Differential Effects of Isoproterenol on Regional Myocardial Mechanics in Rat Using Three-Dimensional Cine DENSE Cardiovascular Magnetic Resonance

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The present study assessed the acute effects of isoproterenol on left ventricular (LV) mechanics in healthy rats with the hypothesis that β -adrenergic stimulation influences the mechanics of different myocardial regions of the LV wall in different ways. To accomplish this, magnetic resonance images were obtained in the LV of healthy rats with or without isoproterenol infusion. The LV contours were

divided into basal, midventricular, and apical regions. Additionally, the midventricular myocardium was divided into three transmural layers with each layer partitioned into four segments (i.e., septal, inferior, lateral, and anterior). Peak systolic strains and torsion were quantified for each region. Isoproterenol significantly increased peak systolic radial strain and circumferential-longitudinal (CL) shear strain, as well as ventricular torsion, throughout the basal, midventricle, and apical regions. In the mid-ventricle, isoproterenol significantly increased peak systolic radial strain, and induced significant increases in peak systolic circumferential strain and longitudinal strain in the septum. Isoproterenol consistently increased peak systolic CL shear strain in all midventricular segments. Ventricular torsion was significantly increased in nearly all segments except the inferior subendocardium. The effects of isoproterenol on LV systolic mechanics (i.e., three-dimensional (3D) strains and torsion) in healthy rats depend on the region. This region dependency is also strain component-specific. These results provide insight into the regional response of LV mechanics to β -adrenergic stimulation in rats and could act as a baseline for future studies on subclinical abnormalities associated with the inotropic response in heart disease.

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1 Introduction

Both clinical and basic research have shown that β -adrenergic agonists can have a significant impact on cardiac function [1–3]. The previous studies on the performance of the left ventricle (LV) have shown that isoproterenol, a β -adrenergic agonist, not only increases myocardial contractility as indicated by higher peak pressure rise rate during systole, but also enhances isovolumic relaxation with faster isovolumic LV pressure fall [2,4,5]. These studies, however, did not include the effects of isoproterenol on regional measures of contraction such as strain and torsion, which are increasingly seen as more sensitive markers of dysfunction compared to global measures like ejection fraction (EF). Because adrenergic agents are often used to unmask dysfunction that may not be appreciable under basal conditions, it is necessary to first investigate the normal, healthy response to adrenergic stimulation in terms of regional strains and torsion.

Recent advances in cardiovascular magnetic resonance (CMR), including three-dimensional (3D) cine displacement encoding with stimulated echoes (DENSE), enable time-efficient noninvasive quantification of not only the LV volume, stroke volume, ejection fraction, and wall thickness, but also the myocardial strain, twist, and torsion with high strain resolution and displacement accuracy [6,7]. We have previously, using 3D cine DENSE CMR, showed that the 3D Lagrangian strains and LV torsion varied from region to region in healthy rats [6]. This finding provides the necessary baseline information for future cardiac studies, especially because the development of heart diseases involves regional differences [8–11]. Moreover, it has been shown that different myocardial regions respond differentially to the same pharmacological treatments, such as antiarrhythmics [9]. Along this line, the inotropic effects of isoproterenol on LV wall motion may also involve regional variations leading to development of new contraction patterns, which remain unclear and need further investigation.

The present study, therefore, aimed to assess the acute effects of isoproterenol on regional cardiac mechanics of healthy rats. We hypothesized that, in healthy rats, isoproterenol influences the mechanics of different myocardial regions of the LV wall in different ways. To test this, 3D cine DENSE CMR was performed on healthy rats with or without isoproterenol infusion. The 3D Lagrangian strains and ventricular torsion from different regions of the LV were assessed. The results of the present study provide insights into the regional response of LV myocardium to β -adrenergic stimulation in rats, which will facilitate future investigation of heart disease using rat models.

