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## Relationship between Left Ventricular Structural and Metabolic Remodelling in Type 2 Diabetes Mellitus

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### Abstract

Concentric left ventricular (LV) remodelling is associated with adverse cardiovascular events and is frequently observed in patients with type 2 diabetes mellitus (T2DM). Despite this, the cause of concentric remodelling in diabetes, per se, is unclear, but may be related to cardiac steatosis and impaired myocardial energetics. Thus, we investigated the relationship amongst myocardial metabolic changes and LV remodelling in T2DM. Forty-six non-hypertensive T2DM patients and twenty matched controls underwent cardiovascular magnetic resonance to assess LV remodelling (LV mass to LV end diastolic volume ratio-LVMVR), function, pre- and post-contrast tissue characterisation using T1 mapping, <sup>1</sup>H-, <sup>31</sup>P-magnetic resonance spectroscopy for myocardial triglyceride content (MTG) and phosphocreatine to ATP ratio (PCr/ATP) respectively. When compared to body mass index and blood pressure matched controls, diabetes was associated with: concentric LV remodelling, higher MTG, impaired myocardial energetics and impaired systolic strain indicating a subtle contractile dysfunction. Importantly, cardiac steatosis independently predicted concentric remodelling and systolic strain. Extracellular volume fraction was unchanged, indicating absence of fibrosis. In conclusion, cardiac steatosis may contribute to LV concentric remodelling and contractile dysfunction in diabetes. As cardiac steatosis is modifiable, strategies

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#### Author Contributions

EL: contributed substantially to conception and design of study; acquired and analyzed the data; drafted the manuscript; MM: contributed to data acquisition, critical revision of the manuscript; SKP: contributed substantially to data analyses; RA: contributed to data acquisition, critical revision of the manuscript; JMF: contributed to data acquisition; CTR: contributed to data acquisition and analyses, critical revision of the manuscript; WTC contributed to data acquisition and analyses; NS: contributed to data acquisition and analyses; JES: contributed to data acquisition and analyses and critical revision of the manuscript; TDK: participated in study design and have critically revised the manuscript; KC: have critically revised the manuscript; OJR: contributed to statistical analyses and helped to draft the manuscript; SN: conceived the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

aimed at reducing myocardial triglyceride may be beneficial in reversing concentric remodelling and improving contractile function in the diabetic heart.

## Keywords

Diabetes; myocardial steatosis; magnetic resonance imaging; magnetic resonance spectroscopy

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## Introduction

Diabetes mellitus (DM) is associated with an increased risk of both heart failure<sup>1</sup> and cardiovascular mortality<sup>2</sup>, even in the absence of coronary artery disease (CAD). The reasons for this, are not clear, but one candidate mechanism that has emerged is concentric LV hypertrophy, which is frequently observed in patients with type 2 diabetes mellitus (T2DM)<sup>3, 4</sup>, preceding the development of clinical heart failure<sup>5</sup> and shown to be a strong predictor of adverse cardiovascular events<sup>6</sup>.

Concentric remodelling of the LV is characterised by an increased LV mass to LV end diastolic volume ratio (LVMVR) but normal LV mass index<sup>7</sup>. The precise underlying mechanism of concentric LV remodelling in patients with DM, in the absence of significant arterial hypertension, remain unclear. One potential driver of LV concentric remodelling in patients with T2DM is cardiac steatosis, where excess myocyte accumulation of triglyceride leads to hypertrophic signaling<sup>8, 9</sup>. The link between lipotoxicity and concentric LV remodelling has been demonstrated in animal models of excess lipid accumulation<sup>7,10</sup> and in humans<sup>11</sup> and particularly patients with generalised lipodystrophy<sup>12</sup>, who exhibit severe concentric LV hypertrophy and significant cardiac steatosis. Proton (<sup>1</sup>H) magnetic resonance spectroscopy (MRS) allows for the non-invasive measurement of cardiac triglyceride content. Using this technique cardiac steatosis has been shown to be a prominent and early feature of diabetic cardiomyopathy<sup>13</sup>.

In addition, impaired myocardial high-energy phosphate metabolism is another important feature of diabetic cardiomyopathy<sup>14</sup>. <sup>31</sup>Phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS) allows for cardiac energetics to be measured non-invasively. Whether a relationship between concentric LV remodelling and impaired myocardial energetics exists in T2DM is unknown, but given the link between PCr/ATP ratio and mortality<sup>15</sup>, this is worthy of investigation.

Interstitial fibrosis has also been implicated in the pathogenesis of LV hypertrophy<sup>16</sup> and has been identified in the more advanced stages of diabetic cardiomyopathy<sup>17</sup>. The role of interstitial fibrosis in the pathogenesis of LV hypertrophy in stable/early diabetic cardiomyopathy is much less clear as abnormal myocyte hypertrophy rather than fibrosis appears to predominate in the early stages<sup>18</sup>. Cardiac magnetic resonance (CMR) T1 mapping for extracellular volume (ECV) quantification allows for non-invasive quantification of fibrosis<sup>19</sup>, correlating closely with collagen proportionate area on histology<sup>20</sup>.

Thus, we used CMR imaging, combined with  $^1\text{H}$ -MRS and  $^{31}\text{P}$ -MRS, to assess the relationship amongst LV concentric remodelling, cardiac fibrosis, steatosis and myocardial energetics in T2DM, and compared the results to non-diabetic age, body mass index (BMI) and blood pressure matched controls.

## Research Design and Methods

The study complies with the Declaration of Helsinki, it was approved by the National Research Ethics Committee (REC Ref 13/SW/0257) and informed written consent was obtained from each participant. All patients were recruited from the general practice surgeries in Oxfordshire, United Kingdom. Forty-six stable T2DM (diagnosed according to the World Health Organization criteria<sup>21</sup>) and 20 controls were recruited to the study.

