European Heart Journal - Cardiovascular Imaging (2017) **0**, 1–8
doi:10.1093/ehjci/jex190

Myocardial tissue characterization by contrast-enhanced cardiac magnetic resonance imaging in subjects with prediabetes, diabetes, and normal controls with preserved ejection fraction from the general population

Corinna Storz[1], Holger Hetterich[2], Roberto Lorbeer[2], Sophia D. Heber[1], Anina Schafnitzel[2], Hanna Patscheider[2], Sigrid Auweter[2], Tanja Zitzelsberger[1], Wolfgang Rathmann[3], Konstantin Nikolaou[1], Maximilian Reiser[2], Christopher L. Schlett[4], Florian von Knobelsdorff-Brenkenhoff[5,6], Annette Peters[7,8,9], Jeanette Schulz-Menger[5,10†], and Fabian Bamberg[1*†]

[1]Department of Diagnostic and Interventional Radiology, University of Tuebingen, Hoppe-Seyler-Straße 3, 72076 Tuebingen, Germany; [2]Institute of Clinical Radiology, Ludwig-Maximilians-University Hospital, Marchioninistraße 15, Munich 81377, Germany; [3]Department of Biometry and Epidemiology, German Diabetes Center, Auf'm Hennekamp 65, Duesseldorf 40225, Germany; [4]Department of Diagnostic and Interventional Radiology, University Hospital Heidelberg, Im Neuenheimer Feld 400, Heidelberg 69120, Germany; [5]Department of Cardiology, Charité, Experimental and Clinical Research Center and HELIOS-Clinics Berlin-Buch Schwanebecker Chaussee 50, 13125 Berlin, Germany; [6]Department of Cardiology, Clinic Agatharied, Ludwig-Maximilians-University Munich, Norbert-Kerkel-Platz, Hausham 83734, Germany; [7]Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany; [8]Institute for Cardiovascular Prevention, Ludwig-Maximilian-University-Hospital, Pettenkoferstraäe 9, Munich 80336, Germany; [9]German Center for Cardiovascular Disease Research (DZHK e.V.), Partnersite Munich, Biedersteiner Straße 29, Munich 80802, Germany; and [10]German Center for Cardiovascular Disease Research (DZHK e.V.), Partnersite Berlin, Oudenarder Straße 16, Berlin 13347, Germany

Received 16 March 2017; editorial decision 2 July 2017; accepted 10 July 2017

Aims To characterize changes in the myocardium in subjects with prediabetes, diabetes, and healthy controls with preserved left ventricular ejection fraction (LVEF) by using cardiac magnetic resonance imaging (CMR) in a sample from the general population.

Methods and results Subjects without history of cardiovascular disease and preserved LVEF but established diabetes, prediabetes, and controls from a population-based cohort underwent contrast-enhanced CMR. Obtained parameters included left ventricular (LV) function and morphology, late gadolinium enhancement as well as T1-mapping and derivation of extracellular volume fraction (ECV) by modified Look-Locker inversion recovery for diffuse fibrosis in a subset of patients. Fibrosis volume and cell volume were calculated and LV remodelling index was calculated by dividing the LV mass by its end-diastolic volume. Among 343 subjects (56.1 ± 9.2 years, 57% males), 47 subjects were classified as diabetes, 78 as prediabetes, and 218 as controls. Haematocrit values and thus ECV parameters were available in 251 subjects. LV remodelling index was significantly higher in participants with prediabetes and diabetes, independent of body mass index (BMI), hypertension, age, and sex. ECV was decreased in subjects with prediabetes and diabetes compared with healthy controls (23.1 ± 2.4% and 22.8 ± 3.0%, both $P < 0.007$). In contrast, cell volume was significantly higher in subjects with prediabetes and diabetes as compared with controls (109.1 ± 23.8 and

† These authors contributed equally to this work.

* Corresponding author. Tel: +49 (0) 7071/29 8 66 76; Fax: +49 (0) 7071/29 58 45. E-mail fabian.bamberg@post.harvard.edu

114.9 ± 32.3 mL vs. 96.5 ± 26.9 mL, both $P < 0.03$, respectively). However, differences in ECV and cell volume attenuated after the adjustment for cardiometabolic risk factors, including age, sex, BMI, and hypertension.

Conclusion Subjects with prediabetes and diabetes but preserved LVEF had higher LV remodelling indices, suggesting early detectable changes in the disease process, while diffuse myocardial fibrosis appears to be less relevant at this stage.

Keywords myocardial fibrosis • diabetic cardiomyopathy • magnetic resonance imaging • T1-mapping • diabetes mellitus

Introduction

The current epidemic of diabetes affects many individuals in developed and developing countries and the prevalence threatens to increase continuously in the future.[1,2] Besides diabetes with the established risk for cardiovascular outcomes, there is also a substantial proportion of individuals with impaired glucose metabolism not satisfying diabetes criteria, classified as subjects with prediabetes.[3] These individuals incur an increased risk for progressing to type 2 diabetes or cardiovascular events.[4,5] One of the major risks associated with diabetes is the development of diabetic cardiomyopathy, defined as myocardial dysfunction in the absence of coronary artery disease and hypertension, which is associated with adverse outcome.[6] In this setting, hyperglycemic states trigger a series of maladaptive stimuli that result in myocardial fibrosis and collagen deposition that manifest as altered myocardial relaxation and diastolic dysfunction that can be assessed with imaging technology.[5,6] Additionally, in a sample of the general population, also other mechanisms such as hypertensive heart disease may trigger the alteration of the myocardium, leading to cardiac hypertrophy and impaired contractile function.[7]

Advanced cardiac magnetic resonance imaging (CMR) allows for detailed, non-invasive characterization of the myocardium, including assessment of systolic and diastolic function, viability, and T1 relaxation times using mapping techniques.[8] In particular, T1-relaxation times of the myocardium may represent early changes to the myocardium with respect to diffuse fibrotic deposition.[6] As such, a shorter post-contrast myocardial T1 time, assuming a higher interstitial diffuse myocardial fibrosis in patients with diabetes, seems to be associated with impaired myocardial systolic and diastolic function.[9] Also, T1-mapping parameters, such as native T1 relaxation, quantification of the myocardial partition coefficient λ, or extracellular volume fraction (ECV) provide valuable indices for the assessment of diffuse myocardial fibrosis.[10–12] In addition, the use of contrast-enhanced T1 mapping enables derivation of the myocardial intracellular compartment, including cardiac cell volume.[13,14] While these changes are well known in subjects with impaired left ventricular (LV) ejection fraction (EF), CMR may provide more detailed assessment in subjects particularly in whom left ventricular ejection fraction (LVEF) is preserved.[15]

The objective of the present study was to characterize myocardial tissue by using a comprehensive contrast-enhanced CMR protocol in subjects with prediabetes, diabetes and controls without known cardiovascular disease and preserved LVEF in a sample from the general population. Our hypothesis was, that CMR allows for characterization of differences in myocardial tissue composition among subjects with prediabetes, diabetes, and healthy controls.

