Multiparametric CMR Imaging of Myocardial Structure and Function Changes in Diabetic Mini-pigs With Preserved LV Function : A Preliminary Study

Guozhu Shao

Wuhan Union Hospital

Yukun Cao

Wuhan Union Hospital

Yue Cui

Wuhan Union Hospital

Xiaoyu Han

Wuhan Union Hospital

Jia Liu

Wuhan Union Hospital

Yumin Li

Wuhan Union Hospital

Na Li

Wuhan Union Hospital

Heshui Shi (✉ heshuishi@hust.edu.cn)

Wuhan Union Hospital <https://orcid.org/0000-0001-7644-3711>

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Abstract

Background: The purpose of this study is to dynamically monitor the myocardial structure and function changes in diabetic mini-pigs by 1.5T cardiac magnetic resonance.

Methods: Cardiac magnetic resonance (CMR) T1 mapping was performed in three male streptozotocin-induced diabetic mini-pigs. T1-mapping and ECV-mapping were acquired at basal, mid and apical segments. CMR feature-tracking (CMR-FT) is used to quantify left ventricle global longitudinal (LVGLS), circumferential (LVGCS) and radial strain (LVGRS). Epicardial adipose tissue (EAT) was evaluated using a commercially available software.

Results: Left ventricular mass (LVM), myocardial T1 value and extracellular volume (ECV) value increased gradually after 3, 4.5 and 6 months of modeling, while LVGLS decreased gradually after 3 months of modeling. *Modeling 3M VS 1.5M*: LVM 34.0 ± 1.9 VS 26.4 ± 1.3 , $P=0.027$; T1 1012.3 ± 9.6 VS 1002.2 ± 11.4 , $P=0.014$; ECV 24.3 ± 1.6 VS 22.4 ± 1.6 , $P=0.014$; GLS -20.8 ± 1.3 VS -23.0 ± 1.6 , $P=0.014$. *Modeling 4.5M VS 3M*: LVM 37.5 ± 1.3 VS 34.0 ± 1.9 , $P=0.005$; T1, 1017.8 ± 9.5 VS 1012.3 ± 9.6 , $P=0.001$; ECV 26.2 ± 1.5 VS 24.3 ± 1.6 , $P=0.037$; GLS -19.4 ± 1.4 VS -20.8 ± 1.3 , $P=0.016$. *Modeling 6M VS 4.5M*: LVM $42.9 \pm 1.6 \pm 1.9$ VS 37.5 ± 1.3 , $P=0.008$; T1 1026.6 ± 10.2 VS 1017.8 ± 9.5 , $P=0.003$; ECV 28.6 ± 1.8 VS 26.2 ± 1.5 , $P=0.016$; GLS -17.9 ± 1.1 VS -19.4 ± 1.4 , $P=0.019$. EAT did not increase significantly until the sixth month (*Modeling 6M VS 4.5M*, EAT: 24.1 ± 3.1 VS 20.2 ± 2.4 , $P=0.043$).

Conclusion: The progressive impairments in LV structure and myocardial deformation occurs in diabetic mini-pigs. T1 mapping and CMR-FT technology are promising to monitor abnormal changes of diabetic myocardium in early stage of diabetic cardiomyopathy.

Background

In 2010, the prevalence of diabetes among adults (20–79 years old) was 6.4%, reaching 285 million people. By 2030, the prevalence rate will increase to 7.7%, and the number will reach 439 million[1]. Diabetes mellitus (DM) is an important risk factor for the morbidity and mortality of cardiovascular diseases, which seriously endangers human health and aggravates the social burden. Diabetic cardiomyopathy (DCM) is a specific myocardial disease that excludes hypertension or coronary artery disease and causes cardiac structural and functional abnormalities in patients with long-term insulin resistance or hyperglycemia[2, 3]. Many pathological mechanisms promote apoptosis, necrosis and diffuse fibrosis of diabetic cardiomyocytes, and eventually lead to cardiac dysfunction and heart failure[4]. Epicardial adipose tissue (EAT) also plays a particularly important role in diabetes mellitus and cardiovascular physiology. EAT excretes several pro-inflammatory chemokines and cytokines, collectively called adipokines, been shown to impair cardiomyocyte contractile function and fat oxidation[5]. Therefore, early detection of myocardial fibrosis, cardiac dysfunction and EAT and its effective intervention is very important for the prevention and treatment of diabetic cardiomyopathy.

