Association of Imaging Markers of Myocardial Fibrosis with Metabolic and Functional Disturbances in Early Diabetic Cardiomyopathy

Jellis et al: Myocardial Fibrosis in Diabetes

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Abstract

Background—Metabolic and vascular disturbances contribute to diabetic cardiomyopathy (DCM), but the role of interstitial fibrosis in early disease is unproven. We sought to assess the relationship between imaging markers of diffuse fibrosis with myocardial dysfunction, and to link this to possible etiologies of early DCM.

Methods and Results—Hemodynamic and metabolic data were measured in 67 subjects with T2DM (60±10 years) with no cardiac symptoms. Myocardial function was evaluated with standard echocardiography and myocardial deformation; ischemia was excluded by exercise echocardiography. Calibrated integrated backscatter (cIB) was calculated from parasternal long axis views. T1 mapping was performed post-contrast with a modified Look-Locker technique using saturation recovery images. Amino-terminal propeptides of pro-collagens type I and III (PIIINP), and the carboxy-terminal propeptide of pro-collagen type I, were assayed to determine collagen turnover. Subjects with abnormal early diastolic tissue velocity (Em) had shorter post-contrast T1 values (p=0.042) and higher cIB (p=0.007). They were heavier (p=0.003), had worse exercise capacity (p<0.001), lower insulin sensitivity (p=0.003) and blunted systolic tissue velocity (p=0.05). Post-contrast T1 was associated with diastolic dysfunction (E_m r=-0.28, p=0.020; E/E_m r=-0.24, p=0.049), impaired exercise capacity (r=0.30, p=0.016), central adiposity (r=-0.26, p=0.046), blood pressure (systolic r=-0.30, p=0.012; diastolic r=-0.49, p<0.001) and insulin sensitivity (r=0.30, p=0.037). The association of T1 with E/E_m (β=-0.31, p=0.017) was independent of blood pressure and metabolic disturbance. PIIINP was linked to diastolic dysfunction (Em r=-0.32, p=0.008) and cIB (r=0.30, p=0.015) but not T1 values.

Conclusions—The association between myocardial diastolic dysfunction, post-contrast T1 values and metabolic disturbance supports that diffuse myocardial fibrosis is an underlying contributor to early DCM.

Key Words: diabetic cardiomyopathy, myocardial fibrosis, T1 mapping, diastolic dysfunction
Myocardial dysfunction is common in apparently well subjects with type 2 diabetes mellitus (T2DM). The observed reductions in myocardial function likely reflect direct metabolic effects on the myocyte, disturbances of microvascular structure and function and the effects of autonomic neuropathy. Myocardial fibrosis may be a putative contributor to this process and has plausible connections with hyperglycemia via accumulation of advanced glycation end-products, activation of inflammatory markers and potentiation of neurohormonal cascades. However, while fibrosis has been identified in later stages of diabetic cardiomyopathy (DCM), a recent laboratory study suggested that the preponderant problem in early DCM was abnormal myocyte function and hypertrophy rather than fibrosis. This ambiguity may pertain to the heterogeneity of this condition and its evolution over time. T2DM is often intrinsically linked with other metabolic factors such as obesity, hypertension and abnormal lipid profile. These variables have also been linked to the etiology of diffuse myocardial fibrosis. As such, the specific role of insulin resistance and hyperglycemia in the pathogenesis of DCM may be difficult to elucidate.

The non-invasive recognition of fibrosis remains challenging. The inverse relationship between diastolic and systolic tissue velocity and myocardial collagen content may reflect a direct effect of fibrosis or a common etiology of diastolic dysfunction. Calibrated integrated backscatter (cIB) is based on the comparison of myocardial reflectivity with the pericardium or blood pool as a frame of reference. Picano et al illustrated an association between percentage fibrosis and backscatter on myocardial biopsy, however the technique is technically difficult and may be limited by signal saturation. In contrast to the ischemic setting, where gadolinium uptake in localized scar can be identified relative to an apparently normal reference area, diffuse extracellular matrix remodeling is difficult to recognize with standard contrast-enhanced cardiac magnetic resonance (CMR). Recently, new T1 mapping techniques using inversion recovery, saturation recovery and Look-Locker methods have...
been developed with better spatial and temporal resolution. By removing reliance on contrasting signal intensity between normal and abnormal myocardium, this method enables quantification of diffuse fibrosis. This has enabled post-contrast T1 mapping to be used in the heart failure population to quantify diffuse myocardial fibrosis, where T1 time has been shown to be inversely proportionate to the degree of fibrosis on myocardial biopsy in a post heart transplant population. Further validation of post-contrast T1 mapping against endomyocardial biopsy has also recently been achieved in non-ischemic cardiomyopathy. Given the potential central role of fibrosis in DCM, we hypothesized that a relationship could be established between non-invasive structural and functional markers of myocardial fibrosis in T2DM which in turn are linked to metabolic control. This would support fibrosis as an underlying mechanism for myocardial dysfunction in T2DM and suggest that metabolic derangement is a predisposing factor.

