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Deposited on: 14 May 2018

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# Measures of Cardiac Function in Theraphosidae Spiders using *in vivo* Magnetic Resonance Imaging

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Keywords

MRI, cardiac function, Theraphosidae, tarantula, ejection fraction, *in vivo*, magnetic resonance, heart rate

## Abstract

We present the first *in vivo* cardiac Magnetic Resonance Imaging (MRI) measurements of Theraphosidae spiders. MRI scanning was performed on six spiders under isoflurane-induced anaesthesia. Retrospective Self-Gating Cine-Cardiac MRI (RG-CINE-MRI) was used to overcome the difficulties of prospective cardiac gating in this species. The resulting RG-CINE-MRI images were successfully analysed to obtain functional cardiac parameters from live spiders at rest. Cardiac ejection fraction was found to increase with animal mass (Pearson correlation 0.849, p= 0.03) at a faster rate than myocardial tissue volume, while heart rate stayed constant across animals. Suggesting the spider heart undergoes additional biomechanical loading with age. The acquisition of these results demonstrates the potential for retrospective gating to evaluate aspects of cardiac function in a wide range of previously inaccessible species.

## Introduction

To date, the cardiac physiology of invertebrates in general, and spiders in particular, has been comparatively little studied next to the great volume of cardiac literature amassed for rodents and humans, particularly in the medical sciences. Additionally for spiders the techniques used have been restricted to measurements of heart action potential via electrocardiogram (ECG) (Dunlop et al.,1992) or monitoring exterior cuticle movement as a proxy for cardiac motion (Bromhall, 1987; Coelho and Amaya 2000) . These latter observations have included both visual observation and the use of attached magnets and sensors to gauge movement. Many of these methods involve either attaching experimental apparatus to the animal, penetrating the outer cuticle (which could potentially lead to lowering of the internal hydrodynamic pressure) or indirect observations of heart function. Therefore, there is little quantitative information on spider cardiac function and outputs in the literature.

This is in contrast to many vertebrates where cardiovascular Magnetic Resonance Imaging (MRI) routinely provides a non-invasive method of assessing both function and structure in live subjects. MRI is widely used for clinical assessment of cardiovascular disease in humans, providing measurements such as myocardial mass, ventricular volumes, stroke volume, ejection fraction (Epstein, 2007) and even quantitative myocardial perfusion (Jerosch-Herold, 2010) and blood flow maps (Markl M, 2007) . Applications of cardiovascular MRI to other vertebrates, such as rodents, are predominately focussed on biomedical research using disease models. This research has driven the development of specialised MRI systems optimised for mice and rats. The data needed for an MR image generally needs to be acquired over several separate acquisitions. For cardiac MRI where the heart is in continual motion, the MRI scanner typically needs to be triggered/gated by an ECG signal identifying the phase of the cardiac cycle. This is called prospective gating.

Although MRI has been used previously to acquire basic MRI images of spider anatomy (Pohlmann et al., 2007) it has never been used to study the cardiac function of spiders. Indeed, conventional prospective gating with its need for ECG electrodes and respiration probes is not practical for spiders due to the difficulty of

attaching electrode pads or needles. However, the recent development of retrospectively gated cardiac cine-MRI (RG-CINE-MRI) (Heijman et al., 2006) has simplified cardiac MRI experiments by removing the need for ECG and respiratory contact probes (Holmes 2008, 2009). Here we seek to demonstrate this technology to acquire the first *in vivo* cardiac images of a spider heart and obtain quantitative measures of cardiac function. The Theraphosidae family and *Grammostola* genus were chosen as subjects, being a good fit in terms of physical size for existing commercial rat cardiac MRI coils. In addition, possessing hearts of comparable size to rodent hearts helps produce good quality MRI images for quantitative analysis. However it should be noted that the cardiac method described here would be equally applicable to smaller spiders, if an appropriately sized MRI coil was used (Merrifield et al 2017) . With the advent of coil-on-a-chip technology, the size of MRI coils now ranges from several tens of centimetres down to just 50microns diameter (Webb 2013).

RG-CINE-MRI

## Materials and Method

*Animal Ethics*

Experiments were conducted according to UK legislation (UK Animals (Scientific Procedures) Act (1986)). Subjects were anaesthetised throughout scanning for immobilisation and to reduce potential subject stress. Efforts were made to avoid direct handling of subjects.