Cardiac magnetic resonance (CMR) is the gold standard for evaluating myocardial structure and function, and has been widely used in many diseases. T1-mapping techniques is of great importance in the diagnosis and treatment evaluation of various heart diseases for their non-invasive early quantitative and monitoring of myocardial focal and diffuse lesions. In recent years, CMR tissue tracking (CMR-FT) imaging, as a new and sensitive technique to measure myocardial deformation, has been widely used in myocardial strain analysis of different types of cardiomyopathy[6, 7], and can also provide important value for prognosis analysis of cardiomyopathy[8].

So far, the researchs on diabetic cardiomyopathy mainly focus on patients with type 2 diabetes[9]. However, patients with type 1 diabetes mellitus are better candidates because the myocardial changes associated with T1DM are the direct result of insulin deficiency and are less affected by lifestyle factors. Therefore, it is necessary to use mini-pigs to simulate human type 1 diabetes model to evaluate the effect of DM on myocardial structure and function by using a variety of advanced CMR quantitative techniques.

Methods

Experimental animals

All animal experiments were approved by the Animal Center of Tongji Medical College and were executed conforming to the “Guide for the Care and Use of Laboratory Animals” and in compliance with the ARRIVE guidelines. Three male mini-pigs, aged 5-6 months, were provided by the Laboratory Animal Center of Tongji Medical College. Type 1 DM animal models were induced by intravenous injection of streptozotocin at a dose of 150mg/kg[10]. Fasting blood glucose was continuously measured with a glycimeter at pre-determined time points. Sustaining fasting blood glucose levels > 250 mg/dL were considered as having diabetes[11]. Their weight ranged at 20-24kg at the beginning and 28-30kg at the end of this study. At 6 months, after non-invasive imaging the pigs were anaesthesia through intravenous injection of overdoses of 3% pentobarbital sodium (6 mg/kg). The pigs were sacrificed when unconscious (no pedal reflex and no blink reflex, no response to painful stimuli) using the blood-letting method for histologic analyses.

CMR scanning protocol

The pigs were anesthetized through intravenous injection of pentobarbital sodium (0.1 mg/kg.min). The pigs were free-breathing. Hematocrit level (HCT) was measured by collecting blood from ear vein before MR scanning was performed.

A balanced steady-state free precession (b-SSFP) sequence was performed to acquire left ventricular long-axis and short-axis (coverage from the base to the apex segment) cines. The parameters included: repetition time, 2.93 ms; echo time, 1.16 ms; flip angle, 80°; slice thickness, 6 mm; field of view, 340 × 255 mm²; and matrix, 256 × 205. T1 mapping technique was performed at the basal, mid and apical slices of the left ventricular and short axis before and 15 minutes after the administration of gadolinium-diethylenetriamine pentaacetic acid (DTPA) (0.2 mmol/kg, Magnevist; Bayer Healthcare; Germany) using a prototype-modified Look-Locker inversion recovery (MOLLI) sequence. The following parameters were used: repetition time, 3.89 ms; echo time, 1.12 ms; flip angle, 35°; slice thickness, 8 mm; field of view, 360 × 270 mm²; matrix, 256 × 192; PAT factor, 2; and acquisition scheme. After 15 minutes of contrast administration, postcontrast T1 maps were acquired at the same level used for the native T1 mapping. The mapping acquisition parameters are consistent with native T1 mapping acquisition parameters. The ECV mapping image is generated automatically by inputting the value of HCT through a prototype inline processing function from Siemens, based on native and post-contrast T1 mapping at the same slice and the subject-specific HCT value.

MR Data Analysis

Argus software (Syngo MMWP VE30A workstation, Siemens) was used to analyze cardiac structure and function. The basic parameters of cardiac function, including left ventricular end-diastolic volume (LVEDV); Left ventricular end-systolic volume (LVESV); Left ventricular ejection fraction (LVEF); Left ventricular mass (LVM). In order to avoid the influence of partial volume effect, the native T1 and ECV values were measured by manually sketching the the region of interest (ROI) in the interventricular septum myocardium of the left ventricular basal, middle and apical segments(Figure 1). EAT was manually traced on contiguous end-diastolic short axis slices from the base to the apex using a dedicated commercial software (CVI42; Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) (Figure 2). EAT measurement was performed according to the method described by Cai[12].

Myocardial systolic strains were analyzed and processed using commercial cardiovascular postprocessing software (Medis 3.0, Netherlands) to obtain global measurements of LV strain. All the continuous short axis films and two-,three-, and four-chamber long-axis images were imported into Medis 3.0 software offline. At the end of diastole, the left ventricular endocardial and epicardial contours were manually delineated on the short axis and the long axis respectively. The trabecular and papillary muscles were included within the endocardium (Figure 3, A-C). The left ventricle global longitudinal (LVGLS), circumferential (LVGCS) and radial strain(LVGRS) were calculated by automatically tracking the contours in each cardiac cycle.