**Methods**

**Subjects:** We prospectively recruited 67 apparently healthy subjects with T2DM (37 men, 60±10 years) from the hospital clinic and community. Subjects were excluded if they had pre-existing micro or macrovascular complications of diabetes, known valvular, congenital or ischemic heart disease or other significant comorbidities including malignancy, renal failure, significant psychiatric illness or pregnancy. Valvular disease was defined as greater than mild valvular regurgitation or stenosis or a past history of valve surgery. Additional exclusion criteria were a history of hypertensive heart disease manifesting as left ventricular (LV) hypertrophy on echo and contraindications to CMR such as claustrophobia or metallic implants. Subjects were analyzed as a whole study population and then stratified according to evidence of subclinical myocardial dysfunction (Septal E_m>1SD below normal for age) into
predetermined normal and abnormal $E_m$ groups for further assessment. Approval for this study was granted by the Human Research Ethics Committees of Princess Alexandra Hospital, Greenslopes Private Hospital and The University of Queensland, Brisbane, Australia.

**Demographic, anthropometric and metabolic data:** Clinical data were collected regarding subject age, sex, basic anthropometry (to establish body mass index [BMI] and waist to hip circumference ratio as a measure of central adiposity) and duration of T2DM. Serum glucose, insulin, HbA1c, creatinine, estimated glomerular filtration rate (eGFR), B-type natriuretic peptide (BNP) and lipid profile were obtained prior to exercise after fasting for at least 8 hours and before administration of hypoglycemic agents. Insulin sensitivity was determined by the Quantitative Insulin Sensitivity Check Index (QUICKI).24

**Pro-collagen biomarkers:** Additional peripheral venous blood samples pre-exercise were drawn into EDTA and serum specific tubes before being centrifuged (10 minutes at 4°C) and then separated into aliquots for storage at -80°C. Simultaneous analysis of samples was later performed with standard commercially available kits according to the manufacturers’ instructions. Amino-terminal propeptides of pro-collagen type I (PINP) and pro-collagen type III (PIIINP) were measured in serum by radio-immuno assay (Orion Diagnostica, Espoo, Finland). The carboxy-terminal propeptide of pro-collagen type I (PICP) was measured in plasma by enzyme-linked immunosorbent assay (Takara Biochemicals Co, Japan). The assay ranges were: 5-250µg/l for PINP, 1-50 µg/l for PIIINP and 10-640ng/ml for PICP. Assays were run in duplicate with mean values used for analysis.

**Hemodynamic data and exercise capacity:** Antihypertensive medications including β-blockers, calcium channel blockers, angiotensin-converting-enzyme inhibitors (ACEI) and nitrates were withheld for at least 12 hours prior to an exercise stress echocardiogram (ESE).
Baseline resting hemodynamic parameters included: heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP). These parameters were measured throughout the exercise and recovery phases of the ESE. Tonometric pulse wave velocity (PWV; SphygmoCor, AtCor Medical, Sydney, Australia) between carotid and femoral sites was employed to determine aortic stiffness. Cardiorespiratory fitness was assessed by indirect calorimetry (Vmax29c, SensorMedics, CA, USA), which measured maximal oxygen consumption \([\text{VO}_2\text{max} \ (\text{ml/kg/min})]\) via a closed circuit breathing system during the maximal treadmill exercise test. Exercise capacity was also estimated in metabolic equivalents (METS) based on the duration of treadmill exercise achieved using the standard equation:

\[
\text{METs} = \left\{ \text{Speed x [0.1 + (Grade x 1.8)] + 3.5} \right\}/3.5.
\]

**Exercise echocardiography:** A standard commercially available cardiac ultrasound machine (Vivid 7 General Electric Medical Systems, Milwaukee, WI) was used to perform M-mode and 2D resting echocardiograms. In addition to standard baseline parasternal and apical images, color tissue Doppler was captured for offline measurement of tissue velocity and strain parameters. Subjects underwent maximal treadmill exercise using the Bruce protocol before being re-imaged in the same parasternal and apical planes. Images were saved in raw data format for offline analysis.

**Echocardiographic data analysis:** Offline analysis was performed to assess LV wall thickness, valvular morphology and chamber volumes. Ischemia was excluded based on the absence of inducible wall motion abnormalities on a standard 16 segment model at peak stress. The modified Simpson’s biplane method was used to measure LV ejection fraction at rest and peak exercise. Conventional apical views (four-chamber, two-chamber and long-axis) in color TDI formats at rest and peak were used to obtain tissue velocity, strain and strain rate curves from the 6 basal segments with standard commercial software (Echopac, GE Vingmed). Peak systolic tissue velocity \((S_m)\), peak early diastolic tissue velocity \((E_m)\) and
peak late diastolic tissue velocity ($A_m$) were calculated from tissue velocity curves by placing a sample volume at the level of the mitral annulus. An average value from three consecutive tissue velocity curves was established for each of the six basal segments. The resultant values for the six segments were then averaged to determine mean basal longitudinal $E_m$, $A_m$ and $S_m$. Subclinical diabetic heart disease was defined as septal $E_m$ greater than one standard deviation below the normal for age based on previous age adjusted values.23

TDI strain and strain rate curves were derived from the same apical views by placing sample volumes in the mid-myocardium of the six basal segments and tracking the position of the sample volume throughout the cardiac cycle. An average peak value from three consecutive curves was used to calculate both strain and systolic strain rate. In all cases, the angle of incidence between the transducer and the wall of interest was maintained <20 degrees. Calibrated integrated backscatter (cIB) was calculated by measuring the tissue intensity of the pericardium, posterior wall and anteroseptum in a parasternal long-axis view (Echopac, GE Vingmed). cIB was calculated by subtracting mean pericardial IB intensity at end-diastole from mean IB intensity of the posterior wall and the anteroseptum which were then averaged to establish mean cIB.

