopted in clinical practice. This is especially important in the current era where large CMR imaging datasets such as in the UK Biobank imaging sub-study may be utilised to inform normal ranges for GLS and PEDSR in the general population. Although there have been significant advances in the quantification of strain/strain rates with the advent of tracking techniques based on routinely acquired cine images, these issues will continue to hinder their real-world application and limit their diagnostic and prognostic value. In our own PROBE trial we therefore opted to include a nested case-control study, which enabled us to

determine whether PEDSR is indeed abnormal in our cohort with T2D and support our assertion that this group has diastolic dysfunction.

Lastly, in **Chapters 4, 5 and 6** of this thesis, we combined strain measures in subjects with and without T2D acquired at 1.5T and 3T, as has been the case in other studies(292,294,351). However, the agreement of strain measurements between these two field strengths is yet to be established. For this reason, we have recently begun recruitment to a study assessing the inter-field strength agreement of CMR strain measures, using a variety of techniques, in 20 healthy volunteers scanned on the same day at 1.5T and 3T. It is hoped that this will confirm our assumption that strain measures acquired at two different field strengths are consistent. Other work that will be explored in this study are the effects of varying acquisitions, e.g. higher temporal resolution or compressed sensing, on CMR strain measurements at 1.5T and 3T.

8.2 A cross-sectional comparison of cardiovascular function in people with and without type 2 diabetes (Chapter 4)

The purpose of this chapter was to describe presence and nature of subclinical cardiovascular dysfunction associated with T2D in our regional multi-ethnic adult population. Given the numerous inconsistencies amongst descriptions of diabetic cardiomyopathy, variations in techniques used to study the diabetic heart, and heterogeneous patient cohorts in existing literature, it was fundamental to phenotype our own cohort of people with T2D and compare them with matched controls. This provided key insights regarding the cardiovascular perturbations that accompany asymptomatic adults with T2D, representative of our multi-ethnic population in Leicestershire, UK. We found that there is already evidence of subclinical cardiovascular dysfunction, with concentric LV remodelling, increased arterial stiffness, reduced diastolic and systolic function, and lower MPR, when compared with age-, sex- and ethnicity-matched non-diabetic controls.

8.2.1 Original hypothesis

H_0: When matched for age, sex and ethnicity, there will be no difference in cardiovascular structure or function between people with and without T2D, who have no prior history, signs or symptoms of CVD: **rejected**.

8.2.2 Implications and recommendations for future research

Despite having no signs, symptoms or history CVD, our T2D cohort already have evidence of AHA stage B heart failure, confirming findings from existing literature. This group are therefore at impending risk of progression to overt clinical heart failure. Concentric LV remodelling(306), aortic stiffening(145), subclinical diastolic(307) and systolic dysfunction(308,309) have all been independently associated with poorer outcomes in T2D, and lower MPR is associated with impaired LV function in asymptomatic people(310). Furthermore, longitudinal studies have demonstrated the temporal progression of these early manifestations of diabetic cardiomyopathy(72,146,311), with the exception of MPR. What is not known is whether these measures of cardiovascular dysfunction contribute to the development of exercise limitation and heart failure.

A major strength of our large cohort of people with T2D is the high proportion of females and individuals from minority ethnic groups (especially south Asians). Our analyses of cardiovascular function were adjusted for age, sex, and ethnicity as these factors significantly impact upon cardiovascular outcomes (including heart failure) in people with T2D, with females and minority ethnic groups being at highest risk(352,353). Given our cohort is expanding, we are well poised to compare males with females and Caucasians with south Asians, to understand the interplay between sex, ethnicity and cardiovascular function in T2D. These analyses will be undertaken when the cohort has increased in size and will form the basis of my successor's PhD.

Lastly, studying whether the early manifestations of diabetic cardiomyopathy can be reversed, and determining if this leads to improved clinical outcomes, is crucial. Despite a wealth of research studies, it remains unclear whether intensive medical management or lifestyle interventions actually reverse subclinical cardiac dysfunction, lead to sustained improvements in cardiac function, and reduce the risk of heart failure. Some of these questions were addressed in later chapters within this thesis.

8.3 Clinical associations with subclinical cardiovascular dysfunction in adults with type 2 diabetes (Chapter 5)

The aim of this chapter was to identify independent associations between key clinical characteristics and indices of subclinical cardiovascular dysfunction in our

T2D cohort. Despite the wealth of previous research exploring such associations, the clinical factors contributing to early heart failure in people with T2D are incompletely understood. This is partly because previous imaging studies have been hindered by variations in methods of assessing cardiac structure and function, together with small sample sizes not permitting rigorous multivariable analyses. We found that the major clinical determinants of early heart failure in our participants with T2D were increasing age, duration of T2D, systolic BP, BMI and smoking history. Importantly, glycaemic control was not independently associated with any measure of LV remodelling, diastolic or systolic function, arterial stiffening, or myocardial perfusion.

8.3.1 Original hypothesis

H_0: There will be no association between clinical indicators of metabolic dysfunction (e.g. BMI, BP, HbA1c, diabetes duration, and dyslipidaemia) and markers of subclinical cardiovascular dysfunction in adults with T2D: **partially rejected.**

8.3.2 Implications and recommendations for future research

It is well recognised from previous large, multi-centre randomised controlled trials that aggressive glucose lowering management does not herald significant improvements in macrovascular outcomes, although microvascular complications do benefit(354). Even with multifactorial cardiovascular risk factor control, the risk of heart failure development remains unacceptably high in people with T2D(212,258). This is especially relevant in younger adults with T2D, a group that will be living with dysglycaemia for many years and who therefore have the highest lifetime risk of developing heart failure(30). The observed independent association of diabetes duration with GLS and LV diastolic filling suggests that aggressive early interventions to reverse T2D may be key in preventing heart failure development in this patient group and is justification for our selection of working-age adults for the DIASTOLIC study. Similarly, reducing BMI, BP, and smoking cessation are likely to be vital early interventions to attenuate progression of early heart failure in people with T2D. Research targeting younger adults with aggressive risk factor management should therefore become a central focus of efforts to reduce heart failure development in T2D.

8.4 Cardiovascular determinants of aerobic exercise capacity in adults with type 2 diabetes (Chapter 6)

The purpose of this chapter was to evaluate whether those markers of subclinical cardiovascular dysfunction identified in our cohort are independently related to aerobic exercise capacity, a strong surrogate marker of cardiovascular outcomes and mortality in people with T2D. Our T2D cohort had dramatically lower peak VO_2 than controls, comparable to what we may expect in people with symptomatic heart failure. We found that diastolic function and MPR were key determinants of aerobic exercise capacity, independent of age, sex, ethnicity, smoking status, BP, or glycaemic control. These features of early diabetic cardiomyopathy may therefore drive the progression of stage B heart failure.

8.4.1 Original hypothesis

H_0: In people with T2D, there will be no association between CMR markers of subclinical cardiovascular dysfunction and age- and sex-corrected peak VO_2: **rejected**.

8.4.2 Implications and recommendations for future research

Our findings that diastolic function and MPR are disturbed in people with T2D, and that they are intimately linked with aerobic exercise capacity, is highly suggestive that these are key mechanisms driving the development of heart failure. The capability of CMR to assess these parameters in a single examination with excellent inter-study reproducibility is a unique strength of this imaging modality for the assessment of the early stages of diabetic cardiomyopathy. These findings do, however, raise the question of whether some of our T2D cohort already have HFpEF despite our careful screening for signs or symptoms, especially given their strikingly low overall peak VO_2. Very recent longitudinal data from the Look AHEAD trial cohort (n=5,109, mean age 59±7 years, median follow-duration 12 years) have shown that baseline cardiorespiratory fitness is an independent predictor of incident HFpEF (but not HFrEF) after adjustment for traditional cardiovascular risk factors and interval myocardial infarction(355). Our data may therefore indicate that reduced MPR and diastolic dysfunction are major risk factors for HFpEF in people with T2D, and ought to be key targets for intervention (as is the case in the DIASTOLIC study). Although earlier studies have linked diastolic dysfunction to

reduced exercise capacity and subsequent heart failure(309,335), no data exist in an asymptomatic T2D cohort demonstrating an inverse association between MPR and exercise capacity, which makes this finding highly novel.

8.5 Effects of low-energy diet or exercise on cardiovascular function in younger adults with type 2 diabetes (Chapter 7)

The aims of this study were: (1) to confirm the presence and nature of subclinical cardiovascular dysfunction in younger adults with T2D, and (2) to determine if diastolic function can be improved by either a low-energy MRP or a supervised aerobic exercise programme, compared to routine care. We confirmed that, in younger adults with T2D and no prevalent cardiovascular disease, there is already evidence of subclinical diastolic dysfunction, concentric LV remodelling and aortic stiffening. A 12-week supervised aerobic exercise training programme led to improvements in LV diastolic function (PEDSR) without major effects on cardiac remodelling, weight loss, BP, or glycaemic control. Conversely, a low-energy MRP diet led to improvements in glycometabolic profiles, concentric LV remodelling and aortic stiffness, but did not improve measures of diastolic function.

8.5.1 Original hypothesis

H_0: Neither a low-energy MRP diet nor aerobic exercise training will cause improvements in subclinical cardiovascular dysfunction in people with T2D, when compared to standard care: **rejected.**

8.5.2 Implications and considerations for future research

The findings of this study suggest that increased physical activity is central to improving subclinical diastolic dysfunction, whereas short-term improvements in glycometabolic profiles achieved by weight loss are not alone sufficient in this regard. Although diastolic function did not improve, we did observe modest changes in cardiac remodelling and aortic distensibility in the MRP arm of the trial. It is therefore possible that the best approach for improving stage B heart failure in people with T2D is a combination of exercise and dietary restriction to achieve weight loss, given the different effects of these interventions on diastolic function and cardiac remodelling in our study. Further trials are needed to assess the cardiovascular effects of combined exercise with dietary restriction and weight loss in people with T2D and for longer durations to determine whether these measures in turn lead to reductions in heart failure incidence. Other studies are required to

determine whether lifestyle intervention is additive to therapy with SGLT2i, which appear to be able to slow the development of heart failure in patients with T2D either with or at high risk of ASCVD(186-188).