Histological examination

At 6 months, after MR scanning, three pigs were sacrificed, and the hearts were collected and fixed in 10% formalin. After dehydration and embedding, sectioning was conducted and the corresponding part was selected in the interventricular septum myocardium, with a slice thickness of 5µm, followed by Masson stainin.

Statistical analysis

All data were statistically analyzed using standard statistical software (SPSS 21.0 for Windows, IBM, Chicago, IL, USA). The Kolmogorov–Smirnov test was used to check the normality of all continuous data. Normally distributed data and categorical variables are presented as the means $\pm$ standard deviations and frequencies (percentages). Repeated measure analysis of variance (ANOVA) was used to compare baseline and the value of the relevant index at predetermined time points. For all comparisons, statistical significance was considered at a P -value <0.05 .

Reproducibility

The LV strain of three mini-pigs were analyzed independently by two experienced observers. One observer made another analysis after 14 days. The repeatability of parameters within and between observers were calculated respectively. By calculating the inter class correlation coefficient (ICC), the repeatability of multiple measurements were analyzed.

Results

MRI characteristics and myocardial strain indexes of diabetic pigs

All the three miniature pigs were successfully modeled, and there were no statistically significant differences in LVEDV, LVESV, LVEF, LVGRS and LVGCS before and after the DCM model. After 3, 4.5 and 6 months of modeling, LVM, Native T1 and ECV values were gradually increased compared with baseline, while LVGLS decreased gradually after 3 months of modeling. *Modeling 3M VS 1.5M* LVM 34.0 ± 1.9 VS 26.4 ± 1.3 $P=0.027$ T1 1012.3 ± 9.6 VS 1002.2 ± 11.4 , $P=0.014$; ECV 24.3 ± 1.6 VS 22.4 ± 1.6 $P=0.014$ GLS -20.8 ± 1.3 VS -23.0 ± 1.6 $P=0.014$ *Modeling 4.5M VS 3M* LVM 37.5 ± 1.3 VS 34.0 ± 1.9 $P=0.005$ T1, 1017.8 ± 9.5 VS 1012.3 ± 9.6 , $P=0.001$ ECV 26.2 ± 1.5 VS 24.3 ± 1.6 $P=0.037$ GLS -19.4 ± 1.4 VS -20.8 ± 1.3 $P=0.016$ *Modeling 6M VS 4.5M* LVM $42.9 \pm 1.6 \pm 1.9$ VS 37.5 ± 1.3 $P=0.008$ T1 1026.6 ± 10.2 VS 1017.8 ± 9.5 , $P=0.003$ ECV 28.6 ± 1.8 VS 26.2 ± 1.5 $P=0.016$ GLS -17.9 ± 1.1 VS -19.4 ± 1.4 $P=0.019$ (Table 1)(Figure 4A-C, E). EAT did not increase significantly until the sixth month (*Modeling 6M VS 4.5M*, EAT: 24.1 ± 3.1 VS 20.2 ± 2.4 , $P=0.043$) (Table 1)(Figure 4D).

Histological analysis of myocardial alterations

After 6 months of modeling, gross anatomy of DCM model pig heart showed left ventricular wall hypertrophy (Figure 5A, B), Masson staining of myocardium showed diffuse myocardial interstitial fibrosis (Figure 5C).

Repeatability analysis

The intraclass correlation coefficient (ICC) values of nativeT1, ECV, EAT, LVGRS, LVGCS and LVGLS measured by the same physician were 0.974, 0.957, 0.945, 0.985, 0.869 and 0.934 respectively. The ICC values of nativeT1, ECV, EAT, LVGRS, LVGCS and LVGLS were 0.969, 0.973, 0.948, 0.981, 0.893 and 0.926 respectively, measured by two different physicians.

Discussion

This animal model simulates the type I diabetic model of human disease, the advantages of this model not only avoid the effect of various confounding factors in patients on diabetic cardiomyopathy, such as age, the course of the disease, and coexisting diseases, but also obtain the imaging indicators of myocardial fibrosis by non-invasive imaging method and compare them with myocardial histological alterations to insight into the pathogenesis of DCM. The main findings of this study were that 1) When 3 months of diabetic model, a longer duration of diabetes resulted in a higher myocardial T1 value, ECV and LVM, while left ventricular GLS decreased with the prolongation of modeling time, and 2) at 6 months, abnormalities were observed in EAT compared with before modeling.

