Interstrain Comparisons of Murine Global Cardiac Mechanical Function using MRI

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Introduction: Quantitative characterization of ventricular function has become important for the assessment of cardiac performance in heart disease. As the manipulation of the mammalian genome becomes routine, it is now possible to generate animal models to study cardiovascular function and dysfunction [1]. Critical to successful phenotypic screening of mouse models of the cardiovascular system using MRI are highly efficient four-dimensional (4D) acquisition protocols, and reduction of the computational image processing complexity for accurate quantification. The goal of this study is the efficient, quantitative assessment of interstrain cardiac performance in C57BL/6J and DBA/2J mouse hearts under anesthesia, using MRI.

Methods: *Physiology*: Ten C57BL/6J (weight±sd, 26.3±2.7g;age, 9-16 weeks) and five DBA (weight±sd, 25.9±6.5g;age, 8-12 weeks) mice were anesthetized using isoflurane (ISO) mixed with 100% O_2 and were allowed to breathe freely throughout the study through a nose cone. ECG and breathing rates were monitored using and SA instruments recording system (SA Instruments, Edison, NJ, USA). Heart rate was maintained between 450-550 beats per minute by adjusting the mixture of ISO and oxygen. A rectal probe was used to monitor and maintain stable body temperature.

Imaging: Work was performed at 7T with a GE Excite console (EPIC 12.4) using a custom-made 2.5x3cm^2 transmit/receive surface coil. A 4D radial MRI pulse sequence was implemented with TE and TR set to 300μs and 2.4ms respectively, as published in [2]. Eight phases of the heart cycle were acquired at temporal resolution of 9.6ms and spatial resolution of 87-110μm^3 in approximately 31 minutes with BW=±125kHz, and a flip angle=45°. Reconstruction used a non-uniform fast Fourier transformation. Data regridding was implemented with a least squares optimized kernel for interpolation. Raw data were reconstructed offline.

Image Processing and Surface Model Development: Mouse cardiac images were segmented by an expert user using seed-point spline contour segmentation of the left and right ventricular myocardium, and left ventricular blood cavity (Analyze 7.0, (Analyze Inc, Mayo Clinic, USA) from short axis cardiac MRI, spanning the entire heart throughout the entire cardiac cycle. Binary masks were generated, intensity normalized, and converted to the Analyze (.img) format using ImageJ (http://rsbweb.nih.gov/ij NIH, Bethesda, MD, USA). Alignment of all mouse models to a common coordinate system was achieved using a multipoint-landmark affine registration (IRTK software, IXICO, Ltd., London, UK) algorithm [3]. Uncertainties in the apical regions were corrected with the RView program (IXICO) and stored in the standard Analyze (.img) format [4]. Surface models were constructed using the surface extraction module of Analyze.

Quantification of Global Cardiac Function: 3Dvolume renditions of the left (LV) and right ventricular (RV) cavity were generated using Analyze and the LV blood cavity volume estimated using ImageJ. Estimated volumes were converted to absolute volume by multiplication of volume voxel counts with the image voxel volume (110μm^{3}x110μm^{3}x110μm^3). Stroke volume (SV) and ejection fractions (EF) were calculated according to: $SV = EDV - ESV$ and $EF = \frac{SV}{EDV}$, where EDV and ESV represent the end-diastolic (preload), and end-systolic left ventricular blood volumes, respectively.

Generalized Linear Modeling: To assess possible statistical dependence of computed EF and SV on mouse strain or body weight, a statistical parametric generalized linear model (GLM) was constructed for both LV and RV using R [R Development Core Team (2008), http://www.R-project.org.]. The model equations are:

$$EF_{\{LV_{i,j}, RV_{i,j}\}} \sim \text{Weight}_i + \text{Strain}_j$$

$$SV_{\{LV_{i,j}, RV_{i,j}\}} \sim \text{Weight}_i + \text{Strain}_j$$

where i={1,…, n_{C57B}; 1…n_{DBA}} and j={C57B, DBA}. The assumption of normally distributed datasets was confirmed from quantile plots for both mouse populations.