To address some of these outstanding questions, we have recently received industry funding (Astra Zeneca) from for an investigator-initiated PROBE trial. This will assess the impact of a 24-week intervention of exercise training in combination with the SGLT2i dapagliflozin, compared to diet-induced weight loss in a more representative population of 140 subjects with T2D. It is hypothesised that the combination of exercise training and dapagliflozin will result in greater improvements in physical function and cardiac function than dapagliflozin alone or diet-induced weight loss. In addition, we secured a Medical Research Council UK-Canada Diabetes Research Team Grant support for an efficacy trial investigating whether combining a low-energy MRP diet with structured exercise training over 24 weeks leads to improved cardiovascular function in younger adults (aged 18-40 years) with T2D. It is hoped that this further research will address some of the as yet unanswered questions that have emerged from this thesis. Lastly, we have recently commenced a feasibility study to assess the safety, tolerability and cardiovascular effects of a low-energy MRP in 20 adults with T2D and HFpEF. This will enable us to ascertain whether our dietary intervention is feasible in an older, symptomatic patient group and whether weight loss leads to favourable effects on cardiac structure and function in this group.

8.6 Other planned analyses

8.6.1 Sex and ethnic differences in subclinical cardiovascular dysfunction in type 2 diabetes

In our expanding combined cohort of asymptomatic adults with T2D and matched controls, we plan to assess sex- and ethnic-differences in cardiac structure and function, in an effort to better understand whether the differential cardiovascular outcomes observed in these groups are related to underlying differences in the manifestations of diabetic cardiomyopathy.

8.6.2 Biomarker responses to weight loss or exercise in type 2 diabetes

Stored plasma was obtained from all participants of our PROBE trial. This will be used for proteomic and metabolomics analyses. We have also undertaken a panel of 50 fibro-inflammatory biomarkers pre- and post-intervention in the DIASTOLIC

study cohort in conjunction with Bristol Myers Squibb. These biomarker analyses will help us understand the mechanisms of the observed effects of the diet and exercise interventions on cardiac structure and function.

8.6.3 Relationship between adiposity and subclinical cardiovascular dysfunction in type 2 diabetes

Additional CMR sequences for measurement of subcutaneous and visceral fat were acquired in all our participants with and without T2D. These are currently being analysed by a PhD student in our group to examine the associations between adiposity and subclinical cardiac dysfunction, as well as the impact of the diet and exercise arms on measures of adiposity.

8.6.4 Relationship of physical activity levels with subclinical cardiovascular dysfunction in type 2 diabetes

All participants with T2D in this thesis have undergone measurement of physical activity levels with seven-day accelerometry. Analysis of these data is currently underway, to explore the relationship between sedentary time, light physical activity, and moderate-to-vigorous physical activity levels with early heart failure.

8.6.5 Early changes in cardiovascular structure and function with diet or exercise

A subset of participants in the DIASTOLIC study underwent additional CMR scanning at 4-weeks post-intervention, to ascertain the earlier impact of dietary restriction or exercise on cardiac structure and function. These analyses may shed light on how quickly improvements occur with exercise and whether shorter-term adverse effects on diastolic function are seen with the MRP, as has been reported in single group interventions in the first week(233).

8.6.6 Relation of subclinical cardiovascular dysfunction to clinical outcomes

Ethical approval has been granted for long-term follow-up (10 years) of subjects with T2D in this thesis. This will enable us to determine which markers of early heart failure are related to clinical outcomes in these patients.

8.7 Limitations

Although the majority of the limitations of this thesis have been discussed within the relevant results chapters, some warrant further discussion.

The capacity of CMR to study cardiometabolic disease has expanded considerably in recent years. In this thesis, only data on cardiac structure and function, myocardial perfusion, focal fibrosis, and aortic stiffness have been

presented. CMR measures of diffuse fibrosis (native T_1 and ECV were not assessed, myocardial triglyceride content could not be measured due to difficulties in obtaining analysable spectra on our wide-bore 3T scanner, and the unit is not currently equipped with phosphorus MRS for assessment of myocardial energetics. These are likely important mechanisms in the pathogenesis of diabetic cardiomyopathy and the upcoming installation of a new 3T MRI scanner at the University of Leicester will enable these measures to be included in future studies.

Non-invasive assessment of diastolic function, especially in a cohort with obesity, is notoriously challenging. This is reflected in the data presented, where three measures of diastolic function (E/A ratio, E/'e, and PEDSR) were employed. In **Chapter 4**, a cross-sectional comparison of adults with and without T2D, the only measure of diastolic function that differed between patients and controls was E/A ratio, which suggested the T2D cohort overall had grade 1 diastolic dysfunction. However in **Chapter 6**, it was LV diastolic filling (E/e') and neither E/A ratio nor PEDSR, which emerged as an independent determinant of aerobic exercise capacity in subjects with T2D. Lastly, participants in the DIASTOLIC study were working-age adults with T2D and younger than the larger combined population. The nested case-control comparison for DIASTOLIC showed that all three measures of diastolic function were perturbed in patients with T2D. This indicates that, in younger adults with T2D and obesity, diastolic dysfunction is the predominant manifestation of diabetic cardiomyopathy. When combining cohorts from the LYDIA study and the PREDICT study in particular (which included patients with T2D up to age 75 years) as in **Chapter 4**, diastolic dysfunction was still evident, but reductions in GLS also emerged. This suggests: 1) that progressive diastolic dysfunction occurs with normal ageing in individuals with and without T2D, and 2) subclinical systolic dysfunction may not precede diastolic dysfunction in isolation. Nevertheless, the heterogeneity of these findings may well be due to recognised difficulties in diagnosing diastolic dysfunction and this important limitation is acknowledged.

Lastly, the prognostic value of the chosen subclinical measures of cardiovascular dysfunction (particularly PEDSR) is not yet known. Longitudinal follow-up of the T2D patient cohort will help address this question, as will validation against external datasets such as UK Biobank. Cardiac function could not be dichotomised as normal or abnormal in the T2D cohort, as reliable cut-off values for

CMR measures do not yet exist. There is marked heterogeneity across CMR centres with regards to scanning technology, image acquisition, software, and analysis techniques, all of which are likely to introduce variation in measures of cardiac function. Determining cut-offs for abnormal cardiac function will require long-term outcome data and external validation against other datasets, both of which are planned. For the purpose of the present analyses, all CMR measures of cardiovascular structure and function were therefore assessed as continuous variables.

9 References

1. Cho NH, Shaw JE, Karuranga S et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271-281.
2. Wilmot EG, Edwardson CL, Biddle SJ et al. Prevalence of diabetes and impaired glucose metabolism in younger 'at risk' UK adults: insights from the STAND programme of research. Diabet Med 2013;30:671-5.
3. Haines L, Wan KC, Lynn R, Barrett TG, Shield JP. Rising incidence of type 2 diabetes in children in the U.K. Diabetes Care 2007;30:1097-101.
4. NCDRF Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 2016;387:1377-1396.
5. Grinstein G, Muzumdar R, Aponte L, Vuguin P, Saenger P, DiMartino-Nardi J. Presentation and 5-year follow-up of type 2 diabetes mellitus in African-American and Caribbean-Hispanic adolescents. Horm Res 2003;60:121-6.
6. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovasc Diabetol 2018;17:83.
7. American Diabetes Association. 9. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2018. Diabetes Care 2018;41:S86-S104.
8. Uddin SN, Malik F, Bari MA et al. Angiographic severity and extent of coronary artery disease in patients with type 2 diabetes mellitus. Mymensingh Med J 2005;14:32-7.
9. Rana JS, Dunning A, Achenbach S et al. Differences in prevalence, extent, severity, and prognosis of coronary artery disease among patients with and without diabetes undergoing coronary computed tomography angiography: results from 10,110 individuals from the CONFIRM (COronary CT Angiography EvaluatioN For Clinical Outcomes): an InteRnational Multicenter Registry. Diabetes Care 2012;35:1787-94.
10. Kim U, Leipsic JA, Sellers SL et al. Natural History of Diabetic Coronary Atherosclerosis by Quantitative Measurement of Serial Coronary Computed Tomographic Angiography: Results of the PARADIGM Study. JACC Cardiovasc Imaging 2018;11:1461-1471.
11. Deseive S, Straub R, Kupke M et al. Impact of diabetes on coronary artery plaque volume by coronary CT angiography and subsequent adverse cardiac events. J Cardiovasc Comput Tomogr 2019;13:31-37.
12. Kim JJ, Hwang BH, Choi IJ et al. Impact of diabetes duration on the extent and severity of coronary atheroma burden and long-term clinical outcome in asymptomatic type 2 diabetic patients: evaluation by Coronary CT angiography. Eur Heart J Cardiovasc Imaging 2015;16:1065-73.
13. Yang J, Dou G, Tesche C et al. Progression of coronary atherosclerotic plaque burden and relationship with adverse cardiovascular event in asymptomatic diabetic patients. BMC Cardiovasc Disord 2019;19:39.

14. Kwan AC, May HT, Cater G et al. Coronary artery plaque volume and obesity in patients with diabetes: the factor-64 study. Radiology 2014;272:690-9.
15. Almdal T, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. Arch Intern Med 2004;164:1422-6.
16. Lau LH, Lew J, Borschmann K, Thijs V, Ekinci EI. Prevalence of diabetes and its effects on stroke outcomes: A meta-analysis and literature review. J Diabetes Investig 2018.
17. Desilles JP, Meseguer E, Labreuche J et al. Diabetes mellitus, admission glucose, and outcomes after stroke thrombolysis: a registry and systematic review. Stroke 2013;44:1915-23.
18. Li W, Prakash R, Kelly-Cobbs AI et al. Adaptive cerebral neovascularization in a model of type 2 diabetes: relevance to focal cerebral ischemia. Diabetes 2010;59:228-35.
19. Kelly-Cobbs A, Elgebaly MM, Li W, Ergul A. Pressure-independent cerebrovascular remodelling and changes in myogenic reactivity in diabetic Goto-Kakizaki rat in response to glycaemic control. Acta Physiol (Oxf) 2011;203:245-51.
20. Healthcare Quality Improvement Partnership. National Diabetes Audit, 2015-2016. NHS Digital: NHS Digital, 2017.
21. Healthcare Quality Improvement Partnership. Are diabetes services in England and Wales Measuring up? National Diabetes Audit 2011-12. 2012.
22. Kannel W, Hjortland M, Castelli W. Role of Diabetes in Congestive Heart Failure: The Framingham Study. The American Journal of Cardiology 1974;34:29-34.
23. Aronow W, Ahn C. Incidence of Heart Failure in 2,737 Older Persons With and Without Diabetes Mellitus. Chest 1999;115:867-868.
24. Bertoni A, Tsai A, Kasper E, Brancati F. Diabetes and Idiopathic Cardiomyopathy: A nationwide case-control study. Diabetes Care 2003;26:2791-2795.
25. Maisch B, Alter P, Pankuweit S. Diabetic cardiomyopathy--fact or fiction? Herz 2011;36:102-15.
26. Lundbaek K. Diabetic angiopathy: a specific vascular disease. Lancet 1954;266:377-9.
27. MacDonald MR, Petrie MC, Hawkins NM et al. Diabetes, left ventricular systolic dysfunction, and chronic heart failure. European heart journal 2008;29:1224-40.
28. National Diabetes Audit, 2015-16. Report 2b: Complications and mortality. (Associtaions between disease outcomes and preceding care). 2017.
29. Norhammar A, Schenck-Gustafsson K. Type 2 diabetes and cardiovascular disease in women. Diabetologia 2013;56:1-9.
30. Sattar N, Rawshani A, Franzen S et al. Age at Diagnosis of Type 2 Diabetes Mellitus and Associations With Cardiovascular and Mortality Risks Findings From the Swedish National Diabetes Registry. Circulation 2019.
31. Tillquist MN, Maddox TM. Update on diabetic cardiomyopathy: inches forward, miles to go. Curr Diab Rep 2012;12:305-13.