T1 mapping can early monitor myocardial focal and diffuse fibrosis by quantifying myocardial T1 and extracellular volume values, and have been confirmed in previous histological studies[13]. At present, they have important value in the diagnosis, treatment and prognosis evaluation of heart disease. Our results showed that T1 and ECV values of type 1 DM animal models increased gradually with the prolongation of DM course after 3 months of modeling, which was consistent with the previous experimental results on rabbits[14]. There are many factors causing this myocardial damage, including many molecular mechanisms, animal studies in vivo have shown that hyperglycemia leads to cardiomyocyte apoptosis through the activation of reactive oxygen species (ROS)[15],

which in turn can induce the formation of end glycation end products and eventually lead to myocardial fibrosis[16]. Modeling for 3 months, T1 value and ECV value gradually increased with the extension of diabetes duration, which was consistent with the results of Zeng[14], indicating that the longer the course of diabetes, the higher the degree of myocardial fibrosis. One possible explanation is that long term chronic hyperglycemia may aggravate myocardial interstitial matrix expansion, and promote myocardial apoptosis and necrosis, and eventually lead to continuous myocardial injury.

In this study, it was found that the LVM was increased after modeling 1.5 month, and similar results were obtained by Nakai et al[17]. We also found that LV remodeling index (LV mass/volume ratio) was increased after modeling 4.5 month. This may be related to the concentric LV remodeling due to myocardial hypertrophy caused by diabetes[18, 19]. According to relevant reports, the pathogenesis of diabetic left ventricular hypertrophy was related to diabetic myocardial interstitial fibrosis and myocardial triglyceride accumulation[20, 21]. In our this experiment, diffuse fibrosis of myocardial interstitium has been confirmed in the histological analysis, so next we will expand our sample size, and conduct anti-myocardial fibrosis therapy in diabetic pigs to observe the histological changes of isolated heart to verify whether the above treatment can protect or reverse myocardial damage.

EAT comprises adipocytes, stromo-vascular cells, neurons, and immune cells[22, 23], it's is metabolically very active, and the secretome of EAT disrupts cardiomyocyte metabolism[24], depresses cardiomyocyte contractile function[5] and alters expression of adhesion markers of primary cardiac endothelial cells[25]. In our study, we also find the EAT in diabetic model was significantly increased when 6 months of diabetic model. It's been reported previously that the amount of EAT is increased in patients with T2DM[26] and the high level of EAT is associated with cardiac systolic dysfunction in patients with T2DM[27]. Thus, in our study, at 6 months, whether dysfunction of cardiomyocyte contractile function(LVGLS) can be ascribed to the true effect of DM or to a confounding effect of DM and EAT still requires further study.

At 3 months, CMR-FT was used to find that the value of LVGLS began to decreased, while other strain parameters remained unchanged. The myocardium consists of 3 layers[28]. The orientation of left ventricle subendocardial muscle fibers is longitudinal, corresponds to longitudinal strain, and is more susceptible to ischemia and to other mechanical, toxic, or metabolic factors[29, 30]. Thus, the value of LVGLS decreased may document the subendocardial dysfunction although the global cardiac function remains normal, as assessed by standard EF. This shows that CMR-FT is a very sensitive tool to detect subtle endocardial impairment in the early stages of DM. In addition, this study also found that the longer the course of diabetes, the lower the value of LVGLS, indicating the more serious the degree of endocardial damage. Therefore, effective reduction of blood glucose level is essential to protect the myocardium. In the following experiments, we will also conduct hypoglycemic treatment on diabetic pigs to observe whether myocardial mechanical injury can be reversed or ameliorated, so as to provide an important theoretical basis for clinical improvement of cardiac function in patients with DCM.

In this study, two limitations should be considered. First, our animal sample size was small, which greatly reduced the power of the study and did not allow us to draw generalized conclusions. We will continue to expand our sample size in our future studies on this topic. Second, Our study was unable to carry out serial histological validation evaluation at various timepoints in type 1 DM animal models because pigs are large animals and limited by many experimental conditions.

Conclusions

In summary, this study found that 3 months after modeling, LVM, myocardial T1 value and ECV gradually increased, and left ventricular GLS decreased with the continuous extension of modeling time. The duration of DM is related to the degree of myocardial damage. Whether LV systolic dysfunction can be ascribed to the true effect of DM or to a confounding effect of DM and EAT still requires further study. T1 mapping and myocardial strain analysis are promising method in detecting subtle myocardial dysfunction in the early stages of DM.

