Asymptomatic type 2 diabetes mellitus display a reduced myocardial deformation but adequate response during exercise

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Title page

Title: Asymptomatic type 2 diabetes mellitus display a reduced myocardial deformation but adequate response during exercise

Running title: Cardiac function during exercise in asymptomatic T2DM

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DECLARATIONS

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The study protocol was approved by the medical ethical committee of Jessa hospital (Hasselt, Belgium) and Hasselt University (Hasselt, Belgium) and was performed according to the Declaration of Helsinki (2013). The study was part of a clinical trial and registered at Clinicaltrials.gov (NCT number: NCT03299790)

Consent to participate

All participants gave written informed consent, prior to the execution of the tests.

Consent to Publish

Full anonymity is guaranteed in this paper. No personal details such as date of birth, names and contact details are included in this paper.

Availability of data and material

Raw data is available upon request. Requests should be oriented towards the corresponding author or last author.

Code availability

Not applicable

Author contributions

L.V.R. and D.H. conceived and designed the study. L.V.R. included the participants. The cardiologists (I.F., T.P., E.B., S.J., S.S.) executed the echocardiographic assessments and L.V.R assisted (execution of electrocardiogram, ergospirometry). L.V.R. performed the offline measurements of the echocardiographic assessments assisted by J.V. and C.K. analysed the data of the breath-by-breath gas exchange analyses. E.V. assisted in the conduction of the study as part of her internship. L.V.R. and D.H. performed the statistical analyses. L.V.R. and D.H. wrote the manuscript. C.K., J.V., P.D., S.J., E.B. and V.B. critically reviewed the manuscript. All authors gave their final approval of the manuscript to be submitted.

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ABSTRACT

Background and purpose The development of myocardial fibrosis is a major complication of Type 2 diabetes mellitus (T2DM), impairing myocardial deformation and therefore cardiac performance. It remains to be established whether abnormalities in longitudinal strain (LS) exaggerate or only occur in well-controlled T2DM, when exposed to exercise and therefore cardiac stress. We therefore studied left ventricular LS at rest and during exercise in T2DM patients vs. healthy controls.

Methods & Results Exercise echocardiography was applied with combined breath-by-breath gas exchange analyses in asymptomatic, well-controlled (HbA1c: 6.9±0.7%) T2DM patients (n=36) and healthy controls (HC, n=23). Left ventricular LS was assessed at rest and at peak exercise. Peak oxygen uptake ($\dot{V}O_{2peak}$) and workload (W_{peak}) were similar between groups ($p>0.05$). Diastolic (E, e'_s, E/e') and systolic function (left ventricular ejection fraction) were similar at rest and during exercise between groups ($p>0.05$). LS (absolute values) was significantly lower at rest and during exercise in T2DM vs. HC (17.0±2.9% vs. 19.8±2% and 20.8±4.0% vs. 23.3±3.3%, respectively, $p<0.05$). The response in myocardial deformation (the change in LS from rest up to peak exercise) was similar between groups (+3.8±0.6% vs. +3.6±0.6%, in T2DM vs. HC respectively, $p>0.05$).

Multiple regression revealed that HDL-cholesterol, fasted insulin levels and exercise tolerance accounted for 30.5% of the variance in response of myocardial deformation in the T2DM group ($p=0.002$).

Conclusion Myocardial deformation is reduced in well-controlled T2DM and despite adequate responses, such differences persist during exercise.

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Key words: type 2 diabetes mellitus, stress echocardiography, exercise tests, left ventricular longitudinal strain

LIST OF ABBREVIATIONS

A	Late diastolic inflow
AGE's	Advanced glycation end products
ANOVA	Analysis of variance
AP4C	Apical four chamber
AP5C	Apical five chamber
BMI	Body mass index
BPM	Beats per minute
BSA	Body surface area
CO	Cardiac output
CVD	Cardiovascular diseases
Dt	Deceleration time
E	Early diastolic inflow
e'_s	Early diastolic velocity at the septal annulus
E/e' ratio	LV filling pressure
HbA1c	Glycated haemoglobin A1c
HC	Healthy control
HDL	High density lipoprotein
HR	Heart rate
IVSd	Interventricular septum thickness end-diastole
LDL	Low density lipoprotein
LS	Longitudinal strain
LV	Left-ventricular
LVEDV	End-diastolic LV volume
LVEF	LV ejection fraction
LVESV	End-systolic LV volume
LVM	LV mass
LVMi	LV mass indexed for BSA
LVDd	LV diameter end-diastole

LVPWd	LV posterior wall thickness end-diastole
LVOT	LV outflow tract diameter
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PLAX	Parasternal long axis
RER	Respiratory exchange ratio
RWT	Relative wall thickness
SD	Standard deviation
SV	Stroke volume
T2DM	Type 2 diabetes mellitus
TDI	Tissue Doppler imaging
$\dot{V}O_2$	Oxygen uptake

INTRODUCTION

More than 400 million people worldwide are affected by diabetes mellitus, and the prevalence is expected to exceed 600 million people by 2045 [1]. Type 2 diabetes mellitus (T2DM) patients have twice the risk to suffer from cardiovascular diseases (CVD) leading to an elevated incidence of premature cardiovascular death [2]. Among these cardiovascular complications, diabetes-induced heart failure is a significant clinical entity, involving structural and functional changes in the myocardium in the absence of coronary artery disease, valve diseases or hypertension [3]. These changes can result in ventricular adverse remodelling (increased left ventricular wall mass and thickness) and cardiac dysfunction, the latter being estimated to occur in respectively 13% and 23% (systolic and diastolic dysfunction) of T2DM patients [3-6]. Next to hyperglycaemia, hyperinsulinemia and hyperlipidaemia, known as key etiological factors, oxidative stress and accumulation of advanced glycation end products (AGE's) also seem to play an important role in the development of cardiac impairment in T2DM patients [7].

Recently the use of longitudinal strain (LS) as a non-invasive marker of subclinical left-ventricular (LV) dysfunction has gained interest, providing an estimation of myocardial deformation and enabling the detection of subtle wall abnormalities or ischemia in a reproducible manner and showing good interobserver variability both at rest and during exercise [8-10]. Abnormalities in myocardial deformation are estimated to occur in 23-45% of well-controlled T2DM patients without a history of cardiovascular diseases and are associated with adverse outcomes (mortality and hospitalisation) [11-13]. Of interest, such impairments are closely related to disease control (HbA1c) and ventricular remodelling in T2DM, an important hallmark of the diabetic heart [12,14].

However, when using conventional echocardiography at rest, a significant amount of these reductions in LS are likely to remain undetected. Up to 33% of T2DM patients report symptoms of dyspnoea or chest pain but have a normal echocardiography at rest [5]. Hence, in daily life, symptoms related to cardiac dysfunction (e.g. dyspnoea, symptomatic cardiac arrhythmias and exercise intolerance) often only occur or worsen/exaggerate during stress in T2DM [15].

It therefore remains to be investigated whether cardiac (dys)function worsens during exercise in T2DM patients and whether such responses are related to metabolic parameters and exercise performance. Furthermore, if such responses would be affected by ventricular remodelling and/or resting systolic/diastolic dysfunction (hallmarks of the diabetic heart), stress testing would be warranted for specific indications. We hypothesized that LS is impaired in T2DM and that such abnormalities would exaggerate during exercise and would relate to impaired exercise performance.