*Animal Details and Housing*

Six captive bred adult spiders (gender undetermined, exact age unknown but of adult size, n = 4 *Grammostola rosea (Walcknenaer), n* = 2 *Grammostola porteri (Mello-Leitao)*, Mean Mass = 15.7 g ± 1.75) were obtained from a UK-based supplier (Virginia Cheeseman, High Wycombe, UK). Spiders were individually housed in plastic vivariums (Length = 29 cm, Width = 19 cm, Height = 23 cm). Sterilised vermiculite substrate was provided (~4 cm deep) along with a retreat. A one week acclimatisation period followed delivery of spiders before subsequent scanning. Free

access to water was provided. Food was withheld until after full recovery from scanning and anaesthesia.

*Animal Anaesthesia, Handling and Positioning*

Following standard procedures in animal MRI research, subjects were anaesthetised using 5% isoflurane delivered in a 30%/70% mixture of $O_2$/$N_2O$ gas (1000 ml $min^{-1}$) to minimise subject motion derived imaging artefacts (Fig 1B). Under anaesthesia all measurements would also be taken at a physiological baseline, eliminating variation due to involuntary movement or behaviour.

Subjects were positioned in an MRI compatible animal cradle, lying supine with the heart close to the MRI coil (Fig. 1C). Restraints were cushioned by folded medical gauze swabs that were placed along the length and width of the spider. The assembly was then enclosed in a sealed clear plastic chamber to allow maintenance of anaesthesia and for visual observation of the subject (Fig. 1D). This was then placed into the MRI scanner.

After scanning was complete (>1 hour) subjects were in an unresponsive state suggesting deep anaesthesia had been achieved. Locator scans performed before and after cardiac scanning confirmed animals had remained in position during the scanning procedure. Activity returned to normal over a subsequent 24-48 hour period. After recovery no adverse health effects or anomalous behaviours were noted.

*MRI Scanning*

Animals were scanned in a 7T Bruker Biospec MRI scanner (Figure 1A) equipped with a 400 $mTm^{-1}$ gradient insert and 4-channel phased array cardiac coil (Rapid Biomedical GmbH, Germany). Ambient room temperature during scanning was 18-21ºC. The animal was not directly heated by any additional equipment in this time.

Anatomical MRI scans were obtained to set up imaging slice prescriptions for Fast Low Angle Shot (FLASH) based retrospective RG-CINE-MRI scans (repetition time

$T_R$ = 8.00ms, echo time $T_E$ = 3.30ms, field of view (FOV) = 30.0mm x 30.0mm, matrix = 256 x 256, in-plane resolution 117μm x117μm, slice yhickness = 1.50mm, 14-18 slices depending on size of individual spider, 300 continuous k-space acquisitions, 5mins 7secs imaging time per slice). These were obtained using an in-slice navigator echo as part of the Intragate software package on the scanner (Paravision v.4, Bruker).This navigator is used retrospectively to determine the phase of the cardiac cycle associated with each k space acquisition, allowing images to be created for 10 different phases of the cardiac cycle [Heijman et al., 2006; Bovens SM et al., 2011].Axial image slices along the length of the heart were obtained sequentially until the whole heart was scanned marked by the distal and proximal aortas.

*Image Reconstruction and Analysis*

Cardiac images were reconstructed using the software tools available in Bruker's Intragate software. Residual navigator pulse trace discontinuities were excluded. Heart rates for each acquired slice were individually outputted as part of this process. Images were analysed using Image J (Schneider et al., 2012). Three independent researchers were trained in cardiac image analysis and then each conducted a separate analysis of all images. Researchers were blinded to which subjects the images came from.

For each slice, images were acquired for 10 different phases of the cardiac cycle. From the 10 images of the cardiac cycle for the central chamber, the images corresponding to the diastolic and systolic phases were identified. For each slice at diastole and systole, a region of interest was manually drawn around the heart perimeter giving the area of the ventricle at diastole and systole. This area is then converted to a volume for that slice by multiplying by the image slice thickness of 1.5mm. The total ventricular volume of the heart, the end diastolic volume (EDV) and end systolic volume (ESV) are then given by summing the volumes from each slice. The cardiac ejection fraction (EJ) was then determined by,

$$EJ = \frac{(EDV - ESV)}{EDV}$$

. Calculations of global heart parameters were then made from these measurements.