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Fig. 1 Representative end-systolic 3D cine DENSE CMR images and strain maps from one midventricular short-axis slice of a rat left ventricle. ((a)–(c)) Representative images from an isoproterenol-treated rat, including a magnitude-reconstructed image (a), original phase image encoded for x - (b), unwrapped phase image encoded for x - (c), and vector field of displacement (d); ((e)–(g)) example of E_{cc} (e), E_{c1} (f), and E_{c1} (g) strains; the top panels show strains from an untreated rat (CTL) and the bottom panels show strains from an isoproterenol-treated rat (ISO). Note regarding panel (e): S = septum, I = inferior, L = lateral, and A = anterior.

2 Methods

2.1 Animal Preparations. Nine female Sprague–Dawley rats (~6 months; Harlan, Indianapolis, IN) were anesthetized with 2.5% isoflurane in oxygen at a rate of 1.5 L/min. Cardiac cycle and respiration were monitored with cutaneous electrocardiogram (ECG) electrodes and a diaphragm placed underneath the abdomen, respectively, for cardiac and respiratory gating. Body temperature was monitored with a rectal thermometer and maintained at $36.7 \pm 0.2^\circ\text{C}$ with a customized heating pad. All vital signs including heart rate (HR), respiratory rate, and body temperature were monitored with a fiber optic system (SA Instruments, Stony Brook, NY). To apply acute inotropic stress on the rat LV, isoproterenol (Sigma-Aldrich, St. Louis, MO) was administered to animals through intraperitoneal infusion of 30 $\mu\text{g/g/min}$ using a magnetic resonance imaging compatible syringe pump (Harvard Apparatus, Holliston, MA). Ten independent rats without isoproterenol infusion scanned in a previous study [6] were used as controls. It should be noted that these animals were also female Sprague–Dawley rats and ~6 months of age. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky and were in agreement with the National Institute of Health's guidelines for the care and use of laboratory animals (NIH Publication 85-23, revised 1996).

2.2 Cardiovascular Magnetic Resonance Image Acquisition. Multislice 3D encoded spiral cine DENSE (i.e., 3D cine DENSE) CMR was performed on a 7-Tesla Bruker ClinScan system (Bruker, Ettlingen, Germany) equipped with a 2×2 hydrogen

phased-array coil and a gradient system (strength: 450 mT/m; slew rate: 4500 T/m/s) as described previously [6,7,12]. Briefly, immediately following an ECG R-wave trigger detection, a displacement encoding module, which consists of radiofrequency and gradient pulses, was applied to store position-encoded longitudinal magnetization. This was followed by successive applications of a readout module, which employed a radiofrequency excitation pulse (constant flip angle = 20 deg), a displacement encoding gradient, and an interleaved spiral k -space trajectory. As a result, a total of 12–22 frames per cardiac cycle depending on heart rate were acquired with one magnitude image and three displacement-encoded phase images (i.e., x , y , and z) (Fig. 1) via balanced encoding [13]. Other relevant acquisition parameters included repetition time = 7.4 ms, echo time = 1 ms, pixel size = 0.357×0.357 mm, field of view = 50×50 mm, slice thickness = 1.3 mm, spiral interleaves = 36, and displacement encoding frequency = 0.3 cycles/mm (chosen based on pilot studies).

For each animal, 5–6 short-axis (SA) slices (without slice gaps) and 2 long-axis (LA) slices (4-chamber and 2-chamber views) were defined for DENSE image acquisition [6]. The short-axis slices were evenly distributed to cover the majority of the end-systolic LV length. After slice planning, isoproterenol was infused into the peritoneal cavity of rats for 10–20 min to reach a stable HR prior to DENSE image acquisition. Once imaging began, isoproterenol was continuously infused until the acquisition was complete. On average, the total isoproterenol infusion time was less than 1 h. Instant adjustments were made to ECG gating to correct abnormal triggers, for example, due to changes in HR.

2.3 Image Analysis. The DENSE phase images were analyzed using an open-source application *DENSEanalysis*³ [14]. The myocardium was segmented from the blood pool and surrounding tissue with papillary muscles excluded using a semi-automated motion-guided segmentation method followed by manual correction [15]. As described in the previous studies [6,7], the 3D Lagrangian finite strain tensor of each pixel, relative to end diastole (ED; i.e., the first frame), was derived in polar coordinates throughout the cardiac cycle. The strain tensor consists of six components: radial strain E_r , circumferential strain E_{cc} , longitudinal strain E_{ll} , and three shear strains (E_{cl} , E_{rl} , and E_{cr}). The basic steps included semi-automated motion-guided segmentation of the myocardium [15], phase unwrapping, spatial smoothing, and temporal fitting of displacements [7,14].

