# Multimodal imaging shows fibrosis architecture and action potential dispersion are predictors of arrhythmic risk in spontaneous hypertensive rats

## Supporting Image Data

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## Global LV morphology

Closed contours were fitted to the inner and outer LV surfaces of the composite WGA confocal images of short-axis rings. The images were acquired using a Nikon TE2000 inverted confocal microscope system (excitation wavelength 488 nm; 4× objective: NA 0.13, WD 16.4 mm; 6.22 × 6.22 μm$^2$ pixels). Areas bounded by both contours were determined, as well as the estimated total LV wall cross-section area, average inner and outer diameters and wall thickness for equivalent circular contours. The dimensions from the center of the inter-ventricular septum (IVS) to the center of the LV free wall (Figure 1) and the LV wall thickness at the center of LV free wall were also calculated. The results are given in Table 1 and the short axes slices for all animals across all cohorts are given in Figure 1. The measured dimensions align with expectation of increasing wall thickness from 6-12 months and then decreasing again in the 18 month animals. Simple statistics suggest that changes in wall thickness are real but mostly non-significant.

**Table 1.** Global LV morphology of short-axis sections across age cohorts. Highlighted cells are significantly different, p=0.03680, for a one tailed test, but not a two tailed test (p=0.07354).

| | 6 month (n=6) | 12 month (n=6) | 18 month (n=5) |
|---|---|---|---|
| Wall area (mm$^2$) | 82.96±10.56 | 92.80±8.89 | 82.96±13.86 |
| Average outer diameter (mm) | 12.87±1.05 | 13.40±0.59 | 13.14±0.96 |
| Average inner diameter (mm) | 7.68±1.50 | 7.79±1.09 | 8.20±0.88 |
| Average wall thickness (mm) | 2.59±0.39 | 2.81±0.37 | 2.47±0.34 |
| LV freewall wall thickness (mm) | 2.71±0.48 | 3.18±0.59 | 2.60±0.27 |
| Distance IVS to LV freewall (mm) | 7.34±1.95 | 7.29±1.32 | 7.59±0.97 |

