Layer-specific myocardial strain analysis: investigation of regional deformation in a rabbit model of diabetes mellitus during different stages

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Abstract

Aim: The purpose of the present study was to determine the characteristics of myocardial damage at different stages of diabetes mellitus (DM) using layer-specific myocardial strain. **Material and methods:** Thirty six New Zealand white rabbits were randomly divided into either the control group (n=18) or the DM group (induced with alloxan) (n=18). For the myocardial deformation studies echocardiography and layer-specific strain were performed at baseline and after 3, 6, and 9 months in all of the rabbits. Three-layer longitudinal strain (LS) was calculated in the apical 4-chamber view, and three-layer circumferential strain (CS) in the short-axis view at the level of mitral valve. Layer-specific longitudinal and circumferential strains were assessed from endocardium, mid-myocardium and epicardium. For histomorphological study of the heart structure, the rabbits were sacrificed at 3, 6 and 9 months. Routine hematoxylin and eosin staining was performed. **Results:** The highest absolute values of left ventricular longitudinal strain (LS) and circumferential strain (CS) were registered in the endocardium and the lowest in the epicardium in both groups. At 3 months, there was no significant difference in three-layer LS and CS ($p>0.05$), but at 6 months the LS of endocardium (LSendo) and CS of endocardium (CSendo) were lower in the DM group compared with the control group; at 9 months, the rest of the parameters were also decreased ($p<0.05$). Moreover, in ROC analysis at 6 months LSendo yielded better sensitivity and specificity in the detection of diabetic cardiomyopathy (AUC of LSendo was 0.897 and AUC of CSendo was 0.617). With the progression of untreated diabetes, the histopathological abnormalities intensified gradually beginning at 6 months. **Conclusion:** The progressive impairments in LV myocardial deformation and structure occurs early in diabetic rabbits, the myocardial damage may be nontransmural, and endocardial function is more susceptible to be affected by DM. Layer-specific myocardial strain echocardiography may identify subtle myocardial dysfunction in the early stages of DM.

Keywords: layer-specific myocardial strain analysis, rabbits, diabetic cardiomyopathy

Introduction

Diabetes mellitus (DM) is associated with an increased risk of cardiovascular complications, including hypertension, coronary artery disease and the development of heart failure (HF) [1,2]. However, there is grow-

ing recognition of a primary myocardial disease process or “diabetic cardiomyopathy” that predisposes diabetic patients to ventricular dysfunction in the absence of clinically significant coronary, valvular, or hypertensive disease [3-4]. Diabetic cardiomyopathy (DCM) is defined as either systolic or diastolic left ventricular dysfunction.

To prevent the progression of HF in diabetic patients, a sensitive method to quantify the presence and extent of DCM is important [5]; however, the noninvasive tools for detection of early myocardial damage in diabetes are not yet well established [2].

By assessing the myocardial deformation in any direction, the myocardial strain echocardiography can

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detect myocardial damage more accurately than indicated by the ejection fraction (EF). Furthermore, newly developed layer-specific speckle tracking imaging can evaluate myocardial deformation layer-by-layer [6]. This technique could allow the noninvasive evaluation of layer-specific myocardial damage. Accordingly, the present study aimed to evaluate the extent of myocardial damage at different stages of DM by measurement of layer-specific myocardial strain.

Materials and methods

Ethics

All studies were approved by the Ethics Committee of the Beijing Anzhen Hospital and performed in accordance with international ethical standards.

Experimental animals

A total of 36 male New Zealand rabbits weighing 2.50 ± 1.34 kg were used in this study. Animals were randomly divided into a DM group ($n=18$) and a control group ($n=18$). DM was induced by a single injection of alloxan (ALX) (150 mg/kg) via the ear vein after fasting for 12 h. In the control group, animals were injected with an equal volume of normal saline (NS). Rabbits were given ad libitum access to food and water. Blood glucose was measured weekly until sacrifice (3, 6, and 9 months after the model was induced). In the DM rabbits group we defined the blood glucose values higher than 16 mmol/L for more than three successive weeks as successful models.