### Statistical Analysis

Where group averages are given they are presented with plus/minus the standard deviation. In Figure 3a the error bars represent the range of values of the measurement made by the three independent researchers. Researchers were blinded to which spider the images had come from and slice ordering was randomised for each researcher. From the RG-CINE-MRI navigator signal a heart rate is measured for each of the (14 to 18) slices acquired. The heart rate was then presented as the mean and standard deviation of these values (figure 3b). Correlations were performed using Pearson correlation coefficient and a 2-tailed test of significance (OriginPro 8, OriginLab Corporation).

## Results

### Cardiac Anatomy

Scans revealed anatomy matching that broadly outlined for spiders in existing literature (Paul et al. 1994, Foelix 1996, Huckstorf K 2013). Figures 2a and 2b show typical image slices acquired from the RG-CINE-MRI scans. Figure 2c shows a set of ostia in the open position as the heart chamber fills with blood. Figure 2d shows the same slice but with the heart now filled and the ostia closed. Blood pooling between the myocardium and pericardium prior to injection to the interior of the myocardium was also visible.

### Heart Rate

Resting heart rates were measured via the RG-CINE-MRI technique as outputted by navigator signal data. Multiple samplings on each spider were yielded by measuring

the heart rate in each image slice. The mean heart rate for the group of spiders was 20 bpm ± 2 and showed good consistency between subjects using this method (Fig. 3B). No significant correlation was found between body mass and heart rate (Pearson correlation -0.256, p=0.62).

Cardiac function

Table 1 shows quantitative measurements of cardiac function derived from the RG-CINE-MRI datasets for each spider. As may be expected there was a significant correlation between body mass and total heart volume (Pearson correlation 0.882, p=0.02) and between body mass and end diastolic ventricular volume (EDV) (Pearson correlation 0.956, p= 0.006). The fraction of blood ejected from the heart with each heart beat (the cardiac ejection fraction (EJ)) was successfully measured in each spider as a measure of cardiac function (Fig. 3A). These are the first measurements of *in vivo* ejection fraction in spiders that we were able to find in the literature. Interestingly, we find a significant correlation between body mass and EJ (Pearson correlation 0.849, p= 0.03). The difference in measurement of EJ between the three researchers gave a mean observer difference of EJ = 5.9%. This mirrors accepted levels of observer variability in corresponding rodent cardiac MRI (Heijman et al., 2008). The motion of the heart over the full cardiac cycle at different positions along the axis of the heart can clearly be seen in Supplementary Information 1-3.

## Discussion

The spider heart differs in many structural and biochemical aspects to those of vertebrates previously studied with MRI. However, the spider's contiguous nested two chamber system - the heart myocardium surrounded by a pericardium (Paul et al., 1994; Huckstorf et al., 2013) - provides a resultant MRI image similar to that of a standard human/rodent short-axis view (Figure 2). Hence we used similar image analysis methods to quantify cardiac function.

As might be expected we found a significant positive correlation between body mass and both heart volume and ventricular volume. However, we also found a significant

positive correlation between body mass and ejection fraction. This is interesting as in humans there is no significant correlation between body mass and ejection fraction (Dorbala S. et al., 2006; Seo J.et al., 2017), but there is some correlation between age and ejection fraction (Gebhard C. et al., 2012). A possible explanation for this is that as spiders grow by iterative stages of moulting, a spider's mass can serve as an approximate proxy for age (Foelix 1996). Therefore the correlation observed between EJ and mass can also be broadly considered to be one of EJ and age.

While the cardiac ventricular volume and to a lesser extent the total heart volume changes between diastolic and systolic phases of the cardiac cycle as expected, the total volume of the myocardial tissue remains largely unchanged in each spider and between systolic and diastolic phases of the cardiac cycle. This suggests that the material is being expanded and compressed across the cardiac cycle as would be expected. No obvious trend for increase in myocardial material over animal mass was observed in contrast to the trend observed for ventricular ejection fraction.

It can be speculated that if the growth of the heart volume and/or myocardial tissue from one moulting instar to the next is less than that of the overall animal's volume then a corresponding increase in EJ would be required. This means that the heart would have to pump a larger volume of blood as it aged in order to maintain adequate cardiac function, just as we observed. Further assessment of spiders of different masses (and so at different ages or developmental instars at least) would provide a more complete understanding of this relationship as well as potentially revealing any additional physiological costs of this increased output as subjects age. These costs might mirror mechanical heart degradation and disease in vertebrate animals but could be of novel interest to cardiac researchers given the evolutionary independent nature of the spider cardiac system as an invertebrate. Additionally, if measurements of the mechanical properties of the spider heart could be conducted ex vivo, then it would be possible to combine these with MRI to calculate the biomechanical forces at work in the spider heart *in vivo*. Spider growth gradually tapers off both physically and with frequency dependent on species, age and food availability (Foelix 1996). It is conceivable that the end point to this process might be marked or triggered by the condition of the heart, precluding further growth when

specific mechanical limits have been reached. The MRI technique we have demonstrated here would be suitable for investigating this further.

