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

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

Methods and Results—Hemodynamic and metabolic data were measured in 67 subjects with type 2 diabetes mellitus (age 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 was calculated from parasternal long-axis views. T1 mapping was performed after contrast with a modified Look-Locker technique using saturation recovery images. Amino-terminal propeptides of procollagens type I and III, as well as the carboxy-terminal propeptide of procollagen type I, were assayed to determine collagen turnover. Subjects with abnormal early diastolic tissue velocity (E\textsubscript{m}) had shorter postcontrast T1 values (P=0.042) and higher calibrated integrated backscatter (P=0.007). They were heavier (P=0.003) and had worse exercise capacity (P<0.001), lower insulin sensitivity (P=0.003), and blunted systolic tissue velocity (P=0.05). Postcontrast T1 was associated with diastolic dysfunction (E\textsubscript{m} r=0.28, P=0.020; E/E\textsubscript{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\textsubscript{m} (β=−0.31, P=0.017) was independent of blood pressure and metabolic disturbance. Amino-terminal propeptide of procollagens type III was linked to diastolic dysfunction (E\textsubscript{m} r=−0.32, P=0.008) and calibrated integrated backscatter (r=0.30, P=0.015) but not T1 values.

Conclusions—The association between myocardial diastolic dysfunction, postcontrast T1 values, and metabolic disturbance supports that diffuse myocardial fibrosis is an underlying contributor to early diabetic cardiomyopathy. (Circ Cardiovasc Imaging. 2011;4:693-702.)

Key Words: diabetic cardiomyopathies • fibrosis • magnetic resonance • 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, although 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 origin of diffuse myocardial fibrosis. As such, the specific role of insulin resistance and hyperglycemia in the pathogenesis of DCM may be difficult to elucidate.

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The noninvasive 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 origin of diastolic dysfunction. Calibrated integrated backscatter (cIB) is based on the comparison of myocardial reflectivity, with the peri...
cardium or blood pool as a frame of reference. Picano et al.16 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, in which 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.17-20 By removing reliance on contrasting signal intensity between normal and abnormal myocardium, this method enables quantification of diffuse fibrosis. This has enabled postcontrast 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 transplantation population.21 Further validation of postcontrast T1 mapping against endomyocardial biopsy has also been achieved recently in nonischemic cardiomyopathy.22

Given the potential central role of fibrosis in DCM, we hypothesized that a relationship could be established between noninvasive 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 would suggest that metabolic derangement is a predisposing factor.

Methods

Subjects

We prospectively recruited 67 apparently healthy subjects with T2DM (37 men, 60±10 years old) from the hospital clinic and community. Subjects were excluded if they were pregnant or had preexisting microvascular or macrovascular complications of diabetes, known valvular, congenital or ischemic heart disease, or other significant comorbidities, including malignancy, renal failure, or significant psychiatric illness. 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 echocardiography and contraindications to CMR, such as claustrophobia or metallic implants. Subjects were analyzed as an entire study population and then stratified according to evidence of subclinical myocardial dysfunction (septal Em >1 SD below normal for age)23 into predetermined normal and abnormal Em 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 and waist-to-hip circumference ratio as a measure of central adiposity), and duration of T2DM. Serum glucose, insulin, hemoglobin A1c, creatinine, estimated glomerular filtration rate, B-type natriuretic peptide, and lipid profile were obtained before exercise after subjects had fasted for at least 8 hours and before administration of hypoglycemic agents. Insulin sensitivity was determined by the Quantitative Insulin Sensitivity Check Index (QUICKI).24

