

Speckle-tracking Echocardiography is Preferable Measurement for Detecting the Early and Subtle Alterations of Cardiac Dysfunction in Diabetic Rats

Supplementary Materials

Methods

Quantitative real time PCR (qRT-PCR)

Total mRNA of the samples from animal hearts were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol[1]. QRT-PCR was performed by using SYBR Green to detect PCR products in real time with ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The relative expression levels of the genes were calculated by the $2^{-\Delta\Delta CT}$ method and β -actin served as a housekeeping gene. The sequences of the primers were used as follow:

Table S1 The sequences of the primers used in this study.

Gene (rat)	Forward primer (5'->3')	Reverse primer (5'->3')
Mfn1	GCTGCATACAGACAGACAGCCT	GGTAATGACCTGTCTCAGGGCT
Mfn2	CACTACCACATCGGACACCCTA	GAACTTGTGTCTTGCATTTGGC
Drp1	AGGAACCGACAACAGGCAACT	CAACTGGAAGTGGCACATCTAGC
Fis1	TACCCCGAGGCTGTCCTAAG	CAGGACATTAGGCCAGAGC
β-actin	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC

1. Xiao H, Li H, Wang JJ, Zhang JS, Shen J, An XB, Zhang CC, Wu JM, Song Y, Wang XY *et al.* IL-18 cleavage triggers cardiac inflammation and fibrosis upon beta-adrenergic insult. *Eur Heart J.* 2018;39(1):60-69.

Figures:

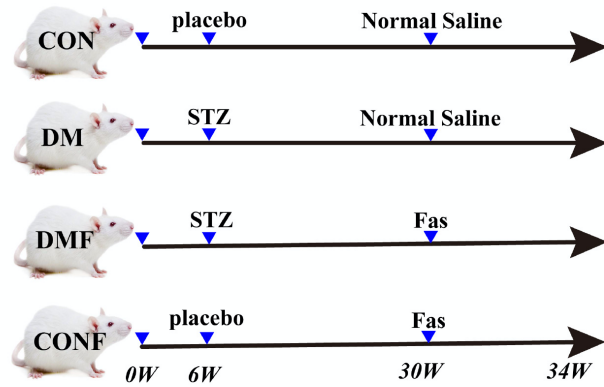


Fig.S1 A schematic of the experimental protocol. CON: control rats, DM: diabetic rats, DMF: diabetic rats and fasudil, CONF: control rats and fasudil.

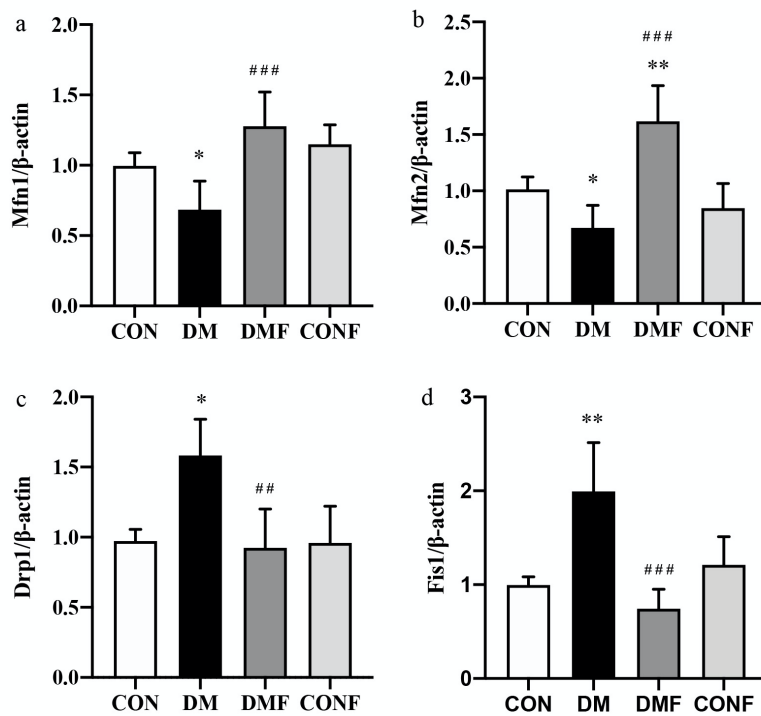


Fig.S2 Effect of ROCK inhibition on mitochondrial fusion and fission. The expression levels of the genes (a) Mfn1 and (b) Mfn2 were reduced in the DM group but increased in the DMF group. The transcripts of the genes (c) Drp1 and (d) Fis1 were upregulated in diabetic rats but reduced after treated with fasudil. Values are presented as the mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs CON group; # $P < 0.05$, ## $P < 0.001$, ### $P < 0.001$ vs DM group. CON: control rats, DM: diabetic rats, DMF: diabetic rats and fasudil, CONF: control rats and fasudil.

Tables:

Table S2. Conventional echocardiographic parameters of cardiac function.