Left ventricular torsion was calculated as the circumferential-longitudinal (CL) shear angle α_{CL} using the following equation [6,16]:

$$\alpha_{CL} = \sin^{-1} \frac{2E_{cl}}{\sqrt{(1 + 2E_{cc})(1 + 2E_{ll})}} \quad (1)$$

In order to evaluate the regional response of cardiac mechanics to isoproterenol, the three slices located at the basal, midventricular, and apical regions, respectively, were analyzed. For a more in-depth analysis, the myocardium at the midventricle was divided into four wall segments (i.e., septal, inferior, lateral, and anterior segments) as described previously [6]. Each segment was partitioned equally into three transmural layers (i.e., subendocardium, midmyocardium, and subepicardium). The strain and CL shear angle were then averaged within each region of interest.

To calculate the ventricular volumes and ejection fraction, the DENSE magnitude images were segmented at ED and end systole (ES). The LV volume was reconstructed using the contours of both SA and LA slices. The apical SA contours absent from the DENSE images were approximated using the Brent-Powell minimization method [17].

2.4 Left Ventricular Pressure Catheterization. After the CMR scan, LV pressure of the rats was measured following a standard LV pressure catheterization protocol [18]. Briefly, the carotid artery of anesthetized rats was isolated, and a 1.4F nonferrous catheter (SPR-903, Millar Instruments, Houston, TX) coated with heparin (Sigma-Aldrich, St. Louis, MO) was placed into the LV cavity through the carotid artery. The rectal temperature, heart rate, and LV pressure were monitored using LabChart (ADInstruments, Colorado Springs, CO). To increase heart rate to the level observed during the CMR scan, isoproterenol infusion resumed after the placement of LV pressure catheter. Pilot studies were conducted to show that the LV pressure remained consistent before, during, and after CMR scan when the vital signs (i.e., HR, body temperature) were maintained at similar levels.

2.5 Statistics. Data were represented as mean $\pm$ standard error of the mean. Single comparisons for the differences in hemodynamic parameters between control and isoproterenol-treated rats were conducted with unpaired student *t*-test. Multiple comparisons related with strain components or CL shear angle in each transmural layer were conducted using two-way analysis of variance (ANOVA) with post hoc Bonferroni *t*-tests. A value of $p < 0.05$ was considered significant.

3 Results

3.1 Effects of Isoproterenol on Ventricular Global Function. The comparisons between isoproterenol-treated and untreated (control) rats, in terms of global LV function, were listed in Table 1. As compared to controls, rats infused with isoproterenol

Table 1 Hemodynamic parameters in untreated and isoproterenol-infused rats

	CTL	ISO
HR (bpm)	334 $\pm$ 7	432 $\pm$ 8*
EDP (mmHg)	12.5 $\pm$ 0.9	13.3 $\pm$ 0.9
P_{max} (mmHg)	115.1 $\pm$ 2.5	121.8 $\pm$ 2.7*
peak (+)dP/dt (mmHg/s)	9213 $\pm$ 399	13,553 $\pm$ 858*
peak (−)dP/dt (mmHg/s)	10,867 $\pm$ 325	15,354 $\pm$ 1631*
EDV (μ l)	311 $\pm$ 11	291 $\pm$ 13
ESV (μ l)	130 $\pm$ 6	87 $\pm$ 6*
SV (μ l)	181 $\pm$ 11	204 $\pm$ 9
EF (%)	58 $\pm$ 2	70 $\pm$ 1*

Note: CTL, control (untreated) rats; ISO, isoproterenol-treated rats; HR, heart rate; EDP, LV end-diastolic pressure; P_{max} , peak LV systolic pressure; peak (+)dP/dt and peak (−)dP/dt, maximum rates of LV pressure rise and fall, respectively; EDV, LV end-diastolic volume; ESV, LV end-systolic volume; SV, LV stroke volume; EF, LV ejection fraction. * $p < 0.05$ compared to CTL.

