Magnetic resonance imaging accurately estimates LV mass in a transgenic mouse model of cardiac hypertrophy

FATIMA FRANCO,1 SUSAN K. DUBOIS,2 RONALD M. PESHOCK,1,2 AND RALPH V. SHOHE2
Departments of 1Radiology and 2Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Franco, Fatima, Susan K. Dubois, Ronald M. Pesheck, and Ralph V. Shohet. Magnetic resonance imaging accurately estimates LV mass in a transgenic mouse model of cardiac hypertrophy. Am. J. Physiol. 274(Heart Circ. Physiol. 43): H679–H683, 1998.—Transgenic mice with a dysfunctional guanylyl cyclase A gene (GCA –/−) are unable to transduce the signals from atrial natural peptide and develop hypertension and cardiac hypertrophy. Magnetic resonance imaging (MRI) was performed to assess cardiac hypertrophy in these animals, using wild-type siblings as controls. Anesthetized mice were studied by gated multislice, multiphase cine MRI at 1.5 T. Simpson’s rule was used to estimate left ventricle (LV) mass and volumes from short-axis images. Correlation between LV mass evaluated by MRI and at necropsy was excellent, with LVnecropsy = 1.04 × LVMRI + 4.69 mg (r² = 0.95). By MRI, GCA –/− LV mass was significantly different when compared with isogenic controls [GCA –/–, 226 ± 43 mg (n = 14) vs. controls, 156 ± 14 mg (n = 10); P < 0.0001]. LV volumes and ejection fraction in the two groups were not significantly different. MRI provides an accurate means for the noninvasive assessment of murine cardiac phenotype and may be useful in following the effects of genetic modification.

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MATERIALS AND METHODS

Animal Protocol

The study was approved by the Institutional Review Board for Animal Experimentation at the University of Texas Southwestern Medical Center at Dallas. The transgenic mouse model was previously described (11). Twenty-four mice, weighing 25–44 g, were studied, including 14 transgenic (GCA –/−) and 10 isogenic controls (GCA +/-) with ages between 3 and 16 mo. Animals were weighed before MRI and anesthetized with serial intraperitoneal injections of Avertin (2.5% tribromoethanol and 0.8% 2-methyl-2-butanol in water; Sigma, St. Louis, MO) and were allowed to breathe spontaneously. After the scan, the mice were killed, and the heart was dissected and weighed. To test interstudy reproducibility, three additional mice were scanned twice on the same day and again the following day.

MRI Technique

MRI was performed using a 1.5-T Philips Gyroscan NT whole body imaging system (Philips Medical Systems, Shelton, CT). The mouse was positioned supine, head up on a plastic petri dish, and electrocardiograph leads were attached to both front paws and one hindpaw. A standard endorectal coil (4 × 8 cm) was placed under the animal and used for imaging. All MRI scans used a 6-channel electrocardiogram gating. Heart rates were 350–550 beats/min.

Multislice, multiphase cine MRI was performed. Each study included a scout, coronal plane long axis of the left ventricle and a set of short-axis acquisitions. Multiframe, short-axis gradient-echo sequences were used to measure LV end-systolic volume (LVESV) and end-diastolic volume (LVEDV) and estimate LV mass. Four or five slices perpendicular to the long axis were obtained for each heart spanning apex to base. The slice thickness was 1.6 mm with a 0.2-mm gap between slices. The matrix was 256 × 256, with a field of view of 50 mm (yielding voxel sizes of 0.19 × 0.19 × 1.6 mm). Flip angle of 30°, repetition time of 39 ms, and echo time of 14 ms.

The pulse sequence was set for a heart rate of 250/min with five cardiac phases and a temporal resolution of 39 ms. Depending on the heart rate and R-R interval length, a trigger delay was introduced. Thus end-diastolic and end-systolic images were captured over two contiguous cardiac cycles. The frame with the largest chamber dimensions was used as end diastole for mass and volume measurements, and
the image with the smallest chamber volume was taken as the systolic image.

Data Analysis

Image analysis. For LV mass determination, the epicardial and endocardial border of each slice was identified during diastole and traced by hand. The LV wall volume was calculated as follows:

\[ \text{LV wall volume} = \sum (\text{epicardial area} - \text{endocardial area}) \times (\text{distance between slice centers}) \]

where the distance between slice center equals slice thickness plus slice gap.

Myocardial mass was estimated by multiplying the LV wall volume obtained with Simpson’s rule by the specific gravity of the myocardium, 1.05 g/cm³. Systolic images were also analyzed, and similar determinations of LVEDV and LVESV permitted calculation of stroke volume (SV = LVEDV − LVESV) and ejection fraction (EF = SV/LVEDV). To determine interobserver and intraobserver variability, the MRI data were analyzed by two independent observers (F. Franco and R. V. Shohet) and twice by a single observer (F. Franco). MRIs were stored on optical disks for subsequent recall and analysis.