**Cardiac Magnetic Resonance Imaging:** All subjects underwent CMR imaging on a 1.5T Signa HDxT scanner (General Electric Healthcare, Milwaukee, WI) using VCG gating, a dedicated 8 channel cardiac surface coil, and inspiratory breath holds. After determining the cardiac axes with localizers, vertical and horizontal long-axis and short-axis cine MRI was performed using a balanced steady state free precession sequence (FIESTA). T1 mapping was then performed in 3 standard short axis slices - basal, mid-cavity and apical. These standardized slice planes were established and maintained throughout the CMR by dividing the left ventricle into 5 equally spaced end-diastolic tomographic images being careful to exclude the LV outflow tract from the most basal slice. The 2 outermost slices, at the tip of
the LV apex and mitral annulus were then excluded; leaving 3 central slices in basal, mid-
cavity and apical positions for analysis. Slices were maintained at 10 mm in thickness at
each level. The sequence used for T1 mapping was a prototype modified Look-Locker
FIESTA technique using saturation recovery imaging. A single breath-hold at each slice
position yielded 8 images, each with a consecutively longer inversion time, upon which a
curve-fitting technique was used to generate a T1 map. The mean inversion times for the 8
images were: 100±0.4 ms, 201±8.7 ms, 302±18 ms, 967±140 ms, 1069±138 ms, 1170±136
ms, 2032±289 ms and 2895±446 with a range of 97-4300 ms. Imaging parameters were: TR
4.0 ms, TE 1.8 ms, flip angle 45°, acquisition matrix 256x160 and 38cm field of view (FOV).
A total dose of 0.1 mmol/kg of gadobenate dimeglumine (MultiHance, BraccoDiagnostics)
was administered, and 8 minutes later delayed hyperenhancement imaging was performed in
LV short axis using a standard inversion-recovery sequence with the inversion time set to null
normal myocardium (slice thickness 8 mm, acquisition matrix 256 x 160, 40cm FOV, NEX =
2). The T1 mapping sequence was repeated in the 3 previously determined slice positions, 12
minutes after contrast.

Prototype Vizpack software (General Electric Healthcare) was used to analyze the T1 maps
using a 16 segment model.25 The epicardial and endocardial borders of the LV were
manually traced on each short-axis slice taking care to avoid any non-myocardial structures
including blood pool. Using the anterior insertion point of the right ventricle into the septum
as a reference point, the myocardium was then divided into 6 (basal and mid-cavity) or 4
(apical) equal segments. Exponential recovery curves of signal intensity were then created on
a pixel by pixel basis to extrapolate the T1 time for each of the 16 segments [Figure 1].
Segments were visually graded as good, acceptable and poor by two observers (C.J. and
J.W.). Poor segments were deemed to be non-evaluable and were excluded from subsequent
analysis. Segmental T1 values were combined for each slice to give basal, mid-cavity and
apical mean T1 values, which were then averaged to determine the mean myocardial T1. This process was performed on the T1 maps acquired before and after contrast administration. Heart rate (HR) is a documented physiologic covariate of T1 values due to imaging dependency on ECG triggering.26 Hence, HR and other predetermined potential covariates (weight, height, renal function and contrast dose) were screened as predictors of post-contrast T1 values using univariable linear regression analysis. T1 values were corrected for established covariates prior to data analysis.

Statistical analysis: Results are expressed as mean ± standard deviation, median and interquartile range (IQR) or subject number and percentage. The normality of continuous data was verified using a Kolmogorov-Smirnov test. Analysis of normally distributed variables was performed with Pearson bivariate correlations whilst Spearman correlations were used for nonparametric variables. Analysis between defined categorical groups was performed using Student’s independent t test for parametric continuous variables, Wilcoxon test for nonparametric continuous variables and X² for categorical variables. Independent associations were sought with linear and logistic regression models of independent variables. Candidate variables for the models were selected on clinical grounds, guided by univariable correlation with p<0.10 and the absence of co-linearity. Statistical analysis was performed using standard statistical computer software (SPSS 17, SPSS Inc., Chicago, Illinois). A p value of 0.05 was deemed to be statistically significant.