The consistency of mean heart rate across subjects supports the use of non-contact methods to assess heart function in spiders. Although some reports suggest that heart rate across all spider species is correlated with animal mass (Carrel and Heathcote 1976) this was not found to be the case for the subjects involved in our study. However, the Carrel and Heathcote study treats multiple spider types in a collective fashion, combining measurements from them all into a single trend for heart rate and mass. This is despite known differences between these spiders in terms of behaviour, environmental conditioning and physiology. This variation between types is visible in the Carrel and Heathcote study itself. Our study suggests that within groupings of spider type heart rate does not vary - certainly within the Theraphosidae species. Comparative study of different types of spiders using MRI would clarify this situation further.

A wider range of heart rate values was observed in two subjects in this study. These two animals were scanned first. This greater range of obtained heart rates could be explained by a poorer quality Intragate navigator signal resulting from increased animal motion. In turn this could be due to less restrictive restraints used on these initial two subjects as we finalised the experimental set up. However, mean values are still acceptably consistent with those found in the subsequent scanned subjects.

We were unable to absolutely determine the gender of the subjects scanned, but their large size (indicating greater age) and continued life-span post-scanning (years) suggests they were all female. This would also tally with the assessment of the supplier. It is possible that our group of subjects included a mix of both male and female subjects which may have resulted in some of the variation in results. However, sexing of spiders is notoriously difficult until males reach sexual maturity in the final instar of growth and so may continue to be difficult to determine.

Despite reports of the effectiveness of isoflurane-based inhalation anaesthesia induction in spiders (Zachariah et al., 2009; Dombrowski et al., 2013) we found it to have variable performance (Pizzi 2012). Some spiders were rendered lethargic after

5-10 minutes. In others it appeared to have minimal effect even for induction times greater than 30 minutes. The brief time needed to move the animal from the induction chamber to the scanning chamber (<15 seconds) was often sufficient for recovery from the initial lethargic state.

Although alternative injectable anaesthetics have been studied recently in spiders (Gjeltema 2014) more effective inhalation anaesthesia agents should be investigated for future use. Additionally, the design and use of a combined induction/scanning chamber is recommended to avoid the need to remove spiders from the anaesthesia environment. Placement of the spiders in an oxygen enriched environment post-scanning is recommended to potentially accelerate recovery from the deep level of anaesthesia induced in subjects over the scan duration.

The RG-CINE-MRI sequence used was designed for rodent hearts beating at much higher rates compared to spiders (~20 beats per minute (bpm) compared to ~350bpm in rats and ~550 bpm in mice). Therefore, a potential concern was that the spider heart rate would be insufficient and provide too few complete cardiac cycles for the reconstruction algorithms of the RG-CINE-MRI software to work. This did not prove as problematic as originally thought and the reconstruction appeared robust and consistent with experience in house.. Our own particular scan settings may not be optimal in terms of balancing total scan time (and so cost) against the obvious benefits of *in vivo* MRI as a technique. Fewer image averages and Intragate cardiac cycles should be possible to speed up the imaging process without compromising the final measurement quality. Our high imaging resolution enabled us to see the operation of the heart ostia, but for simple analysis of heart function the image resolution could also be reduced, speeding up image acquisition further. It should be noted that in these experiments the RG-CINE-MRI for each slice was acquired sequentially. We found this gave stable pseudo cardiac and respiratory gating signals that are needed for the retrospective reconstruction of the cardiac images. However, a more efficient multi-slice approach is often used in rodent cardiac studies, which could be potentially applied to spiders (Heijman et al., 2006).

These results suggest that RG-CINE-MRI can potentially be applied to spiders and

other invertebrates. The availability of MRI micro-coils, from just 50um diameter (Webb 2013), would allow even small invertebrates to be studied. However, it is worth noting that the centralised, cohesive heart structure of spiders is comparatively rare. Amongst both arachnids and insects, a chain of small 'pseudo-hearts' that act collectively is more the norm (Klowden 2007). Though, the application of RG-CINE-MRI to such a chain of pseudo hearts should be technically possible, it would need to be practically tested.