METHODS

Study design and subjects

This cross-sectional study was performed at REVAL (Rehabilitation Research Centre), Faculty of Rehabilitation Sciences, Hasselt University, Belgium and the Department of Cardiology, Jessa hospital (Hasselt, Belgium). Fifty-three T2DM patients and 30 healthy controls (HC) were included. Participants had no evidence of CVD and were asymptomatic (no symptoms of dyspnoea or chest pain (during exercise)).

We included T2DM patients (aged 18-81 years), diagnosed according to the criteria of the American Diabetes Association, who were able to perform a maximal incremental exercise test and who were stable on pharmacologic treatment for at least three months (e.g. anti-hypertensive, glucose- and lipid-lowering drugs). Patients were excluded if renal disease, retinopathy, neurological, orthopaedic, oncologic or pulmonary diseases prohibiting the performance of an exercise test, and/or heart diseases (e.g. valve disease, coronary artery disease, congenital heart disease) was present. Except for the diagnostic criteria of T2DM, the same in- and exclusion criteria were applied for the HC group.

Anthropometric measures and body composition were assessed in a fasted state, followed by blood sample collection for evaluation of glycaemic control and lipid profile. Echocardiography was performed during exercise for the evaluation of exercise-related cardiac performance (stress echocardiography).

The study protocol was approved by the medical ethical committee of Jessa hospital (Hasselt, Belgium) and Hasselt University (Hasselt, Belgium) and was performed according to the Declaration of Helsinki (2013). All participants gave written informed consent, prior to the execution of the tests. The study was part of a clinical trial and registered at Clinicaltrials.gov (NCT number: NCT03299790)

Patient and Public Involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Body composition

Anthropometric measures (body length and weight) were assessed using a wall-mounted Harpenden stadiometer (ICD 250DW, De Grood Metaaltechniek, Nijmegem, The Netherlands) and a digital-balanced weighing scale (Seca 770, Seca Hamburg, Germany). Body mass index (BMI, kg/m^2) and body surface area (BSA, m^2) were calculated. Body composition was analysed by using a Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer, Vilvoorde, Belgium) from which whole-body lean tissue mass, fat mass and percentage were determined.

Blood parameters

Fasting blood samples (lithium heparin and sodium fluoride tubes) were collected between 8-9AM and stored for 30 minutes at room temperature and thereafter for 120 minutes at 4°C. Afterwards, samples were centrifuged (1650g, for 15 min) and plasma was stored at -80°C until analyses at the clinical laboratory (Jessa Hospital, Hasselt, Belgium) for glucose, insulin, total cholesterol, high-density lipoprotein (HDL-) and low-density lipoprotein (LDL)-cholesterol and triglycerides (Roche Cobas 8000, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Non-fasted blood samples (prior to the echocardiographic assessments) were collected and immediately transferred to the clinical laboratory for the analyses of; glycated haemoglobin A1c (HbA1c, Menarini HA-8180 HbA1c auto-analyser, Menarini Diagnostics, Diegem, Belgium) and N-terminal pro-B-type natriuretic peptide (NT-proBNP, electrochemiluminescence immunoassay, Cobas e 801 immunoassay analyser, Menarini Diagnostics, Diegem, Belgium).

Echocardiography

Echocardiography using a phased array probe (Vivid E90 and GE M5S 1.5-4.5 MHz, GE Health Medical, Milwaukee, Wisconsin, USA) was performed by a trained cardiologist in exercise imaging.

Resting echocardiography involved the following analyses: LV outflow tract diameter (LVOT) was determined at the aortic leaflet points at the ventricular side in the parasternal long axis (PLAX) view in mid-systole, dimensions

of the LV (interventricular septum thickness end-diastole (IVSd), LV posterior wall thickness end-diastole (LVPWd), LV diameter end-diastole (LVDd) and relative wall thickness (RWT). LV mass (LVM) was calculated via the formula of Devereux and indexed for BSA (LVMi) [16]. Diastolic function was evaluated according to the latest guidelines and included: mitral inflow pattern (early (E) and late (A) diastolic inflow, deceleration time (Dt)) using pulsed wave Doppler at the tips of the mitral leaflets [17]. Pulsed wave tissue Doppler imaging (TDI) was used for early diastolic velocity (e'_s) at the septal annulus. The E/e' ratio was measured as an indicator for LV filling pressures.

The exercise echocardiographic assessment included 2 stages of evaluation; rest and peak exercise. Breath-by-breath gas exchange analyses (CS-200 Ergo-Spiro, Schiller AG, Switzerland) were simultaneously performed for evaluation of respiratory exchange ratio (RER) and oxygen uptake ($\dot{V}O_2$). Prior to every test, a volume and gas calibration were executed according to the manufacturer's recommendations. A standardized ramp-stage protocol (initial workload of 20W, gradually increased by 10W/min) was applied on a semi-supine bicycle (Ergocouch erg 911 LS, Ergosana, Rotterdam, The Netherlands). A cycling frequency of 60-65 revolutions per minute was applied during the test and participants were encouraged to achieve maximal effort. Peak exercise evaluation was performed when RER exceeded 1.03 (steady state cycling workload). Systolic and diastolic blood pressure were measured prior to the echocardiographic evaluation and monitored during the exercise echocardiography, using an electronic sphygmomanometer (Omron®, Omron Healthcare, IL, USA). Continuous 12-lead ECG monitoring was applied during the test (CardioSoft v6.7, Acertys, Aartselaar, Belgium).

Images were digitally stored in a cine-loop format containing at least three cardiac cycles for each measure. Apical four- and five-chamber (AP4C, AP5C) views were obtained. Analyses were performed via the EchoPAC software version 201 (General Electric Vingmed, Horten, Norway). 2D Speckle tracking analyses were performed in the AP4C for left ventricular LS and defined in accordance with the consensus on strain measurements and reported as absolute values [18]. Contractile reserve in LS was expressed as the absolute increase from rest to high intensity exercise. End-systolic and end-diastolic LV volumes (LVESV, LVEDV) were assessed in combination with LV ejection fraction (LVEF) using the Simpson's biplane method [19]. Cardiac output (CO) was measured using the velocity time integral of the flow through the aortic valve in the AP5C view via pulsed wave Doppler, LVOT and heart rate (HR). Diastolic function was evaluated as previously described [20]. For each state, a time frame of two to four minutes was required for the assessment of all measurements.

Statistical analyses

Statistical analyses were performed in SPSS V.24 (IBM SPSS Statistics for Windows, Chicago, IL, USA). Participants were only included if assessment of the exercise echocardiography was successful for the prior outcome (left ventricular LS) at rest and peak exercise. Data are expressed as mean±standard deviation (SD). Normality was checked via the Shapiro-Wilk test. Descriptive statistics included independent sample T-tests and Mann-Whitney U tests (non-parametric alternative). Differences in proportions between groups were evaluated using the Fisher's exact test. A two-way mixed Analysis of variance (ANOVA) was executed for the analysis of LS at different stages (group, intensity and interaction effects). Outliers and normality were assessed via boxplots and Shapiro-Wilk Test. Homogeneity was assessed via the Box's M test and Levene's test. Sphericity was checked via Mauchly's test. Pearson correlations were calculated for LS and blood parameters and indicators for physical fitness ($\dot{V}O_{2peak}$ and W_{peak}). Level of statistical significance was set at $p<0.05$ (two-tailed).