Methods

Study design

The study was designed as a case control study nested in a prospective cohort from the Cooperative Health Research in the Region of Augsburg (KORA).

Subjects and recruitment procedure

Details on the study design and protocol have been provided elsewhere.[16] Briefly, subjects were recruited from the FF4 follow-up of the KORA S4 study (second follow-up 2013/14 and baseline survey 1999–2001, respectively), a large sample from the general population in the region of Augsburg, Germany.[17] Eligible subjects without prior cardiovascular disease were enrolled in a CMR sub-study, if they had no contraindications to either CMR or gadolinium contrast administration. The study was approved by the institutional review board of the medical faculty of Ludwig-Maximilian University Munich and all participants provided written informed consent.

Covariates

Subjects of the KORA S4 cohort were re-examined between June 2013 and September 2014 at the KORA study centre.[16] An oral glucose tolerance test (OGTT) was administered to all participants and established definitions of diabetes and prediabetes were applied.[18] Specifically, prediabetes was defined as impaired glucose tolerance and/or impaired fasting glucose, healthy controls were defined as normal glucose metabolism by OGTT.[19]

Blood pressure was measured in the course of the KORA health study examinations, independent of the time of CMR acquisition. Systolic and diastolic blood pressure (BP) measurements were obtained three times at the right arm of seated participants after a 5-min resting period. The resting period between readings was 3 min. An oscillometric digital BP monitor (HEM-705CP, Omron Corporation, Tokyo, Japan) was used and one of two cuff sizes was applied according to the circumference of the participant's arm. The mean of the second and third BP measurements was used for the present analyses. Hypertension was defined as increased systolic blood pressure ≥140 mmHg, increased diastolic blood pressure ≥90 mmHg or use of antihypertensive medication under awareness of having hypertension. Other established risk factors were collected in standardized fashion as part of the KORA study design and described elsewhere.[17]

In a subset of subjects, blood samples were taken at the time of the examination for the calculation of volume parameters.

Cardiac magnetic resonance imaging

The CMR protocol was embedded in a comprehensive, whole-body exam using a 3 T Magnetom Skyra (Siemens AG, Healthcare Sector, Erlangen, Germany) and details have been described elsewhere.[16] The

cardiovascular imaging protocol comprised cine steady-state free precession (SSFP) sequences in the short axis covering the left ventricle and a four-chamber view followed by pre- and post-contrast T1 ECG-gated steady-state free-precession-based modified Look-Locker inversion-recovery technique (MOLLI) with 5(3)3 pattern (acquiring 5 images after the first inversion, followed by a 3 heartbeat pause and then acquire 3 images after the second inversion)[20] and late gadolinium enhancement (LGE) sequences 10 min after administration of gadopentetate dimeglumine (0.2 mmol/kg, Gadovist, Bayer Healthcare, Berlin, Germany).[21]

The MOLLI sequences were acquired on short axis at the mid-ventricular and basal short-axis plane before and 10 min after the application of contrast agent, using the following parameters: Slice thickness 8 mm, spatial resolution: 1.5 × 1.5 mm^2, acquired voxel size: 2.25 × 1.5 mm^2, FOV: 323 × 380 mm using a 256 × 144 mm matrix, TE: 1.1, TI: 100–3500 with a 35° flip angle (*Figure 1*). T1 relaxation times were calculated per segment (1–12 of the 17 segments of AHA classification[22]) LGE was acquired on Fast Low Angle Shot (FLASH) inversion recovery sequences with the following parameters: Slice thickness 8 mm, FOV 300 × 360 mm, Matrix 256 × 140, TR 700–1000 ms, TE 1.55 ms, FA 20–55°.

All analyses were performed in blinded fashion by independent readers unaware of the diabetic status and clinical covariates on dedicated offline workstations.

Left ventricular function

Cine SSFP sequences were evaluated semi-automatically (analysis performed by A.S.) using commercially available software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada). Following automatic contour detection of the LV endocardium, all borders were corrected manually, if necessary (100% of cases). Established LV volumetric data were derived according to current guidelines excluding the papillary muscles.[23] The LV remodelling index was obtained by calculating the ratio of the LV mass to the LV end-diastolic volume.[12]

Late gadolinium enhancement

Analysis of LGE was performed visually in short-axis stack and a four-chamber view by two experienced readers (HH, TZ) using commercially available software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada) for the presence and distribution pattern (sub-endocardial, mid-myocardial, and epicardial) of LGE using the 17-segment model of the American Heart Association (AHA).[22] In case of disagreement a consensus reading including a senior investigator was performed.

T1-mapping

The myocardium of the LV (inner and outer contour) was segmented in the basal slice and the mid-ventricular slice of the short-axis images (*Figure 1*) following recommendations to omit influence of surrounding fat or blood (AS, HP). Segments affected with obvious artifacts or presence of LGE were omitted in order to avoid distortions of the measured values.[24,25] The myocardium was divided in a total of 17 segments using AHA classification;[22] measurements were obtained in segment 1–12 (basal and mid-ventricular level) in the acquired short-axis images.[22] Another region of interest was placed in the blood volume. ECV as a measure of the amount of the extracellular matrix was calculated from the pertaining T1 relaxation times native and 10 min after the application of the contrast medium by taking into account the haematocrit level. Fibrosis volume and the total cell volume were derived using the following formula:[11,13,14,24,26]

- $\Delta R1\text{myocardium} = 1/T1_{\text{myo-post}} - 1/T1_{\text{myo-pre}}$
- $\Delta R1\ \text{blood} = 1/T1_{\text{blood-post}} - 1/T1_{\text{blood-pre}}$
- Myocardial partition coefficient (λ) = ($\Delta R1\text{myocardium}/\Delta R1$ blood)
- ECV = (1 - haematocrit level) × λ
- Fibrosis volume = ECV*LVmass/myocardial density [myocardial density = 1.05 g/mL]
- Cell volume = ((1 - ECV)*LVmass)/myocardial density

Statistical analysis

Subject demographics, cardiovascular risk factors and CMR outcomes are presented as arithmetic means and standard deviations for continuous variables and counts and percentages for categorical variables. Differences in baseline characteristics according to diabetes status groups were evaluated by one-way ANOVA and χ^2 test, respectively, with Bonferroni-adjustment. Differences between study sample and excluded subjects according to age, sex, and body mass index (BMI) were tested by multivariable logistic regression.