Procollagen Biomarkers

Additional peripheral venous blood samples preexercise 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 procollagen type I (PINP) and procollagen type III (PIIINP) were measured in serum by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The carboxy-terminal propeptide of procollagen type I (PICP) was measured in plasma by ELISA (Takara Biochemicals Co, Osaka, Japan). The assay ranges were as follows: 5 to 250 μg/L for PINP, 1 to 50 μg/L for PIIINP, and 10 to 640 ng/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, and nitrates, were withheld for at least 12 hours before an exercise stress echocardiogram. Baseline resting hemodynamic parameters included heart rate (HR), systolic blood pressure, and diastolic blood pressure. These parameters were measured throughout the exercise and recovery phases of the exercise stress echocardiogram. Tonometric pulse wave velocity (SphygmoCor; AtCor Medical, Sydney, Australia) between carotid and femoral sites was used to determine aortic stiffness. Cardiorespiratory fitness was assessed by indirect calorimetry (Vmax29c; SensorMedics, Yorba Linda, CA), which measured maximal oxygen consumption (V0₂ max, in mL·kg⁻¹·min⁻¹) 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 with the standard equation: METs = (Speed × [0.1 + (grade×1.8)] + 3.5)/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 2-dimensional resting echocardiograms. In addition to standard baseline parasternal and apical images, color tissue Doppler imaging was captured for offline measurement of tissue velocity and strain parameters. Subjects underwent maximal treadmill exercise with the Bruce protocol before being reimaged 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 on the basis of the absence of inducible wall motion abnormalities on a standard 16-segment model at peak stress. The modified Simpson biplane method was used to measure LV ejection fraction at rest and peak exercise. Conventional apical views (4-chamber, 2-chamber, and long-axis views) in color tissue Doppler imaging 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, Horten, Norway). Peak systolic tissue velocity (Sm), peak early diastolic tissue velocity (Em) and peak late diastolic tissue velocity (Am) were calculated from tissue velocity curves by placing a sample volume at the level of the mitral annulus. An average value from 3 consecutive tissue velocity curves was established for each of the 6 basal segments. The resultant values for the 6 segments were then averaged to determine mean basal longitudinal Em, Am, and Sm. Subclinical diabetic heart disease was defined as septal Em >1 SD below the normal for age based on previous age-adjusted values.23 Tissue Doppler imaging strain and strain rate curves were derived from the same apical views by placing sample volumes in the midmyocardium of the 6 basal segments and tracking the position of the sample volume throughout the cardiac cycle. An average peak value from 3 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 at <20 degrees. cIB was calculated by measuring the tissue intensity of the pericardium, posterior wall, and anteroseptum in a parasternal long-axis view (Echopac). Mean cIB was calculated by subtracting mean pericardial integrated backscatter intensity at end-diastole from mean integrated backscatter intensity of the posterior wall and the anteroseptum, which were then averaged to establish mean cIB.

CMR Imaging
All subjects underwent CMR imaging on a 1.5T Signa HDxT scanner (General Electric Healthcare, Milwaukee, WI) with VCG gating, a dedicated 8-channel cardiac surface coil, and inspiratory breath holds. After the cardiac axes were determined with localizers, vertical and horizontal long-axis and short-axis cine MRI was performed with a balanced steady-state free-procession sequence (FIESTA). T1 mapping was then performed in 3 standard short-axis slices: basal, midcavity, and apical. These standardized slice planes were established and maintained throughout the CMR by dividing the LV 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; this left 3 central slices in basal, midcavity, and apical positions for analysis. Slices were maintained at 10-mm thickness at each level. The sequence used for T1 mapping was a prototype modified Look-Locker FIESTA technique with saturation recovery imaging. A single breath hold at each slice position yielded 8 images, each with a consecutively longer inversion time, on which a curve-fitting technique was used to generate a T1 map. The mean inversion times for the 8 images were as follows: 100±0.4, 201±8.7, 302±18, 967±140, 1069±138, 1170±136, 2032±289, and 2895±446 ms, with a range of 97 to 4300 ms. Imaging parameters were repetition time 4.0 ms, echo time 1.8 ms, flip angle 45°, acquisition matrix 256×160, and 38 cm field of view. A total 0.1-mmol/kg dose of gadobenate dimeglumine (MultiHance; Bracco Diagnostics, Milan, Italy) was administered, and 8 minutes later, delayed hyperenhancement imaging was performed in the LV short axis with a standard inversion-recovery sequence with the inversion time set to null normal myocardium (slice thickness 8 mm, acquisition matrix 256×160, 40 cm field of view, number of excitations = 2). The T1 mapping sequence was repeated in the 3 previously determined slice positions, 12 minutes after administration of contrast.

