Developmental changes of cardiac function and mass assessed with MRI in neonatal, juvenile, and adult mice

FRANK WIESMANN,1 JAN RUFF,2 KARL-HEINZ HILLER,2 EBERHARD ROMMEL,2 AXEL HAASE,2 AND STEFAN NEUBAUER1

1Department of Cardiology, 2Institute of Biophysics, University of Würzburg, 97080 Würzburg, Germany

Wiesmann, Frank, Jan Ruff, Karl-Heinz Hiller, Eberhard Rommel, Axel Haase, and Stefan Neubauer. Developmental changes of cardiac function and mass assessed with MRI in neonatal, juvenile, and adult mice. Am. J. Physiol. Heart Circ. Physiol. 278: H652–H657, 2000.—Cardiovascular transgenic mouse models with an early phenotype or even premature death require noninvasive imaging methods that allow for accurate visualization of cardiac morphology and function. Thus the purpose of our study was to assess the feasibility of magnetic resonance imaging (MRI) to characterize cardiac function and mass in newborn, juvenile, and adult mice. Forty-five C57bl/6 mice from seven age groups (3 days to 4 mo after birth) were studied by MRI under isoflurane anesthesia. Electrocardiogram-gated cine MRI was performed with an in-plane resolution of (78–117 µm)². Temporal resolution per cine frame was 8.6 ms. MRI revealed cardiac anatomy in mice from all age groups with high temporal and spatial resolution. There was close correlation between MRI- and autopsy-determined left ventricular (LV) mass (r = 0.95, SE of estimate = 9.5 mg). The increase of LV mass (range 9.6–101.3 mg), cardiac output (range 1.1–14.3 ml/min), and stroke volume (range 3.2–40.2 µl) with age could be quantified by MRI measurements. Ejection fraction and cardiac index did not change with aging. However, LV mass index decreased with increasing age (P < 0.01). High-resolution MRI allows for accurate in vivo assessment of cardiac function in neonatal, juvenile, and adult mice. This method should be useful when applied in transgenic mouse models.

magnetic resonance imaging; mouse heart; neonatal physiology; ventricular mass

Transgenic mouse models are gaining widespread popularity in cardiovascular research. This is because of the well-characterized genome of mice and the ability to perform distinct genetic modifications, such as gene overexpression, mutation, and knockout. Studies of the pathophysiological consequences of gene modulations, however, require appropriate technology to characterize cardiac anatomy and function in mice. Because the consequences of gene alterations may become manifest at an early age (1, 6), noninvasive, sequential characterization of the cardiac phenotype during development is crucial.

Invasive hemodynamic measurements in newborn and very young mice have so far not been reported. Even if they were achievable, the invasive nature of such measurements would make serial assessment during growth difficult. Measurement of cardiac function in adult mice has been successfully demonstrated by M-mode echocardiography (7, 10). Recently, attempts to study cardiac function in mouse embryos by Doppler ultrasound have been described (3, 8).

Magnetic resonance imaging (MRI) has proven to be highly accurate and reproducible in the assessment of cardiac morphology and function in both animals (14) and humans (13). Because of its high temporal and spatial resolution, the MRI method is able to meet the requirements of the small-sized, fast-beating mouse heart, resulting in detailed time-resolved visualization of cardiac morphology and function. Whereas visualization of the murine heart by conventional (slow) MRI techniques has been reported (15, 16), we were recently able to demonstrate the application of a faster MRI method for assessment of left ventricular (LV) volumes and mass in adult mice (12), in which data acquisition with high temporal resolution and microscopic spatial resolution (in-plane pixel size <100 µm) is achievable. Thus the purpose of this study was to investigate the feasibility of fast high-resolution MRI for the in vivo assessment of physiological alterations of cardiac morphology and function in newborn, juvenile, and adult mice.