In summary, we acquired the first *in vivo* cinematic cardiac MRI images from spiders. From these images we were able to directly measure common cardiac functional parameters for the first time in an identical manner to existing human and rodent cardiac MRI. Cardiac ejection fraction was found to increase with animal mass at a faster rate than myocardial tissue volume while heart rate stayed constant across animals, suggesting the spider heart undergoes additional biomechanical loading with age. The RG-CINE-MRI technique provides much potential for *in vivo* cardiac MRI research to be expanded into a wider range of novel species.

**Acknowledgements**

The authors thank Dr M. A. Jansen and Prof. W. N. McDicken for advice throughout this project.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

Movie M1 Example CINE cardiac movie

Movie M2 Example CINE cardiac Movie

Movie M3 Example CINE cardiac Movie

**Contributions**

GM and WMH conceived and designed the experiments. GM, JM, LG, RP and WMH performed the experiments. GM, NB and LH analysed the resultant images and data. GM, NB and WMH prepared the manuscript

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| Spider | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± std |
|---|---|---|---|---|---|---|---|
| Body Mass (g) | 11.7 | 12.00 | 12.00 | 15.10 | 16.09 | 18.8 | 14 ± 3 |
| Heart Rate (beats/min) | 20.2 (±1) | 20.6 (±1) | 21.1 (±2) | 22.9 (±4) | 16.0 (±3) | 20.6 (±6) | 20 ± 2 |
| End systolic ventricular Volume (ESV) ($mm^3$) | 29.6 | 50.0 | 30.7 | 28.6 | 53.7 | 49.4 | 40 ± 12 |
| End Diastolic ventricular Volume (EDV) ($mm^3$) | 42.5 | 57.5 | 51.1 | 59.9 | 96.5 | 115.1 | 70 ± 29 |
| Ejection Fraction (EJ) | 0.25 (±0.04) | 0.13 (±0.02) | 0.37 (±0.04) | 0.52 (±0.02) | 0.48 (±0.04) | 0.57 (±0.01) | 0.38 ± 0.17 |
| Heart volume (systole) ($mm^3$) | 114 | 134 | 91.8 | 135 | 176 | 184 | 139 ± 35 |
| Heart volume (diastole) ($mm^3$) | 122 | 140 | 114 | 170 | 213 | 232 | 165 ± 48 |
| Volume of Myocardium (systole) ($mm^3$) | 84.0 | 83.7 | 61.1 | 106 | 123 | 135 | 99 ± 28 |
| Volume of Myocardium (diastole) ($mm^3$) | 79.3 | 82.6 | 63.1 | 110 | 116 | 117 | 95 ± 23 |
| Change in myocardium Thickness ($mm^3$) | 0.06 | 0.01 | -0.02 | -0.03 | 0.08 | 0.15 | 0.04 ± 0.07 |

Table 1. Cardiovascular physiological measurements made on each of the six spiders from the cine MRI datasets. The mean values from all six spiders is given in the end column

## Figure Legends

**Figure 1. Experimental set up for scanning. A**. 7T preclinical MRI scanner used in the experiments, **B.** Spider in anaesthetic chamber undergoing anaesthesia

induction, **C.** Anaesthetised spider lying prone on back in place above MRI coil, **D.** Spider ready for scanning now with restraints in place and with plastic sleeve in place over the coil and cradle.