32. Lindman BR, Davila-Roman VG, Mann DL et al. Cardiovascular phenotype in HFpEF patients with or without diabetes: a RELAX trial ancillary study. J Am Coll Cardiol 2014;64:541-9.
33. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am J Cardiol 1972;30:595-602.
34. Galderisi M. Echocardiographic Evidence for the Existence of a Distinct Diabetic Cardiomyopathy (The Framingham Heart Study). Am J Cardiol 1991;68:85-89.
35. Skali H, Shah A, Gupta DK et al. Cardiac structure and function across the glycemic spectrum in elderly men and women free of prevalent heart disease: the Atherosclerosis Risk In the Community study. Circ Heart Fail 2015;8:448-54.
36. De Marco M, de Simone G, Roman MJ et al. Cardiac geometry and function in diabetic or prediabetic adolescents and young adults: the Strong Heart Study. Diabetes Care 2011;34:2300-5.
37. Larghat AM, Swoboda PP, Biglands JD, Kearney MT, Greenwood JP, Plein S. The microvascular effects of insulin resistance and diabetes on cardiac structure, function, and perfusion: a cardiovascular magnetic resonance study. Eur Heart J Cardiovasc Imaging 2014;15:1368-76.
38. Khan JN, Wilmot EG, Leggate M et al. Subclinical diastolic dysfunction in young adults with Type 2 diabetes mellitus: a multiparametric contrast-enhanced cardiovascular magnetic resonance pilot study assessing potential mechanisms. Eur Heart J Cardiovasc Imaging 2014;15:1263-9.
39. Bertoni A, Goff D, D'Agostino R et al. Diabetic Cardiomyopathy and Subclinical Cardiovascular Disease. Diabetes Care 2006;29:588-594.
40. Gottdiener J, Arnold A, Aurigemma G et al. Predictors of Congestive Heart Failure in the Elderly: The Cardiovascular Health Study. Journal of the American College of Cardiology 2000;35:1628-1637.
41. Savage DD, Levy D, Dannenberg AL, Garrison RJ, Castelli WP. Association of echocardiographic left ventricular mass with body size, blood pressure and physical activity (the Framingham Study). Am J Cardiol 1990;65:371-6.
42. Foppa M, Duncan BB, Rohde LE. Echocardiography-based left ventricular mass estimation. How should we define hypertrophy? Cardiovasc Ultrasound 2005;3:17.
43. de Simone G, Daniels SR, Devereux RB et al. Left Ventricular Mass and Body Size in Normotensive Children and Adults: Assessment of Allometric Relations and Impact of Overweight. Journal of the American College of Cardiology 1992;20:1251-1260.
44. Armstrong AC, Gidding S, Gjesdal O, Wu C, Bluemke DA, Lima JA. LV mass assessed by echocardiography and CMR, cardiovascular outcomes, and medical practice. JACC Cardiovasc Imaging 2012;5:837-48.
45. Jensen MT, Fung K, Aung N et al. Changes in Cardiac Morphology and Function in Individuals With Diabetes Mellitus: The UK Biobank Cardiovascular Magnetic Resonance Substudy. Circ Cardiovasc Imaging 2019;12:e009476.
46. Levelt E, Mahmod M, Pichnik SK et al. Relationship between Left Ventricular Structural and Metabolic Remodelling in Type 2 Diabetes Mellitus. Diabetes 2015;ePub:1-26.

47. Ernande L, Bergerot C, Girerd N et al. Longitudinal myocardial strain alteration is associated with left ventricular remodeling in asymptomatic patients with type 2 diabetes mellitus. J Am Soc Echocardiogr 2014;27:479-88.
48. Moir S, Hanekom L, Fang ZY et al. Relationship between myocardial perfusion and dysfunction in diabetic cardiomyopathy: a study of quantitative contrast echocardiography and strain rate imaging. Heart 2006;92:1414-9.
49. Ofstad AP, Urheim S, Dalen H et al. Identification of a definite diabetic cardiomyopathy in type 2 diabetes by comprehensive echocardiographic evaluation: A cross-sectional comparison with non-diabetic weight-matched controls. J Diabetes 2014.
50. Saglam H, Seyfeli E, Gul I, Duru M, Gokce C. Index of myocardial performance in patients with type 2 diabetes without hypertension and its relationship with clinical and echocardiographic parameters. J Diabetes 2009;1:50-6.
51. Gjesdal O, Bluemke DA, Lima JA. Cardiac remodeling at the population level--risk factors, screening, and outcomes. Nat Rev Cardiol 2011;8:673-85.
52. Bluemke DA, Kronmal RA, Lima JA et al. The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study. J Am Coll Cardiol 2008;52:2148-55.
53. Salton CJ, Chuang ML, O'Donnell CJ et al. Gender Differences and Normal Left Ventircular Anatomy in an Adult Population Free of Hypertension. Journal of the American College of Cardiology 2002;39:1055-1060.
54. Rodriguez CJ, Sciacca RR, Diez-Roux AV et al. Relation between socioeconomic status, race-ethnicity, and left ventricular mass: the Northern Manhattan study. Hypertension 2004;43:775-9.
55. Rider OJ, Francis JM, Ali MK et al. Determinants of left ventricular mass in obesity; a cardiovascular magnetic resonance study. J Cardiovasc Magn Reson 2009;11:9.
56. Verdecchia P, Carini G, Circo A et al. Left ventricular mass and cardiovascular morbidity in essential hypertension: the MAVI study. J Am Coll Cardiol 2001;38:1829-35.
57. Jorgensen PG, Jensen MT, Biering-Sorensen T et al. Burden of Uncontrolled Metabolic Risk Factors and Left Ventricular Structure and Function in Patients With Type 2 Diabetes Mellitus. J Am Heart Assoc 2018;7:e008856.
58. Gaasch WH, Zile MR. Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry. J Am Coll Cardiol 2011;58:1733-40.
59. Armstrong AC, Liu K, Lewis CE et al. Left atrial dimension and traditional cardiovascular risk factors predict 20-year clinical cardiovascular events in young healthy adults: the CARDIA study. Eur Heart J Cardiovasc Imaging 2014;15:893-9.
60. Khan MA, Yang EY, Zhan Y et al. Association of left atrial volume index and all-cause mortality in patients referred for routine cardiovascular magnetic resonance: a multicenter study. J Cardiovasc Magn Reson 2019;21:4.
61. Gardin JM, McClelland R, Kitzman D et al. M-mode echocardiographic predictors of six- to seven-year incidence of coronary heart disease, stroke,

congestive heart failure, and mortality in an elderly cohort (the Cardiovascular Health Study). Am J Cardiol 2001;87:1051-7.
62. Stritzke J, Markus MR, Duderstadt S et al. The aging process of the heart: obesity is the main risk factor for left atrial enlargement during aging the MONICA/KORA (monitoring of trends and determinations in cardiovascular disease/cooperative research in the region of Augsburg) study. J Am Coll Cardiol 2009;54:1982-9.
63. Kadappu KK, Boyd A, Eshoo S et al. Changes in left atrial volume in diabetes mellitus: more than diastolic dysfunction? Eur Heart J Cardiovasc Imaging 2012;13:1016-23.
64. Ponikowski P, Voors AA, Anker SD et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2016;18:891-975.
65. Boonman-de Winter LJ, Rutten FH, Cramer MJ et al. High prevalence of previously unknown heart failure and left ventricular dysfunction in patients with type 2 diabetes. Diabetologia 2012;55:2154-62.
66. Solomon SD, McMurray JJV, Anand IS et al. Angiotensin-Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction. N Engl J Med 2019;381:1609-1620.
67. McMurray JJ, Packer M, Desai AS et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med 2014;371:993-1004.
68. Boyer JK, Thanigaraj S, Schechtman KB, Perez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. Am J Cardiol 2004;93:870-5.
69. Yazici M, Ozdemir K, Gonen MS et al. Is there any relationship between metabolic parameters and left ventricular functions in type 2 diabetic patients without evident heart disease? Echocardiography 2008;25:675-82.
70. Seyfeli E, Duru M, Saglam H et al. Association of left ventricular diastolic function abnormalities with aortic elastic properties in asymptomatic patients with type 2 diabetes mellitus. A tissue doppler echocardiographic study. Int J Clin Pract 2008;62:1358-65.
71. Faden G, Faganello G, De Feo S et al. The increasing detection of asymptomatic left ventricular dysfunction in patients with type 2 diabetes mellitus without overt cardiac disease: data from the SHORTWAVE study. Diabetes Res Clin Pract 2013;101:309-16.
72. Bergerot C, Davidsen ES, Amaz C et al. Diastolic function deterioration in type 2 diabetes mellitus: predictive factors over a 3-year follow-up. Eur Heart J Cardiovasc Imaging 2018;19:67-73.
73. Fang ZY, Leano R, Marwick TH. Relationship between longitudinal and radial contractility in subclinical diabetic heart disease. Clinical Science 2004;106:53-60.
74. Andersen NH, Poulsen SH, Eiskjaer H, Poulsen PL, Mogensen CE. Decreased left ventricular longitudinal contraction in normotensive and normoalbuminuric patients with Type II diabetes mellitus: a Doppler Tissue tracking and strain rate echocardiography study. Clinical Science 2003;105:59-66.