RESULTS

General characteristics and tolerance of the test

Fifty-three T2DM patients and 30 HC's entered the study, after which 36 T2DM patients and 23 HC's were included in the analyses. Reasons for exclusion are presented in Figure 1. Two T2DM patients and one HC reported symptoms of dyspnoea during the test and mild ST-depression was observed in one T2DM patient. None of the tests were terminated because of contraindications (Supplementary table 1). Groups were comparable regarding age. Body weight, BMI, BSA and lean mass were greater in the T2DM vs. HC group ($p<0.05$ Table 2). Detailed comparisons revealed that differences in body composition were related to an unequal proportion of males and females between groups (Supplementary table 2).

Detailed comparisons between males and females for all the outcome measures are reported as supplementary material (Supplementary tables 2-3 and Supplementary figure 4).

Table 2: General characteristics

	HC (n= 23)	T2DM (n= 36)	P value
Demographics			
Age (years)	57±13	62±8	0.124
Disease duration (years)		9.6±6.9	
Smoking (n)	2	5	0.475
Anthropometrics			
Body weight (kg)	75.3±13.4	86.7±14.6	**0.005***
Body length (cm)	170.9±9.2	175.2±7.4	0.056
BMI (kg/m²)	25.6±3.2	28.2±4.2	**0.018***
BSA (m²)	1.86±0.21	2.04±0.2	**0.001***
Body composition			
Fat mass (%)	29.4±7	28.9±5.3	0.782
Fat mass (kg)	21.7±6.2	25±7	0.076
Lean mass (kg)	50.2±10.7	57.8±9.1	**0.005***
Blood sample analyses			
HbA1c (%)	5.3±0.3	6.9±0.7	**<0.001***
NT-proBNP (ng/µL)	62±27	69±38	0.49
Blood sample analyses – fasted state			
Glucose (mg/dL)	89±8	142±33	**<0.001***
Insulin (pmol/L)	47±28	88±52	**0.001***
HDL-cholesterol (mg/dL)	62±19	49±16	**0.007***
LDL-cholesterol (mg/dL)	145±26	82±31	**<0.001***
Total cholesterol (mg/dL)	198±33	156±36	**<0.001***
Triglycerides (mg/dL)	114±46	123±61	0.552
Medication			
Insulin	0	7	**0.028***
Metformin	0	31	**<0.001***
Insulin secretion stimulation drugs	0	9	**0.009***
Incretin mimetics and DPP4-inhibitors	0	11	**0.003***
SGLT2-inhibitors	0	6	**0.048***
Statins	4	20	**0.006***
Fibrates	1	2	0.381
B-blocker	2	9	0.844
ACE-inhibitor	1	6	0.346
Diuretics	0	4	0.365
Sartans	0	3	0.231
Calcium antagonists	0	6	**0.048***
Anticoagulation/antithrombotics	3	7	0.112

General characteristics. Data are presented as means ± SD. BMI; body mass index, BSA; body surface area, HbA1c; blood glycated haemoglobin A1c, NT-proBNP; N-terminal pro-B-type natriuretic peptide, HDL; high-density lipoprotein, LDL; low-density lipoprotein, DPP4; dipeptidylpeptidase-4, SGLT-2; sodium-glucose co-transporter-2, ACE; angiotensin converting enzyme. a: Data abnormally distributed, Mann-Whitney U test used. Significant differences between two groups at *$P < 0.05$.

Blood parameters

As would be expected, HbA1c was higher in the T2DM group vs. HC (6.9±0.7% vs. 5.3±0.3%, respectively, $p<0.001$), as well as fasting plasma glucose (142±33mg/dL vs. 89±8mg/dL, $p<0.001$) and fasting plasma insulin concentrations (88±52pmol/L vs. 47±28pmol/L, $p=0.001$). Total cholesterol (156±36mg/dL vs. 198±33mg/dL, $p<0.001$), LDL- (82±31mg/dL vs. 145±26mg/dL, $p<0.001$) and HDL-cholesterol (49±16mg/dL vs. 62±19mg/dL, respectively, $p=0.007$) were significantly lower in the T2DM group vs. HC group.

Cardiac function at rest

LS was lower in the T2DM group (17.0±2.9% vs. 19.8±2.0% in HC group, Table 3, $p<0.001$) indicating a lower cardiac deformation/early impairment of cardiac function, demonstrated in both males and females (Supplementary table 3). Systolic blood pressure and resting HR were elevated in the T2DM group vs. HC group (149±16mmHg vs. 140±14mmHg and 71±8bpm vs. 65±9bpm, respectively, $p=0.045$ and $p=0.008$, Table 3). IVSd (10±1mm vs. 9±2mm, $p=0.011$) was significantly elevated and E/A (0.8±0.2 vs. 1.1±0.05, $p=0.008$) significantly reduced in the T2DM group vs. HC group. However, LVM was not significantly different in the T2DM group compared to HC. LVEDV, LVESV, systolic function and other parameters for diastolic function were similar between groups. The proportion of some grade of diastolic dysfunction (grade 1 and 2) was not significantly different between both groups (respectively 30.4% in the HC and 38.9% in the T2DM group, $p>0.05$).

Table 3: Echocardiography and breath-by-breath gas exchange analyses during exercise

	HC (n= 23)	T2DM (n= 36)	P value
Echocardiography at rest			
Blood pressure			
BPsys (mmHg)	140±14	149±16	**0.045 ***
BPdia (mmHg)	86±9	85±10	0.71
HR (bpm)	65±9	71±8	**0.008 ***
Cardiac structure and dimensions			
IVSd (mm)	9±2 [a]	10±1 [a]	**0.011 ***
LVPWd (mm)	9±1 [a]	10±1 [a]	0.195
LVDd (mm)	44±5	42±4 [a]	0.203
LVM (g)	129±29 [a]	135±34	0.762
LVMi (g/m²)	68.7±12.7 [a]	65.6±12.8	0.273
RWT	0.43±0.07	0.47±0.08 [a]	0.124
LVOT (cm)	2.1±0.2	2.1±0.1 [a]	0.179
LVEDV (mL)	93±28	96±21	0.637
LVESV (mL)	32±15 [a]	33±12	0.586
Diastolic function			
E (m/sec)	0.54±0.14	0.58±0.15	0.149
A (m/sec)	0.69±0.23	0.73±0.14	0.454
E/A	1.1±0.5	0.8±0.2 [a]	**0.008** [*]
Dt (ms)	186±55	186±37	0.972
e's (m/sec)	0.07±0.02 [a]	0.06±0.01 [a]	0.279
E/e'	9±3	10±3	0.94
Systolic function			
LVEF (%)	66±8	66±9 a	0.81
SV (mL)	61±19 a	64±17 a	0.355
CO (L/min)	4.3±0.9	4.8±1.2	0.093
Strain analyses			
LS (%)	19.8±2 [a]	17±2.9 [*]	**<0.001** [*]
Echocardiography at peak exercise			
HR (bpm)	138±16	134±17 [a]	0.34
Blood pressure			
BPsys (mmHg)	185±17	202±22	0.037
Workload (watts)	123±53	111±26	0.254
Ergospirometry			
$\dot{V}O_{2peak}$ (mL/min)	1505±650	1558±353 [a]	0.577
$\dot{V}O_{2\ peak}$ (mL/kg/min)	20.1±7.5	18.4±4.8 [a]	0.184
RER peak	1.05±0.02	1.06±0.05 [a]	0.041
Systolic function			
LVEF (%)	78±8	78±8 a	0.835
SV (mL)	69±24	78±18	0.111
CO (L/min)	11.5±3.2 a	11.8±2.6	0.361
Diastolic function			
E (m/sec)	1.09±0.15	1.06±0.19	0.571
e' s (m/sec)	0.13±0.03	0.12±0.03	0.333
E/e'	9±3 a	9±3 [a]	0.511
Left ventricular volumes			
LVEDV (mL)	93±25	101±25	0.261
LVESV (mL)	21±9	23±13 [a]	0.797
Strain analyses			
LS (%)	23.3±3.3	20.8±4	**0.015** [*]
$LS_{\text{delta peak exercise-rest}}$ (%)	3.6±0.6 a	3.8±0.6	0.895
Other breath-by-breath gas exchange analyses			
$\dot{V}O_2$ peak-predicted (%)	77.9±18.1	77.3±15	0.888
VEpeak (L/min)	45.5±19.1	50.2±9.6	0.216
$HR/\dot{V}O_2$slope	4.4±1.7 a	4.2±1.5 a	0.54
$VE/\dot{V}CO_2$slope	25.6±2.3	27.6±4.6 a	0.094