Echocardiographic measurements

All the rabbits underwent conventional echocardiography and layer-specific strain echocardiography at 0, 3, 6, and 9 months. The procedures were performed by the same experienced operator who was blinded to the rabbits belonging to the diabetic or control group. All of the rabbits were anesthetized by an intraperitoneal injection of diazepam at 1 mg/kg and xylazine hydrochloride at 2.5 mg/kg and placed in a supine position with removal of anterior chest hair. The scan was performed by a Vivid7 Ultrasound cardiovascular system (GE Healthcare USA) using a 10-S phased array transducer and a cardiac application. The transmission frequency was 7-10 MHz, the depth was 4-6 cm, and the frame rate was 90-100 frames/s. It is noteworthy that unlike the human heart, ultrasound scans of the rabbit heart do not suffer from artifacts due to the ribs and lungs. During rabbit parasternal short-axis scanning, the width of the probe covers two to three ribs, which do not affect the image due to their thinness. Moreover, the lungs do not impose artifacts on the scan when the rabbit is placed on its left side.

The standard two-dimensional (2D) views of the heart were obtained to evaluate the cardiac structure. At

least three consecutive heartbeats were analyzed and the means obtained. Left atrial diameter (LAD), left ventricular end-systolic and end-diastolic diameters (LVIDd and LVIDs, respectively), interventricular septum (IVS), and left ventricular posterior wall (LVPW) thickness were measured by M-mode echocardiography. Left ventricular ejection fraction (LVEF) was assessed with biplane Simpson method using the manual tracing of digital images. Routine grayscale 2D cine loops from 3 consecutive beats were obtained from standard parasternal short-axis view of the left ventricular (LV) at the level of the mitral valve and from the apical 4-chamber view.

Layer-specific myocardial strain-speckle tracking echocardiography (LS-STE)

Scans were post-processed by the LS-STE program. The program uses a commercial STE program (EchoPAC Dimension '08, GE Healthcare) that requests the user's validation of the endocardial border and the width of the myocardium. The endocardial borders were traced in the end-systolic frame of the 2D images from the apical 4-chamber view for analyses of longitudinal endocardial, mid-myocardial and epicardial strains (fig 1). Analyses of layer-specific circumferential strains were obtained from the parasternal short-axis view at the mitral valve level. Peak negative systolic longitudinal and circumferential strains (LS and CS, respectively) from 3 layers were assessed. Segments that failed to automatically track were manually adjusted by the operator.

Histomorphological study of the heart

After ultrasound evaluation, the rabbits were sacrificed. The hearts were harvested, fixed in 10% formalin,

Fig 1. Layer-specific longitudinal strain in DM rabbits (a) and age-matched control rabbits (b) at 6 months

embedded in paraffin and processed for histomorphological examination. Tissue blocks were sectioned at a thickness of 5 μ m and were stained with Harris hematoxylin and eosin (H&E).

Statistical analysis

All analyses were performed with SPSS version 17.0. Data were expressed as mean $\pm$ standard deviation (SD). Data was tested for normal distribution ($p < 0.05$). Differences in conventional echocardiographic parameters and strain echocardiographic parameters between the DM group and control group were determined with the unpaired t test. One-way analysis of variance (ANOVA) with the LSD post-hoc correction for multiple comparisons was used to test all parameters at different time points from the same group. A value of $p < 0.05$ was considered statistically significant. The areas under the receiver-operating characteristic (ROC) curves (AUC) were calculated for endocardial longitudinal strain (LSendo) and endocardial circumferential strain (CSendo) at 6 months. The value closest to the upper left corner of the ROC curves determined optimal sensitivity and specificity for the ability of LSendo and CSendo to predict the presence of DCM.

Results

Echocardiographic findings

The standard ultrasound parameters of left ventricle structure and systolic function in DM group and control group are shown in Table I. The thickness of interventricular septum and posterior wall were similar at 3, 6, and 9 months ($p > 0.05$). No significant differences in the LVIDd, LVIDs, LVEF, and LAD at any time point were registered between the two groups ($p > 0.05$); in both subgroups the aforementioned parameters remained unchanged during study ($p > 0.05$).