75. Ng AC, Delgado V, Bertini M et al. Findings from left ventricular strain and strain rate imaging in asymptomatic patients with type 2 diabetes mellitus. The American journal of cardiology 2009;104:1398-401.
76. Liu X, Yang ZG, Gao Y et al. Left ventricular subclinical myocardial dysfunction in uncomplicated type 2 diabetes mellitus is associated with impaired myocardial perfusion: a contrast-enhanced cardiovascular magnetic resonance study. Cardiovasc Diabetol 2018;17:139.
77. Levelt E, Rodgers CT, Clarke WT et al. Cardiac energetics, oxygenation, and perfusion during increased workload in patients with type 2 diabetes mellitus. European Heart Journal 2015.
78. Roos CJ, Scholte AJ, Kharagjitsingh AV, Bax JJ, Delgado V. Changes in multidirectional LV strain in asymptomatic patients with type 2 diabetes mellitus: a 2-year follow-up study. Eur Heart J Cardiovasc Imaging 2014;15:41-7.
79. Liu JH, Chen Y, Yuen M et al. Incremental prognostic value of global longitudinal strain in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2016;15:22.
80. Jorgensen PG, Biering-Sorensen T, Mogelvang R et al. Predictive value of echocardiography in Type 2 diabetes. Eur Heart J Cardiovasc Imaging 2018.
81. Leung M, Wong VW, Hudson M, Leung DY. Impact of Improved Glycemic Control on Cardiac Function in Type 2 Diabetes Mellitus. Circ Cardiovasc Imaging 2016;9:e003643.
82. Ceyhan K, Kadi H, Koc F, Celik A, Ozturk A, Onalan O. Longitudinal left ventricular function in normotensive prediabetics: a tissue Doppler and strain/strain rate echocardiography study. J Am Soc Echocardiogr 2012;25:349-56.
83. Stefanidis A, Bousboulas S, Kalafatis J et al. Left ventricular anatomical and functional changes with ageing in type 2 diabetic adults. Eur J Echocardiogr 2009;10:647-53.
84. Muranaka A, Yuda S, Tsuchihashi K et al. Quantitative assessment of left ventricular and left atrial functions by strain rate imaging in diabetic patients with and without hypertension. Echocardiography 2009;26:262-71.
85. Enomoto M, Ishizu T, Seo Y et al. Subendocardial Systolic Dysfunction in Asymptomatic Normotensive Diabetic Patients. Circ J 2015;79:1749-55.
86. Zoroufian A, Razmi T, Taghavi-Shavazi M, Lotfi-Tokaldany M, Jalali A. Evaluation of subclinical left ventricular dysfunction in diabetic patients: longitudinal strain velocities and left ventricular dyssynchrony by two-dimensional speckle tracking echocardiography study. Echocardiography 2014;31:456-63.
87. Ernande L, Bergerot C, Rietzschel ER et al. Diastolic dysfunction in patients with type 2 diabetes mellitus: is it really the first marker of diabetic cardiomyopathy? J Am Soc Echocardiogr 2011;24:1268-1275 e1.
88. Almeida JG, Fontes-Carvalho R, Sampaio F et al. Impact of the 2016 ASE/EACVI recommendations on the prevalence of diastolic dysfunction in the general population. Eur Heart J Cardiovasc Imaging 2018;19:380-386.
89. Harmancey R, Taegtmeyer H. The complexities of diabetic cardiomyopathy: lessons from patients and animal models. Curr Diab Rep 2008;8:243-8.

90. Ernande L, Derumeaux G. Diabetic cardiomyopathy: myth or reality? Arch Cardiovasc Dis 2012;105:218-25.
91. Brooks BA, Franjic B, Ban CR et al. Diastolic dysfunction and abnormalities of the microcirculation in type 2 diabetes. Diabetes Obes Metab 2008;10:739-46.
92. Rijzewijk LJ, van der Meer RW, Smit JW et al. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. J Am Coll Cardiol 2008;52:1793-9.
93. Ng AC, Delgado V, Bertini M et al. Myocardial steatosis and biventricular strain and strain rate imaging in patients with type 2 diabetes mellitus. Circulation 2010;122:2538-44.
94. Korosoglou G, Humpert PM, Ahrens J et al. Left ventricular diastolic function in type 2 diabetes mellitus is associated with myocardial triglyceride content but not with impaired myocardial perfusion reserve. J Magn Reson Imaging 2012;35:804-11.
95. Wierzbowska-Drabik K, Hamala P, Kasprzak JD. Delayed longitudinal myocardial function recovery after dobutamine challenge as a novel presentation of myocardial dysfunction in type 2 diabetic patients without angiographic coronary artery disease. Eur Heart J Cardiovasc Imaging 2015;16:676-83.
96. Zhang X, Wei X, Liang Y, Liu M, Li C, Tang H. Differential changes of left ventricular myocardial deformation in diabetic patients with controlled and uncontrolled blood glucose: a three-dimensional speckle-tracking echocardiography-based study. J Am Soc Echocardiogr 2013;26:499-506.
97. Nakai H, Takeuchi M, Nishikage T, Lang RM, Otsuji Y. Subclinical left ventricular dysfunction in asymptomatic diabetic patients assessed by two-dimensional speckle tracking echocardiography: correlation with diabetic duration. Eur J Echocardiogr 2009;10:926-32.
98. Scholte AJ, Nucifora G, Delgado V et al. Subclinical left ventricular dysfunction and coronary atherosclerosis in asymptomatic patients with type 2 diabetes. Eur J Echocardiogr 2011;12:148-55.
99. Babyak MA. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. Psychosom Med 2004;66:411-21.
100. Maack C, Lehrke M, Backs J et al. Heart failure and diabetes: metabolic alterations and therapeutic interventions: a state-of-the-art review from the Translational Research Committee of the Heart Failure Association-European Society of Cardiology. Eur Heart J 2018.
101. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. Prostaglandins, leukotrienes, and essential fatty acids 2004;70:309-19.
102. Mazumder PK, O'Neill BT, Roberts MW et al. Impaired Cardiac Efficiency and Increased Fatty Acid Oxidation in Insulin-Resistant ob/ob Mouse Hearts. Diabetes 2004;53:2366-2374.
103. Neubauer S. The Failing Heart — An Engine Out of Fuel. New England Journal of Medicine 2007;356:1140-1151.

104. Scheuermann-Freestone M, Madsen PL, Manners D et al. Abnormal Cardiac and Skeletal Muscle Energy Metabolism in Patients With Type 2 Diabetes. Circulation 2003;107:3040-3046.
105. Shivu GN, Phan TT, Abozguia K et al. Relationship Between Coronary Microvascular Dysfunction and Cardiac Energetics Impairment in Type 1 Diabetes Mellitus. Circulation 2010;121:1209-1215.
106. Levelt E, Rodgers CT, Clarke WT et al. Cardiac energetics, oxygenation, and perfusion during increased workload in patients with type 2 diabetes mellitus. Eur Heart J 2016;37:3461-3469.
107. Neubauer S, Horn M, Cramer M et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation 1997;96:2190-6.
108. Garvey WT, Maianu L, Huecksteadt TP, Birnbaum MJ, Molina JM, Ciaraldi TP. Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. J Clin Invest 1991;87:1072-81.
109. Poornima IG, Parikh P, Shannon RP. Diabetic cardiomyopathy: the search for a unifying hypothesis. Circulation research 2006;98:596-605.
110. Murthy VK, Shipp JC. Accumulation of Myocardial Triglycerides in Ketotic Diabetes: Evidence for Increased Biosynthesis. Diabetes 1977;26:222-229.
111. Paulson DJ, Crass MF. Endogenous triacylglycerol metabolism in diabetic heart. American Journal of Physiology - Heart and Circulatory Physiology 1982;242:H1084-H1094.
112. Robertson RP, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 2004;53 Suppl 1:S119-24.
113. Brindley DN, Kok BP, Kienesberger PC, Lehner R, Dyck JR. Shedding light on the enigma of myocardial lipotoxicity: the involvement of known and putative regulators of fatty acid storage and mobilization. Am J Physiol Endocrinol Metab 2010;298:E897-908.
114. Sharma S, Adrogue JV, Golfman L et al. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J 2004;18:1692-700.
115. Zib I, Jacob AN, Lingvay I et al. Effect of pioglitazone therapy on myocardial and hepatic steatosis in insulin-treated patients with type 2 diabetes. J Investig Med 2007;55:230-6.
116. Glenn DJ, Cardema MC, Ni W et al. Cardiac steatosis potentiates angiotensin II effects in the heart. American Journal of Physiology - Heart and Circulatory Physiology 2015;308:H339-H350.
117. Levelt E, Mahmod M, Piechnik SK et al. Relationship between Left Ventricular Structural and Metabolic Remodelling in Type 2 Diabetes Mellitus. Diabetes 2015.
118. Utz W, Engeli S, Haufe S et al. Myocardial steatosis, cardiac remodelling and fitness in insulin-sensitive and insulin-resistant obese women. Heart 2011;97:1585-9.
119. McGavock JM, Lingvay I, Zib I et al. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. Circulation 2007;116:1170-5.
120. Monji A, Mitsui T, Bando YK, Aoyama M, Shigeta T, Murohara T. Glucagon-like peptide-1 receptor activation reverses cardiac remodeling via