METHODS

Forty-five male C57bl/6 mice from seven age groups (3 days, 10 days, 3 wk, 4 wk, 5 wk, 10 wk, or 16 wk after birth; body wt 1.8–33.2 g) were studied using MRI (Fig. 1A). Mice were anesthetized with isoflurane (1.5–2.5 vol% at 1-liter oxygen flow) via a nose cone. Electrocardiogram (ECG) wires...
were attached to the front paws. The ECG trigger signal was taken from a laboratory-built ECG unit. During the experiment, the mouse was positioned supine on a warming pad to keep body temperature constant. Mean heart rate (HR) for the entire study group was 396 ± 12 beats/min. To demonstrate the capability of repeated MRI studies for follow-up in individual animals, four separate mice were repetitively studied 10 days, 4 wk, 5 wk, and 16 wk after birth. Animal experimental procedures were in accordance with institutional guidelines.

In vivo MRI. Experiments were performed on a horizontal-bore, 7.05-T magnetic resonance scanner equipped with a magnetic field gradient system (Bruker, Germany) capable of 870 mT/m maximum gradient strength and 280-µs rise time. For NMR signal transmission and reception, laboratory-built birdcage probe heads (5) with varying inner diameters were used. These inner diameters were optimized for the different mouse sizes from measurements of the maximal murine circumference and ranged from 16 to 35 mm to yield optimal coil filling. The homogeneity of the radio frequency field (B1) in the z direction just fitted the size of the corresponding mouse heart. This allowed for high image quality represented by clear definition of anatomic structures and good contrast between static and dynamic tissue compartments in mice of all studied ages and sizes. MRI was performed using an ECG-triggered fast gradient echo (FLASH) cine sequence (4) with the following imaging parameters: echo time 1.5 ms, repetition time 4.3 ms, flip angle 40°, field of view varying from (20 mm)² to (30 mm)², acquisition matrix 256 × 256, in-plane pixel size ranging from (78 µm)² to (117 µm)², and slice thickness ranging from 0.5 mm (neonatal mice) to 1.0 mm (adult mice, ages 10 wk and 16 wk). With this in-plane and through-plane resolution, we were able to resolve the LV myocardial wall into 8–10 pixels and to cover the LV along its long axis with 7–9 slices. To yield sufficient image quality, two consecutive echoes were averaged per k-space line, resulting in an acquisition window per cine frame of 8.6 ms. To further increase signal-to-noise ratios, the experiment for each acquired slice was repeated after acquisition of all 256 phase-encoding steps. After the image plane orientation from coronal and oblique LV long-axis images was positioned, MRI data acquisition was performed in multiple contiguous short-axis slices (no interslice gap) to cover the entire LV. The total acquisition time per slice for one cine sequence ranged from 60 to 90 s, depending on the HR. Typically, 7–9 short-axis slices were acquired, resulting in a total scan time in the range of 10–15 min.

Ex vivo measurements. After MRI studies were completed, mice were killed, and hearts were excised (Fig. 1B). Both atria, aortic tissue, and the free right ventricular wall were removed, and the LV was gently blotted with cellulose tissue without squeezing. Autopsy LV mass was determined as LV wet weight.

Data analysis. For LV mass measurements, epicardial borders were manually delineated. Because of the intrinsic strong contrast between the blood pool and myocardium, LV cavity volume could be segmented as a second compartment by applying a threshold and regional-growth algorithm. End-diastolic parameters were determined from the first frame after onset of the QRS-complex; end systole was defined as the frame with visual estimation of the minimal LV cavity volume. This visual estimation of the minimal volume for the end-systolic cine frame was done in each slice acquired. Total LV volumes were calculated as the sum of all slice volumes (multislice volumetric method). In situations in which the LV cavity was visible during diastole but not in the end-systolic frame, the LV cavity compartment delineated in the end-diastolic frame was included in the LV cavity volume (EDV), as suggested by Lorenz et al. (9). Stroke volume (SV) was calculated as EDV minus end-systolic volume (ESV), and ejection fraction (EF) was given as SV divided by EDV. To calculate cardiac output (CO), SV was multiplied by HR, which was determined from multiple averaged R-R intervals. Myocardial mass was defined as the weight within the LV cavity without the myocardium, multiplied by a factor of 1.05 (assuming myocardial specific gravity of 1.05 g/cm³) (10). By definition, papillary muscles were included in the myocardial mass measurement. LV mass index was calculated by normalizing LV mass to body weight (mg/g).