Necropsy analysis. The animals were killed with ether and cervical dislocation, and the ventricles were dissected free of atria, fat, and large blood vessels. During initial experiments, the weight of the right ventricular free wall was found to be <10% of the total ventricular weight. Because variation in right ventricle mass contributed only marginally to total ventricular mass and the dissection of the free wall was prone to error, we included the right ventricle in all necropsy mass determinations.

Statistics

Data are expressed as means ± SD. LV mass and EF from transgenic (GCA −/−) and control mice were assessed for differences using the Student’s t-test. Least-squares linear regression was used to relate the MRI estimate of LV mass to necropsy mass. Analysis of the difference of the measurements with MRI and necropsy was performed according to the technique of Bland and Altman (2). LV mass index was determined in each animal [LVMI (mg/g) = LV mass determined by MRI (mg)/body weight (g)]. Figure 4 demonstrates the time course for development of cardiac hypertrophy in GCA −/− mice as determined by MRI. The regression equation for the 14 GCA −/− mice is

\[ \text{LVMI}_{\text{GCA}−/−} = 5.47 + 0.137 \times \text{time (mo)} \]

Thus, for each month of age, the LVMI increases by 0.137 mg·g body wt⁻¹·mo⁻¹. The regression equation for the 10 GCA +/+ mice is

\[ \text{LVMI}_{\text{GCA}+/+} = 4.5 + 0.0032 \times \text{time (mo)} \]

For this group, the increase in LVMI is much less, only 0.0032 mg·g body wt⁻¹·mo⁻¹. The t-test performed to compare the two coefficients was not significant (P = 0.252). However, there is a trend present that could reach significance with a larger sample size.

LV volumes and EF were calculated for each mouse (Table 1). There were no significant differences between the two groups in LVEDV, LVESV, SV, or EF.

DISCUSSION

The results of this study indicate that 1) MRI provides an accurate means for the noninvasive estimate of myocardial mass in the intact mouse; 2) MRI can noninvasively detect differences in myocardial mass between wild-type mice and siblings lacking the GCA gene; 3) GCA −/− mice have increased myocardial mass as compared with GCA +/+ mice, with no significant differences in LV volumes or ejection fraction; and 4) GCA −/− mice demonstrate a trend toward increasing myocardial mass with age.

Over the last decade, transgenic manipulation of the mouse has allowed dramatic progress in our understanding of cellular and molecular mechanisms of disease. Unfortunately, the application of these techniques to cardiovascular biology has been limited by the difficulty of evaluating cardiac phenotype in an animal with a heart rate of 450 beats/min and LV mass of <200 mg.

Echocardiography has been used extensively in studies of cardiac mass in humans (3) and large animal models (10, 13, 16). In the mouse, with the use of
high-frequency transducers (7.5 and 9.0 MHz) (4, 12, 19), there was a good correlation between echocardiographic estimation of LV mass and LV mass at autopsy. Also, echocardiography has been used to evaluate ventricular function in transgenic mice (5). Previous studies performed in rodents to assess LV mass used geometric assumptions to estimate mass (4, 12, 13), and such assumptions may not be valid in hearts with segmental wall motion abnormalities or nonhomogeneous distributions of LV hypertrophy (4).

MRI has also been used extensively in the evaluation of ventricular mass in human (7, 8) and large animal models (16, 20, 23). An important feature of MRI in these studies has been the lack of assumptions regarding LV shape. Application of MRI methods to small animal models has been limited. Rose et al. (15) imaged mouse hearts using high-field magnets (7 and 9.4 T); however, no quantification or functional studies were performed. Recently, Siri et al. (18) performed gated MRI and M-mode echocardiography in normal and hypertrophied murine hearts. Again, imaging was performed at 9.4 T, and LV mass was estimated using a truncated ellipsoid model. LV mass was estimated more accurately by MRI than echocardiography in this study. Our study is the first to perform imaging using a conventional imaging system at 1.5 T, which would facilitate broad application of this method. In addition, we used Simpson’s rule to calculate LV mass and volumes, which is not based on geometric assumptions and has been shown to be the most accurate method in other animal models (21).