normalizing cardiac steatosis and oxidative stress in type 2 diabetes. American Journal of Physiology - Heart and Circulatory Physiology 2013;305:H295-H304.
121. Ramírez E, Klett-Mingo M, Ares-Carrasco S et al. Eplerenone attenuated cardiac steatosis, apoptosis and diastolic dysfunction in experimental type-II diabetes. Cardiovascular Diabetology 2013;12:172-172.
122. Hammer S, Snel M, Lamb HJ et al. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. J Am Coll Cardiol 2008;52:1006-12.
123. Steadman CD, Jerosch-Herold M, Grundy B et al. Determinants and functional significance of myocardial perfusion reserve in severe aortic stenosis. JACC Cardiovasc Imaging 2012;5:182-9.
124. Yang J, Park Y, Zhang H et al. Feed-forward signaling of TNF-alpha and NF-kappaB via IKK-beta pathway contributes to insulin resistance and coronary arteriolar dysfunction in type 2 diabetic mice. Am J Physiol Heart Circ Physiol 2009;296:H1850-8.
125. Woodman RJ, Watts GF, Playford DA, Best JD, Chan DC. Oxidized LDL and small LDL particle size are independently predictive of a selective defect in microcirculatory endothelial function in type 2 diabetes. Diabetes Obes Metab 2005;7:612-7.
126. Murthy VL, Naya M, Foster CR et al. Association between coronary vascular dysfunction and cardiac mortality in patients with and without diabetes mellitus. Circulation 2012;126:1858-68.
127. Potier L, Chequer R, Roussel R et al. Relationship between cardiac microvascular dysfunction measured with 82Rubidium-PET and albuminuria in patients with diabetes mellitus. Cardiovasc Diabetol 2018;17:11.
128. Kawata T, Daimon M, Miyazaki S et al. Coronary microvascular function is independently associated with left ventricular filling pressure in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2015;14:98.
129. Sorensen MH, Bojer AS, Broadbent DA, Plein S, Madsen PL, Gaede P. Cardiac perfusion, structure, and function in type 2 diabetes mellitus with and without diabetic complications. Eur Heart J Cardiovasc Imaging 2019.
130. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991;83:1849-65.
131. Jellis C, Wright J, Kennedy D et al. Association of imaging markers of myocardial fibrosis with metabolic and functional disturbances in early diabetic cardiomyopathy. Circulation Cardiovascular imaging 2011;4:693-702.
132. Ugander M, Oki AJ, Hsu LY et al. Extracellular volume imaging by magnetic resonance imaging provides insights into overt and sub-clinical myocardial pathology. European heart journal 2012;33:1268-78.
133. Cao Y, Zeng W, Cui Y et al. Increased myocardial extracellular volume assessed by cardiovascular magnetic resonance T1 mapping and its determinants in type 2 diabetes mellitus patients with normal myocardial systolic strain. Cardiovasc Diabetol 2018;17:7.

134. Ng AC, Auger D, Delgado V et al. Association between diffuse myocardial fibrosis by cardiac magnetic resonance contrast-enhanced T(1) mapping and subclinical myocardial dysfunction in diabetic patients: a pilot study. Circ Cardiovasc Imaging 2012;5:51-9.
135. Rao AD, Shah RV, Garg R et al. Aldosterone and myocardial extracellular matrix expansion in type 2 diabetes mellitus. The American journal of cardiology 2013;112:73-8.
136. Wong TC, Piehler KM, Kang IA et al. Myocardial extracellular volume fraction quantified by cardiovascular magnetic resonance is increased in diabetes and associated with mortality and incident heart failure admission. Eur Heart J 2014;35:657-64.
137. Storz C, Hetterich H, Lorbeer R et al. Myocardial tissue characterization by contrast-enhanced cardiac magnetic resonance imaging in subjects with prediabetes, diabetes, and normal controls with preserved ejection fraction from the general population. Eur Heart J Cardiovasc Imaging 2018;19:701-708.
138. Catalano M, Scandale G, Carzaniga G et al. Increased aortic stiffness and related factors in patients with peripheral arterial disease. J Clin Hypertens (Greenwich) 2013;15:712-6.
139. van der Meer RW, Diamant M, Westenberg JJ et al. Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus. J Cardiovasc Magn Reson 2007;9:645-51.
140. Stacey RB, Bertoni AG, Eng J, Bluemke DA, Hundley WG, Herrington D. Modification of the effect of glycemic status on aortic distensibility by age in the multi-ethnic study of atherosclerosis. Hypertension 2010;55:26-32.
141. Laurent S, Boutouyrie P, Asmar R et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236-41.
142. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999;99:2434-9.
143. Mitchell GF, Hwang SJ, Vasan RS et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation 2010;121:505-11.
144. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106:2085-90.
145. Cardoso CR, Ferreira MT, Leite NC, Salles GF. Prognostic impact of aortic stiffness in high-risk type 2 diabetic patients: the Rio deJaneiro Type 2 Diabetes Cohort Study. Diabetes Care 2013;36:3772-8.
146. Ferreira MT, Leite NC, Cardoso CR, Salles GF. Correlates of aortic stiffness progression in patients with type 2 diabetes: importance of glycemic control: the Rio de Janeiro type 2 diabetes cohort study. Diabetes Care 2015;38:897-904.
147. Bibra H, Siegmund T, Ceriello A, Volozhyna M, Schumm-Draeger PM. Optimized postprandial glucose control is associated with improved cardiac/vascular function - comparison of three insulin regimens in well-controlled type 2 diabetes. Horm Metab Res 2009;41:109-15.

148. Cherney DZ, Perkins BA, Soleymanlou N et al. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. Cardiovasc Diabetol 2014;13:28.
149. Kiyici S, Ersoy C, Kaderli A et al. Effect of rosiglitazone, metformin and medical nutrition treatment on arterial stiffness, serum MMP-9 and MCP-1 levels in drug naive type 2 diabetic patients. Diabetes Res Clin Pract 2009;86:44-50.
150. Nakamura T, Matsuda T, Kawagoe Y et al. Effect of pioglitazone on carotid intima-media thickness and arterial stiffness in type 2 diabetic nephropathy patients. Metabolism 2004;53:1382-6.
151. Eren M, Gorgulu S, Uslu N, Celik S, Dagdeviren B, Tezel T. Relation between aortic stiffness and left ventricular diastolic function in patients with hypertension, diabetes, or both. Heart 2004;90:37-43.
152. Gulsin GS, Swarbrick DJ, Hunt WH et al. Relation of Aortic Stiffness to Left Ventricular Remodeling in Younger Adults With Type 2 Diabetes. Diabetes 2018;67:1395-1400.
153. Ripley DP, Negrou K, Oliver JJ et al. Aortic remodelling following the treatment and regression of hypertensive left ventricular hypertrophy: a cardiovascular magnetic resonance study. Clin Exp Hypertens 2015;37:308-16.
154. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-65.
155. ACCORD Study Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-72.
156. Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59.
157. Duckworth W, Abraira C, Moritz T et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360:129-39.
158. Turnbull FM, Abraira C et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. Diabetologia 2009;52:2288-98.
159. Castagno D, Baird-Gunning J, Jhund PS et al. Intensive glycemic control has no impact on the risk of heart failure in type 2 diabetic patients: evidence from a 37,229 patient meta-analysis. Am Heart J 2011;162:938-948 e2.
160. Costantino S, Paneni F, Mitchell K et al. Hyperglycaemia-induced epigenetic changes drive persistent cardiac dysfunction via the adaptor p66(Shc). Int J Cardiol 2018;268:179-186.
161. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007;356:2457-71.
162. US Food and Drug Administration Diabetes Mellitus - Evaluating Cardiovascular Risk in New Antidiabetic Therapies to Treat Type 2 Diabetes. 2008.
163. European Medicines Agency. Guideline on clinical investigation of medicinal products in the treatment or prevention of diabetes mellitus. European Medicines Agency, 2012.

164. Davies MJ, D'Alessio DA, Fradkin J et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 2018.
165. Arnett DK, Blumenthal RS, Albert MA et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol 2019.
166. Grant PJ, Cosentino F. The 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: New features and the 'Ten Commandments' of the 2019 Guidelines are discussed by Professor Peter J. Grant and Professor Francesco Cosentino, the Task Force chairmen. Eur Heart J 2019;40:3215-3217.
167. Nauck MA, Heimesaat MM, Behle K et al. Effects of Glucagon-Like Peptide 1 on Counterregulatory Hormone Responses, Cognitive Functions, and Insulin Secretion during Hyperinsulinemic, Stepped Hypoglycemic Clamp Experiments in Healthy Volunteers. The Journal of Clinical Endocrinology & Metabolism 2002;87:1239-1246.
168. Marso SP, Daniels GH, Brown-Frandsen K et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2016;375:311-22.
169. Marso SP, Bain SC, Consoli A et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 2016;375:1834-1844.
170. Hernandez AF, Green JB, Janmohamed S et al. Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease (Harmony Outcomes): a double-blind, randomised placebo-controlled trial. Lancet 2018.
171. Gerstein HC, Colhoun HM, Dagenais GR et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. Lancet 2019.
172. Pfeffer MA, Claggett B, Diaz R et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. N Engl J Med 2015;373:2247-57.
173. Holman RR, Bethel MA, Mentz RJ et al. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2017;377:1228-1239.
174. Husain M, Birkenfeld AL, Donsmark M et al. Oral Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 2019.
175. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care 2005;28:1092-100.
176. Prasad-Reddy L, Isaacs D. A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. Drugs Context 2015;4:212283.
177. Buse JB, Rosenstock J, Sesti G et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet 2009;374:39-47.

178. Wysham C, Blevins T, Arakaki R et al. Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). Diabetes Care 2014;37:2159-67.
179. Sun F, Wu S, Guo S et al. Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: A systematic review and network meta-analysis. Diabetes Res Clin Pract 2015;110:26-37.
180. Sun F, Wu S, Wang J et al. Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: a systematic review and network meta-analysis. Clin Ther 2015;37:225-241 e8.
181. Dar S, Tahrani AA, Piya MK. The role of GLP-1 receptor agonists as weight loss agents in patients with and without type 2 diabetes. Practical Diabetes 2015;32:297-300b.
182. Bizino MB, Jazet IM, Westenberg JJM et al. Effect of liraglutide on cardiac function in patients with type 2 diabetes mellitus: randomized placebo-controlled trial. Cardiovasc Diabetol 2019;18:55.
183. Paiman EHM, van Eyk HJ, van Aalst MMA et al. Effect of Liraglutide on Cardiovascular Function and Myocardial Tissue Characteristics in Type 2 Diabetes Patients of South Asian Descent Living in the Netherlands: A Double-Blind, Randomized, Placebo-Controlled Trial. J Magn Reson Imaging 2019.
184. Webb DR, Htike ZZ, Swarbrick DJ et al. A randomised, open label, active comparator trial assessing the effects of 26 weeks of liraglutide or sitagliptin on cardiovascular function in young obese adults with type 2 diabetes. Diabetes Obes Metab 2020.
185. Sattar N, Petrie MC, Zinman B, Januzzi JL, Jr. Novel Diabetes Drugs and the Cardiovascular Specialist. J Am Coll Cardiol 2017;69:2646-2656.
186. Wiviott SD, Raz I, Bonaca MP et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2018.
187. Zinman B, Wanner C, Lachin JM et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-28.
188. Neal B, Perkovic V, Mahaffey KW et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 2017;377:644-657.
189. Kato ET, Silverman MG, Mosenzon O et al. Effect of Dapagliflozin on Heart Failure and Mortality in Type 2 Diabetes Mellitus. Circulation 2019;139:2528-2536.
190. McMurray JJV, Solomon SD, Inzucchi SE et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. N Engl J Med 2019;381:1995-2008.
191. Verma S, Garg A, Yan AT et al. Effect of Empagliflozin on Left Ventricular Mass and Diastolic Function in Individuals With Diabetes: An Important Clue to the EMPA-REG OUTCOME Trial? Diabetes Care 2016;39:e212-e213.
192. Soga F, Tanaka H, Tatsumi K et al. Impact of dapagliflozin on left ventricular diastolic function of patients with type 2 diabetic mellitus with chronic heart failure. Cardiovasc Diabetol 2018;17:132.