Prototype Vizpack software (General Electric Healthcare) was used to analyze the T1 maps with a 16-segment model.25 The epicardial and endocardial borders of the LV were manually traced on each short-axis slice, with care taken to avoid any nonmyocardial 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 midcavity) or 4 (apical) equal segments. Exponential recovery curves of signal intensity were then created on a pixel-by-pixel

Figure 1. Postcontrast T1 maps of the basal, midcavity, and apical left ventricle in short-axis view 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 postcontrast myocardial T1 value for each anterior segment (red line): basal 476 ms, midcavity 439 ms, and apical 418 ms. This process was repeated before and after contrast for all 16 segments to determine the mean T1 value as a quantitative measure of diffuse fibrosis.
basis to extrapolate the T1 time for each of the 16 segments (Figure 1). Segments were visually graded as good, acceptable, or poor by 2 observers (C Jellis and JW). Poor segments were deemed to be nonevaluable and were excluded from subsequent analysis. Segmental T1 values were combined for each slice to give basal, midcavity, 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. HR is a documented physiological covariate of T1 values because of 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 postcontrast T1 values by univariable linear regression analysis. T1 values were corrected for established covariates before data analysis.

Statistical Analysis
Results are expressed as mean±SD, median and interquartile range, or subject number and percentage. The normality of continuous data was verified with a Kolmogorov-Smirnov test. Analysis of normally distributed variables was performed with Pearson bivariate correlations, whereas Spearman correlations were used for nonparametric variables. Analysis between defined categorical groups was performed with Student’s independent t test for parametric continuous variables, Wilcoxon test for nonparametric continuous variables, and χ² 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 collinearity. Statistical analysis was performed with standard statistical computer software (SPSS 17; SPSS Inc, Chicago, Illinois). P<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, preexisting infarction, or hemodynamically significant valvular disease. Of the predetermined clinically relevant factors, HR was the only variable noted to be a significant covariate of postcontrast T1 values in the present study population (β=−0.31, P=0.012). Hence, postcontrast 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. B-type natriuretic peptide levels were <100 pg/mL in all subjects, with 85% of subjects having levels below the lower detection limit of the assay range (<50 pg/mL). Subject characteristics are listed in Table 1.

Clinical and Biochemical Correlates of Abnormal Function
When subjects were stratified into normal and abnormal Em 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 postcontrast T1 values (Figure 2).

Both groups had similar mean age, blood pressure, renal function, central adiposity (waist-to-hip circumference ratio), and lipid profile. Abnormal subjects were heavier and had lower exercise capacity and lower insulin sensitivity, with a trend toward a higher hemoglobin A1c. 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD (n=67)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60±10</td>
<td>−0.04</td>
<td>0.78</td>
</tr>
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<td>Duration of diabetes, y</td>
<td>9±7</td>
<td>0.07</td>
<td>0.59</td>
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<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>
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<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>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140±22</td>
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<td>0.012</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82±10</td>
<td>−0.49</td>
<td>&lt;0.001</td>
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<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>
</tbody>
</table>