Results

Subject characteristics: All subjects had a resting ejection fraction of >60% (mean 64±7%) and no evidence of inducible ischemia, pre-existing infarction or hemodynamically significant valvular disease. HR was the only variable, of the predetermined clinically
relevant factors, noted to be a significant covariate of post-contrast T1 values in our study population ($\beta=-0.31$, $p=0.012$). Hence, post-contrast T1 values were corrected for HR and used for subsequent data analysis. All subjects remained in sinus rhythm throughout the testing protocol and had normal renal function. BNP levels were $<100\text{pg/mL}$ in all subjects, with 85% of subjects having levels below the lower detection limit of the assay range ($<50\text{pg/mL}$). Subject characteristics are listed in Table 1.

**Clinical and biochemical correlates of abnormal function:** When subjects were stratified into normal and abnormal $E_m$ groups, both quantitative imaging parameters of myocardial signal intensity were significantly different between groups [Table 2]. Patients with DCM demonstrated higher (less negative) cIB and shorter post-contrast T1 values [Figure 2].

Both groups had similar mean age, blood pressure, renal function, central adiposity (waist:hip circumference ratio) and lipid profile. Abnormal subjects were heavier, had lower exercise capacity and lower insulin sensitivity with a trend towards a higher HbA1c. There was no significant association between abnormality and duration of diabetes, which suggests that glycemic control may be a more important factor in the pathophysiology of DCM than duration of diabetes [Table 3]. Ejection fraction was preserved in both groups, although $S_m$ was lower in the abnormal subjects along with a trend towards reduced strain perhaps reflective of a mild degree of coexistent early systolic dysfunction [Table 3]. Overall, 69% of subjects were hypertensive or normotensive on antihypertensive therapy however this did not differ between groups. Importantly, subjects with DCM did not demonstrate features of long-standing hypertensive heart disease, with similar LV mass to the normal subjects and no difference in arterial stiffness when measured by aortic pulse wave velocity. There was also no difference between groups with respect to: smoking history, antihypertensive use (including ACEI or angiotensin 2 receptor blockers [AR2B]), HMG-CoA reductase inhibitor therapy or anti-hyperglycemic agents (including metformin or insulin). Pro-collagen
biomarker levels (including PINP, PIIINP and PICP) were not different when stratified according to normal or abnormal \( E_m \) groups [Table 3]. On multivariable logistic regression analysis of clinically relevant structural imaging parameters from Table 2, cIB was a better independent predictor of septal \( E_m \) (OR=1.20, \( p=0.029 \)) than post-contrast T1 (OR=0.97, \( p=0.13 \)).

**Myocardial T1 Values:** Pre- and post-contrast T1 values are given in a 16 segment model in basal, mid-cavity and apical short-axis slices [Figure 3] for the complete study population. Both data-sets had a relatively similar spread of results across all 16 segments which compared favorably to those previously published.\(^{26} \) Both pre- and post-contrast T1 values were normally distributed. Pre- and post-contrast sequences each yielded 201 maps (each comprising 1072 segments). The mean pre-contrast T1 was 830±168 ms (range 600-1569 ms) and the mean post-contrast T1 was 434±64 ms (range 338-686 ms). Correction for HR reduced the spread of data with a corrected mean T1 value of 434±20 ms (range 388-472 ms). Uniformity of signal intensity was variable and dependent on segment location. Post-contrast, 86% of segments were graded as good (920 segments), 8% acceptable (82 segments) and 6% poor (70 segments). Non-evaluable segments occurred most frequently in the postero-lateral region (69% of all excluded segments), especially in the apical slice, where ventricular motion artifacts were greatest. Regional delayed enhancement on post-contrast imaging was noted in two subjects. Both subjects demonstrated a small region of delayed enhancement at the insertion point of the right ventricle into the basal infero-septum. There were no subjects with regional delayed enhancement in a typical coronary distribution.

**Correlates of post-contrast T1 values:** Post-contrast T1 values were related to several markers of metabolic control including: central adiposity (waist:hip circumference ratio), blood pressure, exercise capacity and insulin sensitivity. A link between diastolic dysfunction and post-contrast T1 was established with a trend noted between T1 values and calibrated
integrated backscatter which was of borderline statistical significance [Table 1]. On separate segmental analysis, septal \( E_m \) demonstrated the strongest relationship with post-contrast T1 values \((r=0.31, p=0.011)\). [Figure 4] On multivariable analysis, independent associates of post-contrast T1 values were \( E/E_m \) \((\beta=-0.31, p=0.017)\) and DBP \((\beta=-0.48, p<0.001)\) but not SBP \((\text{Model } R^2=0.30)\).

**Correlates of pro-collagen biomarkers:** All pro-collagen biomarkers failed to demonstrate a relationship with T1 values. PIIINP demonstrated an association with markers of metabolic derangement including: age \((r=0.27, p=0.028)\), BMI \((r=0.30, p=0.013)\), waist circumference \((0.31, p=0.012)\), serum triglycerides \((r=0.31, p=0.010)\) and insulin sensitivity \((r=-0.39, p=0.005)\) of which age \((\beta=0.30, p=0.024)\) and insulin sensitivity \((\text{QUICKI } \beta=-0.43, p=0.002)\) were independently related \((\text{Model } R^2=0.24)\). PIIINP was also inversely associated with exercise performance (peak HR \(r=-0.36, p=0.002\); exercise capacity in METS \(r=-0.37, p=0.002\)). A relationship was noted between PIIINP and myocardial functional and structural parameters \((\text{mean } E_m r=-0.32, p=0.008; \text{cIB } r=0.30, p=0.015)\) of which \( E_m \) was independently associated \((\beta=-0.32, p=0.008, \text{Model } R^2=0.11)\) on multivariable linear regression analysis. PINP also demonstrated an association with age \((r=0.33, p=0.007)\), waist circumference \((r=-0.29, p=0.019)\) and LV strain \((r=-0.32, p=0.009)\). However, PICP was poorly associated with metabolic function or echo parameters of DCM.