Statistical analysis. All results are given as means ± SE. For correlation of LV mass results acquired from MRI with ex vivo LV mass results, a regression analysis was performed. For comparison of the seven age groups, factorial ANOVA was performed. P values < 0.05 were considered significant.
RESULTS

Developmental changes of cardiac physiology. High-resolution MRI allowed for visualization of murine cardiovascular morphology with great detail (Fig. 2). Satisfactory image quality was obtained in all mice with good contrast between the blood pool and myocardium. This allowed for clear delineation of epicardial and endocardial borders and, hence, accurate quantification of LV volumes and mass. Table 1 summarizes the results of the MRI LV volume and mass measurements of the entire study population, demonstrating the increase of these parameters with age. Although, as expected, LV mass increased with age, ANOVA revealed a significant decrease for LV mass index with aging (P < 0.01) (Fig. 3). Comparison of HR (396 ± 12 beats/min, mean ± SE for entire study group), EF (72.2 ± 1.8%), and cardiac index (0.67 ± 0.05) among the studied age groups revealed no significant differences (Table 1). There was high correlation of LV autopsy mass and LV mass measurements acquired from MR images (r = 0.95, SE of estimate = 9.5 mg) (Fig. 4).

Follow-up study with repeated MRI measurements. The studies described in Developmental changes of cardiac physiology were performed at single time points. For assessment of feasibility of repeated MRI experiments, a follow-up study was performed in a separate group of mice. Repeated MRI studies at four defined

Table 1. MRI results for the entire study population

<table>
<thead>
<tr>
<th>Age</th>
<th>3 d</th>
<th>10 d</th>
<th>3 w</th>
<th>4 w</th>
<th>5 w</th>
<th>10 w</th>
<th>16 w</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>2.2±0.3</td>
<td>7.4±0.4</td>
<td>9.9±0.3</td>
<td>13.2±0.4</td>
<td>17.8±0.3</td>
<td>23.1±0.6</td>
<td>26.8±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV autopsy mass, mg</td>
<td>10.0±0.4</td>
<td>35.3±1.8</td>
<td>44.4±1.2</td>
<td>54.8±3.4</td>
<td>72.8±3.5</td>
<td>82.9±2.7</td>
<td>100.2±5.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MRI LV mass, mg</td>
<td>9.6±0.9</td>
<td>35.9±3.6</td>
<td>43.0±1.3</td>
<td>57.5±3.6</td>
<td>73.1±4.5</td>
<td>83.6±2.4</td>
<td>101.3±7.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV long-axis length, mm</td>
<td>2.9±0.1</td>
<td>5.1±0.2</td>
<td>5.7±0.2</td>
<td>5.8±0.2</td>
<td>6.3±0.3</td>
<td>6.8±0.2</td>
<td>7.2±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV EDV, µl</td>
<td>4.2±0.6</td>
<td>19.9±1.9</td>
<td>27.3±0.8</td>
<td>32.3±2.3</td>
<td>45.2±3.3</td>
<td>51.8±2.0</td>
<td>63.6±6.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV ESV, µl</td>
<td>1.1±0.5</td>
<td>4.9±0.5</td>
<td>6.5±0.9</td>
<td>8.4±1.3</td>
<td>14.6±2.0</td>
<td>15.9±1.6</td>
<td>23.5±4.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV SV, µl</td>
<td>3.2±0.4</td>
<td>15.0±1.5</td>
<td>20.8±0.8</td>
<td>23.9±1.6</td>
<td>30.5±1.6</td>
<td>38.6±1.4</td>
<td>40.2±2.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>76.8±8.6</td>
<td>75.6±1.1</td>
<td>76.2±3.1</td>
<td>74.2±2.8</td>
<td>68.6±2.3</td>
<td>69.6±2.4</td>
<td>64.6±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>LV CO, ml/min</td>
<td>1.1±0.1</td>
<td>5.3±0.8</td>
<td>8.7±0.3</td>
<td>9.3±0.9</td>
<td>11.2±0.8</td>
<td>15.7±0.5</td>
<td>14.3±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV CI, ml·min⁻¹·g body wt¹⁻</td>
<td>0.52±0.09</td>
<td>0.70±0.17</td>
<td>0.88±0.06</td>
<td>0.71±0.08</td>
<td>0.63±0.05</td>
<td>0.69±0.03</td>
<td>0.53±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>372±54</td>
<td>418±18</td>
<td>422±26</td>
<td>390±32</td>
<td>366±14</td>
<td>442±15</td>
<td>360±19</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mice. LV, left ventricular; MRI, magnetic resonance imaging; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output; CI, cardiac index; d, days; w, weeks; NS, not significant.
time points (10 days, 4 wk, 5 wk, and 16 wk after birth) were well tolerated in all mice. After the MRI experiment was completed, mice recovered within minutes from isoflurane anesthesia without any noticeable behavioral alteration. The time course of MRI parameters in the follow-up study was consistent with the results from Table 1. Figure 5 shows the individual courses of LV mass, SV, and CO with age. As in the study described in Developmental changes of cardiac physiology, there was no significant change of LV EF with increasing age. However, comparison of HR among the four time points revealed an increase of HR with age that was of borderline statistical significance ($P < 0.03$) (Fig. 6).