exhibited significantly (* $p < 0.05$) faster HR. While there were no detectable changes in the end-diastolic pressure (EDP), the peak LV pressure (P_{max}) generated during systole was significantly (* $p < 0.05$) elevated in isoproterenol-treated rats. Moreover, the peak rates of LV pressure rise and fall, i.e., peak (+)dP/dt and (−)dP/dt, in isoproterenol-treated rats were significantly (* $p < 0.05$) higher than those observed in untreated animals. As for LV volume, isoproterenol-treated rats exhibited comparable end-diastolic volume (EDV) but significantly (* $p < 0.05$) reduced end-systolic volume (ESV), resulting in greater (* $p < 0.05$) EF, as compared to controls. It should be noted that the LV volumes of the control animals in the present study were in close agreement with those found in the previous studies that used a steady-state free precession sequence to assess the LV volumes in female Sprague–Dawley rats [19].

3.2 Effects of Isoproterenol on the Mean Peak Systolic Strains and Torsion Averaged Over the Entire Slice in the Basal, Midventricular, and Apical Regions. Compared to untreated rats, isoproterenol-treated animals exhibited significantly (* $p < 0.05$) elevated peak systolic E_r but similar levels of peak systolic E_{cc} at either basal, midventricular, or apical regions (Table 2). Isoproterenol-treated rats also exhibited significantly (* $p < 0.05$) reduced basal peak systolic E_{ll} . Peak systolic E_{cl} was significantly (* $p < 0.05$) elevated in isoproterenol-treated rats at all the three longitudinal locations, while E_{rl} and E_{cr} were either not affected or only increased (* $p < 0.05$) at the base and apex in the treated animals. Moreover, the ventricular torsion, represented as CL shear angle, in isoproterenol-treated rats was significantly (* $p < 0.05$) higher than that in untreated ones at all the three longitudinal locations.

3.3 Effects of Isoproterenol on Peak Systolic Strain E_{cc} in the Midventricle. Overall, isoproterenol-treated rats exhibited peak systolic E_{cc} strains, in each transmural layer, that were similar to the levels measured in untreated animals ($p > 0.05$ for treatment in each layer; Fig. 2). However, these treated animals exhibited significantly (* $p < 0.05$) elevated peak systolic E_{cc} in the septal segment of all the three transmural layers, as compared to untreated ones. Contrary to the septal segment, the other three segments of each transmural layer exhibited a reduction in the magnitude of E_{cc} with significant differences (* $p < 0.05$) detected in the lateral and/or anterior segments between isoproterenol-treated and untreated animals. Two-way ANOVA detected significant treatment X segment interactions in all three transmural layers, i.e., subendocardium ($p = 0.005$), midmyocardium ($p = 0.001$), and subepicardium ($p < 0.001$). With isoproterenol treatment, there were more significant differences in peak E_{cc} between different cardiac segments compared to untreated animals (as detected by post hoc tests shown in the inset box of each

³<https://github.com/denseanalysis/denseanalysis>.

Table 2 Effects of isoproterenol on the mean peak systolic strains and circumferential-longitudinal shear angles in basal, midventricular, and apical myocardium

	Base		Mid-LV		Apex	
	CTL	ISO	CTL	ISO	CTL	ISO
E_{cc}	-0.19 ± 0.01	-0.18 ± 0.00	-0.21 ± 0.01	-0.19 ± 0.01	-0.19 ± 0.01	-0.19 ± 0.01
E_{ll}	-0.15 ± 0.00	$-0.11 \pm 0.01^*$	-0.16 ± 0.01	-0.15 ± 0.01	-0.14 ± 0.01	-0.14 ± 0.01
E_{lr}	0.46 ± 0.02	$0.55 \pm 0.02^*$	0.54 ± 0.02	$0.66 \pm 0.02^*$	0.45 ± 0.04	$0.66 \pm 0.02^*$
E_{cl}	0.04 ± 0.00	$0.08 \pm 0.01^*$	0.05 ± 0.00	$0.09 \pm 0.01^*$	0.00 ± 0.01	$0.06 \pm 0.00^*$
E_{rl}	0.09 ± 0.00	0.09 ± 0.01	-0.05 ± 0.01	0.00 ± 0.02	-0.17 ± 0.01	-0.14 ± 0.02
E_{rc}	-0.05 ± 0.00	$-0.07 \pm 0.01^*$	0.04 ± 0.00	0.04 ± 0.01	0.09 ± 0.01	$0.16 \pm 0.01^*$
CL shear angle (deg)	7.47 ± 0.38	$12.70 \pm 1.10^*$	9.09 ± 0.51	$14.92 \pm 0.73^*$	1.36 ± 0.91	$9.99 \pm 0.66^*$