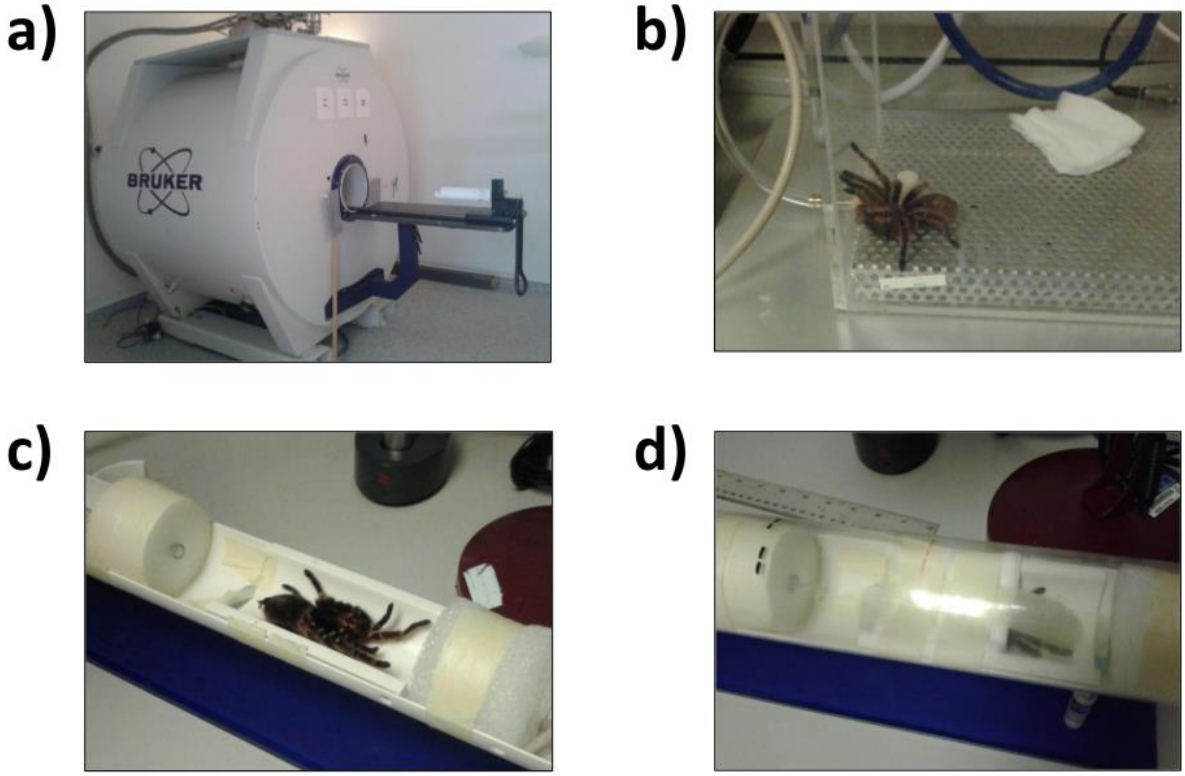



**Figure 2. Example cardiac images from RG-CINE-MRI data.** Axial image slices showing **A**. Diastolic phase (heart arrowed), B**.** Systolic phase (heart arrowed), **C.** Heart with ostia (arrowed) in the open position, **D.** Heart with ostia (arrowed) in the closed position.