### Inclusion and Exclusion Criteria

The diabetes population assessed in this study is comprised of T2DM patients only, with stable disease and no known diabetes complications. Subjects were excluded if they had a history of cardiovascular disease, chest pain, smoking, hypertension (resting systolic blood pressure (BP) >140 mmHg and diastolic BP >90 mmHg), contraindications to MR imaging, ischemic changes on 12-lead ECG, or renal impairment (estimated glomerular filtration rate below 30 mL/min). T2DM participants were excluded if they had HBA1C >9% or were taking insulin.

### Anthropometric Measurements

Height and weight were recorded and BMI calculated. Blood pressure was recorded as an average of 3 supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Corp). Fasting venous blood was drawn for glucose, triglyceride, HBA1c, renal function and free fatty acids (FFA), full blood count tests as previously described<sup>22</sup>. Fasting insulin was also recorded in all diabetic patients. In agreement with the diabetes management guidelines<sup>23</sup> spot (random) urine sample albumin levels and the albumin: creatinine ratio was assessed in the majority of patients with T2DM, (~69%, n=32).

### Coronary computed tomographic angiography

An optional scan of coronary computed tomographic angiography (CCTA) were offered to diabetic patients to exclude obstructive coronary artery disease (>50% of luminal stenosis). CCTA scans were performed on 64-slice CT scanner (GE Healthcare, Discovery 690) in accordance to performance guidelines from the Society of Cardiovascular Computed Tomography<sup>24</sup>. Participants received beta-blockade (intravenous Metoprolol) and sublingual GTN (if necessary and safe) prior to the scan to achieve a heart rate of <65 beats per minute. During the CTCA acquisition, 80ml of iodinated contrast (Visipaque, GE Healthcare, Princeton, NJ) was injected followed by a 50ml saline flush.

### Echocardiography

Transthoracic echocardiography was performed with the subjects at rest using commercially available ultrasound transducer and equipment (Philips iE33 Medical Systems, The Netherlands). All images were digitally stored on hard disks for offline analysis (EchoPAC

version 108.1.5, GE-Vingmed). Measurements of LV diastolic function were performed according to the guidelines of the American Society of Echocardiography.<sup>25</sup> The following diastolic indices were obtained: transmitral early (E) and late (A) diastolic velocities and E/A ratio.

### Cardiac Magnetic Resonance Protocol

All LV imaging were performed on a 3.0 Tesla MR system (Siemens, Germany). Images for LV volumes and diastolic function were acquired using a steady state free precession (SSFP) sequence and analysed using cmr42© (Circle Cardiovascular Imaging Inc, Canada) as previously described<sup>26</sup>.

To determine mid-ventricular peak systolic circumferential strain and diastolic strain rate, myocardial tagging was performed as described previously<sup>27 28</sup>. Tagged images were analysed using Cardiac Image Modeller software (CimTag2D v7 Auckland Medical Research, Auckland, New Zealand). Semi-automated analysis was performed by aligning a grid to the myocardial tagging planes at end-diastole.

T1 mapping and ECV quantification was performed using a Shortened Modified Look-Locker Inversion Recovery (ShMOLLI) sequence<sup>29</sup>. T1 maps were generated from the mid short-axis images as described previously<sup>29</sup>. Consistent with earlier reports on ECV estimation<sup>19, 30</sup>, we measured pre-contrast and post-contrast myocardial and blood T1 values, and the estimation of ECV and lambda was based on multipoint regression, incorporating all available pre-contrast and post-contrast points, to increase the robustness of the estimates by increasing the number of underlying data points. ECV was calculated as:  $(1 - \text{hematocrit})$ . For calculation of post-contrast T1 values, the post-contrast T1 map acquired at 15 min was utilized for ECV calculation. Baseline and 15 min post-contrast images were contoured by two observers (EL and SKP) in a blinded fashion, using dedicated software, as previously described<sup>31</sup>.

Late gadolinium enhancement (LGE) imaging was performed to exclude presence of previous silent myocardial infarction or regional fibrosis and it was acquired according to standard clinical protocols and analyzed qualitatively.

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

<sup>31</sup>P MR spectroscopy was performed to obtain the rest PCr/ATP from a voxel placed in the mid-ventricular septum, with the subjects lying prone with their heart over the centre of the <sup>31</sup>P heart/liver coil in the isocentre of the magnet as previously described<sup>32</sup>. <sup>31</sup>P-MRS post processing analysis was performed using in house software within Matlab version R2012a (Mathworks, Natick, Massachusetts) as previously described<sup>32</sup>.

### Cardiac <sup>1</sup>H-MRS

Myocardial <sup>1</sup>H-MR spectra were obtained from the mid-interventricular septum as previously described<sup>33</sup>. Spectroscopic acquisitions were performed using ECG trigger at end-expiration to minimize motion artefacts. Water-suppressed spectra were acquired to measure myocardial lipid content, and spectra without water suppression were acquired as

an internal standard. Spectra were analyzed using Matlab and the AMARES algorithm in Java-based Magnetic Resonance User Interface as previously described<sup>33</sup>. Myocardial lipid content was calculated as a percentage relative to water: (signal amplitude of lipid/signal amplitude of water)×100.

## Statistical Analysis

All statistical analysis was performed with commercially available software packages (IBM SPSS Statistics, version 20). All data were checked for normality using the Kolmogorov–Smirnov test and presented as mean ± standard deviations, and median (interquartile range) as appropriate. Normally distributed data sets were analysed with the independent Student *t* test. The chi-square test was used to compare discrete data as appropriate. Bivariate correlations were performed using Pearson's or Spearman's method as appropriate. To assess the associations concentric remodeling and metabolic parameters, linear regression across all subjects was performed. Linearity was assessed visually. Variables with  $P < 0.05$ , and the strongest relationship with concentric remodelling were then included in multiple linear regression models by a stepwise selection method to assess the "best" subset in predicting cardiovascular remodelling. Significance was assumed at  $P < 0.05$ .

## Results

### Participant Characteristics

Demographic, clinical, and biochemical data are shown in Table 1.