**Discussion**

Diffuse, non-ischemic fibrosis contributes to the myocardial dysfunction associated with obesity\(^{12}\), hypertension\(^{13}\) and aging\(^{27}\). The results of this study show an association between post-contrast T1 values and diastolic dysfunction \((E_m)\) in T2DM. While a direct relationship between T1 values and pro-collagen biomarkers was not shown, pro-collagen biomarkers
demonstrated a relationship with $E_m$ as well as cIB and metabolic derangement. These associations support that diffuse myocardial fibrosis may be a contributor to early DCM.

**T1 Mapping:** CMR has become the gold-standard non-invasive cardiac imaging test for assessment of a multitude of pathologies including scar, inflammation and infiltration. To date, the identification of diffuse interstitial fibrosis has been problematic. Detection of myocardial fibrosis content based on contrast volume of distribution has been recently demonstrated but in its current form remains practically difficult due to long and complicated protocols.\(^2^8\) T1 mapping theoretically provides an alternative method of diffuse fibrosis quantification which can be relatively simply incorporated into any standard contrast CMR scan without lengthy additions to total scan time. Although its use has been demonstrated and validated against biopsy in previous studies, its sensitivity in a relatively healthy population such as ours had not been tested. Despite the relatively good health of our subjects and asymptomatic nature of their myocardial dysfunction we were still able to demonstrate a relationship between myocardial dysfunction and tissue signal intensity in early DCM suggestive of underlying myocardial fibrosis. This supports the sensitivity of the T1 mapping technique in a relatively well population such as ours. Had we recruited less well subjects with more advanced DCM, we may have detected a more robust association between T1 values, myocardial function and perhaps backscatter. The relative absence of delayed enhancement in our study population is in contrast to another cohort with T2DM\(^2^9\) where clinically-requested CMR was performed in patients with cardiac symptoms. However, our study group specifically excluded individuals with either a cardiac history or a positive functional test for ischemia prior to CMR in an attempt to minimize the confounding effect of ischemic cardiomyopathy on myocardial fibrosis.

In this study, post-contrast T1 values correlated with functional markers of diastolic dysfunction typical of early DCM. This suggests that impaired LV relaxation, manifesting as
blunted diastolic tissue velocity and elevated filling pressures, may be related to myocardial fibrosis but requires further supporting histological evidence. The associations between imaging parameters, metabolic factors and pro-collagen biomarkers support the hypothesis that myocardial fibrosis in T2DM is linked to metabolic derangement. This most likely relates to increased advanced glycation end products in the setting of hyperglycemia which not only alter extracellular matrix composition (by causing collagen crosslinking and increased myocardial stiffness) but also effect enzymatic activity and impair collagen turnover.\textsuperscript{30, 31} This combination of metabolic and myocardial factors appears to clinically manifest as impaired exercise capacity, which was also associated with shorter post-contrast T1 values.

BNP levels were normal overall for the study population which is not surprising, given the asymptomatic profile of our participants. We attempted to minimize the confounding effect of hypertension on myocardial fibrosis by excluding subjects with evidence of LV hypertrophy. Although post-contrast T1 values correlated with acute measures of blood pressure, multivariable analysis demonstrated that diastolic function was independently related to post-contrast T1 values but not systolic blood pressure. There was also no relationship between post-contrast T1 values and arterial stiffness (aortic pulse valve velocity [APVW]) or LV mass as measures of chronic hypertension. Additionally, when subjects were stratified according to diastolic function there was no difference between groups with respect to blood pressure, LV mass, APWV or history of hypertension. Importantly, there was no difference between groups with respect to use of antihypertensive agents with potential antifibrotic properties such as ACEI or AR2B. There was also no association between pro-collagen biomarkers and parameters of acute or chronic hypertension in our population. These findings support that our results were independent of the effects of chronic systolic hypertension. In our study population there was an association found between insulin
sensitivity and T1 valves but not weight or lipid profile. This supports that the link between T2DM and T1 values is distinct from the effect of other potential confounding metabolic variables.