To assess the association between diabetes status and parameters of diffuse myocardial fibrosis, a linear regression model crude and subsequently adjusted for age, sex, BMI, and hypertension was calculated. Diabetes status entered the model as a categorical variable with the three levels control, prediabetes, and diabetes with the control group as the reference group. For each outcome, predicted means with 95% confidence intervals were calculated. For LGE, predicted proportions with

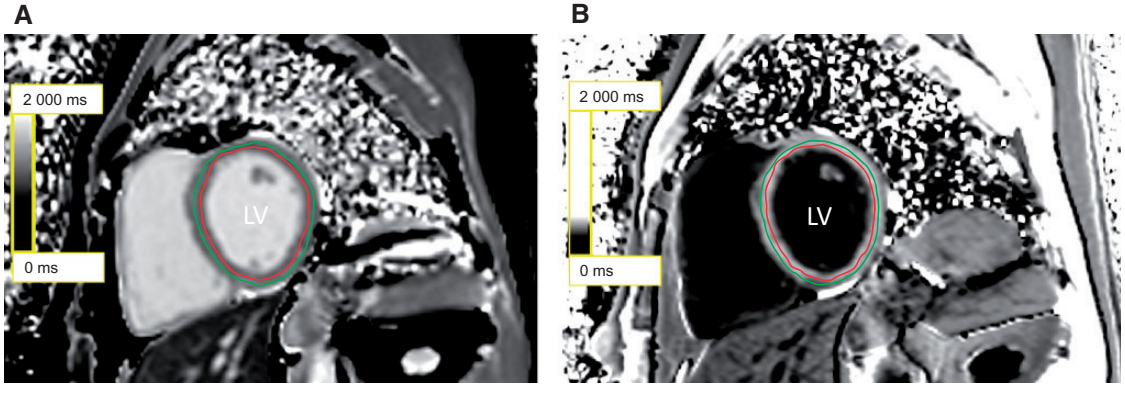

Figure 1 Pre- and Post-contrast T1 mapping of the myocardium. Mid-ventricular short axis T1 maps of the myocardium (*A*) prior and (*B*) post-administration of gadolinium, acquired with modified Look-Locker inversion recovery sequence (MOLLI) in a 54 years old patient with diabetes.

95% confidence intervals were estimated by penalized logistic regression to reduce bias due to rare events.[27] In sensitivity analyses, all multivariable analyses were further adjusted for sampling weights considering differences in age, sex, and diabetic status between the study sample and the entire KORA cohort with no substantially changed findings.

P-values <0.05 were considered to indicate statistical significance. Analyses were conducted with Stata 14.1 (Stata Corporation, College Station, TX, USA).

Results

Among 400 subjects enrolled in the study, the cardiovascular protocol was completed in 368 subjects. After excluding one subject with T1 mapping affected by artefacts in all segments, 17 subjects with incomplete measurements for LV functional parameter and 7 subjects with LVEF < 50%, 343 subjects were included in the analysis; 78 subjects were classified as having prediabetes and 47 subjects had established diabetes mellitus (23 and 14%, respectively). Haematocrit values and thus ECV parameters were available in 251 subjects (184 controls, 39 subjects with prediabetes and 28 subjects with diabetes, respectively), who were younger and less obese than the overall cohort ($P = 0.04$ and $P = 0.01$, respectively).

Demographic and risk factors according to diabetes status

Demographic and risk profiles of the study participants are provided in *Table 1*. The population were predominantly middle aged males (mean age: 56.1 ± 9.2 years, 57% male). Diabetes status was associated with male sex and age ($P = 0.006$ and $P < 0.001$, respectively) and subjects with prediabetes and diabetes had significantly higher BMI than controls (29.9 ± 4.4 and 30.0 ± 5.0 kg/m^2 vs. 26.7 ± 4.2 kg/m^2, both $P < 0.001$, respectively). Prevalence of hypertension was significantly higher in subjects with prediabetes or diabetes as compared with healthy controls (44 and 70% vs. 22%, both $P < 0.001$).

Characterization of myocardial tissue by CMR

Cardiovascular findings stratified by prediabetes, diabetes and controls are provided in *Table 2*. The end-diastolic volume index was significantly lower in prediabetes and diabetes as compared with healthy controls (61.0 ± 12.3 and 57.1 ± 12.6 mL/m^2 vs. 69.9 ± 14.5 mL/m^2; both $P < 0.001$). Due to decreased end-diastolic LV volume ($P < 0.001$) and elevated LV mass in patients with prediabetes and diabetes compared with the control group ($P = 0.97$ and $P = 0.05$, respectively), LV remodelling index was significantly higher in participants with prediabetes and diabetes as compared with controls (1.21 ± 0.27 and 1.37 ± 0.38 g/mL, vs. 1.03 ± 0.24 g/mL, both $P < 0.001$) and elevated in subjects with diabetes as compared with subjects with prediabetes ($P = 0.01$).

Overall prevalence of subjects with LGE was low but significantly higher in subjects with prediabetes and diabetes as compared with controls (7 and 5%, vs. 2%, $P = 0.02$ and $P = 0.009$, respectively). LGE was detected in 26 segments of the overall 8 subjects with presence of LGE. LGE was detected in the sub-endocardial and mid-myocardial wall layer or transmurally (6/8, 75%) whereas subepicardial or exclusive mid-myocardial enhancement was detected in 25% (2/8). There was no difference with respect to native T1 relaxation times or the myocardial partition coefficient λ between subjects with prediabetes, diabetes, and healthy controls (all $P > 0.05$; *Table 2*).