Forty-six patients (24 male, mean age  $55 \pm 9$  years, body mass index (BMI)  $29.6 \pm 5.7 \text{ kg/m}^2$ ) with T2DM, median diabetes duration 7 years [IQR: 1-8] and mean HBA1c of  $7.5 \pm 1.2\%$ , and twenty controls (9 male, mean age  $54 \pm 10$  years, BMI  $28.6 \pm 2.8 \text{ kg/m}^2$ ) were studied. Patients were of similar age, gender, weight, resting heart rate and blood pressure with controls. As expected, diabetes was associated with higher fasting blood glucose, HBA1c, free fatty acids (FFA), triglyceride levels and lower high-density lipoprotein cholesterol. About 74% of the diabetic patients were on statin therapy, hence the lower total cholesterol and low-density lipoprotein cholesterol levels were detected in patients compared to controls. Urine albumin: creatinine ratio and urine albumin results were recorded in 32 patients with T2DM in the study and all were within normal limits.

### CCTA

Of the 46 patients with T2DM, in 76% significant CAD was excluded by CCTA and the remaining 11 did not consent to having CCTA.

### The Effect of Diabetes on LV Geometry and Function

In agreement with previous reports, diabetes was associated with concentric LV remodelling. Although LV mass was not significantly different between T2DM and controls ( $P = 0.183$ ), LV end-diastolic volume was 16% smaller in T2DM ( $P = 0.004$ ). As a result, T2DM was associated with increased LV mass to volume ratio (LVMVR) by 31%, ( $0.97 \pm 0.17$ , vs  $0.74 \pm 0.14 \text{ g/ml}$ ,  $P < 0.001$ ; Figure 1, A; Table 2), suggesting significant concentric remodelling.

Importantly, this concentric remodelling was not correlated with blood pressure, which was within normal limits in both groups, ( $R = -0.002$ ,  $P = 0.989$ ).

Despite normal LV ejection fraction (LVEF), mid-ventricular peak systolic circumferential strain was impaired in patients with T2DM (T2DM  $14.5 \pm 3.5\%$ , vs controls  $18.3 \pm 2.6\%$ ,  $P < 0.001$ ; Figure 1B), indicating subtle contractile dysfunction. The differences in diastolic strain rate between the T2DM patients and controls did not reach statistical significance, but there was a strong trend (T2DM  $60 \pm 24s^{-1}$ , vs controls  $65 \pm 13s^{-1}$ ,  $P = 0.057$ ). LVMVR showed a negative correlation with peak systolic circumferential strain ( $R = -0.430$ ,  $P < 0.001$ ), but not with diastolic strain rates ( $R = -0.121$ ,  $P = 0.341$ ). The echocardiographic assessment of mitral inflow E/A ratio was significantly lower in T2DM patients (T2DM  $0.99 \pm 0.25$ , vs controls  $1.17 \pm 0.38$ ,  $P = 0.038$ ). In-keeping with the dissociation of diastolic strain rates and the myocardial triglycerides, there was no significant correlation between the mitral inflow E/A ratio and the myocardial triglyceride ( $R = -0.135$ ,  $P = 0.393$ ).

### Cardiac Steatosis, Myocardial Energetics, Concentric Remodeling and Strain

As described before, diabetes was associated with an almost two-fold elevation of myocardial triglyceride (T2DM  $1.13 \pm 0.78$ , vs controls  $0.64 \pm 0.52$ ,  $P = 0.017$ ; Figure 1, C) and was also associated with ~18% reduction in the myocardial PCr/ATP ratio (T2DM  $1.68 \pm 0.28$ , vs controls  $2.05 \pm 0.34$ ,  $P < 0.001$ ; Figure 1, D). When investigating all study subjects, there was a positive correlation between the MTG and concentric LV remodelling ( $R = 0.41$ ,  $P = 0.003$ ) and a negative correlation between myocardial energetics and LVMVR ( $R = -0.30$ ,  $P < 0.020$ ). Stepwise multivariable regression revealed myocardial triglyceride ( $\beta = 0.473$ ,  $P = 0.001$ ) to be the only independent predictor of concentric remodelling (overall  $R^2$  of the model = 0.304,  $P = 0.001$ ). Furthermore, myocardial triglyceride also negatively correlated with systolic strain ( $R = -0.40$ ,  $P = 0.003$ ), and it was also the only independent predictor of systolic strain ( $\beta = -0.400$ ,  $P = 0.003$ ) on stepwise multivariable regression analysis. However, there was no correlation between diastolic strain rate and steatosis ( $R = 0.158$ ,  $P = 0.263$ ). Figure 2 shows representative examples of cardiac  $^{31}P$ -MRS,  $^1H$ -MRS and cine images in a control and a patient with T2DM.

### T1 mapping , extracellular volume (ECV) quantification and late Gadolinium Enhancement

There was no significant difference in native myocardial T1 values between the T2DM patients and controls (T2DM  $1194 \pm 32$  ms, vs controls  $1184 \pm 28$  ms;  $P = 0.23$ ). Similarly, ECV did not differ between the groups (T2DM  $29 \pm 2\%$ , vs controls  $29 \pm 3\%$ ,  $P = 0.773$ ), suggesting absence of interstitial fibrosis. On visual assessment of the LGE images, no areas of enhancement indicative of scarring in either ischaemic or non-ischaemic patterns were identified in any of the participants.

### Discussion

Concentric LV remodelling is an adverse prognostic marker of cardiovascular events<sup>34</sup>, and is linked to contractile dysfunction<sup>35</sup>. Using CMR and MRS we show here that diabetes, in the absence of hypertension, is associated with concentric LV remodelling and we confirm the findings of previous studies, showing pronounced cardiac steatosis<sup>13</sup> and decreased



energetics<sup>14,36</sup> in patients with T2DM. We have also shown that despite normal LVEF, peak systolic strain was significantly impaired in diabetics, indicating a subtle contractile dysfunction, which was negatively correlated with both reduced myocardial energetics and concentric LV remodelling. Importantly, we have shown here for the first time, that the degree of myocardial triglyceride accumulation is predictive of concentric LV remodelling and cardiac contractile function in patients with T2DM. The correlation of myocardial concentric remodelling with myocardial triglyceride accumulation and peak systolic strain in diabetic hearts suggest a link between these; however, the causality of these relationships will need to be investigated in future studies. From these initial cross-sectional studies, we clearly cannot determine whether the observed correlations between myocardial triglyceride and LVMVR and function are causal. As myocardial steatosis has been shown to be modifiable<sup>37,38</sup>, this provides the potential for novel therapies aimed at reducing concentric LV remodelling and improving cardiac function in diabetes. Finally, as no significant difference in ECV and native (pre-contrast) T1 mapping was found between the patients with T2DM and controls, it is unlikely that interstitial fibrosis plays a significant role in the pathogenesis of concentric LV remodelling in this well controlled, stable T2DM population. This suggests that the process of concentric remodelling is not limited to poorly controlled diabetics, or those with renal dysfunction<sup>17</sup>, and occurs in the absence of significant systemic hypertension.