**Integrated backscatter:** Backscatter has been used in various research populations as a non-invasive measure of myocardial fibrosis and has been validated against myocardial biopsy. However, there are significant limitations (related to signal saturation) which have limited its clinical use and its application as a screening tool in pathologies such as early DCM. This may, at least in part, explain the lack of correlation between backscatter results and T1 values. Although both techniques measure tissue signal saturation they may also be measuring separate tissue signals from different elements of the fibrotic myocardial skeleton or other structural components within the myocardium. Therefore directly comparing the two techniques may not yield the same results. However, both modalities maintain relationships with $E_m$ which suggests that regardless of the specific cause of the increased signal intensity, the myocardial structural factors are directly related to the functional impairment detected in DCM. These structural factors may represent different elements of myocardial extracellular matrix deposition. T1 mapping may prove to be a more reliable tool for the detection of underlying diffuse fibrosis than cIB which could lead to a greater crossover of the technique from research into clinical application.

**Pro-collagen biomarkers:** Types I and III collagen predominate in the heart and have exaggerated accumulation in DCM where type III synthesis is proportionally increased. The association between PIIINP and diastolic dysfunction in our cohort supports the negative effect of type III collagen accumulation on myocardial function even in early DCM. In contrast, type I collagen appears to have a less significant role in the etiology of subclinical diastolic myocardial dysfunction. This may be related to the evolving distribution of different collagen types between the perimysium of the muscle bundle and endomysium of the muscle
fibers as initial cardiomyocyte hypertrophy is gradually replaced with the more prominent fibrosis seen in later disease. The link between PIIINP and cIB, but not T1 values, may reflect that the cIB technique detects signal intensity of the fibrotic milieu and specifically type III collagen differently to the T1 mapping technique. The mechanistic difference between these two techniques warrants further investigation. Overall, the pro-collagen peptides did not appear to be as sensitive or clinically relevant as imaging markers in this study of early DCM.

**Limitations:** There are obvious issues with the use of a functional parameter (E_m) as a reference standard instead of histological evidence of fibrosis. However, myocardial biopsy is an invasive procedure, requires a significant degree of technical expertise, hospital resources and financial cost. This could not be ethically or practically justified in an asymptomatic population group as a screening tool for the detection of underlying interstitial fibrosis. The use of exercise stress echocardiography to exclude macrovascular ischemic heart disease in our population means that false-negative findings are possible. However, our center has both high sensitivity and specificity in stress imaging interpretation, making it unlikely that hemodynamically significant coronary lesions were underappreciated. The absence of delayed enhancement further supports this assumption. Invasive coronary angiography of this asymptomatic population could also not be justified on ethical grounds.

The number of subjects having CMR was limited by cost and scanner accessibility. Broadly applicable normal T1 values are yet to be established, with variation noted in T1 values depending on the scanner vendor and exact sequences used. Whilst previous results have shown good agreement between T1 measured by saturation recovery and inversion recovery modified Look-Locker sequences, as yet, histological validation of a saturation recovery T1 sequence such as ours has not been performed. Additionally, both histological validation studies employed gadolinium-DTPA contrast whilst our study used gadobenate.
dimeglumine which may also make direct comparison of our results with the biopsy studies
difficult. Standardization of the technique will enable greater consistency and reproducibility
of findings, allowing comparison of candidate populations and between imaging techniques.
Automatic correction for heart rate in analysis software may also create more uniformity in
measurements and allow more consistent application in the general population. Although pro-
collagen biomarkers are surrogate measures of myocardial fibrosis, there is a graded
association between levels in peripheral and coronary sinus blood which has been correlated
with myocardial collagen content\textsuperscript{33} thereby validating their use in a population such as ours
where biopsy is not feasible. This study is limited by its observational nature and hence while
associations have been found, direct causal relationships cannot be attributed. Whilst our
findings are attributable to well subjects with early subclinical DCM, the absence of
participants with more advanced T2DM means that our results cannot be generalized to all
subjects with DCM. The risk of an alpha error is acknowledged in an observational study
such as this where multiple comparisons have been performed on the one study population.

Conclusions: T1 mapping is a useful technique for the investigation of subclinical non-
ischemic heart disease in T2DM. The associations between post-contrast T1 values,
metabolic factors and myocardial dysfunction support that diffuse myocardial fibrosis may be
significant underlying mechanism of early DCM and is linked to the degree of metabolic
derangement. This process appears to be indolent and often asymptomatic in the initial
phases. Early detection may enable institution of therapies to reverse or halt the progression
of this reactive process. At the very least, identification of this underlying disease may
prompt patients and clinicians to institute lifestyle and pharmacological therapies sooner than
would otherwise have been done. Potentially this may have long-term benefits to both
morbidity and mortality. Validation of T1 mapping against myocardial biopsy in DCM would
further verify this technique. Future investigation should also examine the value of T1 mapping in guiding management.