Exercise capacity
- METS                          | 9.9±3.2        | 0.30 | 0.016|
- V̇O₂max, mL·kg⁻¹·min⁻¹       | 28±7           | 0.27 | 0.03|
- Hemoglobin, g/L              | 143±11         | −0.14| 0.26|
- eGFR, mL/min                 | 85 (IQR 74–90)† | 0.220 | 0.07|
- Creatinine, mmol/L           | 73±14          | −0.08 | 0.53|
- Total cholesterol, mmol/L    | 4.7±0.9        | −0.10| 0.43|
- Triglycerides, mmol/L        | 1.9±1.8        | −0.005| 0.97|
- Fasting glucose, mmol/L      | 8.7±2.9        | −0.07| 0.59|
- HbA1c, %                     | 7.7±1.6        | 0.05 | 0.69|
- Insulin sensitivity (QUICKI)  | 0.32±0.03      | 0.30 | 0.037|
- PINP, µg/L                   | 39±21          | 0.10 | 0.44|
- PIIINP, µg/L                 | 3.4±1.5        | −0.10| 0.41|
- PICP, ng/mL                  | 249±86         | 0.02 | 0.86|
- Ejection fraction, %         | 64±7           | 0.07 | 0.60|
- E/A ratio                    | 0.88±0.25      | 0.25 | 0.046|
- Calibrated integrated backscatter, dB | −17.1±5.0 | −0.24 | 0.053|
- Mean Em, cm/s                | −5.1±1.4       | 0.28 | 0.02|
- Mean An, cm/s                | −7.1±1.4       | −0.01| 0.92|
- Mean Sm, cm/s                | 5.7±1.2        | 0.20 | 0.10|
- E/Em ratio                   | 12.3±5.1       | −0.24| 0.049|
- Mean strain, %               | −17.8±3.3      | 0.17 | 0.16|
- Mean systolic strain rate, s⁻¹ | −1.3±0.2      | 0.19 | 0.13|

**METS** indicates metabolic equivalents; eGFR, estimated glomerular filtration rate; IQR, interquartile range; HbA1c, hemoglobin A1c; QUICKI, Quantitative Insulin Sensitivity Check Index; PINP, amino-terminal propeptide of procollagen type I; PIIINP, amino-terminal propeptide of procollagen type III; and PICP, carboxy-terminal propeptide of procollagen type I.

*Measurements derived from magnetic resonance imaging parameters.
†Median value and IQR given nonparametric distribution.

both groups, although Sm was lower in the abnormal subjects, along with a trend toward reduced strain, perhaps reflective of a mild degree of coexistent early systolic dysfunction (Table 3). Overall, 69% of subjects were hypertensive or normotensive while undergoing antihypertensive therapy; however, this did not differ between groups. Importantly, subjects with DCM did not demonstrate features of long-standing hyper-
Correlates of Postcontrast T1 Values

Postcontrast T1 values were related to several markers of metabolic control, including central adiposity (waist-to-hip circumference ratio), blood pressure, exercise capacity, and insulin sensitivity (Figure 4). A link between diastolic dysfunction and postcontrast T1 was established, with a trend noted between T1 values and cIB that was of borderline statistical significance (Table 1). On separate segmental analysis, septal E\textsubscript{m} demonstrated the strongest relationship with postcontrast T1 values ($r=0.31, P=0.011$). On multivariable analysis, independent associates of postcontrast T1 values were $E/E_{m}$ ($β=-0.31, P=0.017$) and diastolic blood pressure ($β=-0.48, P<0.001$) but not systolic blood pressure (model $R^2=0.30$).

Correlates of Procollagen Biomarkers

All procollagen biomarkers failed to demonstrate a relationship with T1 values. PIIINP demonstrated an association with markers of metabolic derangement including age ($r=0.27, P=0.028$), body mass index ($r=0.30, P=0.013$), 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 ($β=0.30, P=0.024$) and insulin sensitivity (QUICKI $β=0.43, P=0.002$) were independently related (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 not between PIIINP and myocardial functional and structural parameters (mean $E_{m}$ $r=-0.32, P=0.008$; cIB $r=0.30, P=0.015$), with $E_{m}$ showing an independent association ($β=-0.32, P=0.008$, model $R^2=0.11$) on multivariable linear regression analysis. PNP 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 echocardiographic parameters of DCM.