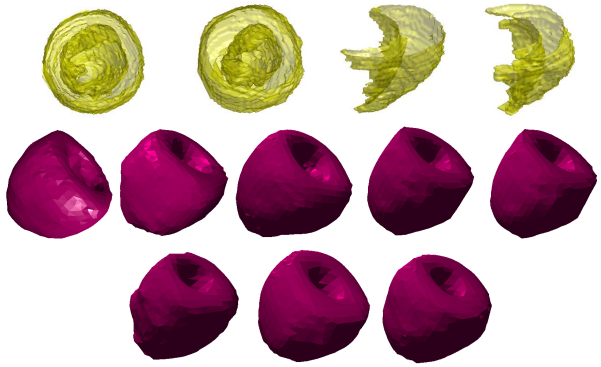

Figure 1: *(Top; left to right) Representative 3D renderings of the LV and RV end-diastolic and end-systolic timeframes; (Bot) Dynamic reconstruction of 3D surface models of the C57BL/6J mouse hearts.*

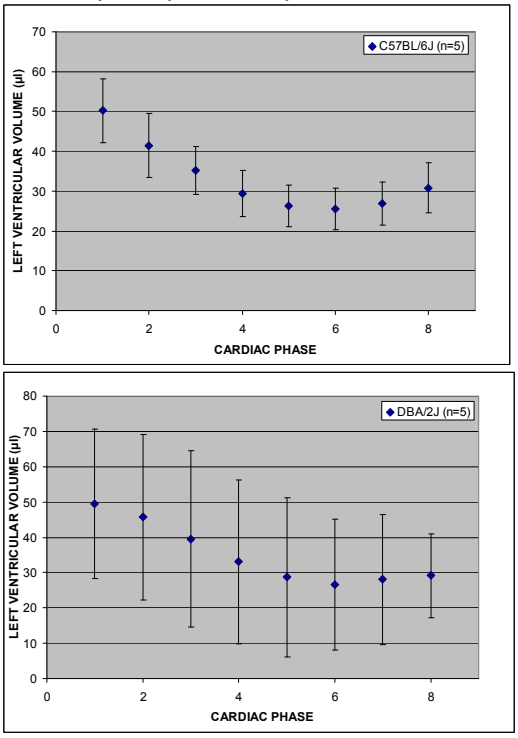

Figure 2: *Left ventricular volume variation over eight phases of the cardiac cycle for (left) 10 male **C57BL/6J** and (right) 5 **DBA/2J** mice.*

Results and Discussion: Figure 1 shows renditions of the end-diastolic and end-systolic phases for the LV and the RV, as well as a representative dynamic series of surface model over the eight reconstructed cardiac phases. Figure 2 depicts the temporal evolution of left ventricular blood volume over the eight cardiac phases of the cardiac cycle. Table 1 lists global cardiac mechanical functional indices for the LV and RV for C57BL/6J and DBA/2J mice, in agreement with recent prior attempts [5; 6]. Increased variability in estimated indices for DBA mice reflects cumulative segmentation and image processing errors as well as the limited size of the mouse population. Indicative are the end-diastolic (Phase 1) and the end-systolic (Phase 6) phases of the cardiac cycle. Interstrain statistically significant differences were found between SV values for RV and LV at the 5% significance level. GLM confirmed no statistical significance of EF and SV dependence on mouse strain or weight for both LV and RV at the 1% significance level. This study confirms that interstrain cardiac functional, dynamic characterization in mice has become possible with MRI, establishing the platform for highly efficient phenotypic screening of transgenic mouse models of animal pathology.

Strain	N	L EDV (μl)	L ESV (μl)	L SV (μl)	L EF (%)	R EDV (μl)	R ESV (μl)	R SV (μl)	R EF (%)
CB57BL/6J	10	46.4±12.3	22.8±8.0	23.6±5.4†	51.9±7.5	37.8±6.7	24.9±5.8	13.0±4.2†	34.5±10.2
DBA/2J	5	68.5±19.6	33.7±16.3	34.7±6.6†	53.3±15.0	40.0±12.6	25.9±13.5	14.1±2.9†	37.8±14.5

Table 1: *Interstrain comparison of global mechanical functional parameters of the right and left ventricles from MRI (†statistical significance at 5%).*

References: **1**) Jeanne F, et. al. Circ. Res. 82:407-415, 1998. **2**) Bucholz E, et. al. 60:111-118, 2008. **3**) Rueckert D, et. al. IEEE TMI, 18(8):712-721, 1999. **4**) Schnabel JA, et. al., MICCAI'01,573-581, 2001. **5**) Weissman F, et. al., Circ. Res. 88:563-569, 2001. **6**) Bucholz E, et. al. MRM 2009 (in press).

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