Exercise echocardiography and breath-by-breath gas exchange analyses. Data are presented as means ± SD. BPsys; systolic blood pressure, BPdia; diastolic blood pressure, HR; heart rate, IVSd; interventricular septum thickness end-diastole, LVPWd; left ventricular posterior wall thickness end-diastole, LVDd; left ventricular diameter end-diastole, LVM; left ventricular mass, LVMi; left ventricular mass indexed for BSA, RWT; relative wall thickness, LVOT; left ventricular outflow tract diameter, LVEDV; left ventricular end-diastolic volume, LVESV; left ventricular end-systolic volume, E; peak velocity of early diastolic filling phase, A; peak velocity of late diastolic filling phase, Dt; deceleration time, e's; early diastolic velocity at the septal annulus, E/e'; left ventricular filling pressure, LVEF; left ventricular ejection fraction, SV; stroke volume, CO; cardiac output, LS; longitudinal strain, $\dot{V}O_2$; oxygen uptake, RER; respiratory exchange ratio, $\dot{V}O_{2\ peak}$; peak oxygen uptake, RERpeak; peak respiratory exchange ratio, $LS_{\text{delta peak exercise-rest}}$; change in longitudinal strain between rest and peak exercise, $\dot{V}O_2$ peak-predicted; percentage of predicted oxygen uptake, VEpeak; peak ventilation, $\dot{V}CO2$; carbon dioxide. a: Data abnormally distributed, Mann-Whitney U test used. Significant differences between two groups at $^*P < 0.05$

Cardiac function during exercise and exercise capacity

LS was significantly lower in the T2DM group vs. HC group at peak exercise (20.8±4% vs. 23.3±3.3%, p=0.015) and such differences were mainly dominated by male T2DM patients (Supplementary table 3). LS increased significantly during exercise ($p_{Intensity}$<0.005) in both groups, although 22% of the HC and 26% of the T2DM displayed an absolute response <2% for LS. There was no statistically significant difference in the proportion of such an impaired response (p>0.05) between groups nor an interaction effect between stages of evaluation on LS (p>0.05) (Figure 4). Contractile reserve in LS ($LS_{\text{delta peak exercise-rest}}$) was similar between the T2DM group vs. HC group (3.8±0.6% vs. 3.6±0.6%, p>0.05).

At peak exercise, W_{peak}, $\dot{V}O_{2peak}$ and HR_{peak} were similar at group level, although male T2DM displayed a lower absolute and relative $\dot{V}O_{2peak}$ compared to HC (1611±345ml/min vs 1985±530ml/min and 18.5±5.1mL/kg/min vs 24.1±6.9mL/kg/min respectively, p=0.012 and p=0.002).

Diastolic function was similar between groups at peak exercise and the proportion of positive diastolic stress tests was not significantly different between both groups (1 HC (4.3%) and 4 T2DM (11.1%), p>0.05).

Other cardiac parameters such as LVEF and CO were similar both at group level and when comparing groups based on gender (Table 3 and Supplementary table 3).

After 1min of (active) recovery (steady state cycling at a low-resistance), HR was not significantly different between the T2DM group and HC group (111±14bpm vs 109±17bpm respectively, p=0.544)

Exercise tolerance according diastolic function and myocardial deformation

Exercise tolerance (achieved percentage of peak predicted oxygen uptake) was significantly lower in the T2DM patients displaying resting diastolic dysfunction compared to those without diastolic dysfunction (69.1±14.9% vs. 82.7±12.7%, p=0.007). There was no difference in exercise tolerance between T2DM displaying a positive diastolic stress test compared to those displaying a normal diastolic stress test (p>0.05). In T2DM patients displaying an impaired response in LS (absolute increase <2%), exercise tolerance was significantly lower compared to T2DM patients displaying an adequate response in LS (69.9±13.2% vs. 80.7±14.8%, p=0.048). No such differences were observed in the control group.

Correlations and regression

Pearson correlations between $LS_{\text{delta peak exercise-rest}}$, exercise performance and blood sample parameters revealed significant associations with achieved percentage of predicted $\dot{V}O_2$ ($\dot{V}O_2$ peak-predicted), blood HDL-cholesterol and insulin levels in the T2DM group (r=0.509, r=0.40 and r=-0.386 respectively, p=0.002, p=0.016 and p=0.02, Figure 5). Multiple linear regression for these parameters was statistically significant (F(3.31)=10.363, p<0.005) and explained 30.5% of the variance in $LS_{\text{delta peak exercise-rest}}$ (Figure 5). Post-hoc analyses revealed this multiple regression model to show a large effect size (0.438) with sufficient statistical power (0.894). No such relations were observed in the HC group.

DISCUSSION

In the present study we compared cardiac function, in particular left ventricular longitudinal strain (LS) between asymptomatic, well-controlled (HbA1c<7.5%) T2DM patients and healthy controls at rest and during exercise. Exercise echocardiography is a cost- and risk-effective imaging modality to non-invasively diagnose myocardial dysfunction, during which hemodynamic responses of stress (such as increased systemic blood pressure) are preserved, therefore reflecting the patients' functional status [20].

To the best of our knowledge, the simultaneous evaluation of longitudinal myocardial deformation (strain) and exercise performance ($\dot{V}O_2$ uptake) has not been reported yet in this patient population. Previous exercise echocardiographic studies with T2DM patients evaluated exercise capacity separately (and in the standard upright position) and/or evaluated cardiac function after termination of exercise [21-26]. However, controlling for exercise intensity is crucial, as for instance CO greatly depends on the posture-dependent stroke volume (CO = HR x stroke volume) and therefore influences oxygen uptake ($\dot{V}O_2$ = CO x peripheral oxygen extraction) [27]. Exercise intolerance is a predictor of premature death in T2DM, signifying the clinical importance of this parameter [28]. Furthermore, exercise intensity affects myocardial deformation (and therefore LS) as such echocardiographic parameters are load-dependent and should therefore be evaluated during exercise itself [29].