ECV was decreased in subjects with prediabetes and diabetes as compared with controls (23.1 ± 2.4 and 22.8 ± 3.0% vs. 24.2 ± 2.8%, $P = 0.007$ and $P = 0.003$, respectively). In addition, there was no difference in fibrosis volume between subjects with prediabetes, diabetes, and controls. In contrast, cell volume was significantly higher in subjects with prediabetes or diabetes as compared with controls (109.1 ± 23.8 and 114.9 ± 32.3 mL vs. 96.2 ± 26.9 mL, $P = 0.03$ and $P = 0.003$, respectively; *Figure 2*).

Among available covariates, BMI and hypertension were predictive for ECV (β: -0.23, 95% CI: -0.30 to -0.16 and β: -1.25, 95% CI: -2.00 to -0.51, respectively) and cell volume (β: 2.35, 95% CI: 1.66–3.03 and β: 14.06, 95% CI: 6.81–21.31).

After adjusting for age and sex, the diabetes group maintained a significantly higher cell volume (adjusted mean: 109.8, 95% CI: 102.3–117.4) as compared with controls (adjusted mean: 98.4, 95% CI: 95.5–101.3, $P = 0.006$; *Table 3*). After further adjustment for hypertension and BMI, the LV remodelling index remained significantly higher in participants with prediabetes and diabetes (adjusted mean: 1.17, 95% CI: 1.11–1.23 and adjusted mean: 1.28, 95% CI: 1.20–1.36, respectively) compared with healthy controls (adjusted mean: 1.06, 95% CI: 1.03–1.10; *Table 3*). No adjusted differences among diabetes status groups could be observed for ECV, fibrosis volume, cell volume, and LGE.

Discussion

We performed a comprehensive CMR protocol in subjects with prediabetes, diabetes, and normal controls and preserved LVEF fraction from the general population. Our results indicate that subjects with prediabetes and diabetes have an increased LV remodelling index as compared with controls.

Diabetes status as well as hypertensive heart disease and obesity are correlated with myocardial fibrosis, concentric LV remodelling and hypertrophy of the left ventricle, which may lead to systolic or diastolic dysfunction.[7,28] There is a large body of evidence that even prediabetes is associated with mild diastolic dysfunction and cardiac hypertrophy due to abnormal glucose metabolism.[5,29,30]

In a cohort free of clinical cardiovascular disease, Heckbert *et al.*[31] found that diabetes was associated with increased LV mass (3.5 g, 95% CI: 1.2–5.8), and lower LVEF (-0.8%, 95% CI: -1.5 to -0.2). Furthermore, Velagaleti *et al.*[29] showed that concentric LV remodelling index is significantly increased in subjects with prediabetes and diabetes, however, after inclusion of BMI, these associations were attenuated. In contrary, Shah *et al.*[32] demonstrated an association among insulin resistance, central obesity and concentric LV remodelling across BMI, independent of metabolic risk factors. These results support the assumption that even prediabetes status mediates concentric LV remodelling independently of BMI. We confirm these observations of significantly higher values of the LV remodelling index in subjects with prediabetes and diabetes compared with healthy controls, induced by increased LV mass and decreased end-diastolic LV volume in patients with prediabetes and diabetes compared with the control group. Our study results showed that LV remodelling index

Table 1 Patient demographics and risk factors according to diabetes status

	All	Controls	Prediabetes	*P*-value	Diabetes	*P*-value	*P*-value*
	343	218	78		47		
Age	56.1 ± 9.2	54.4 ± 8.9	57.6 ± 8.8	0.02	61.6 ± 8.3	<0.001	0.04
Men	195 (56.9%)	109 (50%)	51 (65.4%)	0.06	35 (74.5%)	0.006	0.86
Body mass index (kg/m^2)	27.9 ± 4.7	26.7 ± 4.2	29.9 ± 4.4	<0.001	30.0 ± 5.0	<0.001	1.00
Hypertension	114 (33.2%)	47 (21.6%)	34 (43.6%)	<0.001	33 (70.2%)	<0.001	0.01
Systolic BP (mmHg)	121 ± 17	117 ± 15	126 ± 16	<0.001	131 ± 22	<0.001	0.175
Diastolic BP (mmHg)	75 ± 10	74 ± 9	79 ± 10	<0.001	78 ± 13	0.034	1.000
HbA1c (%)	5.6 ± 0.7	5.3 ± 0.3	5.6 ± 0.3	0.01	6.7 ± 1.4	<0.001	<0.001
Diabetes duration (years)	8.4 ± 5.7				8.4 ± 5.7		
Total cholesterol (mg/dL)	217.8 ± 36.9	215.4 ± 36.1	226.8 ± 31.7	0.06	214.1 ± 45.9	1.000	0.18
Oral antidiabetic drugs	27 (7.9%)				27 (57.5%)		
Incretin-based therapy	2 (0.6%)				2 (4.3%)		
Insulin therapy	2 (0.6%)				2 (4.3%)		
Triglycerides (mg/dL)	130.9 ± 86.2	107.9 ± 64.5	157.0 ± 88.6	<0.001	194.2 ± 121.4	<0.001	0.04
Beta blockers	44 (12.8%)	14 (6.4%)	12 (15.4%)	0.05	18 (38.3%)	<0.001	0.01
ACE inhibitors	37 (10.8%)	16 (7.3%)	12 (15.4%)	0.11	9 (19.2%)	0.036	1.00
Diuretics	42 (12.2%)	19 (8.7%)	15 (19.2%)	0.04	19 (8.7%)	0.264	1.00
AT-II receptor antagonists	29 (8.5%)	18 (8.3%)	8 (10.3%)	1.00	3 (6.4%)	1.00	1.00
Calcium antagonists	25 (7.3%)	13 (6%)	7 (9.0%)	1.00	5 (10.6%)	0.744	1.00
Lipid-lowering agents	35 (10.2%)	14 (6.4%)	7 (9.0%)	1.00	14 (29.8%)	<0.001	0.01

Data are means and standard deviations for continuous variables and counts and percentages for categorical variables.
*Comparison between prediabetes and diabetes.