**DISCUSSION**

To our knowledge, this is the first report on magnetic resonance (MR) microimaging for assessment of changes of LV morphology and function in neonate and infant mice. The MR microimaging method presented allows for reliable and robust image quality, which is a main requirement for quantitative data evaluation. To gain a fair compromise between total imaging time (mainly influenced by numbers of averages) and spatial resolution with the aim of achieving MR cine images with clear definition of endocardial and epicardial borders and sufficient contrast between blood and myocardium, experiments with different fields of view and image matrices were performed. These showed, for example, that a slice thickness of 0.5 mm in newborn mice is necessary to cover the LV with 7–9 slices and, hence, to preserve the accuracy of quantification. We further verified that with a slice thickness of 0.5 mm, the gradient performance in the slice direction was capable of repeatedly generating the gradient strength needed for acquisition of the cine data.

In parallel, the choice of in-plane resolution was again mainly led by the attempt to gain high spatial resolution with preservation of temporal resolution. Given the maximal switching rate of 280 µs at maximal gradient performance of our microscopy gradient system, it became obvious that the maximum number of readout steps and, hence, pixels in the read direction, should not exceed 256. Thus, with a field of view ranging from 20 (for newborn mice) to 30 mm (for adult mice), we were able to avoid image artifacts caused by wraparound in the phase direction and to obtain an in-plane resolution that was similar to that described for human cardiac function MRI studies (e.g., LV wall thickness resolved into 8–10 pixels).

We were able to demonstrate a progressive increase of LV mass and volumes with increasing age. Comparison of MRI-determined LV mass and LV mass measured at autopsy revealed close correlation for neonatal, juvenile, and adult mice ($r = 0.95$), indicating high accuracy of the noninvasive MRI quantification. Consistent with developmental changes in humans (2, 17), we found a significant decrease in LV mass index and no change in LV EF with physiological growth. The finding of a similar HR for all age groups was surprising, because the inverse relation of HR and age is well known for the human fetal, neonatal, and infant period (11). In the follow-up study, there was even an increase in HR with age, which showed statistical significance ($P = 0.03$). One could speculate that these observations might well be related to a different extent of negative chronotropic effects of isoflurane anesthesia at varying age, although this hypothesis cannot yet be supported by literature findings. However, MRI studies in rodents can at present only be performed under general anesthesia, and even with the use of the inhalation anesthetic isoflurane, which allows close control over the depth of anesthesia, some extent of negative chronotropic and inotropic effects is unavoidable.