We found that LS is significantly reduced at rest in T2DM patients compared to healthy controls, and that such reductions persisted during exercise, although these seem to be dominated by male T2DM patients. Considering the gender-specific lower limits of resting LS, as demonstrated by Sugimoto et al. [30] (16.1% and 17.3% for males and females respectively), cardiac dysfunction was highly prevalent in the T2DM group and with a significantly higher proportion compared to the control group (12 out of 36 T2DM patients (33,3%) and 2 out of 23 healthy controls (8.7%), $p<0.05$). It remains however to be elucidated whether the response in longitudinal strain is gender-related. The findings on longitudinal strain and proportions of impaired strain are in line with previous studies [22,30-32]. However, of interest, the majority of the T2DM patients displayed an adequate response in LS during exercise, and the proportion of patients displaying abnormal responses was not significantly higher in the T2DM group. The adequate glycaemic control of the T2DM patients (HbA1c 6.9±0.7%) might have contributed to these findings, as glycaemic control and the presence of diabetic complications have been reported to affect responses in myocardial deformation at rest or when exposed to stress (exercise) [26,31]. Diabetes-induced changes at the myocardium, such as deposition of AGE's, increase myocardial fibrosis and therefore affect myocardial strain, at least in animal models [33]. Within diabetes patients, the deposition of such AGE's is enhanced and negatively associated with the development of cardiovascular diseases [34]. Of interest, our data revealed that the contractile reserve (response in LS) seemed to be particularly impaired in T2DM with low levels of HDL-cholesterol and hyperinsulinemia, the latter believed to enhance myocardial lipid accumulation [35]. The subsequent lipotoxicity has been associated with the development of diabetic cardiomyopathy [36]. Importantly, our data also indicate that in addition to hyperinsulinemia, exercise intolerance is negatively related to the response in myocardial deformation, which is in contrast to the study of Roberts et al. [24]. However, of interest, the latter applied upright maximal incremental exercise tests implying RER values exceeding the cut-off value of 1.10 whereas in our study, RER values mainly remained below this threshold and exercise tests were considered submaximal. Furthermore, the previously mentioned role of the posture-dependent cardiac output should not be underestimated as ventricular filling has been reported to limit cardiac reserve in T2DM thereby affecting exercise capacity. Indeed, Wilson et al. recently demonstrated that asymptomatic T2DM patients display a worse diastolic function during exercise as compared to healthy controls [21]. Furthermore, our data are in line with the study of Nishi et al., in which 44% of the T2DM patients displayed some grade of diastolic dysfunction at rest and positive diastolic stress tests were observed in 16.1% [22]. Such stress evaluation of diastolic function is of clinical importance, as the contribution of late/atrial diastolic filling is elevated during exercise when LV filling is reduced. Interpreting resting diastolic function might therefore result in false-negative results [37]. Our data, complementary to the previously mentioned studies, indicate that cardiac function and exercise capacity are indeed associated in asymptomatic T2DM patients without a history of cardiovascular diseases, or at least diastolic function and myocardial deformation seem to interact with exercise capacity.

Though, our study did not reveal worsening of cardiac dysfunction during exercise in T2DM patients, which was observed in previous studies [15,21,38]. Of note, such prior studies included patients with macrovascular complications, a longer disease duration, a worse glycaemic control or higher BMI, compared to the patients included in our study [15,21,23,26,38]. So, the added-value of simultaneous evaluation of exercise capacity (via ergospirometry) during exercise echocardiography could be questioned. However, we recently reported that in

asymptomatic T2DM patients, a reduced exercise capacity is mainly dominated by an impaired oxygen extraction rather than cardiac dysfunction, signifying the importance to comprehensively evaluate the cardiovascular system in this patient population [39]. Our study results stipulate that exercise tolerance seems to be related (however not dominated) to myocardial deformation. Taking into account the prognostic value of exercise capacity, exercise echocardiography with combined ergospirometry might be of added-value particularly in T2DM patients displaying exercise intolerance. Such assessments allow to investigate whether the latter can be attributed to rather a disturbed cardiac function or impairments at the peripheral level (oxygen extraction) and could therefore be used to optimize treatment strategies.

Limitations

To fully investigate myocardial contractility using strain, global left ventricular LS is recommended (measurements in the APLAX, AP2C and AP4C view). Due to the limited time frame, especially at peak exercise, ensuring sufficient image quality in three different views is challenging. The main focus was LS, only involving the AP4C view. Use of a catheter (invasive method) could have provided the opportunity to directly measure filling pressures (instead of E/e' as a surrogate) as well as mean pulmonary pressures. Due to the invasive character, this procedure was not applied. External validity is limited due to the disproportion in participating males and females. A larger sample size is warranted to investigate whether the response in longitudinal strain is gender specific.

Conclusion

In asymptomatic, well-controlled T2DM patients, myocardial deformation is significantly reduced compared to healthy controls both at rest and during exercise although T2DM patients generally displayed an adequate response during exercise, indicating a preserved contractile reserve. However, as high levels of fasting insulin and a reduced exercise tolerance were associated with blunted responses in myocardial deformation in T2DM patients, exercise echocardiography could be of added-value for specific conditions.

Figure captions

Fig1: Flowchart of study. CAD; coronary artery disease

Fig4: Two-way mixed ANOVA for longitudinal strain and stages of echocardiography. Data are presented as means ± SD. Panel A; Mean longitudinal strain for both groups, Panel B and C; Individual cases in the Healthy control group and Type 2 Diabetes Mellitus group respectively. Significant differences at *P < 0.05.

Figure 5: Regression analyses for LS responses and clinical parameters within the T2DM group. Panel A; Multiple regression for response in LS and HDL-cholesterol, fasting insulin levels and exercise tolerance (achieved percentage of predicted V̇O2peak). Panel B,C and D: Linear regression for fasting insulin levels, HDL-cholesterol and exercise tolerance. Significance level was set at *p<0.05.

Supplementary figure 4: Two-way mixed ANOVA for longitudinal strain and stages of echocardiography. Data are presented as means ± SD. Panel A; Mean longitudinal strain for both groups, Panel B and C; Individual cases in for males and females respectively. Significant differences at *P < 0.05.

Supplementary tables

Supplementary table 1

	HC (n= 23)	T2DM (n= 36)
Symptoms of dyspnoea during the exercise test	1 (4.3%)	2 (5.6%)
Mild ST depression during the exercise test	0	1 (2.8%)
PAC's during the exercise test	0	8 (22.2%)
PVC's during the exercise test	0	5 (13.9%)

Symptoms during the exercise echocardiographic assessments. PAC; premature atrial contraction, PVC; premature ventricular contraction