Note: CTL, control (untreated) rats; ISO, isoproterenol-treated rats. * $p < 0.05$ compared to CTL.

Fig. 2 Effects of isoproterenol on peak systolic E_{cc} strains in different myocardial regions of midventricle. Peak systolic E_{cc} values were averaged over each segment of subendocardium ((a), Endo), midmyocardium ((b), Mid), and subepicardium ((c), Epi). The text above each figure shows p -values for the main statistical effects. Significant differences between CTL ($n = 10$) and ISO ($n = 9$) groups, tested separately for each segment, are indicated by asterisks (* $p < 0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

figure). Maps of midventricular end-systolic E_{cc} , from representative animals, are shown in Fig. 1(e).

3.4 Effects of Isoproterenol on Peak Systolic Strain E_{ll} in the Midventricle. Overall, isoproterenol-treated rats exhibited significantly reduced levels of peak systolic E_{ll} in the subendocardium ($p = 0.008$) and midmyocardium ($p = 0.044$), but similar levels in the subepicardium, as compared to untreated rats (Fig. 3). Similar to the E_{cc} component, peak systolic E_{ll} in the treated animals was increased in the septal segment, but reduced in the other three segments, as compared to untreated rats, with significant differences (* $p < 0.05$) detected in the lateral and/or anterior segment of each transmural layer. Two-way ANOVA detected a significant treatment X segment interaction ($p = 0.032$) for the subepicardial layer. Post hoc tests detected more significant differences in peak systolic E_{ll} among the four segments in each

transmural layer of the isoproterenol-treated rats (shown in the inset box of each figure), as compared to untreated animals. Maps of midventricular end-systolic E_{ll} , from representative animals, are shown in Fig. 1(f).

3.5 Effects of Isoproterenol on Peak Systolic Strain E_{cl} in the Midventricle. As compared to untreated rats, animals treated with isoproterenol exhibited significantly ($p < 0.001$) elevated peak systolic E_{cl} over the entire myocardium (Fig. 4). For all three transmural layers, the greatest increase consistently occurred in the anterior segment (>100%, * $p < 0.05$), whereas the change that occurred in the inferior segment was less consistent, but still significant (32–53%, * $p < 0.05$). A significant interaction between treatment and segment ($p = 0.015$) was observed in the midmyocardium. Maps of midventricular end-systolic E_{cl} , from representative animals, are shown in Fig. 1(g).

Fig. 3 Effects of isoproterenol on peak systolic E_{II} strains in different myocardial regions of midventricle. Peak systolic E_{II} values were averaged over each segment of subendocardium ((a), Endo), midmyocardium ((b), Mid), and subepicardium ((c), Epi). The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n=10$) and ISO ($n=9$) groups, tested separately for each segment, are indicated by asterisks ($*p<0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

Fig. 4 Effects of isoproterenol on peak systolic E_{cI} strains in different myocardial regions of midventricle. Peak systolic E_{cI} values were averaged over each segment of subendocardium ((a), Endo), midmyocardium ((b), Mid), and subepicardium ((c), Epi). The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n=10$) and ISO ($n=9$) groups, tested separately for each segment, are indicated by asterisks ($*p<0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

Fig. 5 Effects of isoproterenol on peak systolic E_{rr} strains in different myocardial regions of midventricle. Peak systolic E_{rr} values were averaged over each myocardial segment. The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n = 10$) and ISO ($n = 9$) groups, tested separately for each segment, are indicated by asterisks ($*p < 0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