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**Disclosures**

None.
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Table 1. Clinical, biochemical, exercise and imaging characteristics, and their correlation with post-contrast T1 values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD (n=67)</th>
<th>r value</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>60±10</td>
<td>-0.04</td>
<td>0.78</td>
</tr>
<tr>
<td>Duration of diabetes (yrs)</td>
<td>9±7</td>
<td>0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90±16</td>
<td>-0.14</td>
<td>0.27</td>
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<tr>
<td>Height (cm)</td>
<td>170±9</td>
<td>0.08</td>
<td>0.51</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31±5</td>
<td>-0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>106±12</td>
<td>-0.25</td>
<td>0.046</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>111±11</td>
<td>-0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Waist:Hip circumference ratio</td>
<td>0.96±0.08</td>
<td>-0.008</td>
<td>0.95</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140±22</td>
<td>-0.30</td>
<td>0.012</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82±10</td>
<td>-0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial stiffness - Aortic pulse wave velocity (m/s)</td>
<td>9.9±2.6</td>
<td>-0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Left ventricular mass (g)†</td>
<td>105±26</td>
<td>0.06</td>
<td>0.61</td>
</tr>
<tr>
<td>Exercise Capacity</td>
<td>9.9±3.2</td>
<td>0.50</td>
<td>0.016</td>
</tr>
<tr>
<td>VO₂ (ml/kg/min)</td>
<td>28±7</td>
<td>0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>143±11</td>
<td>-0.14</td>
<td>0.26</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>85* (IQR 74-90)</td>
<td>0.220</td>
<td>0.07</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>73±14</td>
<td>-0.08</td>
<td>0.53</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.7±0.9</td>
<td>-0.10</td>
<td>0.43</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.9±1.8</td>
<td>-0.005</td>
<td>0.97</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>8.7±2.9</td>
<td>-0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7±1.6</td>
<td>0.05</td>
<td>0.69</td>
</tr>
<tr>
<td>Insulin sensitivity (QUICKI)</td>
<td>0.32±0.03</td>
<td>0.30</td>
<td>0.037</td>
</tr>
<tr>
<td>PIGNP (µg/L)</td>
<td>39±21</td>
<td>0.10</td>
<td>0.44</td>
</tr>
<tr>
<td>PIINP (µg/L)</td>
<td>3.4±1.5</td>
<td>-0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>PICP (ng/ml)</td>
<td>249±86</td>
<td>0.02</td>
<td>0.86</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>64±7</td>
<td>0.07</td>
<td>0.60</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.88±0.25</td>
<td>0.25</td>
<td>0.046</td>
</tr>
<tr>
<td>Calibrated integrated backscatter (dB)</td>
<td>-17.1±5.0</td>
<td>-0.24</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean Eₘ (cm/s)</td>
<td>-5.1±1.4</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean Aₘ (cm/s)</td>
<td>-7.1±1.4</td>
<td>-0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>Mean Sₘ (cm/s)</td>
<td>5.7±1.2</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>E/Eₘ ratio</td>
<td>12.3±5.1</td>
<td>-0.24</td>
<td>0.049</td>
</tr>
<tr>
<td>Mean strain (%)</td>
<td>-17.8±3.3</td>
<td>0.17</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean systolic strain rate (l/s)</td>
<td>-1.3±0.2</td>
<td>0.19</td>
<td>0.13</td>
</tr>
</tbody>
</table>