Myocardial deformation

The absolute values of the left ventricle LS and CS increased gradually from epicardium, mid-myocardium to endocardium (fig 2). At 3 months, there were no significant differences in the LV LS and CS in 3 myocardial layers between the DM group and control group. However, at 6 months, the absolute values of LSendo and CSendo in DM group were significantly lower than in the control group ($p < 0.05$). Moreover, at 9 months, all 3-layers parameters significantly decreased ($p < 0.05$) (Table II). In general, the myocardial function assessed by

Table I. Glucose plasmatic levels and standard cardiac ultrasound parameters of LV in the study and control group

Items	DM group				Control group			
	Baseline	3 months	6 months	9 months	Baseline	3 months	6 months	9 months
Glu (mmol/l)	5.51 $\pm$ 1.42	21.32 $\pm$ 5.41*	24.56 $\pm$ 4.62*	24.56 $\pm$ 4.62*	5.62 $\pm$ 1.65	5.28 $\pm$ 1.65	5.89 $\pm$ 1.65	5.64 $\pm$ 1.23
LA (mm)	9.56 $\pm$ 0.98	10.56 $\pm$ 0.58	9.97 $\pm$ 0.86	11.36 $\pm$ 0.82	9.95 $\pm$ 0.68	9.79 $\pm$ 0.81	10.61 $\pm$ 0.58	11.56 $\pm$ 0.67
IVS (mm)	2.32 $\pm$ 0.41	2.43 $\pm$ 0.32	2.42 $\pm$ 0.51	2.52 $\pm$ 0.62	2.63 $\pm$ 0.24	2.34 $\pm$ 0.32	2.75 $\pm$ 0.31	2.86 $\pm$ 0.63
LVPW (mm)	2.33 $\pm$ 0.51	2.56 $\pm$ 0.32	2.78 $\pm$ 0.67	2.89 $\pm$ 0.43	2.35 $\pm$ 0.19	2.67 $\pm$ 0.41	2.78 $\pm$ 0.53	2.89 $\pm$ 0.67
LVIDd (mm)	15.67 $\pm$ 0.59	16.67 $\pm$ 0.81	16.65 $\pm$ 0.78	17.67 $\pm$ 0.55	14.75 $\pm$ 0.87	15.68 $\pm$ 0.85	16.64 $\pm$ 0.54	16.98 $\pm$ 0.67
LVIDs (mm)	11.67 $\pm$ 1.21	12.87 $\pm$ 1.16	12.45 $\pm$ 1.13	12.67 $\pm$ 1.41	11.78 $\pm$ 1.32	11.97 $\pm$ 1.21	13.67 $\pm$ 1.29	12.67 $\pm$ 1.26
EF (%)	60.33 $\pm$ 4.51	61.33 $\pm$ 4.23	62.33 $\pm$ 3.57	62.67 $\pm$ 4.53	61.53 $\pm$ 4.23	63.33 $\pm$ 5.51	63.73 $\pm$ 4.35	62.37 $\pm$ 4.54
FS (%)	31.33 $\pm$ 3.16	32.33 $\pm$ 3.23	32.78 $\pm$ 3.45	34.33 $\pm$ 3.32	30.67 $\pm$ 3.15	31.54 $\pm$ 3.24	35.33 $\pm$ 4.51	34.33 $\pm$ 3.36

DM: diabetes mellitus; Glu: Glucose plasmatic; LA: left atrial; IVS: interventricular septum; LVPW: left ventricular post wall; LVIDd: left ventricular end-diastolic inner diameter; LVIDs: left ventricular end-systolic inner diameter; EF: Ejection fraction; FS: fraction shortening; * $p < 0.05$ compared with control group at the same time point