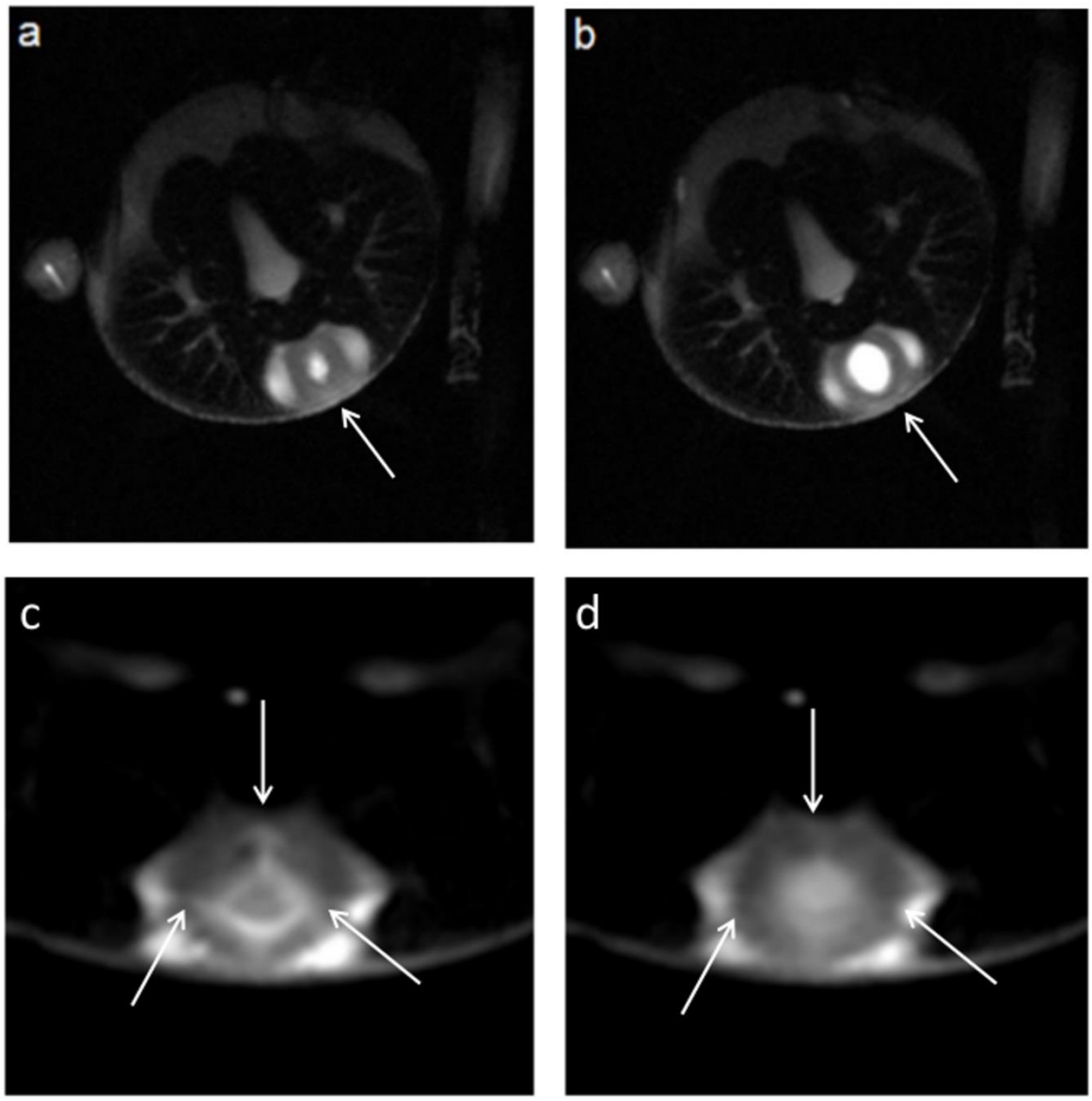
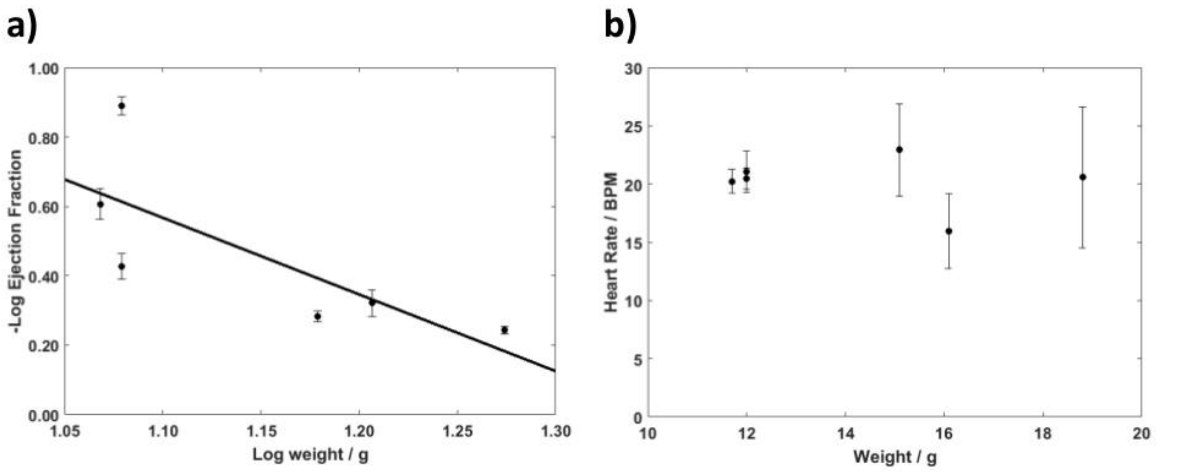



**Figure 3. Results of Cardiac MRI Analysis showing A.** Mean and standard deviation of measurements of cardiac ejection fraction determined between three independent researchers (for n=6 spiders). **B.** RG-CINE-MRI navigator sourced mean heart rates in Beats Per Minute (BPM) with s.d. error bars. After RG-CINE-MRI navigator signal processing the mean heart rate for each slice of cardiac data from an individual subject is estimated and then a mean heart rate is generated for the entire heart (n=6).


a)
b)
-Log Ejection Fraction
Log weight / g
Heart Rate / BPM
Weight / g