193. Matsutani D, Sakamoto M, Kayama Y, Takeda N, Horiuchi R, Utsunomiya K. Effect of canagliflozin on left ventricular diastolic function in patients with type 2 diabetes. Cardiovasc Diabetol 2018;17:73.
194. Verma S, Mazer CD, Yan AT et al. Effect of Empagliflozin on Left Ventricular Mass in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease: The EMPA-HEART CardioLink-6 Randomized Clinical Trial. Circulation 2019;140:1693-1702.
195. Cohen ND, Gutman SJ, Briganti EM, Taylor AJ. Effects of empagliflozin treatment on cardiac function and structure in patients with type 2 diabetes: a cardiac magnetic resonance study. Intern Med J 2019;49:1006-1010.
196. Fernandez-Alonso C, Roffidal D, Sander G, Giles T. Dapagliflozin improves arterial stiffness in patients with type 2 diabetes mellitus. Journal of the American College of Cardiology 2017;69:Supplement.
197. Mudaliar S, Alloju S, Henry RR. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis. Diabetes Care 2016;39:1115-22.
198. Sowers JR. Recommendations for special populations: diabetes mellitus and the metabolic syndrome. Am J Hypertens 2003;16:41S-45S.
199. Grossman A, Grossman E. Blood pressure control in type 2 diabetic patients. Cardiovasc Diabetol 2017;16:3.
200. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. JAMA 2015;313:603-15.
201. Brunstrom M, Carlberg B. Effect of antihypertensive treatment at different blood pressure levels in patients with diabetes mellitus: systematic review and meta-analyses. BMJ 2016;352:i717.
202. National Institute for Health and Care Excellence NICE guideline [NG28] Type 2 diabetes in adults: management. NICE, 2015.
203. James PA, Oparil S, Carter BL et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 2014;311:507-20.
204. Leung AA, Nerenberg K, Daskalopoulou SS et al. Hypertension Canada's 2016 Canadian Hypertension Education Program Guidelines for Blood Pressure Measurement, Diagnosis, Assessment of Risk, Prevention, and Treatment of Hypertension. Can J Cardiol 2016;32:569-88.
205. Group SR, Wright JT, Jr., Williamson JD et al. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. N Engl J Med 2015;373:2103-16.
206. Chobanian AV, Bakris GL, Black HR et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.
207. ACCORD Study Group. Effects of intensive blood-pressure control in type 2 diabetes mellitus. N Engl J Med 2010;362:1575-85.
208. ACCORDION. ACCORDION: long-term follow-up of ACCORD patients. American Heart Association (AHA) 2015 Scientific Sessions. Orlando, Florida, 2015.

209. Cooper-DeHoff RM, Gong Y, Handberg EM et al. Tight blood pressure control and cardiovascular outcomes among hypertensive patients with diabetes and coronary artery disease. JAMA 2010;304:61-8.
210. Navar AM, Gallup DS, Lokhnygina Y et al. Hypertension Control in Adults With Diabetes Mellitus and Recurrent Cardiovascular Events: Global Results From the Trial Evaluating Cardiovascular Outcomes With Sitagliptin. Hypertension 2017.
211. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 2003;348:383-93.
212. Rawshani A, Rawshani A, Franzen S et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 2018;379:633-644.
213. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-53.
214. Inzucchi SE, Bergenstal RM, Buse JB et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care 2015;38:140-9.
215. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes 1995;44:1249-58.
216. Pories WJ, Caro JF, Flickinger EG, Meelheim HD, Swanson MS. The control of diabetes mellitus (NIDDM) in the morbidly obese with the Greenville Gastric Bypass. Ann Surg 1987;206:316-23.
217. Meijer RI, van Wagensveld BA, Siegert CE, Eringa EC, Serne EH, Smulders YM. Bariatric surgery as a novel treatment for type 2 diabetes mellitus: a systematic review. Arch Surg 2011;146:744-50.
218. Sjostrom L, Peltonen M, Jacobson P et al. Bariatric surgery and long-term cardiovascular events. JAMA 2012;307:56-65.
219. Sjostrom L, Peltonen M, Jacobson P et al. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. JAMA 2014;311:2297-304.
220. Aminian A, Zajichek A, Arterburn DE et al. Association of Metabolic Surgery With Major Adverse Cardiovascular Outcomes in Patients With Type 2 Diabetes and Obesity. JAMA 2019.
221. Jackness C, Karmally W, Febres G et al. Very low-calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and beta-cell Function in type 2 diabetic patients. Diabetes 2013;62:3027-32.
222. Viljanen AP, Karmi A, Borra R et al. Effect of caloric restriction on myocardial fatty acid uptake, left ventricular mass, and cardiac work in obese adults. Am J Cardiol 2009;103:1721-6.
223. Steven S, Taylor R. Restoring normoglycaemia by use of a very low calorie diet in long- and short-duration Type 2 diabetes. Diabet Med 2015;32:1149-55.
224. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in

association with decreased pancreas and liver triacylglycerol. Diabetologia 2011;54:2506-14.
225. Jazet IM, Schaart G, Gastaldelli A et al. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. Diabetologia 2008;51:309-19.
226. Lean ME, Leslie WS, Barnes AC et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet 2017.
227. Lean MEJ, Leslie WS, Barnes AC et al. Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. Lancet Diabetes Endocrinol 2019;7:344-355.
228. Steven S, Lim EL, Taylor R. Population response to information on reversibility of Type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2013;30:e135-8.
229. Rider OJ, Francis JM, Ali MK et al. Beneficial cardiovascular effects of bariatric surgical and dietary weight loss in obesity. J Am Coll Cardiol 2009;54:718-26.
230. Rider OJ, Francis JM, Tyler D, Byrne J, Clarke K, Neubauer S. Effects of weight loss on myocardial energetics and diastolic function in obesity. The international journal of cardiovascular imaging 2013;29:1043-50.
231. Utz W, Engeli S, Haufe S et al. Moderate dietary weight loss reduces myocardial steatosis in obese and overweight women. International journal of cardiology 2013;167:905-9.
232. Jonker JT, Djaberi R, van Schinkel LD et al. Very-low-calorie diet increases myocardial triglyceride content and decreases diastolic left ventricular function in type 2 diabetes with cardiac complications. Diabetes care 2014;37:e1-2.
233. Rayner JJ, Abdesselam I, Peterzan MA et al. Very low calorie diets are associated with transient ventricular impairment before reversal of diastolic dysfunction in obesity. Int J Obes (Lond) 2018.
234. Jonker JT, Snel M, Hammer S et al. Sustained cardiac remodeling after a short-term very low calorie diet in type 2 diabetes mellitus patients. Int J Cardiovasc Imaging 2014;30:121-7.
235. de las Fuentes L, Waggoner AD, Mohammed BS et al. Effect of moderate diet-induced weight loss and weight regain on cardiovascular structure and function. J Am Coll Cardiol 2009;54:2376-81.
236. Syed M, Rosati C, Torosoff MT et al. The impact of weight loss on cardiac structure and function in obese patients. Obes Surg 2009;19:36-40.
237. Kosmala W, O'Moore-Sullivan T, Plaksej R, Przewlocka-Kosmala M, Marwick TH. Improvement of left ventricular function by lifestyle intervention in obesity: contributions of weight loss and reduced insulin resistance. Diabetologia 2009;52:2306-2316.
238. Wei M, Gibbons LW, Kampert JB, Nichaman MZ, Blair SN. Low cardiorespiratory fitness and physical inactivity as predictors of mortality in men with type 2 diabetes. Ann Intern Med 2000;132:605-11.

239. Hu FB, Stampfer MJ, Solomon C et al. Physical activity and risk for cardiovascular events in diabetic women. Ann Intern Med 2001;134:96-105.
240. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002;346:793-801.
241. Nylen ES, Kokkinos P, Myers J, Faselis C. Prognostic effect of exercise capacity on mortality in older adults with diabetes mellitus. J Am Geriatr Soc 2010;58:1850-4.
242. Poirier P, Garneau C, Bogaty P et al. Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus. Am J Cardiol 2000;85:473-7.
243. Kemps H, Krankel N, Dorr M et al. Exercise training for patients with type 2 diabetes and cardiovascular disease: What to pursue and how to do it. A Position Paper of the European Association of Preventive Cardiology (EAPC). Eur J Prev Cardiol 2019:2047487318820420.
244. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. Jama 2001;286:1218-27.
245. Umpierre D, Ribeiro PA, Kramer CK et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. Jama 2011;305:1790-9.
246. Hawley JA. Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. Diabetes-Metab Res 2004;20:383-393.
247. Telford RD. Low physical activity and obesity: causes of chronic disease or simply predictors? Medicine and science in sports and exercise 2007;39:1233-40.
248. Brassard P, Legault S, Garneau C, Bogaty P, Dumesnil JG, Poirier P. Normalization of diastolic dysfunction in type 2 diabetics after exercise training. Med Sci Sports Exerc 2007;39:1896-901.
249. Hordern MD, Coombes JS, Cooney LM, Jeffriess L, Prins JB, Marwick TH. Effects of exercise intervention on myocardial function in type 2 diabetes. Heart 2009;95:1343-9.
250. Schuster I, Vinet A, Karpoff L et al. Diastolic dysfunction and intraventricular dyssynchrony are restored by low intensity exercise training in obese men. Obesity 2012;20:134-40.
251. Loimaala A, Groundstroem K, Rinne M, Nenonen A, Huhtala H, Vuori I. Exercise training does not improve myocardial diastolic tissue velocities in Type 2 diabetes. Cardiovasc Ultrasound 2007;5:32.
252. Jonker JT, de Mol P, de Vries ST et al. Exercise and type 2 diabetes mellitus: changes in tissue-specific fat distribution and cardiac function. Radiology 2013;269:434-42.
253. Sacre JW, Jellis CL, Jenkins C et al. A six-month exercise intervention in subclinical diabetic heart disease: effects on exercise capacity, autonomic and myocardial function. Metabolism 2014;63:1104-14.
254. Hollekim-Strand SM, Bjorgaas MR, Albrektsen G, Tjonna AE, Wisloff U, Ingul CB. High-intensity interval exercise effectively improves cardiac function in patients with type 2 diabetes mellitus and diastolic dysfunction: a randomized controlled trial. J Am Coll Cardiol 2014;64:1758-60.