Microalbuminuria is strongly associated with risk for cardiovascular disease, but the nature of this link remains controversial and poorly understood<sup>39</sup>. In this study, in patients with stable T2DM, free of coronary artery disease, no association between the urine albumin excretion and the LV remodelling, contractile dysfunction, cardiac energetics or steatosis was observed.

The diabetes population assessed in this study is comprised of highly selected T2DM patients, with stable disease only and no other significant comorbidities. Although this has the advantage of better demonstrating the pathophysiological relationships between T2DM and cardiovascular remodelling it does reduce the broader applicability to "real world" populations where additional co-morbidities are commonplace. Given the fact we have shown significant abnormalities in myocardial energetics, myocardial triglyceride deposition, myocardial geometry and peak systolic strain in a stable diabetes population, similar or amplified findings may potentially be expected in diabetic patients with more advanced cardiovascular disease, or other significant comorbidities such as hypertension. Future studies are needed to confirm this. Furthermore, whether or not the subtle changes in cardiac geometry, energetics and lipid deposition will predict adverse cardiovascular outcomes in a diabetes cohort remains to be definitively demonstrated by longitudinal studies.

The LVMVR is calculated by dividing the left ventricular mass by the left ventricular end diastolic volume, as an index of wall thickness to cavity size. LVMVR lacks a well-defined normal reference range, therefore age and sex-matched healthy volunteers without co-existent coronary artery disease, hypertension, aortic stenosis or other forms of heart disease were recruited and scanned contemporaneously. LVMVR in this group was  $0.74 \pm 0.10$  g/mL and consistent with previous larger studies of >700 healthy volunteers, carried out in

our centre<sup>40</sup>. The LVMVR in the control group in this small cross sectional study is however lower than the average demonstrated in a multi-ethnic study of atherosclerosis (MESA). This is likely the result of the fact that the MESA population study of >5000 participants did not exclude subjects with hypertension, impaired fasting glucose, diabetes, smoking and other causes of concentric hypertrophy<sup>41</sup>. In addition, in MESA participants were on average 6-8 years older than the participants in our study, and only around 10% had diabetes, making comparisons with this larger population based study more difficult.<sup>41</sup>

### **Relationship between LV Geometric Remodelling and Cardiac Steatosis in the Diabetic Heart**

Ectopic lipid deposition in the diabetic heart is a well-documented process, and non-invasive studies using <sup>1</sup>H-MRS have reported elevated levels of cardiac triglyceride in human diabetes<sup>13</sup>. However, the link between cardiac steatosis and diabetes, above and beyond that observed in obesity, and its potential role in concentric LV remodelling within diabetes, has not been explored in humans. This study now demonstrates that diabetes per se is linked to significant cardiac steatosis, and we show a correlation between myocardial triglyceride and the concentric LV remodelling, suggesting a link between the two; however, the causality of this relationship will need to be investigated in future studies.

The importance of cardiac steatosis in the pathophysiology of concentric LV remodelling has been assessed in experimental models. In diabetes, where fatty acid supply exceeds the oxidative capacity of the heart, this leads to diversion of lipids away from oxidative processes and towards non oxidative processes with the production of lipotoxic intermediates such as ceramide and diacyl-glycerol<sup>42</sup>. These lipotoxic intermediates have been shown to activate signalling pathways affecting ATP production, insulin sensitivity, myo-cellular contractility, and apoptosis<sup>42</sup>. Recently, it has also been demonstrated that cardiac steatosis potentiates the effects of Angiotensin 2 on the heart<sup>43</sup>. Given the fact that Angiotensin 2 is a potent hypertrophic stimulus and that both Angiotensin 2 receptor density and mRNA expression are elevated in the diabetic heart<sup>44</sup>, this is likely to be an important driver for hypertrophy. In addition, animal models of overexpression of fatty acid transporters and increases in triglyceride synthesis both result in severe cardiac steatosis and concentric LV hypertrophy<sup>9,36</sup>. This provides a mechanistic link between cardiac steatosis, lipotoxicity and concentric LV remodelling in diseases of upregulated fatty acid metabolism such as diabetes. Importantly, successful reduction of myocardial steatosis with glucagon-like peptide-1 receptor (GLP-1) agonists<sup>37</sup> and mineralocorticoid receptor blockers<sup>38</sup> have both been shown to reverse concentric LV remodelling.

Here, we have demonstrated that myocardial steatosis is a predictor of concentric LV remodelling and subclinical contractile dysfunction in patients with T2DM. This supports the notion that the development of concentric LV remodelling in T2DM may be mechanistically linked to cardiac steatosis, and that cardiac lipotoxicity represents a component of this process, however an observational study such as ours cannot clearly identify the mechanisms responsible and future studies will need to investigate this. If a causal link is proven, this would suggest that therapies and interventions aimed at reducing myocardial triglyceride may promote beneficial reverse remodelling in humans.