Supplementary table 2: General characteristics

	Males			Females		
	HC (n=12)	T2DM (n=31)	P value	HC (n=11)	T2DM (n=5)	P value
Demographics						
Age (years)	59±13	63±7	0.43	56±12	58±11	0.818
Disease duration (years)		10±7 [a]			9±8	
Smoking (n)	2	5	1.000	0	0	1.000
Anthropometrics						
Body weight (kg)	84±6.9	89.2±13.8	0.114	64.9±11.7	71.3±10.4	0.324
Body length (cm)	177.6±5.8	176.9±6.4	0.735	162.9±4.9	164.8±4.5	0.482
BMI (kg/m²)	26.7±2.8	28.5±4.3	0.182	24.4±3.3	26.3±3.9	0.342
BSA (m²)	2.02±0.08	2.09±0.18	0.102	1.7±0.17	1.8±0.14	0.24
Body composition						
Fat mass (%)	25.1±6	27.9±4.6	0.118	34.6±4	35.7±4.4 [a]	0.859
Fat mass (kg)	21.1±6.4	24.9±7.1	0.115	22.4±6.1	25.5±6.4	0.632
Lean mass (kg)	59±2.6	60.1±7.2	0.43	39.6±5.7	43.2±5.7	0.272
Blood sample analyses						
HbA1c (%)	5.2±0.3	6.9±0.7	**<0.005**	5.4±0.3	7±0.7	**0.008**
NT-proBNP (ng/µL)	56±10 [a]	69±40 [a]	0.81	68±39 [a]	63±25 [a]	0.753
Blood sample analyses – fasted state						
Glucose (mg/dL)	91±7	143±33 [a]	**<0005**	88±9	140±35	**0.027**
Insulin (pmol/L)	52±35 [a]	84±42	**0.017**	41±18	113±95	**0.024**
HDL-cholesterol (mg/dL)	53±17	46±12	0.138	70±17	65±27	0.68
LDL-cholesterol (mg/dL)	114±28	77±26	**<0.005**	115±26	119±35	0.821
Total cholesterol (mg/dL)	188±34	148±30	**0.001**	208±30	205±35	0.86
Triglycerides (mg/dL)	105±37	127±62 [a]	0.445	124±54	103±54	0.49
Medication						
Insulin		7	-		0	-
Metformin		26	-		5	-
Insulin secretion stimulation drugs		8	-		1	-
Incretin mimetics and DPP4-inhibitors		9	-		2	-
SGLT2-inhibitors		6	-		0	-
Statins	2	17	**0.036**	2	3	0.245
Fibrates	0	2	1.000	1	0	1.000
B-blocker	1	8	0.086	1	1	1.000
ACE-inhibitor	0	5	0.303	1	1	1.000
Diuretics	0	3	0.554	0	1	0.313
Sartans	0	3	0.554	0	0	-
Calcium antagonists	0	6	0.172	0	0	-
Anticoagulation/antithrombotics	2	7	1.000	1	0	1.000

Data are presented as means ± SD. BMI; body mass index, BSA; body surface area, HbA1c; blood glycated haemoglobin A1c, NT-proBNP; N-terminal pro-B-type natriuretic peptide, HDL; high-density lipoprotein, LDL; low-density lipoprotein, DPP4; dipeptidylpeptidase-4, SGLT-2; sodium-glucose co-transporter-2, ACE; angiotensin converting enzyme. a: Data abnormally distributed, Mann-Whitney U test used. *; healthy controls vs T2DM, [#]; male HC vs male T2DM and female HC vs female T2DM. Significant differences between two groups at *P < 0.05 and [#] P < 0.05.

Supplementary table 3: Echocardiography and breath-by-breath gas exchange analyses during exercise

	Males			Females		
	HC (n=12)	T2DM (n=31)	P value	HC (n=11)	T2DM (n=5)	P value
Echocardiography at rest						
Blood pressure						
BPsys (mmHg)	142±11	151±15	0.059	139±18	134±16	0.644
BPdia (mmHg)	87±8	86±10	0.751	86±11	82±11	0.505
HR (bpm)	59±7	71±8	**<0.005 ***	71±7	74±6	0.415
Cardiac structure and dimensions						
IVSd (mm)	9±2 [a]	10±1 [a]	**0.04 ***	9±1	9±1 [a]	1
LVPWd (mm)	10±1	10±1 [a]	0.341	9±1	8±1 [a]	0.267
LVDd (mm)	47±4	42±5	**0.005 ***	40±4	39±1	0.619
LVM (g)	145±22 [a]	141±33	0.369	110±24	101±13	0.447
LVMi (g/m²)	72±11 [a]	67±13	0.277	65±14	56±7	0.195
RWT	0.41±0.08	0.47±0.08	**0.027 ***	0.45±0.05	0.43±0.04	0.402
LVOT (cm)	2.2±0.2	2.1±0.1 [a]	0.883	2±0.1	1.9±0.1	0.363
LVEDV (mL)	110±28	101±18	0.261	75±16	65±5	0.075
LVESV (mL)	39±17	34±12	0.351	26±9	22±6	0.394
Diastolic function						
E (m/sec)	0.57±0.17	0.59±0.13	0.718	0.7±0.12	0.52±0.15	**0.029 ***
A (m/sec)	0.69±0.18	0.72±0.14	0.495	0.69±0.28	0.75±0.17	0.666
E/A	1.1±0.6 [a]	0.8±0.2	0.211	1.1±0.4	0.7±0.2	0.056
Dt (ms)	206±57	186±39	0.183	165±45	189±18	0.345
e's (m/sec)	0.06±.02	0.08±0.12 [a]	0.665	0.07±0.02	0.06±0.02	0.124
E/e'	10±3	10±2	0.873	10±3	10±2	0.938
Systolic function						
LVEF (%)	65±10	66±10 [a]	1	67±7	67±9	0.973
SV (mL)	71±20	67±15 [a]	0.64	50±9	44±6	0.194
CO (L/min)	4.3±1	4.9±1.2	0.157	4.3±0.9	4.2±0.3	0.803
Strain analyses						
LS (%)	19.3±1.1	16.9±2.9	**<0.005 ***	20.2±2.6	17.2±2.6	**0.048 ***
Echocardiography at peak exercise						
HR (bpm)	137±16	133±16	0.483	139±17	139±22	0.957

Blood pressure						
BPsys (mmHg)	193±15	204±21	0.292	179±18	-	-
Workload (watts)	158±48 [a]	114±26	**0.002 ***	86±25	94±19	0.51
Ergospirometry						
$\dot{V}O_{2peak}$ (mL/min)	1985±530 [a]	1611±345 [a]	**0.012 ***	1026±314	1244±223	0.186
$\dot{V}O_{2peak}$ (mL/kg/min)	24.1±6.9 [a]	18.5±5.1 [a]	**0.002 ***	15.7±5.5	17.6±2.5 [a]	0.768
RER peak	1.04±0.02	1.06±0.05	0.101	1.06±0.02	1.09±0.02	**0.016 ***
Systolic function						
LVEF (%)	77±8	78±9 [a]	0.498	80±8	83±5	0.375
SV (mL)	77±30	80±17	0.581	61±12	63±15	0.769
CO (L/min)	12.9±4.5	12±2.5	0.634	10.6±1.8	10.4±2.8	0.826
Diastolic function						
E (m/sec)	1.03±0.1	1.06±0.2	0.772	1.13±0.2	1.06±0.2	0.493
e' s (m/sec)	0.12±0.02	0.12±0.03	0.64	0.14±0.03	0.10±0.03	**0.041 ***
E/e'	10±2	9±3 [a]	0.314	8±3 [a]	11±3	0.052
Left ventricular volumes						
LVEDV (mL)	110±23	104±24 [a]	0.35	76±12	75±18	0.939
LVESV (mL)	26±10	24±13 [a]	0.365	15±6	13±6	0.426
LS (%)	23.1±2.5	20.5±3.9	**0.038 ***	23.5±4.2	22.6±4.5	0.707
LS $_{\text{delta peak exercise-rest}}$ (%)	3.8±2.8	3.5±3.6	0.84	3.3±3	5.4±5	0.302
Other breath-by-breath gas exchange analyses						
$\dot{V}O2$ peak-predicted (%)	86.3±0.1	75.8±0.1	**0.049 ***	69.6±0.2	86±0.1	0.102
VEpeak (L/min)	60.3±14.8	51.6±9.3	**0.029 ***	30.7±7.9	41.8±7	**0.018 ***
HR/$\dot{V}O_2$slope	3.7±0.5	4.2±1.6 [a]	0.739	5.1±2.1 [a]	4.4±1.4	0.851
VE/$\dot{V}CO_2$slope	26±2.2	27.8±4.9 [a]	0.365	25.3±2.4	26.5±2	0.327