Table 2 CMR findings according to subjects with prediabetes, diabetes, and controls

	n	All	*n*	Controls	*n*	Prediabetes	*P*-value	*n*	Diabetes	*P*-value	*P*-value*
		343		218		78			47		
End-diastolic volume (mL/m^2)	343	66.1 ± 14.7	218	69.9 ± 14.5	78	61.0 ± 12.3	<0.001	47	57.1 ± 12.6	<0.001	0.38
LVEF (%)	343	69.6 ± 7.2	218	69.1 ± 6.7	78	71.3 ± 7.5	0.06	47	69.0 ± 8.3	1.00	0.26
Myocardial mass (g/m^2)	343	71.0 ± 13.5	218	69.9 ± 14.0	78	71.6 ± 11.2	0.97	47	75.0 ± 14.2	0.053	0.52
LV remodelling index (g/mL)	343	1.12 ± 0.3	218	1.03 ± 0.24	78	1.21 ± 0.27	<0.001	47	1.37 ± 0.38	<0.001	0.007
LGE (%)	337	8 (2.4%)	213	1 (0.5%)	78	4 (5.1%)	0.021	46	3 (6.5%)	0.009	1.00
T1 native (ms)	343	1201.4 ± 45.7	218	1202.2 ± 46.0	78	1200.4 ± 39.5	1.00	47	1199.7 ± 53.9	1.00	1.00
Partition coefficient λ	339	0.42 ± 0.04	217	0.42 ± 0.04	76	0.41 ± 0.05	0.16	46	0.42 ± 0.06	1.00	1.00
ECV (%)	251	24.2 ± 2.8	184	24.6 ± 2.8	39	23.1 ± 2.4	0.007	28	22.8 ± 3.0	0.003	1.00
Fibrosis volume (mL)	251	31.7 ± 8.2	184	31.2 ± 8.4	39	32.6 ± 6.8	1.00	28	33.5 ± 9.1	0.550	1.00
Cell volume (mL)	251	100.5 ± 27.8	184	96.5 ± 26.9	39	109.1 ± 23.8	0.03	28	114.9 ± 32.3	0.003	1.00

Data are means and standard deviations for continuous variables and counts and percentages for categorical variables.
ECV, extracellular volume; LVEF, left ventricular ejection fraction; LGE, late gadolinium enhancement; LV, left ventricular.
*Comparison between prediabetes and diabetes.

was significantly higher in subjects with prediabetes and diabetes, independent of metabolic risk factors like BMI, hypertension, sex, and age. Furthermore, recent research suggested that cardiac steatosis may be an independent predictor of concentric remodelling and systolic strain in patients with diabetes, in the absence of diffuse fibrosis in non-hypertensive patients with diabetes.[33] However, lipomatous metaplasia of the myocardium and mixture of fat and water in the same voxel may cause artefacts in widely used T1 mapping protocols and may affect interpretation of the myocardial tissue composition.[34] Considering the fact that our study population includes rather healthy subjects without prior cardiovascular diseases or impaired LV function, our findings extend current knowledge to indicate that the LV remodelling index may serve as a potential CMR based biomarker of myocardial changes even in subclinical stages of diabetes.

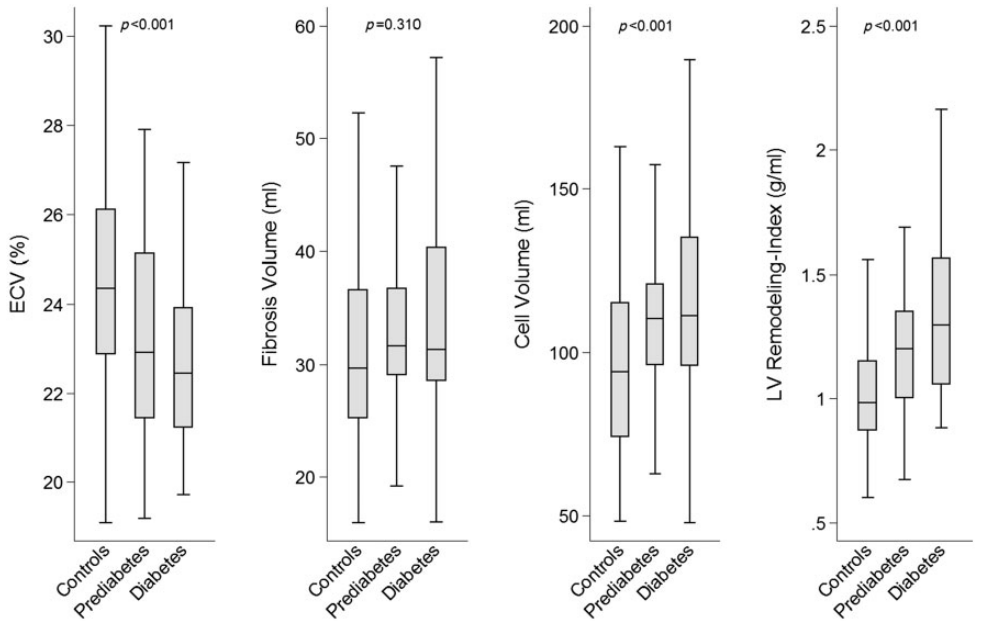

Figure 2 Differences in extracellular volume, fibrosis volume, cell volume, and left ventricular remodelling index between subjects with diabetes, prediabetes and controls. ECV, extracellular volume; LV, left ventricular.