The MRI assessment of the mouse heart has several limitations. First, the typical anesthetized mouse heart rate is ~450 beats/min, with a typical duration of systole of ~50 ms. Given the temporal resolution of 39 ms used in this study, a trigger delay must be used to...
ensure that images are obtained at both end diastole and end systole for the assessment of function. If higher
temporal resolution becomes available, this will re-
move the need for multiple scans to determine the
appropriate trigger delays. Second, the scan protocol
was designed to obtain high spatial resolution and
adequate temporal resolution for a moving object with
an average epicardial volume of \( \sim 300 \mu l \). The LV long
axis, measuring 7–10 mm, was spanned with five
1.6-mm slices with a 0.2-mm gap to minimize total scan
time. For comparison, determination of myocardial
mass in humans using MRI typically uses eight to ten
1-cm-thick slices to span an LV long axis of 8–10 cm.
Thus a relatively limited number of slices was used in
our study, raising the possibility of partial volume
effects. However, the excellent correlation with nec-
ropsy results suggests that the effect on the assessment
of LV mass in the mouse is limited. Third, the right
ventricle was not routinely dissected when necropsy
measurements were done. However, the free wall of the
RV constitutes substantially \(< 10\%\) of the total ventricu-
lar weight, and even a large variation in its mass would
have had a negligible effect on the total measurement.
MRI cardiac mass measurements were consistently
smaller than necropsy, which may be a result of the
dissection technique used. Fourth, our determinations
of intracavitary volumes and derived stroke volume
were slightly larger than a previous invasive study (1),
and an invasive validation of LV volumes was not
performed. The average body weight of the mice used in
our study was higher (35 g) than in the invasive study
(25–30 g), which may explain the larger LV volumes.
Finally, the requirement for anesthesia limits the evalu-
atation of cardiac function in many animal models;
peripheral vasodilation, effects on central sympathetic
output, and the direct chronotropic effect of some
anesthetics may all modify ventricular function. In
addition, no reference measure of function was made
using another method, which would be required to
validate the assessment of LV function.

The principal value of this study is the demonstra-
tion of an accurate, noninvasive, and widely available

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**Fig. 2. Linear regression analysis of left ventricular (LV) mass estimated by magnetic resonance imaging (MRI) and LV mass measured at necropsy.**

**Fig. 3. Scatter plots showing mean LV mass by necropsy and MRI (horizontal axes) and difference between necropsy and MRI measurements (vertical axes). Mean difference (solid line) ± 2SD (dashed lines) is shown. There is a good agreement between MRI and necropsy measurements. MRI measurements are slightly and consistently lower than necropsy measurements.**

**Fig. 4. LV mass index by MRI vs. age for all mice examined in this study. Regression lines for LV mass index vs. age for each group are indicated by solid lines and are further described in text.**

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Table 1. MRI determination of LV mass and volumes in GCA knockout and in control mice

<table>
<thead>
<tr>
<th></th>
<th>LV Mass, mg</th>
<th>LVEDV, µl/beat</th>
<th>LVESV, µl/beat</th>
<th>SV, µl/beat</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCA −/−</td>
<td>226 ± 43</td>
<td>50.2 ± 15.3</td>
<td>12.7 ± 4.7</td>
<td>37.5 ± 11.4</td>
<td>0.74 ± 8.4</td>
</tr>
<tr>
<td>GCA +/+</td>
<td>156 ± 14</td>
<td>51.7 ± 12.8</td>
<td>13.4 ± 6.7</td>
<td>38.3 ± 8.8</td>
<td>0.75 ± 8.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. LV, left ventricle; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; SV, stroke volume; EF, ejection fraction; GCA, guanylyl cyclase A genotype. Differences between mean measurements of GCA −/− and GCA +/+ are assessed by Student’s t-test; NS, not significant.
approach to the assessment of LV mass in transgenic mouse models. In addition, MRI permits the acquisition of functional data only available in vivo; however, further work needs to be done to validate the MRI method against an independent method for assessing function. The ability to assess hypertrophy and function will be valuable in defining the effects of genetic and pharmacological modifications on the heart, especially in longitudinal studies. Also, transgenic technology allows the combination of multiple interacting genetic modifications through simple breeding experiments. For example, mutations conferring an atherosclerotic or diabetic phenotype could be incorporated into the GCA −/− hypertensive mice, allowing study of a combination of cardiovascular insults commonly found in human patients. Moreover, as inducible and tissue-specific transgenic technologies progress, one can envisage sequential addition of cardiac manipulations, even more closely mimicking the evolution of heart disease in the human population. As cardiac evaluation of the mouse becomes easier, the general utility of this genetically modifiable, inexpensive model system should facilitate rapid progress in our understanding of mechanisms of disease and response to therapy.

Conclusion

This study demonstrates that MRI can accurately and noninvasively determine LV mass in both wildtype mice and transgenic mouse models that develop LV hypertrophy. In addition, this technique permits repeated, longitudinal evaluation of cardiac function in mice. It is well suited to evaluate the cardiac effects of genetic and pharmacological modifications over time.

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Address for reprint requests: R. V. Sholet, Div. of Cardiology, Dept. of Internal Medicine, NB 11200, Univ. of Texas Southwestern Medical Center at Dallas, 6000 Harry Hines Blvd., Dallas, TX 75235-8573.

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