Discussion

Diffuse, nonischemic fibrosis contributes to the myocardial dysfunction associated with obesity, hypertension, and aging. The results of the present study show an association between postcontrast T1 values and diastolic dysfunction ($E_{m}$) in T2DM. Although a direct relationship between T1 values and procollagen biomarkers was not shown, procollagen biomarkers demonstrated a relationship with $E_{m}$, 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” noninvasive 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 demonstrated recently but in its current form remains practically difficult because of long and complicated protocols. T1 mapping theoretically provides an alternative method of diffuse fibrosis quantification that can be incorporated relatively simply 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 that in the present study had not been tested. Despite the relatively
good health of subjects in the present study and the 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 the present study population is in contrast to another cohort with T2DM, in which clinically requested CMR was performed in patients with cardiac symptoms. However, the present study group specifically excluded individuals with either a cardiac history or a positive functional test for ischemia before CMR in an attempt to mini-
Table 3. Comparison of Myocardial Diastolic Function With Clinical and Other Imaging Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal E(_m) (n=13)</th>
<th>Abnormal E(_m) (n=54)</th>
<th>(P)</th>
</tr>
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<tbody>
<tr>
<td>Age, y</td>
<td>58±8</td>
<td>60±11</td>
<td>0.39</td>
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<td>Duration of diabetes, y</td>
<td>11±9</td>
<td>8±6</td>
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<td>Weight, kg</td>
<td>84±16</td>
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<td>0.14</td>
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<td>Body mass index, kg/m(^2)</td>
<td>28±4</td>
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<td>Waist-hip circumference ratio</td>
<td>0.98±0.07</td>
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<td>Systolic blood pressure, mm Hg</td>
<td>134±29</td>
<td>142±21</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>Left ventricular mass, g(*)</td>
<td>112±22</td>
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<td>0.27</td>
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<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, n (%)</td>
<td>10 (77)</td>
<td>36 (67)</td>
<td>0.47</td>
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<td>ACEI</td>
<td>5 (38)</td>
<td>14 (26)</td>
<td>0.39</td>
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<tr>
<td>AR2B</td>
<td>3 (23)</td>
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<td>(\beta) blocker</td>
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<tr>
<td>Aldosterone antagonist</td>
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<td>Exercise capacity</td>
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<tr>
<td>METS</td>
<td>11.7±2.9</td>
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<td>0.018</td>
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<td>(V_0), mL·kg(^{-1})·min(^{-1})</td>
<td>34±6</td>
<td>27±6</td>
<td>&lt;0.001</td>
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<td>Hemoglobin, g/L</td>
<td>146±11</td>
<td>143±11</td>
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<tr>
<td>eGFR, mL/min</td>
<td>90 (IQR 81)</td>
<td>84 (IQR 17)†</td>
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<td>Creatinine, mmol/L</td>
<td>73±13</td>
<td>72±14</td>
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<td>Total cholesterol, mmol/L</td>
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<td>9.0±2.8</td>
<td>0.12</td>
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<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>PNP, (\mu)g/L</td>
<td>43±23</td>
<td>38±20</td>
<td>0.49</td>
</tr>
<tr>
<td>PiNP, (\mu)g/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>Mean (A_{mv}), cm/s</td>
<td>(-7.1±1.6)</td>
<td>(-7.2±1.4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Mean (S_{mv}), 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, s(^{-1})</td>
<td>(-1.3±0.2)</td>
<td>(-1.3±0.2)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

ACEI indicates angiotensin-converting enzyme inhibitor; AR2B, angiotensin 2 receptor blocker; NA, not applicable; METS, metabolic equivalents; eGFR, estimated glomerular filtration rate; IQR, interquartile range; Hba1c, hemoglobin A1c; QUICKI, Quantitative Insulin Sensitivity Check Index; PNP, amino-terminal propeptide of procollagene type I; PiNP, amino-terminal propeptide of procollagen type III; and PICP, carboxy-terminal propeptide of procollagen type I.

*Median value and (IQR) given nonparametric distribution.

In the present study, postcontrast 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 this requires further supporting histological evidence. The associations between imaging parameters, metabolic factors, and procollagen 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 cross-linking and increased myocardial stiffness) but also affect enzymatic activity and impair collagen turnover.30,31 This combination of metabolic and myocardial factors appears to clinically manifest as impaired exercise capacity, which was also associated with shorter postcontrast T1 values.