Table 3 Multivariate adjusted differences of myocardial parameters

	n	Controls Mean (95% CI)	*n*	Prediabetes Mean (95% CI)	*P*-value	*n*	Diabetes Mean (95% CI)	*P*-value	*P*-value*
Age and sex adjusted									
ECV (%)	184	24.5 (24.1; 24.8)	39	23.5 (22.7; 24.3)	0.03	28	23.3 (22.4; 24.3)	0.038	0.85
Cell volume (mL)	184	98.4 (95.5; 101.3)	39	104.0 (97.7; 110.3)	0.11	28	109.8 (102.3; 117.4)	0.006	0.24
Fibrosis volume (mL)	184	31.6 (30.6; 32.6)	39	31.5 (29.3; 33.7)	0.93	28	32.7 (30.0; 35.3)	0.459	0.49
LV remodelling-index	218	1.05 (1.01; 1.08)	78	1.19 (1.13; 1.25)	<0.001	47	1.32 (1.24; 1.39)	<0.001	0.01
LGE (proportion, %)**	213	1.0 (0.0; 2.5)	78	5.5 (0.8; 10.3)	0.06	46	5.1 (0.0; 10.7)	0.098	0.91
Age, sex, and hypertension adjusted									
ECV (%)	184	24.4 (24.0; 24.8)	39	23.5 (22.7; 24.3)	0.05	28	23.5 (22.5; 24.5)	0.110	0.99
Cell volume (mL)	184	99.1 (96.2; 102.0)	39	103.2 (97.0; 109.4)	0.24	28	106.6 (98.7; 114.4)	0.086	0.499
Fibrosis volume (mL)	184	31.7 (30.7; 32.8)	39	31.3 (29.1; 33.5)	0.72	28	31.9 (29.2; 34.7)	0.906	0.72
LV remodelling-index	218	1.06 (1.02; 1.09)	78	1.19 (1.13; 1.24)	<0.001	47	1.29 (1.21; 1.37)	<0.001	0.04
LGE (proportion, %)**	213	1.0 (0.0; 2.5)	78	5.8 (0.9; 10.7)	0.06	46	5.7 (0.0; 12.2)	0.094	0.97
Age, sex, hypertension, and BMI adjusted									
ECV (%)	184	24.3 (23.9; 24.7)	39	23.9 (23.1; 24.7)	0.39	28	23.8 (22.9; 24.8)	0.404	0.91
Cell volume (mL)	184	100.3 (97.5; 103.0)	39	99.7 (93.8; 105.5)	0.86	28	103.7 (96.3; 111.0)	0.407	0.39
Fibrosis volume (mL)	184	31.9 (30.9; 32.9)	39	30.8 (28.6; 33.0)	0.37	28	31.5 (28.7; 34.2)	0.781	0.68
LV remodelling-index	218	1.06 (1.03; 1.10)	78	1.17 (1.11; 1.23)	0.002	47	1.28 (1.20; 1.36)	<0.001	0.03
LGE (proportion, %)[a]	213	1.0 (0.0; 2.6)	78	6.1 (0.8; 11.4)	0.06	46	6.1 (0.0; 13.4)	0.098	0.997

Predicted means after linear regression. *P*-values for β-coefficients from linear regression representing differences between diabetes groups and control group.
CI, confidence interval; BMI, body mass index; ECV, extracellular volume; LGE, late gadolinium enhancement; LV, left ventricular.
[a]Predicted proportions after penalized logistic regression with *P*-values for odds ratios representing differences between diabetes groups and control group.
*Comparison between prediabetes and diabetes.
**Predicted proportions after penalized logistic regression with *P*-values for odds ratios representing differences between diabetes groups and control group.

LGE is strongly associated with adverse cardiac events in patients with prediabetes and diabetes status.[30] Kwong *et al.*[35] found a four-fold higher risk in patients with diabetes and present LGE. Our findings are in line with these observations, as we observed a significant difference of LGE between participants with diabetes and healthy controls. Thus, LGE may confer a highly relevant predictive value with an increased risk for adverse cardiac events.

CMR-derived ECV reflects the presence and extent of myocardial fibrosis and correlates well with histological findings.[8,36] Several studies indicate that ECV levels also represent an important prognostic value for risk stratification in patients for example with diabetes mellitus, since myocardial fibrosis measured by ECV is strongly associated with hospitalization for heart failure and death.[10,11,37] There is early evidence that diabetic cardiomyopathy is associated with higher ECV levels, indicating diffuse myocardial fibrosis.[9,38,39] For instance, Wong *et al.*[37] found an association of diabetes and elevated levels of ECV. Similar observations were made by Jellis *et al.*[40] suggesting myocardial fibrosis as a contributor to the pathogenesis of diabetic cardiomyopathy. One explanation for the observed differences may be that the majority of these studies enrolled patients with prior cardiovascular diseases with a high percentage of hypertensive subjects and only a small proportion of patients with subclinical disease states such as prediabetes. Notably, our results showed inverse association between ECV and diabetes status, which attenuated after adjusting for hypertension and other metabolic risk factors. Furthermore, concomitant diseases such as hypertensive heart disease may have influenced differences in ECV, as differences in ECV values between the subgroups attenuated after the adjustment for metabolic risk factors such as hypertension and the proportion of subjects with hypertensive disease was high (70% of patients with diabetes and 44% of patients with prediabetes vs. 22% of controls, respectively). These facts may suggest that also hypertension may explain differences in ECV between subgroups, which is also in line with prior research indicating an association of significantly higher ECV values in patients with hypertensive heart disease as compared with non-hypertensive subjects.[7]

On the contrary, we found higher cell volume levels in subjects with diabetes and prediabetes as compared with healthy controls, however, this association was attenuated after adjusting for cardiometabolic risk factors, including age, sex, BMI, and hypertension. Early findings in biopsied myocardium of diabetic patients with preserved LVEF suggested that beside interstitial myocardial fibrosis, hypertrophy of myocardial cells appears to be associated with diabetes status, even in early stages of the disease.[41,42] These results indicate different stages in the development of diabetic cardiomyopathy. In a priori study, Rodrigues *et al.*[7] found concentric LV hypertrophy resulted from increased cell volume in hypertensive LV phenotypes, suggesting that rather hypertension than diabetic state is associated with increased cell volume in their population. Our results indicate that, after the adjustment for hypertension, LV remodelling index remained significantly higher in patients with diabetes and prediabetes, which indicates a detectable remodelling of the left ventricle even in patients with prediabetes, independent of metabolic risk factors such as age, hypertension, or BMI.

Again, it needs to be highlighted that subjects with diabetes and prediabetes had significantly lower ECV values compared with controls in univariate analysis. Besides the presence of confounding factors, such as hypertension and BMI, one alternative explanation may be attributed to the fact that our study population represents a western population, which is often under appropriate cardioprotective medication, particularly in subjects with diabetes. The majority of the participants were currently treated with cardioprotective medication, including antihypertensive drugs or lipid-lowering agents. As such, these may have significantly impacted on the development of myocardial changes as, for instance, inhibitors of the angiotensin-converting enzyme are associated with regression of myocardial fibrosis and lower ECV levels.[37,43] Moreover, the derived ECV measurements ranged from ~25% in the control group to ~23% in prediabetes and diabetes subjects, corresponding to relatively low ECV values when compared with other studies such as Schelbert *et al.*[10] (median of 28%, range: 17–48%) in a healthy study cohort. As a consequence, it may be assumed that our study population reflects a sample of the general, rather healthy population with preserved cardiac function, even in the diabetes subjects, which may be evident by the fact that nearly all of our subjects had an EF >50%. However, ECV and cell volume parameters were available in 251 subjects only (184 controls, 39 patients with prediabetes and 28 patients with diabetes, respectively) due to missing haematocrit values, which may limit the statistical power concerning the interpretation of the influence of changes in ECV and cell volume parameters to the development of diabetic cardiomyopathy.