Table II. Layer-specific myocardial strain in the DM group and control group

Time points	Group	Layer-specific strain					
		LSendo	LSmid	LSepi	CSendo	CSmid	CSepi
3 months	DM	-18.21 $\pm$ 4.35	-15.60 $\pm$ 4.27	-13.28 $\pm$ 4.43	-15.61 $\pm$ 6.54	-12.93 $\pm$ 3.00	-8.28 $\pm$ 2.23
	Control	-17.45 $\pm$ 3.87	-15.45 $\pm$ 3.60	-13.73 $\pm$ 3.48	-17.80 $\pm$ 5.09	-13.21 $\pm$ 4.09	-8.07 $\pm$ 3.08
	p	0.756	0.937	0.88	0.56	0.747	0.184
6 months	DM	-12.67 $\pm$ 0.55*	-11.03 $\pm$ 0.59	-9.15 $\pm$ 0.67	-13.05 $\pm$ 0.90*	-11.96 $\pm$ 1.48	-7.58 $\pm$ 1.86
	Control	-18.54 $\pm$ 5.52	-14.10 $\pm$ 3.32	-10.15 $\pm$ 1.39	-19.96 $\pm$ 1.79	-14.48 $\pm$ 4.11	-9.53 $\pm$ 2.44
	p	0.02	0.05	0.15	0.01	0.19	0.15
9 months	DM	-14.56 $\pm$ 2.44*	-11.05 $\pm$ 2.22*	-9.83 $\pm$ 1.59*	-12.33 $\pm$ 2.10*	-9.76 $\pm$ 1.72*	-6.35 $\pm$ 1.02*
	Control	-21.56 $\pm$ 2.47	-17.05 $\pm$ 2.34	-15.01 $\pm$ 1.78	-18.78 $\pm$ 3.32	-13.56 $\pm$ 1.57	-10.86 $\pm$ 0.74
	p	0.001	0.012	0.003	0.011	0.004	0.001

LSendo: longitudinal strain of endocardium; LSmid: longitudinal strain of mid-myocardium; LSepi: longitudinal strain of epicardium; CSendo: circumferential strain of endocardium; CSmid: circumferential strain of mid-myocardium; CSepi: circumferential strain of epicardium; * $p < 0.05$ compared with control group at the same time point

Fig 2. The absolute values of longitudinal (a) and circumferential (b) strains decreased progressively from endocardium, mid-myocardium to epicardium. DM3m: diabetes mellitus 3 months; DM6m: diabetes mellitus 6 months; DM9m: diabetes mellitus 9 months; NC3m: normal control 3 months; NC6m: normal control 6 months; NC9m: normal control 9 months.

layer-specific strain echocardiography deteriorated in the DM group.

The ROC analyses of the accuracy of different strain parameters found that AUC of LSendo was 0.897 and AUC of CSendo was 0.617 to identify DCM at 6 months. Comparison of ROC curves showed that LSendo was significantly better for identifying DCM than was CSendo.

Histomorphological study

The heart sections of ALX-induced rabbits showed mild edema and vacuolization in the cytoplasm after 3 months. After 6 months, obvious edema, more vacuolization in the cytoplasm, and a few hemorrhages were observed. Nine months later, in addition to moderate vacuolization and hemorrhage, histiocyte proliferation and interstitial fibrosis appeared, and degenerative changes in the nuclei, severe histiocyte proliferation, interstitial fibrosis, and collagen deposits were observed (fig 3).

Discussions

The main findings of the present study were summarized as follows: 1) at 6 months, abnormalities were observed in only LS and CS of the endocardium, and other parameters remained unchanged; 2) Comparison of ROC curves showed that LSendo was more accurate than CSendo to identify early myocardial impairment in DM; 3) At 9 months, alongside endocardial strain the LS and CS of the mid- and epicardial layers significantly decreased in DM group when compared with control group.