B-type natriuretic peptide 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 postcontrast T1 values correlated with acute measures of blood pressure, multivariable analysis demonstrated that diastolic function was independently related to postcontrast T1 values, but not systolic blood pressure. There was also no relationship between postcontrast T1 values and arterial stiffness (aortic pulse valve velocity) 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, aortic pulse wave velocity, or history of hypertension. Importantly, there was no difference between groups with respect to use of antihypertensive agents with potential antifibrotic properties, such as angiotensin-converting enzyme inhibitors or angiotensin 2 receptor blockers. There was also no association between procollagen biomarkers and parameters of acute or chronic hypertension in the present study population. These findings support that our results were independent of the effects of chronic systolic hypertension. In the present study population, there was an association found between insulin sensitivity and T1 values 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 noninvasive measure of myocardial fibrosis and has been validated against myocardial biopsy.16 However, there are significant limitations (related to signal saturation) that 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, direct comparison of the 2 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.

**Procollagen Biomarkers**

Types I and III collagen predominate in the heart and have exaggerated accumulation in DCM, in which type III synthesis is increased proportionally. The association between PIIINP and diastolic dysfunction in the present 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 origin 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 endomy- sium 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 from the T1 mapping technique. The mechanistic difference between these 2 techniques warrants further investigation. Overall, the procollagen peptides did not appear to be as sensitive or clinically relevant as imaging markers in the present study of early DCM.

**Study Limitations**

There are obvious issues with the use of a functional parameter (E' Rm) as a reference standard instead of histological evidence of fibrosis. However, myocardial biopsy is an invasive procedure and 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 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. Although previous results have shown good agreement between T1 measured by saturation recovery and inversion recovery modified Look-Locker sequences, histological validation of a saturation recovery T1 sequence such as ours has not yet been performed. Additionally, both histological validation studies and used gadolinium-DTPA contrast, whereas the present 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 HR in analysis software may also create more uniformity in measurements and allow more consistent application in the general population. Although procollagen biomarkers are surrogate measures of myocardial fibrosis, there is a graded association between levels in peripheral and coronary sinus blood that has been correlated with myocardial collagen content, thereby validating their use in a population such as ours in whom biopsy is not feasible. This study is limited by its observational nature, and hence, although associations have been found, direct causal relationships cannot be attributed. Although 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 α-error is acknowledged in an observational study such as this in which multiple comparisons have been performed on a single study population.

**Conclusions**

T1 mapping is a useful technique for the investigation of subclinical nonischemic heart disease in T2DM. The associations between postcontrast T1 values, metabolic factors, and myocardial dysfunction support that diffuse myocardial fibrosis may be a significant underlying mechanism of early DCM and that it 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 in terms of 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.

**References**

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CLINICAL PERSPECTIVE

Myocardial dysfunction is common in apparently well subjects with type 2 diabetes mellitus. Myocardial fibrosis is increasingly recognized as a potential contributor to this process and has plausible connections with hyperglycemia and other metabolic factors. Noninvasive identification of myocardial fibrosis is challenging, because endomyocardial biopsy cannot be justified in asymptomatic subjects. Recently, cardiac magnetic resonance T1 mapping techniques have been developed to enable quantification of diffuse fibrosis. Other imaging parameters such as backscatter and tissue Doppler imaging (tissue velocity, strain, and strain rate) are useful in identifying subclinical myocardial structural and functional abnormalities that may be related to underlying fibrosis. Myocardial collagen turnover can also be estimated noninvasively by measuring procollagen biomarker levels in peripheral blood. We hypothesized that a relationship could be established between these noninvasive markers of myocardial fibrosis and metabolic control in type 2 diabetes mellitus. This would support fibrosis as an underlying mechanism for myocardial dysfunction and suggest that metabolic derangement is a predisposing factor. In a multimodality study of 67 subjects with type 2 diabetes mellitus, T1 values on cardiac magnetic resonance were compared with echocardiographic parameters of subclinical diabetic heart disease, procollagen biomarker levels, and metabolic control. The association between diastolic dysfunction and metabolic derangement was confirmed. We also found a relationship between myocardial diastolic dysfunction, postcontrast T1 values, and procollagen biomarkers that supports that diffuse myocardial fibrosis may be an underlying mechanism of early nonischemic diabetic cardiomyopathy.
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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