## LV Concentric Remodeling and Myocardial Energetics

A healthy heart is able to metabolise a range of substrates, to fulfil the demand for ATP production. Depending on the availability of substrates and the physiological conditions, the heart will switch its metabolic preference amongst substrates. This flexibility is lost in diabetes and myocardial substrate selection is shifted almost exclusively to fatty acid metabolism<sup>44</sup>. This over utilisation of fatty acids results in a reduced ATP yield and a loss of mitochondrial efficiency<sup>44</sup>. The interplay between myocardial energetic status and geometrical changes in diabetes has previously not been explored. Here we report that in the context of well controlled diabetes, myocardial energetics, in the form of PCr/ATP ratios, are impaired, and are linked to concentric remodelling.

## Limitations

Of the 46 patients with T2DM, 11 patients (24%) did not consent to have CCTA performed and as such it is possible that occult coronary artery disease could be present in this minority of patients.

Insulin measurements were not taken in the control group. Although diabetes was excluded on fasting glucose measurements, this does preclude the investigation of the role of insulin resistance in the pathogenesis of cardiac steatosis.

Urine albumin levels were recorded only in ~69% (n=32) of the patients with T2DM.

## Conclusions

When compared to BMI matched controls, patients with well controlled T2DM exhibit LV concentric remodelling in the absence of arterial hypertension. This concentric remodelling is associated with cardiac steatosis, myocardial energetic impairment and subclinical systolic dysfunction. As myocardial steatosis is independently predictive of concentric remodelling and cardiac systolic strain, it may play an important role in adverse geometric remodelling in T2DM. Importantly, as myocardial TG content is modifiable, strategies aimed at reversing myocardial steatosis may be beneficial in reversing LV remodelling, and potentially improve contractile function and prognosis in patients with diabetes.

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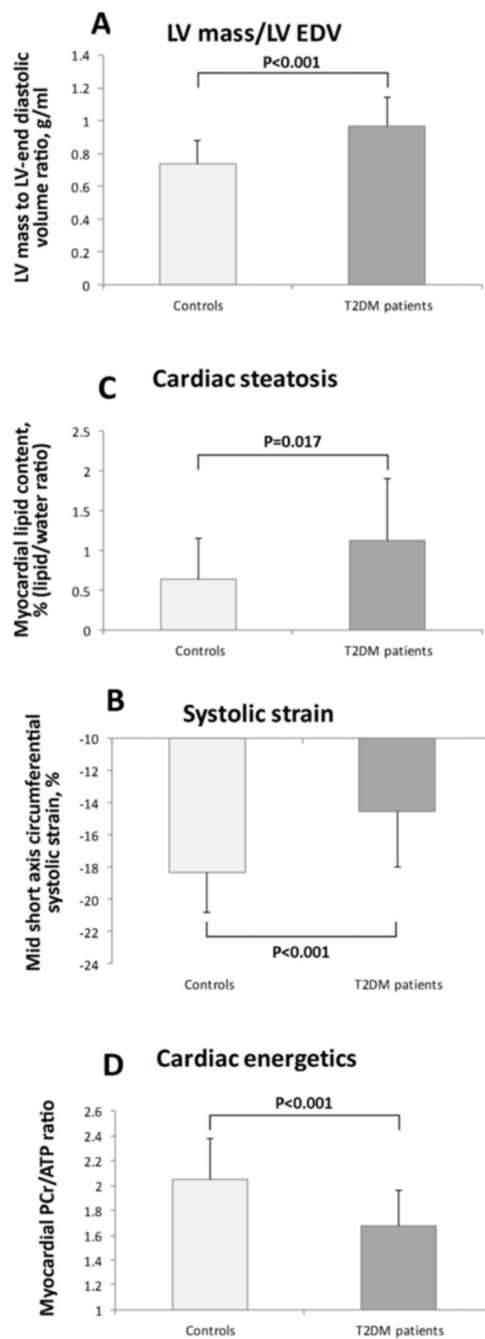
We thank Joanna Sellwood for her help with recruitment and for her support with patient care. Eylem Levelt takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