255. Cugusi L, Cadeddu C, Nocco S et al. Effects of an aquatic-based exercise program to improve cardiometabolic profile, quality of life, and physical activity levels in men with type 2 diabetes mellitus. PM R 2015;7:141-8; quiz 148.
256. Cassidy S, Thoma C, Hallsworth K et al. High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 2016;59:56-66.
257. Smart N, Haluska B, Jeffriess L, Marwick TH. Exercise training in systolic and diastolic dysfunction: effects on cardiac function, functional capacity, and quality of life. American heart journal 2007;153:530-6.
258. Look ARG, Wing RR, Bolin P et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. N Engl J Med 2013;369:145-54.
259. Otten J, Andersson J, Stahl J et al. Exercise Training Adds Cardiometabolic Benefits of a Paleolithic Diet in Type 2 Diabetes Mellitus. J Am Heart Assoc 2019;8:e010634.
260. Otten J, Stomby A, Waling M et al. Benefits of a Paleolithic diet with and without supervised exercise on fat mass, insulin sensitivity, and glycemic control: a randomized controlled trial in individuals with type 2 diabetes. Diabetes Metab Res Rev 2017;33.
261. Gulsin GS, Brady EM, Swarbrick DJ et al. Rationale, design and study protocol of the randomised controlled trial: Diabetes Interventional Assessment of Slimming or Training tO Lessen Inconspicuous Cardiovascular Dysfunction (the DIASTOLIC study). BMJ Open 2019;9:e023207.
262. Htike ZZ, Yates T, Brady EM et al. Rationale and design of the randomised controlled trial to assess the impact of liraglutide on cardiac function and structure in young adults with type 2 diabetes (the LYDIA study). Cardiovasc Diabetol 2016;15:102.
263. ClinicalTrials.gov. Prevalence and Determinants of Subclinical Cardiovascular Dysfunction in Adults with Type 2 Diabetes (PREDICT). 2017.
264. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
265. Kramer CM, Barkhausen J, Flamm SD, Kim RJ, Nagel E, Society for Cardiovascular Magnetic Resonance Board of Trustees Task Force on Standardized P. Standardized cardiovascular magnetic resonance (CMR) protocols 2013 update. J Cardiovasc Magn Reson 2013;15:91.
266. Karamitsos TD, Ntusi NA, Francis JM, Holloway CJ, Myerson SG, Neubauer S. Feasibility and safety of high-dose adenosine perfusion cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2010;12:66.
267. Biglands JD, Magee DR, Sourbron SP, Plein S, Greenwood JP, Radjenovic A. Comparison of the Diagnostic Performance of Four Quantitative Myocardial Perfusion Estimation Methods Used in Cardiac MR Imaging: CE-MARC Substudy. Radiology 2015;275:393-402.

268. Kellman P, Hansen MS, Nielles-Vallespin S et al. Myocardial perfusion cardiovascular magnetic resonance: optimized dual sequence and reconstruction for quantification. J Cardiovasc Magn Reson 2017;19:43.
269. Schulz-Menger J, Bluemke DA, Bremerich J et al. Standardized image interpretation and post processing in cardiovascular magnetic resonance: Society for Cardiovascular Magnetic Resonance (SCMR) board of trustees task force on standardized post processing. J Cardiovasc Magn Reson 2013;15:35.
270. Biglands J, Magee D, Boyle R, Larghat A, Plein S, Radjenovic A. Evaluation of the effect of myocardial segmentation errors on myocardial blood flow estimates from DCE-MRI. Phys Med Biol 2011;56:2423-43.
271. Wharton G, Steeds R, Allen J et al. A minimum dataset for a standard adult transthoracic echocardiogram: a guideline protocol from the British Society of Echocardiography. Echo Res Pract 2015;2:G9-G24.
272. Nauta JF, Hummel YM, van der Meer P, Lam CSP, Voors AA, van Melle JP. Correlation with invasive left ventricular filling pressures and prognostic relevance of the echocardiographic diastolic parameters used in the 2016 ESC heart failure guidelines and in the 2016 ASE/EACVI recommendations: a systematic review in patients with heart failure with preserved ejection fraction. Eur J Heart Fail 2018.
273. Wasserman K. Principles of Exercise Testing and Interpretation: Including Pathophysiology and Clinical Applications: Lippincott Williams & Wilkins, 2005.
274. Das P, Rimington H, Smeeton N, Chambers J. Determinants of symptoms and exercise capacity in aortic stenosis: a comparison of resting haemodynamics and valve compliance during dobutamine stress. Eur Heart J 2003;24:1254-63.
275. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.
276. Cicchetti DV, Sparrow SA. Developing criteria for establishing interrater reliability of specific items: applications to assessment of adaptive behavior. Am J Ment Defic 1981;86:127-37.
277. Puntmann VO, Valbuena S, Hinojar R et al. Society for Cardiovascular Magnetic Resonance (SCMR) expert consensus for CMR imaging endpoints in clinical research: part I - analytical validation and clinical qualification. J Cardiovasc Magn Reson 2018;20:67.
278. Ponikowski P, Voors AA, Anker SD et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37:2129-2200.
279. Cikes M, Solomon SD. Beyond ejection fraction: an integrative approach for assessment of cardiac structure and function in heart failure. Eur Heart J 2016;37:1642-50.
280. Scatteia A, Baritussio A, Bucciarelli-Ducci C. Strain imaging using cardiac magnetic resonance. Heart Fail Rev 2017;22:465-476.

281. Kalam K, Otahal P, Marwick TH. Prognostic implications of global LV dysfunction: a systematic review and meta-analysis of global longitudinal strain and ejection fraction. Heart 2014;100:1673-80.
282. Nesbitt GC, Mankad S, Oh JK. Strain imaging in echocardiography: methods and clinical applications. Int J Cardiovasc Imaging 2009;25 Suppl 1:9-22.
283. Cheng S, Fernandes VR, Bluemke DA, McClelland RL, Kronmal RA, Lima JA. Age-related left ventricular remodeling and associated risk for cardiovascular outcomes: the Multi-Ethnic Study of Atherosclerosis. Circ Cardiovasc Imaging 2009;2:191-8.
284. Kuznetsova T, Herbots L, Richart T et al. Left ventricular strain and strain rate in a general population. Eur Heart J 2008;29:2014-23.
285. Fonseca CG, Dissanayake AM, Doughty RN et al. Three-dimensional assessment of left ventricular systolic strain in patients with type 2 diabetes mellitus, diastolic dysfunction, and normal ejection fraction. Am J Cardiol 2004;94:1391-5.
286. Ng AC, Delgado V, Bertini M et al. Alterations in multidirectional myocardial functions in patients with aortic stenosis and preserved ejection fraction: a two-dimensional speckle tracking analysis. Eur Heart J 2011;32:1542-50.
287. Antoni ML, Mollema SA, Delgado V et al. Prognostic importance of strain and strain rate after acute myocardial infarction. Eur Heart J 2010;31:1640-7.
288. Jeung MY, Germain P, Croisille P, El ghannudi S, Roy C, Gangi A. Myocardial tagging with MR imaging: overview of normal and pathologic findings. Radiographics 2012;32:1381-98.
289. Aletras AH, Ding S, Balaban RS, Wen H. DENSE: Displacement Encoding with Stimulated Echoes in Cardiac Functional MRI. J Magn Reson 1999;137:247-52.
290. Ibrahim el SH. Myocardial tagging by cardiovascular magnetic resonance: evolution of techniques--pulse sequences, analysis algorithms, and applications. J Cardiovasc Magn Reson 2011;13:36.
291. Hor KN, Baumann R, Pedrizzetti G et al. Magnetic Resonance Derived Myocardial Strain Assessment Using Feature Tracking. J Vis Exp, 2011.
292. Amaki M, Savino J, Ain DL et al. Diagnostic concordance of echocardiography and cardiac magnetic resonance-based tissue tracking for differentiating constrictive pericarditis from restrictive cardiomyopathy. Circ Cardiovasc Imaging 2014;7:819-27.
293. Obokata M, Nagata Y, Wu VC et al. Direct comparison of cardiac magnetic resonance feature tracking and 2D/3D echocardiography speckle tracking for evaluation of global left ventricular strain. Eur Heart J Cardiovasc Imaging 2016;17:525-32.
294. Hor KN, Gottliebson WM, Carson C et al. Comparison of magnetic resonance feature tracking for strain calculation with harmonic phase imaging analysis. JACC Cardiovasc Imaging 2010;3:144-51.
295. Moody WE, Taylor RJ, Edwards NC et al. Comparison of magnetic resonance feature tracking for systolic and diastolic strain and strain rate calculation with spatial modulation of magnetization imaging analysis. J Magn Reson Imaging 2015;41:1000-12.
296. Cao JJ, Ngai N, Duncanson L, Cheng J, Gliganic K, Chen Q. A comparison of both DENSE and feature tracking techniques with tagging for the