	CON	DM	DMF	CONF
HR (bmp)	354 ± 29	295 ± 48**	309 ± 20*	346 ± 25
LVPWd (mm)	1.82 ± 0.07	2.11 ± 0.15**	1.95 ± 0.19	1.81 ± 0.19
LVPWs (mm)	3.34 ± 0.37	2.90 ± 0.19*	3.07 ± 0.24	3.09 ± 0.28
LVIDd (mm)	8.01 ± 0.46	8.76 ± 0.75*	9.09 ± 0.76**	8.60 ± 0.40
LVIDs (mm)	4.30 ± 0.41	6.11 ± 0.69***	6.07 ± 0.67***	4.87 ± 0.65
LVEDV (μl)	347 ± 45	425 ± 81	461 ± 90**	405 ± 41
LVESV (μl)	84 ± 18	190 ± 49***	187 ± 49***	114 ± 34
EF (%)	75.39 ± 6.64	55.68 ± 3.95***	59.75 ± 4.33***	72.27 ± 6.52
FS (%)	46.18 ± 6.50	30.41 ± 2.67***	33.39 ± 3.06***	43.45 ± 5.70
CO (ml/min)	94.61 ± 14.74	76.67 ± 15.64*	95.90 ± 11.49#	99.00 ± 16.21
SV (μl)	267 ± 31	259 ± 34	312 ± 46 #	287 ± 47
MV E/A	1.53 ± 0.23	1.22 ± 0.10*	1.18 ± 0.24*	1.57 ± 0.35

Values are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs CON group. CON: control rats, DM: diabetic rats, DMF: diabetic rats and fasudil, CONF: control rats and fasudil. HR: heart rate, LVPWd: diastolic left ventricular posterior wall thickness, LVPWs: systolic left ventricular posterior wall thickness, LVIDd: left ventricular end-diastolic inner dimension, LVIDs: left ventricular end-systolic inner dimension, LVEDV: Left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, EF: ejection fraction, FS: fractional shortening, CO: cardiac output, SV: stroke volume, MV E/A: mitral valve E/A.

Table S3. Speckle tracking echocardiography characteristics of the LV.

		CON	DM	DMF	CONF
Longitudinal	V PK(cm/s)	1.03±0.47	0.72±0.26	0.85±0.34	0.85±0.32
	D PK(cm/s)	0.16±0.06	0.20±0.09	0.29±0.12*	0.25±0.11
	GLS PK(%)	-12.06±1.57	-8.07±0.55***	-12.79±1.81####	-12.10±0.98
	GLSR PK(1/s)	-3.63±0.97	-2.09±0.45***	-2.82±0.18*	-2.92±0.66
Circumferential	V PK(cm/s)	101.60±50.13	62.64±26.23*	70.62±26.00	69.52±12.09
	D PK(cm/s)	2.31±1.55	1.07±0.63	2.17±1.25	1.66±0.77
	GCS PK(%)	-17.08±1.32	-12.82±1.18***	-15.98±1.24##	-16.90±2.38
	GCSR PK(1/s)	-4.27±0.46	-2.70±0.60***	-3.46±0.35*#	-3.96±0.65
Radial	As (mm ²)	21.48±1.46	42.93±4.94***	29.88±4.02****##	26.15±1.74*
	Ad (mm ²)	45.95±2.57	70.37±6.04***	58.18±7.19**##	53.40±3.15*
	FAC (%)	52.67±2.34	38.67±2.80***	48.17±2.64**###	50.50±1.05
	V PK(cm/s)	1.66±0.13	1.36±0.27*	1.45±0.20	1.69±0.23
	D PK(cm/s)	0.75±0.06	0.69±0.06	0.77±0.09	0.81±0.10
	GRS PK(%)	30.69±4.91	28.14±6.65	31.87±3.30	32.82±7.98
	GRSR PK(1/s)	5.55±1.10	4.07±1.16*	4.64±0.38	5.16±1.30

Values are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs CON group; # $P < 0.05$, ## $P < 0.001$, ### $P < 0.001$ vs DM group. CON: control rats, DM: diabetic rats, DMF: diabetic rats and fasudil, CONF: control rats and fasudil. V: velocity, D: displacement, PK: peak value, GLS: global longitudinal strain, GLSR: global longitudinal strain rate, GCS: global circumferential strain, GCSR: global circumferential strain rate, As: area at systole, Ad: area at diastole, FAC: fractional area change, GRS: global radial strain, GRSR: global radial strain rate.

Table S4. The correlations between cardiac functional parameters and cardiac structure parameters.

	EF	FS	CO	SV	E/A	AET	IVCT	IVRT	MPI	FAC	GLS	GLSR	GCS	GCSR	GRS	GRSR
Pearson	-0.631	-0.602	-0.497	-0.123	-0.468	0.061	0.605	0.654	0.742	-0.800	0.569	0.475	0.664	0.644	-0.207	-0.451
Correlation																
CSA																
Sig. (2-tailed)	0.001	0.002	0.014	0.566	0.021	0.779	0.002	0.001	<0.001	<0.001	0.004	0.019	<0.001	0.001	0.331	0.027
Pearson	-0.691	-0.661	-0.496	-0.16	-0.549	-0.112	0.516	0.627	0.749	-0.825	0.622	0.580	0.772	0.829	-0.08	-0.526
Correlation																
CVA																
Sig. (2-tailed)	<0.001	<0.001	0.014	0.457	0.005	0.603	0.01	0.001	<0.001	<0.001	0.001	0.003	<0.001	<0.001	0.712	0.008

EF: ejection fraction; FS, fractional shortening, CO: cardiac output, SV: stroke volume, MV E/A: mitral valve E/A, AET: aortic ejection time, IVCT: isovolumic contraction time, IVRT: isovolumic relaxation time, MPI: myocardial performance index, FAC: fractional area change, GLS: global

longitudinal strain, GLSR: global longitudinal strain rate, GCS: global circumferential strain, GCSR: global circumferential strain rate, GRS: global radial strain, GRSR: global radial strain rate.