## References

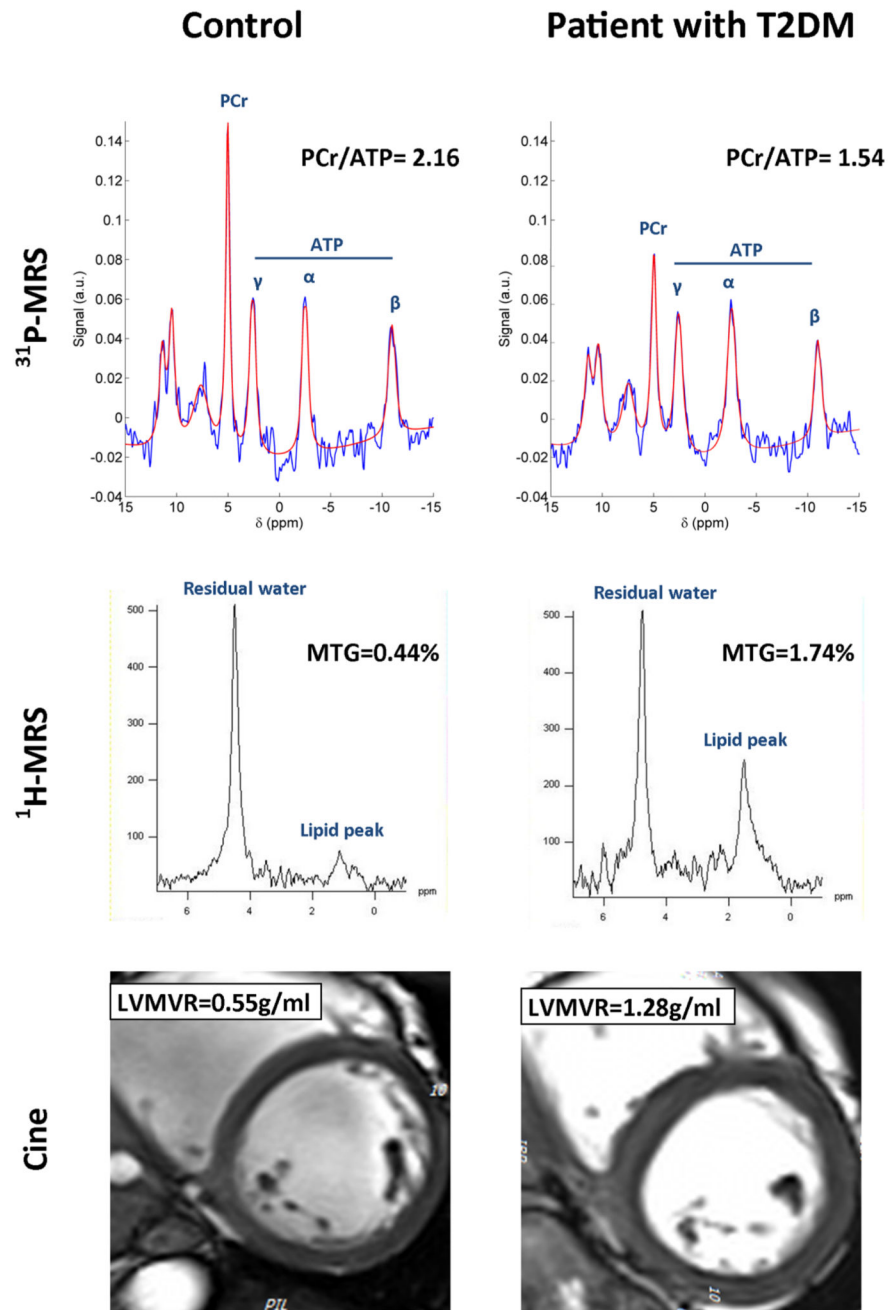
1. Garcia MJ, McNamara PM, Gordon T, Kannel W. Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes*. 1974; 23:105–11. [PubMed: 4359625]
2. Kannel WB, McGee DL. Diabetes and cardiovascular disease: The framingham study. *JAMA*. 1979; 241:2035–2038. [PubMed: 430798]
3. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Howard BV. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation*. 2000; 101:2271–6. [PubMed: 10811594]
4. Bella JN, Devereux RB, Roman MJ, Palmieri V, Liu JE, Paranicas M, Welty TK, Lee ET, Fabsitz RR, Howard BV. Separate and joint effects of systemic hypertension and diabetes mellitus on left ventricular structure and function in American Indians (the Strong Heart Study). *The American journal of cardiology*. 2001; 87:1260–1265. [PubMed: 11377351]
5. Gjesdal O, Bluemke DA, Lima JA. Cardiac remodeling at the population level: risk factors, screening, and outcomes. *Nat Rev Cardiol*. 2011; 8:673–685. [PubMed: 22027657]
6. Bluemke DA, Kronmal RA, Lima JAC, Liu K, Olson J, Burke GL, Folsom AR. The Relationship of Left Ventricular Mass and Geometry to Incident Cardiovascular Events: The MESA Study. *JACC*. 2008; 52:2148–2155. [PubMed: 19095132]
7. Dweck MR, Joshi S, Murigu T, Gulati A, Alpendurada F, Jabbour A, Maceira A, Roussin I, Northridge DB, Kilner PJ, Cook SA, et al. Left ventricular remodeling and hypertrophy in patients with aortic stenosis: insights from cardiovascular magnetic resonance. *Journal of Cardiovascular Magnetic Resonance*. 2012; 14:50. [PubMed: 22839417]
8. Gao H, Feng X-j, Li Z-m, Li M, Gao S, He Y-h, Wang J-j, Zeng S-y, Liu X-p, Huang X-y, Chen S-r, et al. Downregulation of adipose triglyceride lipase promotes cardiomyocyte hypertrophy by triggering the accumulation of ceramides. *Archives of Biochemistry and Biophysics*. 2015; 565:76–88. [PubMed: 25436917]
9. Glenn DJ, Cardema MC, Ni W, Zhang Y, Yeghiazarians Y, Grapov D, Fiehn O, Gardner DG. Cardiac steatosis potentiates angiotensin II effects in the heart. *American Journal of Physiology - Heart and Circulatory Physiology*. 2015; 308:H339–H350. [PubMed: 25485904]
10. Glenn DJ, Wang F, Nishimoto M, Cruz MC, Uchida Y, Holleran WM, Zhang Y, Yeghiazarians Y, Gardner DG. A Murine Model of Isolated Cardiac Steatosis Leads to Cardiomyopathy. *Hypertension*. 2011; 57:216–222. [PubMed: 21220706]
11. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: In vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magnetic Resonance in Medicine*. 2003; 49:417–423. [PubMed: 12594743]
12. Nelson MD, Victor RG, Szczepaniak EW, Simha V, Garg A, Szczepaniak LS. Cardiac Steatosis and Left Ventricular Hypertrophy in Patients With Generalized Lipodystrophy as Determined by Magnetic Resonance Spectroscopy and Imaging. *The American journal of cardiology*. 2013; 112:1019–1024. [PubMed: 23800548]
13. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation*. 2007; 116:1170–5. [PubMed: 17698735]
14. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S, Clarke K. Abnormal Cardiac and Skeletal Muscle Energy Metabolism in Patients With Type 2 Diabetes. *Circulation*. 2003; 107:3040–3046. [PubMed: 12810608]
15. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, Pabst T, Ertl G, Hahn D, Ingwall JS, Kochsiek K. Myocardial Phosphocreatine-to-ATP Ratio Is a Predictor of Mortality in Patients With Dilated Cardiomyopathy. *Circulation*. 1997; 96:2190–2196. [PubMed: 9337189]
16. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation*. 1991; 83:1849–65. [PubMed: 1828192]

17. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *The American journal of cardiology*. 1972; 30:595–602. [PubMed: 4263660]
18. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJJ, Schalkwijk CG, Bronzwaer JGF, Diamant M, Borbély A, et al. Diastolic Stiffness of the Failing Diabetic Heart: Importance of Fibrosis, Advanced Glycation End Products, and Myocyte Resting Tension. *Circulation*. 2008; 117:43–51. [PubMed: 18071071]
19. White SK, Sado DM, Fontana M, Banyersad SM, Maestrini V, Flett AS, Piechnik SK, Robson MD, Hausenloy DJ, Sheikh AM, Hawkins PN, et al. T1 Mapping for Myocardial Extracellular Volume Measurement by CMR: Bolus Only Versus Primed Infusion Technique. *JACC: Cardiovascular Imaging*. 2013; 6:955–962. [PubMed: 23582361]
20. Liu S, Han J, Nacif M, Jones J, Kawel N, Kellman P, Sibley C, Bluemke D. Diffuse myocardial fibrosis evaluation using cardiac magnetic resonance T1 mapping: sample size considerations for clinical trials. *Journal of Cardiovascular Magnetic Resonance*. 2012; 14:90. [PubMed: 23272704]
21. Alberti KG, Zimmet P. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998; 15:539–53. [PubMed: 9686693]
22. Rider OJ, Tayal U, Francis JM, Ali MK, Robinson MR, Byrne JP, Clarke K, Neubauer S. The effect of obesity and weight loss on aortic pulse wave velocity as assessed by magnetic resonance imaging. *Obesity (Silver Spring)*. 2010; 18:2311–6. [PubMed: 20360756]
23. American Diabetes Association. Standards of Medical Care in Diabetes—2007. *Diabetes Care*. 2007; 30:S4–S41. [PubMed: 17192377]
24. Abbara S, Arbab-Zadeh A, Callister TQ, Desai MY, Mamuya W, Thomson L, Weigold WG. SCCT guidelines for performance of coronary computed tomographic angiography: A report of the Society of Cardiovascular Computed Tomography Guidelines Committee. *Journal of Cardiovascular Computed Tomography*. 2009; 3:190–204. [PubMed: 19409872]
25. Baumgartner H, Hung J, Bermejo J, Chambers JB, Evangelista A, Griffin BP, Iung B, Otto CM, Pellikka PA, Quiñones M. Echocardiographic Assessment of Valve Stenosis: EAE/ASE Recommendations for Clinical Practice. *Journal of the American Society of Echocardiography*. 2009; 22:1–23. [PubMed: 19130998]
26. Rider OJ, Lewandowski A, Nethononda R, Petersen SE, Francis JM, Pitcher A, Holloway CJ, Dass S, Banerjee R, Byrne JP, Leeson P, et al. Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. *Eur Heart J*. 2013; 34:292–9. [PubMed: 23053174]
27. Lawton JS, Cupps BP, Knutsen AK, Ma N, Brady BD, Reynolds LM, Pasque MK. Magnetic resonance imaging detects significant sex differences in human myocardial strain. *Biomedical engineering online*. 2011; 10:76. [PubMed: 21859466]
28. Stuber M, Spiegel MA, Fischer SE, Scheidegger MB, Dianas PG, Pedersen EM, Boesiger P. Single breath-hold slice-following CSPAMM myocardial tagging. *MAGMA*. 1999:85–91. [PubMed: 10555178]
29. Piechnik SK, Ferreira VM, Dall'Armellina E, Cochlin LE, Greiser A, Neubauer S, Robson MD. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. *Journal of Cardiovascular Magnetic Resonance*. 2010; 12:69–69. [PubMed: 21092095]
30. Flett AS, Hayward MP, Ashworth MT, Hansen MS, Taylor AM, Elliott PM, McGregor C, Moon JC. Equilibrium Contrast Cardiovascular Magnetic Resonance for the Measurement of Diffuse Myocardial Fibrosis: Preliminary Validation in Humans. *Circulation*. 2010; 122:138–144. [PubMed: 20585010]
31. Ntusi NA, Piechnik SK, Francis JM, Ferreira VM, Rai AB, Matthews PM, Robson MD, Moon J, Wordsworth PB, Neubauer S, Karamitsos TD. Subclinical myocardial inflammation and diffuse fibrosis are common in systemic sclerosis—a clinical study using myocardial T1-mapping and extracellular volume quantification. *J Cardiovasc Magn Reson*. 2014; 4:16–21.
32. Purvis LAB, Clarke WT, Biasioli L, Robson MD, Rodgers CT. Linewidth constraints in Matlab AMARES using per-metabolite T2 and per-voxel B0. *ISMRM*. 2014:2885.

33. Rial B, Robson MD, Neubauer S, Schneider JE. Rapid quantification of myocardial lipid content in humans using single breath-hold 1H MRS at 3 Tesla. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2011; 66:619–24.
34. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Gattobigio R, Zampi I, Santucci A, Santucci C, Reboldi G, Porcellati C. Prognostic value of left ventricular mass and geometry in systemic hypertension with left ventricular hypertrophy. *American Journal of Cardiology*. 78:197–202. [PubMed: 8712142]
35. Rosen BD, Edvardsen T, Lai S, Castillo E, Pan L, Jerosch-Herold M, Sinha S, Kronmal R, Arnett D, Crouse JR, Heckbert SR, et al. Left Ventricular Concentric Remodeling Is Associated With Decreased Global and Regional Systolic Function: The Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2005; 112:984–991. [PubMed: 16103253]
36. Shivu GN, Phan TT, Abozguia K, Ahmed I, Wagenmakers A, Henning A, Narendran P, Stevens M, Frenneaux M. Relationship between coronary microvascular dysfunction and cardiac energetics impairment in type 1 diabetes mellitus. *Circulation*. 2010; 121:1209–15. [PubMed: 20194884]
37. 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. [PubMed: 23709595]
38. Ramírez E, Klett-Mingo M, Ares-Carrasco S, Picatoste B, Ferrarini A, Rupérez FJ, Caro-Vadillo A, Barbas C, Egido J, Tuñón J, Lorenzo Ó. Eplerenone attenuated cardiac steatosis, apoptosis and diastolic dysfunction in experimental type-II diabetes. *Cardiovascular diabetology*. 2013; 12:172–172. [PubMed: 24261558]
39. Damsgaard EM, Frøland A, Jørgensen OD, Mogensen CE. Prognostic value of urinary albumin excretion rate and other risk factors in elderly diabetic patients and non-diabetic control subjects surviving the first 5 years after assessment. *Diabetologia*. 1993; 36:1030–1036. [PubMed: 8243851]
40. Rider OJ, Lewandowski A, Nethononda R, Petersen SE, Francis JM, Pitcher A, Holloway CJ, Dass S, Banerjee R, Byrne JP, Leeson P, et al. Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging. *European Heart Journal*. 2013; 34:292–299. [PubMed: 23053174]
41. Turkbey EB, McClelland RL, Kronmal RA, Burke GL, Bild DE, Tracy RP, Arai AE, Lima JAC, Bluemke DA. The Impact of Obesity on the Left Ventricle: The Multi-Ethnic Study of Atherosclerosis (MESA). *JACC Cardiovascular imaging*. 2010; 3:266–274. [PubMed: 20223423]
42. Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, Han X, Gross RW, Kozak R, Lopaschuk GD, Kelly DP. The cardiac phenotype induced by PPAR $\alpha$  overexpression mimics that caused by diabetes mellitus. *The Journal of Clinical Investigation*. 2002; 109:121–130. [PubMed: 11781357]
43. Schmieder RE, Hilgers KF, Schlaich MP, Schmidt BMW. Renin-angiotensin system and cardiovascular risk. *The Lancet*. 369:1208–1219.
44. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation*. 2007; 115:3213–23. [PubMed: 17592090]