This study has several limitations. First, our study cohort is limited by a relatively small sample size, particularly in subjects in whom ECV, cell volume, and fibrosis volume measurements were available, as haematocrit levels were obtained in 251 subjects only. Post-contrast T1 maps were acquired 10 min after the application of the contrast medium. While this is not in line with currently existing guidelines, which recommend post-contrast T1 map acquisition 15 min after the administration of the contrast agent,[24] the study protocol was designed prior to statement availability. Also, all study participants underwent a similar CMR protocol, which would allow for intergroup comparisons, independent of post-contrast acquisition time. Our study is the largest of its nature and our results are rather hypothesis-generating than of confirmatory nature and further research will be necessary to confirm and extend our findings.

We conclude that in subjects from a western, general population, subjects with prediabetes and diabetes have higher LV remodelling indices as compared with controls, suggesting early detectable changes in the disease process, while diffuse myocardial fibrosis appears to be less relevant at this stage. Furthermore, myocardial tissue changes in patients with hypertensive heart disease or patients with impaired glucose metabolism seem to underlie a complex mechanism, suggesting a progressive alteration of the myocardium in course of disease process. Presumably, the decrease of ECV as well as myocardial cell hypertrophy and concentric LV remodelling with reduced LV end-diastolic volume may be consistent with early disease stages, whereas increased ECV and thus an increase in fibrotic changes mostly appear at later stage of disease process.[7] However, further cohort-based confirmatory studies are clearly warranted.

Conflict of interest: None declared.

Funding

This study was funded by the German Research Foundation (DFG, Bonn, Germany), and the German Centre for Cardiovascular Disease Research (DZHK, Berlin, Germany).