Fig. 6 Effects of isoproterenol on peak systolic E_{rl} strains in different myocardial regions of midventricle. Peak systolic E_{rl} values were averaged over each myocardial segment. The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n = 10$) and ISO ($n = 9$) groups, tested separately for each segment, are indicated by asterisks ($*p < 0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

3.6 Effects of Isoproterenol on Radial-Related Strain Components (i.e., E_{rr} , E_{rl} , and E_{rc}) in the Midventricle. On average, rats with isoproterenol infusion exhibited significantly ($p < 0.001$) higher levels of peak systolic E_{rr} than untreated rats (Fig. 5). Significant increases ($*p < 0.05$) were detected in the septal, inferior, and anterior segments between isoproterenol-treated and untreated groups. In contrast with the comparable levels of peak E_{rr} in all the four segments of untreated rats, significant differences were detected among the segments of isoproterenol-treated rats (shown in the inset box of the figure).

Fig. 7 Effects of isoproterenol on peak systolic E_{rc} strains in different myocardial regions of midventricle. Peak systolic E_{rc} values were averaged over each myocardial segment. The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n = 10$) and ISO ($n = 9$) groups, tested separately for each segment, are indicated by asterisks ($*p < 0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

As for E_{rl} , the peak systolic level was significantly ($*p < 0.05$) increased in the inferior myocardium of isoproterenol-treated rats as compared to untreated animals (Fig. 6). No significance was associated with changes in the other three segments between isoproterenol-treated and untreated groups.

Peak systolic E_{rc} strains in isoproterenol-treated rats were significantly ($*p < 0.05$) reduced and elevated in the inferior and anterior segments, respectively (Fig. 7). There was a significant treatment X segment interaction ($p < 0.001$). In contrast with the comparable levels of peak E_{rc} exhibited in untreated group, significant variations among the four segments were detected in the treated group (shown in the inset box of the figure).

3.7 Effects of Isoproterenol on Midventricular Torsion.

Peak torsion in the midventricle, represented as peak CL shear angle, was significantly ($*p < 0.05$) enhanced over nearly the entire myocardium of isoproterenol-treated rats, with the exception of the inferior subendocardium, as compared to untreated animals (Fig. 8). For all three transmural layers, the isoproterenol-induced increases were greatest in the anterior segment, followed by the septal, lateral, and then inferior segments. A significant treatment X segment interaction ($p = 0.036$) was detected for the subendocardium. Post hoc tests detected either none or fewer significant differences among the four segments in the three transmural layers of isoproterenol-treated rats, as compared to the variations observed in untreated animals (shown in the inset box of the figure).

4 Discussion

The present study investigated the acute response of normal rats to a short time period of isoproterenol stimulation, focusing primarily on LV systolic function. By characterizing the profiles of 3D Lagrangian strains and ventricular torsion in isoproterenol-treated rats, results of the present study provide evidence that isoproterenol influences cardiac mechanics of normal rats in a region-dependent manner.

Isoproterenol, as a nonselective β -adrenergic agonist, previously has been shown to enhance LV pump function in mice [5]. Consistent with this finding, increased EF was observed in rats

Fig. 8 Effects of isoproterenol on peak ventricular torsion in different myocardial regions of midventricle. Peak systolic CL shear angles were averaged over each segment of subendocardium ((a), Endo), midmyocardium ((b), Mid), and subepicardium ((c), Epi). The text above each figure shows p values for the main statistical effects. Significant differences between CTL ($n = 10$) and ISO ($n = 9$) groups, tested separately for each segment, are indicated by asterisks ($*p < 0.05$). Significant differences among the four segments, tested separately for untreated (CTL) and isoproterenol-treated (ISO) rats, are listed in the inset box.

infused with isoproterenol in the present study. Moreover, these isoproterenol-infused animals exhibited enhanced LV contraction and relaxation, reflected as increased peak (+)dP/dt and peak (−)dP/dt, which is in line with the previous reports on isolated murine hearts [5] and/or conscious canine LVs [4]. It should be noted that discrepancies in some measures of global LV function may stem from the species and methodologies used to quantify the acute effects of isoproterenol.