We also demonstrated that MRI can be used for repeated noninvasive measurements of individual mice. Each mouse recovered well within minutes after anesthesia administration was halted, and integration of the mice into the litter society occurred without complications. This makes the MRI method suitable for studying the time course of morphological and functional features in individual animals, thus reducing the number of animal experiments required to demonstrate age-dependent phenomena. This may be particularly important in transgenic models with reduced fertility and, thus, limited availability of animals.

Interestingly, there was a slight but continuous difference in body weight between animals in the
follow-up group (see Fig. 5A) and the other animals (see Table 1). One possible explanation could be that the four animals of the follow-up study came from a litter that was slightly lighter than the other animals, which were mixed from different litters. It might well be that repeated administration of anesthesia had a negative effect on body growth of the mice in the follow-up group. However, the otherwise normal nutritional and social behavior in the litter society as well as the observation of a constant difference in body weight compared with the other animals of same age favors the former explanation of the body weight difference.

With regard to the difference in CO between the follow-up study and the corresponding age groups, it might well be that, because of recurrent administration of isoflurane in the follow-up group, a certain degree of tolerance is induced. This tolerance seems to go along with a higher HR in the repeatedly studied animals, as shown in Fig. 6B in comparison with data in Table 1.

For optimization of overall image quality, a variety of software and hardware adaptations to the mouse model have been performed in this study. To minimize image artifacts from flow or bulk motion, cine imaging was performed with the shortest possible echo time (1.5 ms) achievable with the MRI system. The applied flip angle of the cine FLASH sequence was optimized to gain high contrast between the static myocardium and the inflowing blood in the LV cavity. Important contributions to the image quality were also made by optimization of the radio frequency coil design. To avoid significant losses of sensitivity of the coil and, hence, signal-to-noise ratio of the acquired images, such as that which occurs when the filling factor of a radio frequency coil is not optimized, the inner diameter of the coils was adapted to the different mouse sizes studied. For future work, further improvement of image quality could be obtained by using quadrature-driven birdcage coils.

With the perspective of numerous transgenic and gene knockout mouse models, noninvasive high-resolution MRI allows for fast phenotype evaluation. Such noninvasive colony screening would give the genetically engineering molecular biologist important information for further planning. Applying the described high-resolution MRI method in surgical murine models of heart failure might be of substantial value for the understanding of long-term processes like scar formation and remodeling after myocardial infarction or right and left ventricular hypertrophy caused by pres-
pressure and volume overload. However, the contribution of MRI to the analysis of murine pathophysiology will not end with the characterization of mass and global function. It is conceivable that various MRI methods developed for the study of humans, such as perfusion or flow mapping, will be adapted to the mouse. Thus MRI methods should continue to shed new light on the pathophysiological consequences of gene targeting in mice.

We thank Sabine Voll for technical assistance and Titus Lanz for valuable hardware contributions.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG, WI 1510; SFB 355 “Pathophysiologie der Herzinsuffizienz”, Teilprojekte A1, A3, A6).

Address for reprint requests and other correspondence: F. Wiesmann, Medizinische Universitätsklinik, Josef-Schneider-Str. 2, 97080 Würzburg, Germany (E-mail: f.wiesmann@mail.uni-wuerzburg.de).

Received 5 March 1999; accepted in final form 16 September 1999.

REFERENCES

17. Smith, H. L. The relation of the weight of the heart to the weight of the body and of the weight of the heart to age. Am. Heart J. 4: 79–93, 1928.