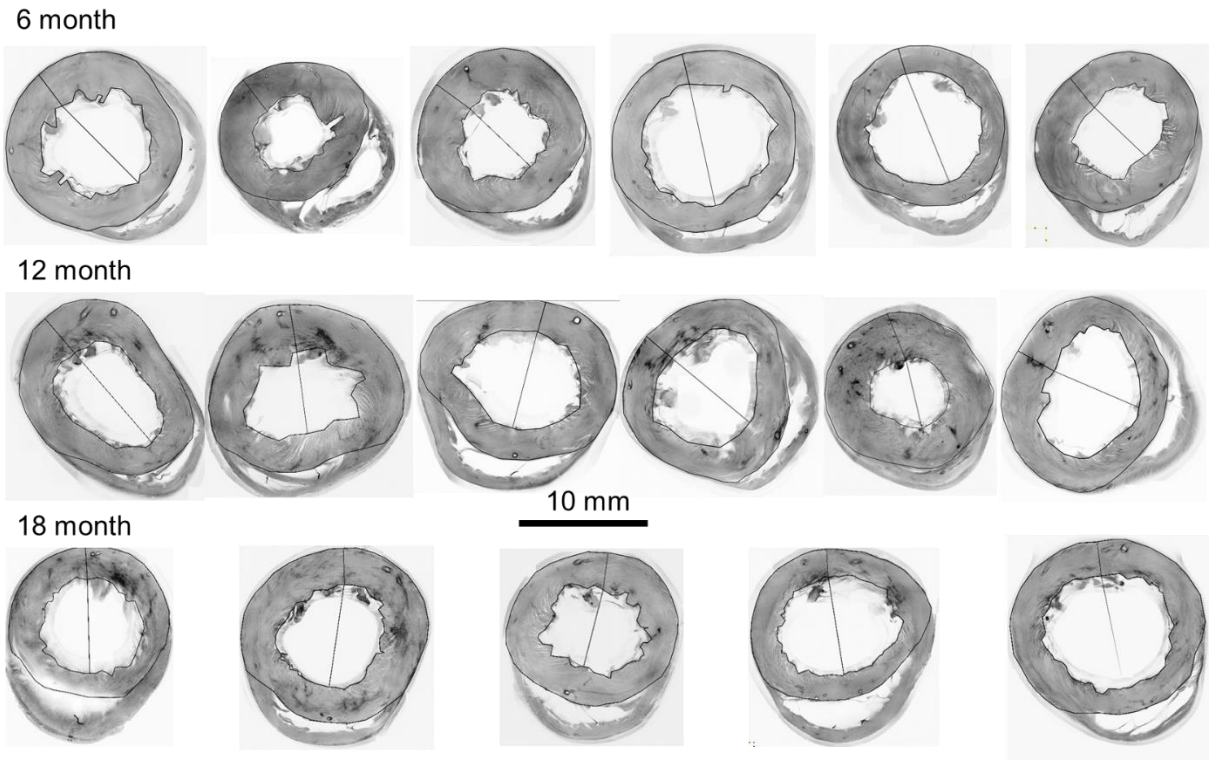


**Figure 1.** Short axis sections from all hearts (6 month, n=6; 12 month, n=6; 18 month, n=5) with inner and outer diameter contours shown, together with LV freewall thickness and distance from IVS to LV freewall.

## Normotensive rat tissue comparisons

Short axis tissue slices were cut from postmortem WKY rat hearts at 18 months of age (n=4), used and euthanized for a different study. The hearts were arrested by perfusion with N-methyl-d-glucamine (NMDG) solution and diffusion fixed in PFA (4% in PBS), according to the protocol of those experiments. The tissue samples were then cleared, labeled and imaged using identical techniques to those of the equivalent SHR animals of this study. The images are shown in Figure 2. There is no evidence of patchy fibrosis, especially toward the endocardium, as was found in age matched SHR animals (Figure 1). SHR hearts were arrested in their relaxed state using St Thomas' solution whereas the WKY rat hearts were arrested using NMDG solution. Consequently, the dimensions of the heart slice images between SHR and WKY are not comparable, but the assessment of replacement fibrosis through WGA labeling and tissue clearing is not affected.

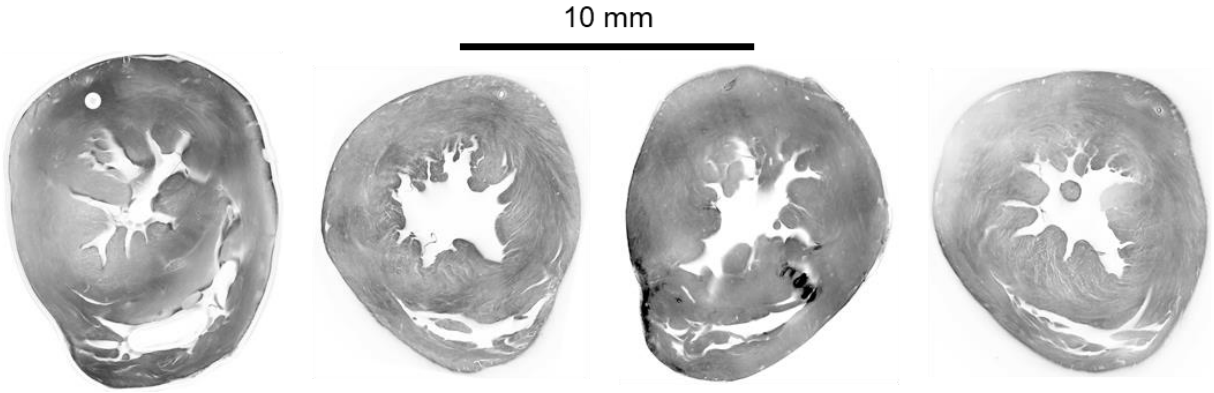


**Figure 2.** Short axis images from 18-month normotensive WKY rats (n=4). There is no evidence of the patchy fibrosis observed in SHR rats at the same time point. The dark blotches in the 3rd panel are from pooled WGA and not related to replacement fibrosis.