The previous studies on mice showed that the effects of dobutamine, a β_1 -adrenergic agonist, on systolic E_{cc} and E_{tr} strains depended on the circumferential (i.e., septal, posterior, lateral, or anterior segment) and longitudinal (i.e., base or apex) locations [20,21]. More specifically, dobutamine was found to increase both of these components of strain in all regions, with significant changes seen primarily in the septal, lateral, and anterior regions. Interestingly, the results of the present study showed a significant decrease in systolic E_{cc} in the lateral and anterior regions, as well as no change in the inferior region (Fig. 2). In order to explore this deeper, we must consider that the analyses performed in Refs. [20,21] used two-dimensional (2D) cine DENSE CMR, whereas in the current study 3D cine DENSE CMR was employed. To elaborate, the 2D analysis in Ref. [20] does not take into account any of the longitudinal components of deformation, in terms of the deformation gradient (F_{ll} , F_{lc} , F_{lr} , etc.). From continuum mechanics, consider the equation for calculating the circumferential component of the Lagrangian strain tensor (E_{cc}) in terms of a 3D analysis

$$E_{cc} = \frac{1}{2} (F_{cc}^2 + F_{lc}^2 + F_{rc}^2 - 1) \quad (2)$$

The 2D analysis does not use the F_{lc} term shown in Eq. (2). The only components of the deformation gradient used in that study were in the circumferential-radial plane. As it turns out, when a β -adrenergic agonist is given to a rat, this value of the deformation

gradient (F_{lc}) becomes much more dominant. In order to better quantify this effect, we went back and reanalyzed our data in order to compare the effects of the 2D assumption on the values of strain relative to a full 3D analysis. As a demonstration, we looked at the average E_{cc} over the entire ventricle using the 2D approach (i.e., ignoring F_{lc} in the calculation) and using the full 3D approach. With the 2D analysis, it was found that $E_{cc} = -0.20 \pm 0.007$ for the baseline animals and $E_{cc} = -0.225 \pm 0.003$ for the isoproterenol animals. This shows a significant increase in E_{cc} , just like the analysis presented in Ref. [20]. With the 3D analysis, it was found that $E_{cc} = -0.195 \pm 0.006$ for baseline animals and $E_{cc} = -0.19 \pm 0.005$ for the isoproterenol animals. This is consistent with the values reported in Table 2 of the present study. This result indicates that care must be taken when interpreting results from a 2D analysis versus a 3D analysis, particularly if one is trying to detect changes due to a drug or disease. Interestingly, the baseline values of E_{cc} were similar between the 2D and the 3D approach, which is in line with the previous reports in healthy normal hearts [22]. However, the 3D analysis in the present study shows that circumferential-longitudinal shear deformation (in terms of strain and torsion) is dramatically increased due to the administration of the β -adrenergic agonist. A 2D analysis may not be able to fully elucidate this due to the lack of longitudinally related deformation.

In terms of regional variation, we detected different effects of isoproterenol on systolic strains, depending on the longitudinal locations and the strain components (i.e., E_{cc} , E_{ll} , E_{tr} , E_{cl} , E_{rl} , and E_{rc}). In the midventricle, isoproterenol-induced effects on different strain components also depended on the circumferential location. These regional differences in the individual strain components could be linked to regional variations in the fiber architecture of the LV wall. In work by Healy et al. [23], it was shown that mice (similar architecture to rats) have regional variation in the fiber angles, with notable differences between the