**Figure 1.** Differences in cardiac geometry and function between patients with T2DM and controls; (A) LV Mass: LV-EDV ratio (g/ml), (B) Systolic strain %, (C) Myocardial triglyceride content (%) and (D) Myocardial energetics (PCr/ATP ratio).



**Figure 2.** Representative examples of cardiac  $^{31}\text{P}$ -MRS,  $^1\text{H}$ -MRS and cine imaging in a control and a patient with T2DM. Top panel: normal control  $^{31}\text{P}$ -MRS with PCr/ATP=2.16, vs patient with T2DM PCr/ATP= 1.54; Second panel: normal control  $^1\text{H}$ -MRS with myocardial lipid to water ratio=0.44%, vs patient with T2DM= 1.74%; Third panel: normal control cine image with LV mass : LV end-diastolic volume ratio=0.55 g/ml, vs patient with T2DM = 1.28g/ml.



**Table 1**  
**Clinical, Biochemical Characteristics and Echocardiographic Features**

Variable	Controls N=20	Type 2 DM patients N=46	P value
Age, y	54 ± 10	55 ± 9	0.583
BMI, kg/m <sup>2</sup>	28.6 ± 2.8	29.6 ± 5.7	0.463
Male, %	45	50	0.714
Diabetes duration, y	...	7[IQR:1-8]	
Heart rate, beats per minute	66 ± 13	68 ± 8	0.377
Systolic blood pressure, mmHg	128 ± 12	129 ± 8	0.583
Diastolic blood pressure, mmHg	74 ± 8	76 ± 7	0.311
Plasma fasting glucose, mmol/L	4.9 ± 0.5	8.9 ± 3.1	<0.001
Glycated hemoglobin, %	5.4 ± 0.3	7.5 ± 0.2	<0.001
Glycated haemoglobin, mmol/mol	37 ± 3	57 ± 15	<0.001
Plasma triglycerides, mmol/L	1.59 ± 0.68	1.60 ± 0.78	0.931
Plasma free fatty acids, mmol/L	0.38 ± 0.23	0.61 ± 0.36	0.017
Total cholesterol, mmol/L	5.6 ± 0.9	3.9 ± 0.9	<0.001
HDL, mmol/L	1.53 ± 0.61	1.18 ± 0.29	0.005
LDL, mmol/L	3.41 ± 0.53	2.04 ± 0.74	<0.001
Creatinine, mmol/L	72 ± 19	65 ± 17	0.228
Hematocrit, %	41 ± 4	42 ± 3	0.501
Urine albumin, mg/L, (N=32)	...	16 ± 30	
Urine albumin: creatinine ratio, mg/mmol, (N=32)	...	1.7 ± 3	
	<b>Medications, n (%)</b>		
Metformin, n (%)	-	41 (89)	
Sulphonylurea	-	14 (30)	
Aspirin	-	5 (11)	
Statin	-	34 (74)	
ACE-I	-	12 (26)	
	<b>Echocardiographic Features</b>		
Mitral in-flow E/A ratio	1.17 ± 0.38	0.99 ± 0.25	0.038

Values are mean  $\pm$ standard deviations or percentages. T2DM indicates type 2 diabetes mellitus; BMI, body mass index; y, years; HDL, high density lipoprotein; LDL, low density lipoprotein; ACE-I angiotensin-converting enzyme inhibitors.

**Table 2**  
**LV Geometry and Function**

	Controls N=20	Type 2 DM N=46	P value
<b>LV end-diastolic volume, ml</b>	148 ± 34	124 ± 27	0.004
<b>LV end-systolic volume (ESV), ml</b>	43 ± 16	37 ± 13	0.351
<b>LV stroke volume (SV), ml</b>	104 ± 21	86 ± 21	0.055
<b>LV ejection fraction, %</b>	71 ± 5	70 ± 7	0.586
<b>LV wall thickness, mm</b>	9.6 ± 1.3	10.4 ± 1.8	0.047
<b>LV mass, g</b>	109 ± 31	120 ± 28	0.183
<b>LV mass index, g/m<sup>2</sup></b>	53.1 ± 15.9	60.2 ± 11.6	0.05
<b>LV mass to LV end-diastolic volume, g/ml</b>	0.74 ± 0.14	0.97 ± 0.17	<0.001
<b>Peak circumferential diastolic strain rate, s<sup>-1</sup></b>	65 ± 13	60 ± 24	0.057
<b>Native myocardial T1 value, ms</b>	1184 ± 28	1194 ± 32	0.23
<b>Extra Cellular Volume fraction, %</b>	29 ± 3	29 ± 2	0.773

Values are mean ± standard deviations or percentages. T2DM indicates type 2 diabetes mellitus; CMR, cardiac magnetic resonance; LV, left ventricular.