In our study at 6 months, the absolute values of LSendo and CSendo significantly decreased, while other parameters remained unchanged. The myocardium consists of 3 layers [7]. The orientation of subendocardial muscle fibers is longitudinal, so a LS decrease along with a reduction of inner myocardial wall thickening may document

Fig 3. Myocardial histomorphological alterations, H&E stain (see details inside the text). Control; b) DM 3 months; c) DM 6 months; d) DM 9 months.

the subendocardial dysfunction. Although there is DM induced myocardial injury, this injury is mild, and only endocardium is involved. This implies that the DCM differently affects three myocardial layers. Thus, it is necessary to analyze the strain of different layers of the LV wall. In addition, this also indicates that the endocardium is more sensitive to injury, which is consistent with previous findings [8]. Thus, there might be endocardial dysfunction although the global cardiac function remains normal, as assessed by standard EF. And this is why the layer-specific myocardial strain analysis more accurately detects the endocardial longitudinal strain impairment, a well-known parameter of early myocardial damage in diabetes.

Our results showed the absolute values of the LV longitudinal and circumferential strain gradually diminish from endocardium, mid-myocardium to epicardium, which was consistent with findings from studies with invasive labeling and studies on layer specific strain of the LV [9].

In addition, at 9 months the LS and CS of all 3 layers in DM group significantly decreased when compared with the control group, suggesting that all 3 myocardial layers are damaged during certain stages of diabetes. The most important reduction in the myocardial function was found in the endocardial layer because of the highest shortening of this layer before diabetes onset. Thus, layer-specific strain analysis may increase the diagnostic accuracy in DM heart involvement. The LV motion consists of longitudinal, radial, and circular motions. The changes in the myocardial strain at different directions may be employed to differentiate the injury of different myocardial layers, which is also helpful for the elucidation of pathophysiology of diseases: the longitudinal fibres localized in the endocardium, corresponds to long-axis strain and are more susceptible to ischemia and to

other mechanical, toxic, or metabolic factors [10-12]. Impairment of both longitudinal and circular strains may be indicative of myocardial diseases with the involvement of the whole ventricular wall (such as transmural myocardial infarction in coronary heart disease as well as certain cardiomyopathies) [13]. This is in accordance with our findings.

Many imaging techniques focused on the evaluation of global and regional LV function. Traditionally, the entire myocardial wall deformation is considered in the analysis of myocardial function without taking into account the differences in the layers of the myocardium. Imaging techniques allowing layer-specific myocardial function analysis are prone to enhance the insights into morphologic and pathophysiologic pathways of DCM and may help to earlier diagnose the patients with DCM.

Although diastolic dysfunction has been described as a marker of early DCM in patients with normal LVEFs, early systolic impairment has also recently been described by myocardial strain. Labombarda et al study [14] demonstrated that the global longitudinal strain was significantly lower in the type 1 DM children and correlated with HbA1c. Sveen et al [15] also reported LV longitudinal strain was significantly reduced in patients with type 1 DM compared to controls. In an experimental rat study, Weytjens et al [16] detected, at 6 weeks after Streptozotocin injection, a lower myocardial velocity and systolic strain rate and delayed time-to-peak deformation on Doppler myocardial imaging. These findings suggest that systolic LV myocardial performance were impaired in the early stage of DM, which was in agreement with our histological results.

Longitudinal and circumferential, but not radial strain was assessed in the present study. We chose not to analyze radial strain because it has methodological limitations and has been shown to be inferior to longitudinal and circumferential strain in identifying some diseases [17]. In addition, longitudinal and circumferential strains that have been well validated against ejection fraction in previous studies [18-19], are reproducible, and are easily obtained with only a minor increase of procedure duration.

Study limitations

The gradient of strain across the myocardium is a nonlinear phenomenon, and the definition of the layers is arbitrary and is based on simple division into 3 parts. Because the spatial resolution of ultrasound is limited, there will always be a certain degree of overlap.

Conclusion

The LSendo and the CSendo are sensitive parameters that can be used as indicators for the evaluation of early

left ventricular myocardial damage in DM. Layer-specific myocardial strain echocardiography may identify subtle myocardial dysfunction in the early stages of DM.

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Conflict of interest: none

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