References

1. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998;**21**:1414–31.
2. Collaboration NCDRF. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**:1513–30.
3. Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM *et al.* Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes Care* 2006;**29**:1263–8.
4. Danaei G, Lawes CM, Vander Hoorn S, Murray CJ, Ezzati M. Global and regional mortality from ischaemic heart disease and stroke attributable to higher-than-optimum blood glucose concentration: comparative risk assessment. *Lancet* 2006;**368**:1651–9.
5. Koncsos G, Varga ZV, Baranyai T, Boengler K, Rohrbach S, Li L *et al.* Diastolic dysfunction in prediabetic male rats: Role of mitochondrial oxidative stress. *Am J Physiol Heart Circ Physiol* 2016;**311**:H927–H43.
6. Aneja A, Tang WH, Bansilal S, Garcia MJ, Farkouh ME. Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. *Am J Med* 2008;**121**:748–57.
7. Rodrigues JC, Amadu AM, Dastidar AG, Szantho GV, Lyen SM, Godsave C *et al.* Comprehensive characterisation of hypertensive heart disease left ventricular phenotypes. *Heart* 2016;**102**:1671–9.
8. Schelbert EB, Messroghli DR. State of the art: clinical applications of cardiac T1 mapping. *Radiology* 2016;**278**:658–76.
9. Ng AC, Auger D, Delgado V, van Elderen SG, Bertini M, Siebelink HM *et al.* Association between diffuse myocardial fibrosis by cardiac magnetic resonance contrast-enhanced T(1) mapping and subclinical myocardial dysfunction in diabetic patients: a pilot study. *Circ Cardiovasc Imaging* 2012;**5**:51–9.
10. Schelbert EB, Piehler KM, Zareba KM, Moon JC, Ugander M, Messroghli DR *et al.* Myocardial fibrosis quantified by extracellular volume is associated with subsequent hospitalization for heart failure, death, or both across the spectrum of ejection fraction and heart failure stage. *J Am Heart Assoc* 2015;**4**:1–14.
11. Liu S, Han J, Nacif MS, Jones J, Kawel N, Kellman P *et al.* Diffuse myocardial fibrosis evaluation using cardiac magnetic resonance T1 mapping: sample size considerations for clinical trials. *J Cardiovasc Magn Reson* 2012;**14**:90.
12. Lamb HJ, Beyerbacht HP, de Roos A, van der Laarse A, Vliegen HW, Leujes F *et al.* Left ventricular remodeling early after aortic valve replacement: differential effects on diastolic function in aortic valve stenosis and aortic regurgitation. *J Am Coll Cardiol* 2002;**40**:2182–8.
13. Flett AS, Sado DM, Quarta G, Mirabel M, Pellerin D, Herrey AS *et al.* Diffuse myocardial fibrosis in severe aortic stenosis: an equilibrium contrast cardiovascular magnetic resonance study. *Eur Heart J Cardiovasc Imaging* 2012;**13**: 819–26.
14. Fontana M, Banypersad SM, Treibel TA, Abdel-Gadir A, Maestrini V, Lane T *et al.* Differential myocyte responses in patients with cardiac transthyretin amyloidosis and light-chain amyloidosis: a cardiac MR imaging study. *Radiology* 2015;**277**: 388–97.
15. Lee SP, Lee W, Lee JM, Park EA, Kim HK, Kim YJ *et al.* Assessment of diffuse myocardial fibrosis by using MR imaging in asymptomatic patients with aortic stenosis. *Radiology* 2015;**274**:359–69.
16. Bamberg F, Hetterich H, Rospleszcz S, Lorbeer R, Auweter SD, Schlett CL *et al.* Subclinical disease burden as assessed by whole-body MRI in subjects with prediabetes, subjects with diabetes, and normal control subjects from the general population: the KORA-MRI study. *Diabetes* 2017;**66**:158–69.
17. Holle R, Happich M, Lowel H, Wichmann HE, Group MKS. KORA–a research platform for population based health research. *Gesundheitswesen* 2005; **67**(Suppl. 1):S19–25.
18. Authors/Task Force M, Ryden L, Grant PJ, Anker SD, Berne C, Cosentino F *et al.* ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2013;**34**:3035–87.
19. World Health Organization. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia*. Geneva: World Health Organization; 2006.
20. Kellman P, Wilson JR, Xue H, Bandettini WP, Shanbhag SM, Druey KM *et al.* Extracellular volume fraction mapping in the myocardium, part 2: initial clinical experience. *J Cardiovasc Magn Reson* 2012;**14**:64.
21. Messroghli DR, Greiser A, Frohlich M, Dietz R, Schulz-Menger J. Optimization and validation of a fully-integrated pulse sequence for modified look-locker inversion-recovery (MOLLI) T1 mapping of the heart. *J Magn Reson Imaging* 2007;**26**: 1081–6.
22. American College of Cardiology Foundation Task Force on Expert Consensus D, Hundley WG, Bluemke DA, Finn JP, Flamm SD, Fogel MA *et al.* ACCF/ACR/AHA/NASCI/SCMR 2010 expert consensus document on cardiovascular magnetic resonance: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents. *J Am Coll Cardiol* 2010;**55**:2614–62.
23. Schulz-Menger J, Bluemke DA, Bremerich J, Flamm SD, Fogel MA, Friedrich MG *et al.* Standardized image interpretation and post processing in cardiovascular magnetic resonance: Society for Cardiovascular Magnetic Resonance (SCMR) board of trustees task force on standardized post processing. *J Cardiovasc Magn Reson* 2013;**15**:35.
24. Moon JC, Messroghli DR, Kellman P, Piechnik SK, Robson MD, Ugander M *et al.* Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the European Society of Cardiology consensus statement. *J Cardiovasc Magn Reson* 2013;**2013**:92.
25. von Knobelsdorff-Brenkenhoff F, Prothmann M, Dieringer MA, Wassmuth R, Greiser A, Schwenke C *et al.* Myocardial T1 and T2 mapping at 3 T: reference values, influencing factors and implications. *J Cardiovasc Magn Reson* 2013;**15**:53.
26. Everett RJ, Stirrat CG, Semple SI, Newby DE, Dweck MR, Mirsadraee S. Assessment of myocardial fibrosis with T1 mapping MRI. *Clin Radiol* 2016;**71**:768–78.
27. Firth D. Bias reduction of maximum likelihood estimates. *Biometrika* 1993;**80**: 27–38.
28. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol* 1974;**34**:29–34.
29. Velagaleti RS, Gona P, Chuang ML, Salton CJ, Fox CS, Blease SJ *et al.* Relations of insulin resistance and glycemic abnormalities to cardiovascular magnetic resonance measures of cardiac structure and function: the Framingham Heart Study. *Circ Cardiovasc Imaging* 2010;**3**:257–63.
30. Yoon YE, Kitagawa K, Kato S, Nakajima H, Kurita T, Ito M *et al.* Prognostic significance of unrecognized myocardial infarction detected with MR imaging in patients with impaired fasting glucose compared with those with diabetes. *Radiology* 2012;**262**:807–15.
31. Heckbert SR, Post W, Pearson GD, Arnett DK, Gomes AS, Jerosch-Herold M *et al.* Traditional cardiovascular risk factors in relation to left ventricular mass, volume, and systolic function by cardiac magnetic resonance imaging: the Multiethnic Study of Atherosclerosis. *J Am Coll Cardiol* 2006;**48**:2285–92.
32. Shah RV, Abbasi SA, Heydari B, Rickers C, Jacobs DR Jr, Wang L *et al.* Insulin resistance, subclinical left ventricular remodeling, and the obesity paradox: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2013;**61**:1698–706.
33. Levelt E, Mahmod M, Piechnik SK, Ariga R, Francis JM, Rodgers CT *et al.* Relationship between left ventricular structural and metabolic remodeling in type 2 diabetes. *Diabetes* 2016;**65**:44–52.
34. Kellman P, Bandettini WP, Mancini C, Hammer-Hansen S, Hansen MS, Arai AE. Characterization of myocardial T1-mapping bias caused by intramyocardial fat in inversion recovery and saturation recovery techniques. *J Cardiovasc Magn Reson* 2015;**17**:33.
35. Kwong RY, Sattar H, Wu H, Vorobiof G, Gandla V, Steel K *et al.* Incidence and prognostic implication of unrecognized myocardial scar characterized by cardiac magnetic resonance in diabetic patients without clinical evidence of myocardial infarction. *Circulation* 2008;**118**:1011–20.
36. de Meester de Ravenstein C, Bouzin C, Lazam S, Boulif J, Amzulescu M, Melchior J *et al.* Histological validation of measurement of diffuse interstitial myocardial fibrosis by myocardial extravascular volume fraction from Modified Look-Locker imaging (MOLLI) T1 mapping at 3 T. *J Cardiovasc Magn Reson* 2015;**17**:48.
37. Wong TC, Piehler KM, Kang IA, Kadakkal A, Kellman P, Schwartzman DS *et al.* Myocardial extracellular volume fraction quantified by cardiovascular magnetic resonance is increased in diabetes and associated with mortality and incident heart failure admission. *Eur Heart J* 2014;**35**:657–64.
38. Rao AD, Shah RV, Garg R, Abbasi SA, Neilan TG, Perlstein TS *et al.* Aldosterone and myocardial extracellular matrix expansion in type 2 diabetes mellitus. *Am J Cardiol* 2013;**112**:73–8.
39. Zeng M, Zhang N, He Y, Wen Z, Wang Z, Zhao Y *et al.* Histological validation of cardiac magnetic resonance T1 mapping for detecting diffuse myocardial fibrosis in diabetic rabbits. *J Magn Reson Imaging* 2016;**44**:1179–85.
40. Jellis C, Wright J, Kennedy D, Sacre J, Jenkins C, Haluska B *et al.* Association of imaging markers of myocardial fibrosis with metabolic and functional disturbances in early diabetic cardiomyopathy. *Circ Cardiovasc Imaging* 2011;**4**:693–702.
41. Nunoda S, Genda A, Sugihara N, Nakayama A, Mizuno S, Takeda R. Quantitative approach to the histopathology of the biopsied right ventricular myocardium in patients with diabetes mellitus. *Heart Vessels* 1985;**1**:43–7.
42. Fischer VW, Barner HB, Larose LS. Pathomorphologic aspects of muscular tissue in diabetes mellitus. *Hum Pathol* 1984;**15**:1127–36.
43. Brilla CG, Funck RC, Rupp H. Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. *Circulation* 2000;**102**:1388–93.