Exercise echocardiography and breath-by-breath gas exchange analyses. Data are presented as means ± SD. BPsys; systolic blood pressure, BPdia; diastolic blood pressure, HR; heart rate, IVSd; interventricular septum thickness end-diastole, LVPWd; left ventricular posterior wall thickness end-diastole, LVDd; left ventricular diameter end-diastole, LVM; left ventricular mass, LVMi; left ventricular mass indexed for BSA, RWT; relative wall thickness, LVOT; left ventricular outflow tract diameter, LVEDV; left ventricular end-diastolic volume, LVESV; left ventricular end-systolic volume, E; peak velocity of early diastolic filling phase, A; peak velocity of late diastolic filling phase, Dt; deceleration time, e's; early diastolic velocity at the septal annulus, E/e'; left ventricular filling pressure, LVEF; left ventricular ejection fraction, SV; stroke volume, CO; cardiac output, LS; longitudinal strain, $\dot{V}O2$; oxygen uptake, RER; respiratory exchange ratio, LS deltahigh intensity-rest; change in longitudinal strain between rest and high-intensity exercise, $\dot{V}O2$ peak; peak oxygen uptake, $\dot{V}O2$ peak-predicted; percentage of predicted oxygen uptake, VEpeak; peak ventilation, RERpeak; peak respiratory exchange ratio, $\dot{V}CO2$; carbon dioxide. a: Data abnormally distributed, Mann-Whitney U test used. *: male HC vs male T2DM and female HC vs female T2DM. Significant differences between two groups at *P < 0.05

Supplementary table 5. Echocardiographic images used for analyses

	HC	T2DM
Cardiac structure and dimensions		
IVSd	23 (100%)	36 (100%)
LVDd	23 (100%)	36 (100%)
LVPWd	23 (100%)	36 (100%)
LVOT	23 (100%)	36 (100%)
LVEDV	23 (100%)	36 (100%)
LVESV	23 (100%)	36 (100%)
Diastolic function at rest		
E	21 (91.3%)	36 (100%)
A	21 (91.3%)	36 (100%)
Dt	21 (91.3%)	36 (100%)
e'_s	21 (91.3%)	36 (100%)
Diastolic function at exercise		
E	18 (78.2%)	33 (91.7%)
e'_s	21 (91.3%)	33 (91.7%)
Systolic function		
CO at rest	23 (100%)	36 (100%)
CO at exercise	18 (78.2%)	34 (94.4%)
LVEF at rest	23 (100%)	36 (100%)
LVEF at exercise	22 (95.7%)	36 (100%)
SV at rest	23 (100%)	36 (100%)
SV at exercise	22 (95.7%)	36 (100%)
LS at rest	23 (100%)	36 (100%)
LS at exercise	23 (100%)	36 (100%)
Ergospirometry		
$\dot{V}O_2$peak	22 (95.7%)	35 (97.2%)

IVSd; Interventricular septum thickness end-diastole, LVDd; left ventricular diameter end-diastole, LVPWd; left ventricular posterior wall thickness end-diastole, LVOT; left ventricular outflow tract, LVEDV; left ventricular end-diastolic volume, LVESV; left ventricular end-systolic volume, E; early diastolic inflow, e's; early diastolic velocity at septal annulus, Dt; deceleration time, CO; cardiac output, LVEF; left ventricular ejection fraction, SV; stroke volume, LS; longitudinal strain, $\dot{V}O2$; oxygen uptake