Abbreviations

DCM: diabetic cardiomyopathy; LV: left ventricle; T2DM: type 2 diabetes mellitus; CMR-FT: cardiovascular magnetic resonance feature tracking; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LAV, left atrial volume; LVGRS, left ventricular global radial strain; LVGCS, left

ventricular global circumferential strain; LVGLS, left ventricular global longitudinal strain; EAT, epicardial adipose tissue; ECV, extracellular volume; ICC: intra-class correlation coefficient.

Declarations

1. Ethics approval and consent to participate

All animal experiments were executed conforming to the 'Guide for the Care and Use of Laboratory Animals' and in compliance with the ARRIVE guidelines.

This study was approved by the Animal Center of Tongji Medical College.

2. Consent for publication

Not Applicable

3. Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

4. Competing interests

All authors declared that: there is no conflict of interest existing in the submission of this manuscript, and all authors approved the article for publication.

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6. Authors' contributions

Study designing: H-SS, G-ZS and Y-KC; data search: G-ZS, Y-KC; data extraction: G-ZS, Y-KC, YC, X-YH, JL, Y-ML and NL; data analysis and interpretation: G-ZS, Y-KC, H-SS; Manuscript drafting: G-ZS, Y-KC and H-SS; manuscript critical intellectual content revision: G-ZS, Y-KC and H-SS. All authors read and approved the final version of the manuscript.

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Tables

Table 1 MRI characteristics of the diabetic pigs

Characteristics	Modeling 0M	Modeling 1.5M	Modeling 3M	Modeling 4.5M	Modeling 6M	P values			
						0M vs 1.5M	1.5M vs 3M	3M vs 4.5M	4.5M vs 6M
LVEDV(mL/m ²)	27.8±2.5	28.3±1.7	28.8±2.2	28.4±3.8	28.6±2.1	0.423	0.696	0.702	0.850
LVESV(mL/m ²)	12.9±3.8	12.6±3.1	12.8±2.8	13.0±3.9	13.0±2.8	0.574	0.463	0.803	1.000
LVEF(%)	60.5±3.4	62.1±4.4	61.5±6.3	62.1±3.8	62.6±4.7	0.114	0.674	0.861	0.803
LVM(g/m ²)	24.9±1.7	26.4±1.3	34.0±1.9	37.5±1.3	42.9±1.6	0.102	0.027	0.005	0.008
LV remodeling index	0.8±0.1	0.9±0.1	1.0±0.1	1.4±0.6	1.6±0.1	0.478	0.184	0.010	0.035
Native T1(ms)	1002.9±9.3	1002.2±11.4	1012.3±9.6	1017.8±9.5	1026.6±10.2	0.844	0.014	□ 0.001	0.003
EAT(ml)	16.3±1.9	18.0±1.7	17.2±1.5	20.2±2.4	24.1±3.1	0.190	0.608	0.286	0.043
ECV(%)	21.8±1.9	22.4±1.6	24.3±1.6	26.2±1.5	28.6±1.8	0.103	0.014	0.037	0.016
LVGRS(%)	58.5±2.2	60.9±2.4	58.8±2.2	59.4±2.8	58.5±3.4	0.280	0.464	0.828	0.826
LVGCS(%)	-23.0±0.8	-21.8±1.5	-23.8±0.4	-22.2±1.1	-22.5±1.9	0.233	0.081	0.086	0.681
LVGLS(%)	-22.7±1.3	-23.0±1.6	-20.8±1.3	-19.4±1.4	-17.9±1.1	0.667	0.014	0.016	0.019

LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; EAT, epicardial adipose tissue; ECV, extracellular volume; LVGRS, left ventricular global radial strain; LVGCS, left ventricular global circumferential strain; LVGLS, left ventricle global longitudinal.

LV remodeling index = LV mass/volume ratio.

Figures

Figure 1

A, The middle section of the left ventricular short axis was scanned; B, T1 mapping image in mid short axis views after the mold was made; C, ECV mapping in mid short axis views after the mold was made.

Figure 2

Representative images of epicardial adipose tissue (A) and 3D model of Epicardial adipose tissue drawn by CVI software (B).

Figure 3

Representative contour of the endocardium and endocardium of the left ventricular in the short axis, 2- , 4-chamber (A, B and C, respectively).

Figure 4

Comparison of LVM (A), ECV (B), GLS (C), EAT (D), Native T1 (E) and values among the pre-determined time points. LVM, left ventricular mass; ECV, extracellular volume. GLS, global longitudinal. EAT, epicardial adipose tissue. * p<0.05, ** p<0.01, *** p<0.001.

Figure 5

A, General morphological anatomy of pig heart; B, Section anatomy in base, mid, apical short axis views were presented; C, After modeling 6M, Masson staining of the left ventricular myocardial base segment (blue = fibrosis, red = myocardial fiber).