septal and anterior segments. The average fiber distributions in those regions were found to be approximately $-59^\circ/71^\circ$ and $-36^\circ/90^\circ$ (epi/endo), respectively. As shown in Figs. 2 and 3 of the current study, these regions also exhibited differential responses to isoproterenol in terms of E_{cc} and E_{ll} . Probing the results of our study further, what is more fascinating is that regardless of whether E_{cc} or E_{ll} goes up or down in a given region, the overall effect of isoproterenol is that the torsion increases significantly everywhere. This is primarily driven by a significant increase in E_{cl} throughout the ventricular wall. Recall that E_{cc} , E_{ll} , and E_{cl} are the strain components used to calculate torsion (see Eq. (1)). Upon further inspection of these strain components in each region, it can be seen that the amount of increase or decrease in the strain components seems to change in a synergistic way. Consider the midmyocardial layer of the midventricle as an example. In the anterior segment, the percent change in the magnitude of those strain components was found to be -15% , -50% , and $+200\%$ for E_{cc} , E_{ll} , and E_{cl} , respectively. In the septal segment, the percent change in the magnitude of those strain components was $+18\%$, $+20\%$, and $+62\%$ for E_{cc} , E_{ll} , and E_{cl} , respectively. It can be seen that when E_{cc} and E_{ll} decrease in the anterior segment, the value of E_{cl} increases dramatically in order to generate a net increase in torsion. Whereas in the septal segment, E_{cl} does not increase as much since both E_{cc} and E_{ll} increase. It should also be noted that the torsion in those two regions of the LV wall was nearly identical, despite the difference in strain components (Fig. 8(b)). Thus, it appears that isoproterenol increases torsion globally, which leads to a significant increase in pump function, via increased ejection fraction.

Interestingly, although changes in strains induced by isoproterenol were different or even opposite among the four segments in the three transmural layers, consistent increases particularly in the three axial strain components (i.e., E_{cc} , E_{ll} , and E_{rr}) were observed in the septal segment of each transmural layer. This is of significant physiological importance considering the pivotal role of the septum in the pump function of the heart [24]. Finally, the isoproterenol-treated rats showed more regional variation in E_{cc} , E_{ll} , and E_{rr} when compared within the same transmural layer. It should be noted that the longitudinal components of the deformation gradient (F_{lr}) did not affect the calculation of E_{rr} when using the 2D or 3D approach. Both ways led to the same significant increase in E_{rr} , which is reported in both the present study and Ref. [20].

Ventricular torsion has previously been related with the regional distributions of contractility and systolic strains [25–27], and is expanding as a potential clinical measure of LV function [28]. As calculated by CL shear angle, peak LV torsion was dramatically increased in isoproterenol-treated rats throughout the entire LV. In the mid-LV, peak torsion was increased to similar levels over almost the entire myocardium due to the differential responses of the three related strain components to isoproterenol stimulation. This finding is consistent with reports showing that dobutamine stimulation increased systolic torsion in mice [20], as well as in healthy human subjects (by as much as a factor of 2) [29]. Additionally, the current results agree with another study that linked increases in torsion to increases in EF, despite similar or decreased levels of E_{cc} and E_{ll} [30]. The increased torsion with higher uniformity observed in the present study may serve to coordinate the global LV deformation during systole for more efficient blood ejection.

One limitation of the present study was that we did not relate our findings to any disease conditions. However, since heart disease and/or treatments are associated with changes in some specific myocardial regions [8–11], a detailed characterization of regional cardiac response to isoproterenol stimulation in healthy rats is necessary and can facilitate future studies using rat models for the understanding of heart disease and development of effective treatments. Moreover, in order to limit the total exposure time to anesthetic gas for the entire experimental procedure (including animal preparations, 3D cine DENSE image acquisition, and LV

catheterization) within 3 h, isoproterenol infusion was performed separately on an independent set of rats rather than control animals. In addition, the effects of transmural location were not included for radial-related strain components (i.e., E_{rr} , E_{rl} , and E_{re}), because measurements on these components in smaller partitions using 3D cine DENSE CMR were associated with lower reproducibility [6]. Finally, instead of the standard 6 LV wall segments used at the midventricle [31], only four segments were assessed in order to increase the power to detect regional differences. In the current study, the ventricle was partitioned in the transmural direction, whereas the standard segments are composed of the full transmural thickness. Thus, it was necessary to increase the size to the segments, which is why four segments were employed.

5 Conclusion

In healthy rats, isoproterenol influences systolic cardiac mechanics (i.e., the Lagrangian strains and ventricular torsion) in a region-dependent way. The degree of this dependency varied among the six strain components. Changes in cardiac mechanics induced by isoproterenol may serve to coordinate the global LV deformation for an enhanced pump function. These results, therefore, provide the necessary normative information for regional response to isoproterenol stimulation for rat hearts and could act as a baseline for future studies on detecting subclinical abnormalities in heart disease by using adrenergic stimulation to unmask dysfunction.

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