†measurements derived from MRI parameters

*median value and interquartile range [IQR] given nonparametric distribution

Standard deviation [SD], estimated glomerular filtration rate [eGFR], amino-terminal propeptide of pro-collagen type I [PINP], amino-terminal propeptide of pro-collagen type III [PIINP], carboxy-terminal propeptide of pro-collagen type I [PICP].
Table 2. Comparison of myocardial diastolic function with imaging markers of fibrosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal E\textsubscript{m} (n=13)</th>
<th>Abnormal E\textsubscript{m} (n=54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pre-contrast T1 value (ms)</td>
<td>786±43</td>
<td>841±185</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean post-contrast T1 value (ms)</td>
<td>444±17</td>
<td>432±20</td>
<td>0.042</td>
</tr>
<tr>
<td>Calibrated integrated backscatter (dB)</td>
<td>-20.4±4.2</td>
<td>-16.3±4.8</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Table 3. Comparison of myocardial diastolic function with clinical and other imaging characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal E\textsubscript{m} (n=13)</th>
<th>Abnormal E\textsubscript{m} (n=54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>58±8</td>
<td>60±11</td>
<td>0.39</td>
</tr>
<tr>
<td>Duration of diabetes (yrs)</td>
<td>11±9</td>
<td>8±6</td>
<td>0.37</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84±16</td>
<td>91±15</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>28±4</td>
<td>32±5</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist:Hip circumference ratio</td>
<td>0.98±0.07</td>
<td>0.96±0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134±29</td>
<td>142±21</td>
<td>0.38</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79±13</td>
<td>83±10</td>
<td>0.19</td>
</tr>
<tr>
<td>Left ventricular mass (g)\textsuperscript{†}</td>
<td>112±22</td>
<td>103±27</td>
<td>0.27</td>
</tr>
<tr>
<td>Arterial stiffness - Aortic pulse wave velocity (m/s)</td>
<td>9±2</td>
<td>10±3</td>
<td>0.26</td>
</tr>
<tr>
<td>History of hypertension or current antihypertensive therapy</td>
<td>10 (77%)</td>
<td>36 (67%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Antihypertensive therapy</td>
<td>ACEI 5 (38%)</td>
<td>14 (26%)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>A2RB 3 (23%)</td>
<td>15 (28%)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Beta blocker 0</td>
<td>2 (4%)</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Calcium channel blocker 0</td>
<td>11 (21%)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Aldosterone Antagonist 0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Exercise Capacity</td>
<td>METS 11.7±2.9</td>
<td>9.4±3.1</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>VO\textsubscript{2} (ml/kg/min)</td>
<td>34±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>146±11</td>
<td>143±11</td>
<td>0.36</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>90* (IQR 8)</td>
<td>84* (IQR 17)</td>
<td>0.67</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>73±13</td>
<td>72±14</td>
<td>0.84</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.6±0.7</td>
<td>4.7±1.0</td>
<td>0.84</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.5±0.8</td>
<td>2.0±1.9</td>
<td>0.37</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>7.6±2.8</td>
<td>9.0±2.8</td>
<td>0.12</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0±1.1</td>
<td>7.9±1.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin sensitivity (QUICKI)</td>
<td>0.35±0.04</td>
<td>0.32±0.03</td>
<td>0.003</td>
</tr>
<tr>
<td>PINP (µg/L)</td>
<td>43±23</td>
<td>38±20</td>
<td>0.49</td>
</tr>
<tr>
<td>PIINP (µg/L)</td>
<td>3.1±1.4</td>
<td>3.5±1.5</td>
<td>0.39</td>
</tr>
<tr>
<td>PICP (ng/ml)</td>
<td>269±132</td>
<td>244±71</td>
<td>0.51</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>65±5</td>
<td>64±7</td>
<td>0.45</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.1±0.3</td>
<td>0.8±0.2</td>
<td>0.015</td>
</tr>
<tr>
<td>Deceleration time (ms)</td>
<td>204±54</td>
<td>234±58</td>
<td>0.09</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1</td>
<td>Value 2</td>
<td>p-value</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>Mean $\Delta_m$ (cm/s)</td>
<td>-7.1±1.6</td>
<td>-7.2±1.4</td>
<td>0.90</td>
</tr>
<tr>
<td>Mean $\Delta_m$ (cm/s)</td>
<td>6.2±1.3</td>
<td>5.5±1.2</td>
<td>0.050</td>
</tr>
<tr>
<td>Mean strain (%)</td>
<td>-17.8±1.9</td>
<td>-16.6±1.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean systolic strain rate (1/s)</td>
<td>-1.3±0.2</td>
<td>-1.3±0.2</td>
<td>0.75</td>
</tr>
</tbody>
</table>

†measurements derived from MRI parameters

*median value and interquartile range [IQR] given nonparametric distribution

Not applicable [NA], estimated glomerular filtration rate [eGFR], amino-terminal propeptide of pro-collagen type I [PINP], amino-terminal propeptide of pro-collagen type III [PIIINP], carboxy-terminal propeptide of pro-collagen type I [PICP], angiotensin-converting-enzyme inhibitor [ACEI], angiotensin-2-receptor blocker [A2RB].
Figure Legends

Figure 1. Post-contrast T1 maps of the basal, mid-cavity and apical left ventricle in short-axis are demonstrated for the anterior segment (MS06 or MS04) highlighted in red. An exponential recovery curve of signal intensities at different inversion times (TI) is produced to determine a post-contrast myocardial T1 value for each anterior segment (red line): basal 476ms, mid-cavity 439ms and apical 418ms. This process was repeated pre- and post-contrast for all 16 segments to determine the mean T1 value as a quantitative measure of diffuse fibrosis.

Figure 2. Characteristics of 2 typical study subjects demonstrating a Normal (left column) vs. Abnormal (right column) multimodality imaging profile - suggests abnormal pattern is related to underlying myocardial fibrosis
A. Normal mitral inflow (E/A) ratio vs. Abnormal pattern of impaired relaxation
B. Normal early diastolic tissue velocity for age (Em=7.5cm/s) vs. Abnormal diastolic tissue velocity (Em=3.5cm/s)
C. Normal calibrated integrated backscatter (cIB=-24.3dB) vs. Abnormal higher backscatter (cIB=-12.9dB)
D. Normal post-contrast T1 value (T1=468.85ms) vs. Abnormal shorter post-contrast T1 value (T1=333.81ms)

Figure 3. Pre- & post-contrast T1 values (uncorrected) listed as means ± standard deviations (in milliseconds) in a standard 16 segment model of the left ventricular myocardium.

Figure 4. Relationship between post-contrast T1 values and (A) diastolic tissue velocity [Em], (B) exercise capacity and (C) insulin sensitivity.
Post-contrast

Basal

Mid-cavity

Apical
Pre-contrast T1 values
(Mean ± standard deviation)

Post-contrast T1 values
(Mean ± standard deviation)
A. Relationship between post-contrast T1 values and Em

B. Relationship between post-contrast T1 values and exercise capacity

C. Relationship between post-contrast T1 values and insulin sensitivity
Association of Imaging Markers of Myocardial Fibrosis with Metabolic and Functional Disturbances in Early Diabetic Cardiomyopathy
Christine Jellis, Jeremy Wright, Dominic Kennedy, Julian Sacre, Carly Jenkins, Brian Haluska, Jennifer Martin, John Fenwick and Thomas H. Marwick

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