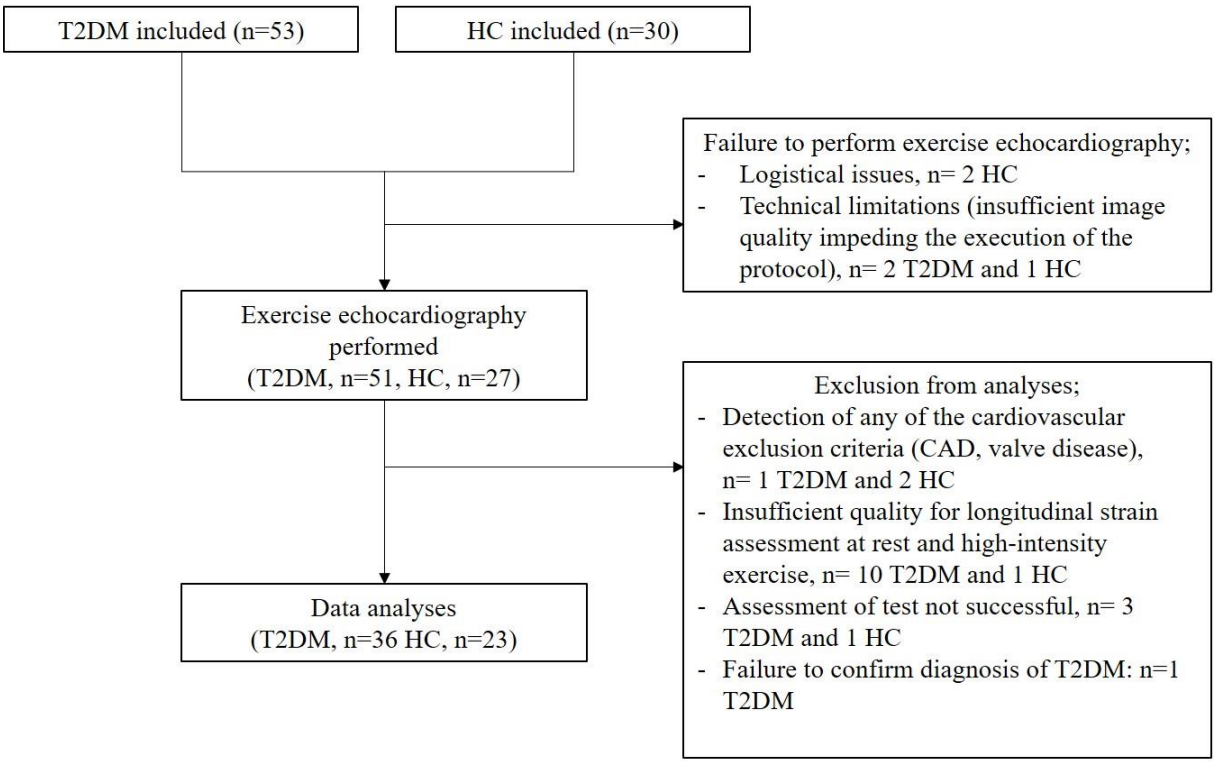


Figure 1: Flowchart of study. CAD; coronary artery disease

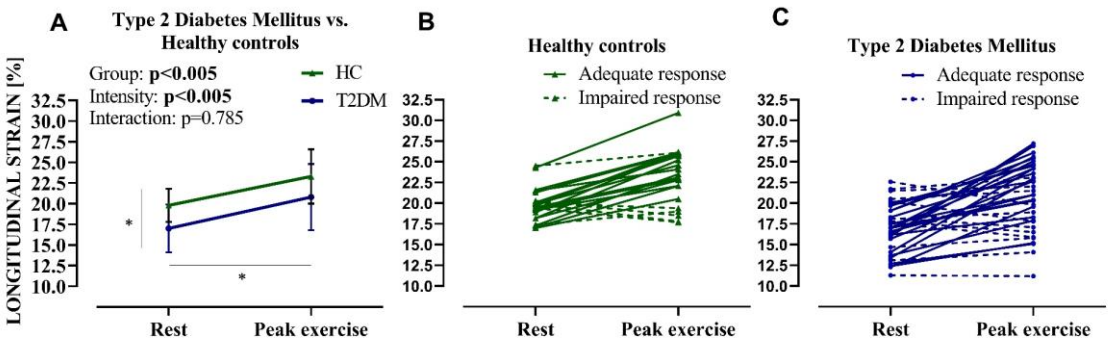


Figure 4: Two-way mixed ANOVA for longitudinal strain during stress echocardiography. Data are presented as means ± SD. Panel A; Mean longitudinal strain for both groups, Panel B and C; Individual cases in the Healthy control group and Type 2 Diabetes Mellitus group respectively. Significant differences at *P < 0.05.

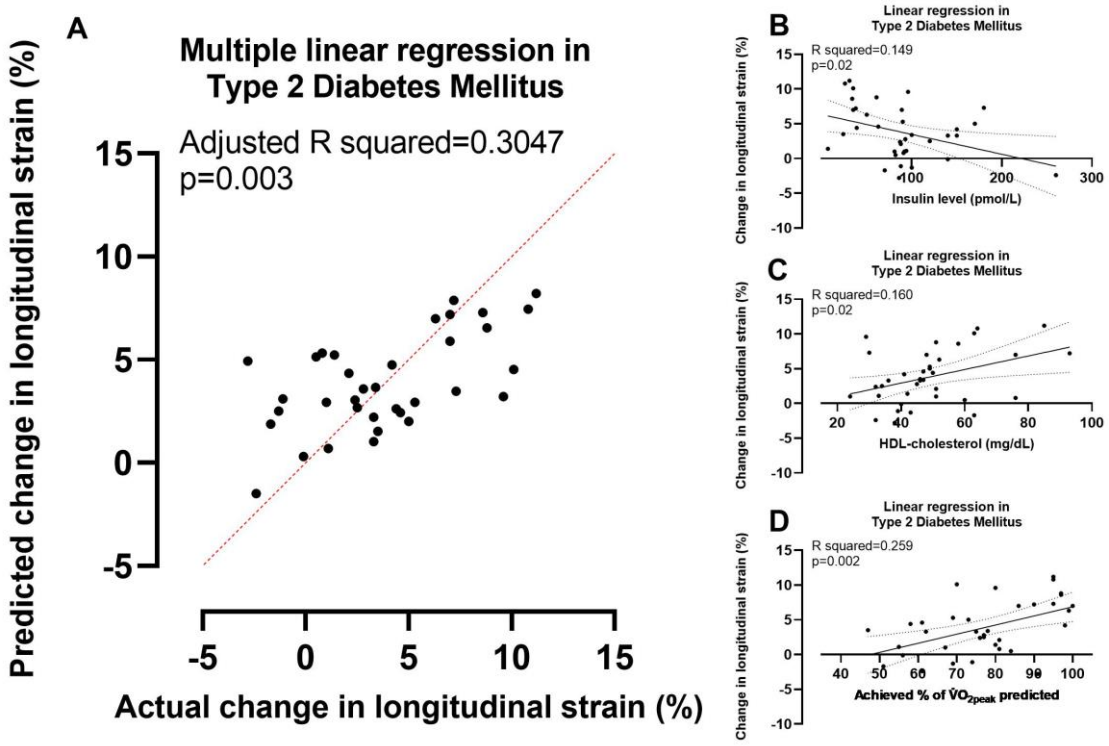
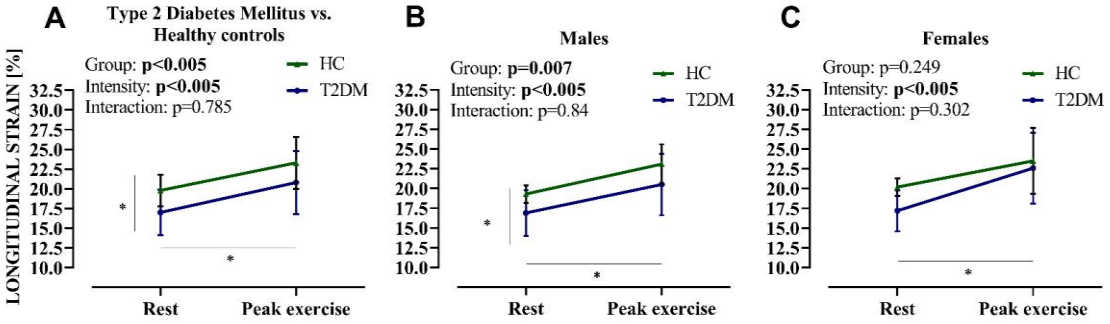
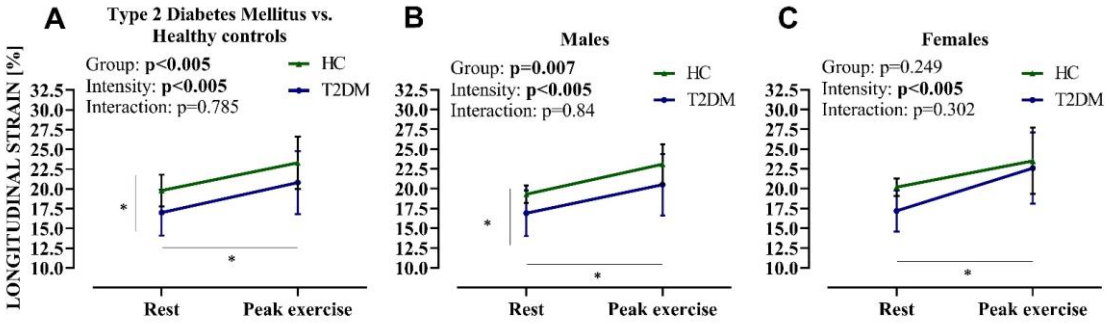


Figure 5: Regression analyses for LS responses and clinical parameters within the T2DM group. Panel A; Multiple regression for response in LS and HDL-cholesterol, fasting insulin levels and exercise tolerance (achieved percentage of predicted V̇O2peak). Panel B,C and D: Linear regression for fasting insulin levels, HDL-cholesterol and exercise tolerance. Significance level was set at *p<0.05.



Supplementary figure 1: Two-way mixed ANOVA for longitudinal strain and stages of echocardiography. Data are presented as means ± SD. Panel A; Mean longitudinal strain for both groups, Panel B and C; Individual cases in for males and females respectively. Significant differences at *P < 0.05.



Supplementary figure 4: Two-way mixed ANOVA for longitudinal strain and stages of echocardiography. Data are presented as means ± SD. Panel A; Mean longitudinal strain for both groups, Panel B and C; Individual cases in for males and females respectively. Significant differences at *P < 0.05.

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