cardiovascular magnetic resonance assessment of myocardial strain. J Cardiovasc Magn Reson 2018;20:26.
297. Hor KN, Baumann R, Pedrizzetti G et al. Magnetic resonance derived myocardial strain assessment using feature tracking. J Vis Exp 2011.
298. Grothues F, Smith GC, Moon JC et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 2002;90:29-34.
299. Voigt JU, Pedrizzetti G, Lysyansky P et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. J Am Soc Echocardiogr 2015;28:183-93.
300. Plana JC, Galderisi M, Barac A et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2014;15:1063-93.
301. Singh A, Steadman CD, Khan JN et al. Intertechnique agreement and interstudy reproducibility of strain and diastolic strain rate at 1.5 and 3 Tesla: a comparison of feature-tracking and tagging in patients with aortic stenosis. J Magn Reson Imaging 2015;41:1129-37.
302. Khan JN, Singh A, Nazir SA, Kanagala P, Gershlick AH, McCann GP. Comparison of cardiovascular magnetic resonance feature tracking and tagging for the assessment of left ventricular systolic strain in acute myocardial infarction. Eur J Radiol 2015;84:840-8.
303. Graham-Brown MP, Gulsin GS, Parke K et al. A comparison of the reproducibility of two cine-derived strain software programmes in disease states. Eur J Radiol 2019;113:51-58.
304. Goldberg LR, Jessup M. Stage B heart failure: management of asymptomatic left ventricular systolic dysfunction. Circulation 2006;113:2851-60.
305. Wang Y, Yang H, Huynh Q, Nolan M, Negishi K, Marwick TH. Diagnosis of Nonischemic Stage B Heart Failure in Type 2 Diabetes Mellitus: Optimal Parameters for Prediction of Heart Failure. JACC Cardiovasc Imaging 2018;11:1390-1400.
306. Rosen BD, Edvardsen T, Lai S et al. Left ventricular concentric remodeling is associated with decreased global and regional systolic function: the Multi-Ethnic Study of Atherosclerosis. Circulation 2005;112:984-91.
307. Kane GC, Karon BL, Mahoney DW et al. Progression of left ventricular diastolic dysfunction and risk of heart failure. JAMA 2011;306:856-63.
308. Ng ACT, Bertini M, Ewe SH et al. Defining Subclinical Myocardial Dysfunction and Implications for Patients With Diabetes Mellitus and Preserved Ejection Fraction. Am J Cardiol 2019;124:892-898.
309. Holland DJ, Marwick TH, Haluska BA et al. Subclinical LV dysfunction and 10-year outcomes in type 2 diabetes mellitus. Heart 2015;101:1061-6.
310. Rosen BD, Lima JA, Nasir K et al. Lower myocardial perfusion reserve is associated with decreased regional left ventricular function in asymptomatic participants of the multi-ethnic study of atherosclerosis. Circulation 2006;114:289-97.

311. Cauwenberghs N, Knez J, Thijs L et al. Relation of Insulin Resistance to Longitudinal Changes in Left Ventricular Structure and Function in a General Population. J Am Heart Assoc 2018;7.
312. Webb DR, Khunti K, Silverman R et al. Impact of metabolic indices on central artery stiffness: independent association of insulin resistance and glucose with aortic pulse wave velocity. Diabetologia 2010;53:1190-8.
313. Striepe K, Jumar A, Ott C et al. Effects of the Selective Sodium-Glucose Cotransporter 2 Inhibitor Empagliflozin on Vascular Function and Central Hemodynamics in Patients With Type 2 Diabetes Mellitus. Circulation 2017;136:1167-1169.
314. Barinas-Mitchell E, Kuller LH, Sutton-Tyrrell K et al. Effect of weight loss and nutritional intervention on arterial stiffness in type 2 diabetes. Diabetes Care 2006;29:2218-22.
315. Lean MEJ, Leslie WS, Barnes AC et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. The Lancet 2017.
316. Gulsin GS, Athithan L, McCann GP. Diabetic cardiomyopathy: prevalence, determinants and potential treatments. Ther Adv Endocrinol Metab 2019;10:2042018819834869.
317. Kozakova M, Morizzo C, Fraser AG, Palombo C. Impact of glycemic control on aortic stiffness, left ventricular mass and diastolic longitudinal function in type 2 diabetes mellitus. Cardiovasc Diabetol 2017;16:78.
318. Pandey A, Garg S, Khunger M et al. Dose-Response Relationship Between Physical Activity and Risk of Heart Failure: A Meta-Analysis. Circulation 2015;132:1786-94.
319. Greenwood JP, Maredia N, Younger JF et al. Cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC): a prospective trial. Lancet 2012;379:453-60.
320. Haaf P, Garg P, Messroghli DR, Broadbent DA, Greenwood JP, Plein S. Cardiac T1 Mapping and Extracellular Volume (ECV) in clinical practice: a comprehensive review. J Cardiovasc Magn Reson 2016;18:89.
321. Soufi Taleb Bendiab N, Meziane-Tani A, Ouabdesselam S et al. Factors associated with global longitudinal strain decline in hypertensive patients with normal left ventricular ejection fraction. Eur J Prev Cardiol 2017;24:1463-1472.
322. Kuznetsova T, Herbots L, Lopez B et al. Prevalence of left ventricular diastolic dysfunction in a general population. Circ Heart Fail 2009;2:105-12.
323. Lee DS, Gona P, Vasan RS et al. Relation of disease pathogenesis and risk factors to heart failure with preserved or reduced ejection fraction: insights from the framingham heart study of the national heart, lung, and blood institute. Circulation 2009;119:3070-7.
324. Russo C, Jin Z, Homma S et al. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. J Am Coll Cardiol 2011;57:1368-74.
325. Haass M, Kitzman DW, Anand IS et al. Body mass index and adverse cardiovascular outcomes in heart failure patients with preserved ejection fraction: results from the Irbesartan in Heart Failure with Preserved Ejection Fraction (I-PRESERVE) trial. Circ Heart Fail 2011;4:324-31.

326. Packer M. Leptin-Aldosterone-Neprilysin Axis: Identification of Its Distinctive Role in the Pathogenesis of the Three Phenotypes of Heart Failure in People With Obesity. Circulation 2018;137:1614-1631.
327. Yokoyama I, Momomura S, Ohtake T et al. Reduced myocardial flow reserve in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 1997;30:1472-7.
328. Marciano C, Galderisi M, Gargiulo P et al. Effects of type 2 diabetes mellitus on coronary microvascular function and myocardial perfusion in patients without obstructive coronary artery disease. Eur J Nucl Med Mol Imaging 2012;39:1199-206.
329. Reusch JE, Bridenstine M, Regensteiner JG. Type 2 diabetes mellitus and exercise impairment. Rev Endocr Metab Disord 2013;14:77-86.
330. Regensteiner JG, Bauer TA, Reusch JE et al. Cardiac dysfunction during exercise in uncomplicated type 2 diabetes. Med Sci Sports Exerc 2009;41:977-84.
331. American Thoracic Society, American College of Chest P. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med 2003;167:211-77.
332. Laukkanen JA, Kurl S, Salonen R, Rauramaa R, Salonen JT. The predictive value of cardiorespiratory fitness for cardiovascular events in men with various risk profiles: a prospective population-based cohort study. Eur Heart J 2004;25:1428-37.
333. Khan H, Kunutsor S, Rauramaa R et al. Cardiorespiratory fitness and risk of heart failure: a population-based follow-up study. Eur J Heart Fail 2014;16:180-8.
334. Zeiher J, Ombrellaro KJ, Perumal N, Keil T, Mensink GBM, Finger JD. Correlates and Determinants of Cardiorespiratory Fitness in Adults: a Systematic Review. Sports Med Open 2019;5:39.
335. Fang ZY, Sharman J, Prins JB, Marwick TH. Determinants of exercise capacity in patients with type 2 diabetes. Diabetes Care 2005;28:1643-8.
336. Meyer M, McEntee RK, Nyotowidjojo I, Chu G, LeWinter MM. Relationship of exercise capacity and left ventricular dimensions in patients with a normal ejection fraction. An exploratory study. PLoS One 2015;10:e0119432.
337. Singh A, Jerosch-Herold M, Bekele S et al. Determinants of Exercise Capacity and Myocardial Perfusion Reserve in Asymptomatic Patients With Aortic Stenosis. JACC Cardiovascular imaging 2020;13:178-180.
338. Broadbent DA, Biglands JD, Ripley DP et al. Sensitivity of quantitative myocardial dynamic contrast-enhanced MRI to saturation pulse efficiency, noise and t1 measurement error: Comparison of nonlinearity correction methods. Magn Reson Med 2016;75:1290-300.
339. Colberg SR, Sigal RJ, Yardley JE et al. Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. Diabetes Care 2016;39:2065-2079.
340. Verboven M, Van Ryckeghem L, Belkhouribchia J et al. Effect of Exercise Intervention on Cardiac Function in Type 2 Diabetes Mellitus: A Systematic Review. Sports Med 2019;49:255-268.
341. Nagi D, Hambling C, Taylor R. Remission of Type 2 Diabetes: A Position Statement from the Association of British Clinical Diabetologists (ABCD) and the Primary Care Diabetes Society (PCDS). 2019.

342. Rider OJ, Lewandowski A, Nethononda R et al. Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. European heart journal 2013;34:292-9.
343. National Institute for Health and Care Excellence. The management of type 2 diabetes. NICE clinical guideline 87., 2010.
344. Carlo Agostoni RBC, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Scientific Opinion on the essential composition of total diet replacements for weight control. EFSA Journal 2015;13:52.
345. Hunt SA, Abraham WT, Chin MH et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. Circulation 2005;112:e154-235.
346. Gambardella J, Morelli MB, Wang XJ, Santulli G. Pathophysiological mechanisms underlying the beneficial effects of physical activity in hypertension. J Clin Hypertens (Greenwich) 2020.
347. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985) 2005;98:1154-62.
348. Phan TT, Abozguia K, Nallur Shivu G et al. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. J Am Coll Cardiol 2009;54:402-9.
349. Wang TJ, Larson MG, Levy D et al. Impact of obesity on plasma natriuretic peptide levels. Circulation 2004;109:594-600.
350. Baldassarre S, Fragapani S, Panero A et al. NTproBNP in insulin-resistance mediated conditions: overweight/obesity, metabolic syndrome and diabetes. The population-based Casale Monferrato Study. Cardiovasc Diabetol 2017;16:119.
351. Wehner GJ, Jing L, Haggerty CM et al. Comparison of left ventricular strains and torsion derived from feature tracking and DENSE CMR. J Cardiovasc Magn Reson 2018;20:63.
352. Malmborg M, Schmiegelow MDS, Norgaard CH et al. Does type 2 diabetes confer higher relative rates of cardiovascular events in women compared with men? Eur Heart J 2019.
353. Bank IEM, Gijsberts CM, Teng TK et al. Prevalence and Clinical Significance of Diabetes in Asian Versus White Patients With Heart Failure. JACC Heart Fail 2017;5:14-24.
354. Fox CS, Golden SH, Anderson C et al. Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. Diabetes Care 2015;38:1777-803.

355. Pandey A, Patel KV, Bahnson JL et al. Association of Intensive Lifestyle Intervention, Fitness and Body Mass Index with Risk of Heart Failure in Overweight or Obese Adults with Type 2 Diabetes Mellitus: An Analysis from the Look AHEAD Trial. Circulation 2020.