Magnetic Resonance Imaging and Invasive Evaluation of Development of Heart Failure in Transgenic Mice With Myocardial Expression of Tumor Necrosis Factor-α

Fatima Franco, MD; Gail D. Thomas, PhD; Brett Giroir, MD; Debora Bryant, BS; M. Craig Bullock, BSEE; Michael C. Chwialkowski, PhD; Ronald G. Victor, MD; Ronald M. Peshock, MD

Background—Transgenic mice expressing tumor necrosis factor-α (TNF-α) in cardiac myocytes develop dilated cardiomyopathy, but the temporal progression to cardiac dysfunction is not well characterized. We asked (1) Does magnetic resonance imaging (MRI) provide a reproducible assessment of cardiac output in mice that correlates with invasive measurements obtained with thermodilution? (2) What is the time course of left ventricular (LV) remodeling in transgenic mice with myocardial expression of TNF-α?

Methods and Results—Transgenic mice from 2 different lineages with differing amounts of myocardial TNF-α expression (lineage 1 (L1) and lineage 2 (L2)) and littermate controls (LC) were studied. In protocol 1, cardiac output (CO) and stroke volume (SV) were measured by MRI and thermodilution (TD) in 15 mice (3 L1, 4 L2, 8 LC). In protocol 2, 23 mice (7 L1, 8 L2, 8 LC) were scanned at 1 month of life and every 4 weeks thereafter. In both protocols, cine-MRI was performed with the use of a 1.5-T clinical system (1.5-mm slices, 195×195 μm in-plane resolution). MRI CO and SV correlated well with TD [CO_TD (mL/min)=0.94*CO_MRI+0.72, r=0.84; SV_TD(μL)=1.01*SV_MRI−1.07, r=0.94]. Serial MRI studies showed significant increase in LV mass and volumes over time and a significant decrease in ejection fraction in transgenic mice when compared with littermate controls. Compared with lineage 2, lineage 1 showed significantly larger LV mass and volumes and significantly lower ejection fraction.

Conclusions—MRI assessment of cardiac function in mice correlates well with invasive measurements. Serial MRI studies in the TNF-α mouse model demonstrate that the rate of progression and severity of LV dysfunction are dependent on the degree of TNF-α overexpression. (Circulation. 1999;99:448-454.)

Key Words: heart failure ■ systole ■ magnetic resonance imaging ■ cardiac output

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine detected in several human cardiac-related conditions, including congestive heart failure.1,2 There is increasing evidence that cardiac expression of TNF-α may be a common response to different types of cardiac stress.3–5 However, the role played by TNF-α in the development and progression of ventricular dilatation and cardiac decompensation is not known.

Recently, a transgenic mouse model has been developed in which TNF-α is overexpressed specifically in the myocardium, resulting in dilated cardiomyopathy and cardiac failure.6,7 A method that permits longitudinal evaluation of structure and function in individual animals would be of value to define the phenotypic consequences of TNF-α overexpression. However, repeated, reproducible assessment of in vivo cardiac function in mice remains a challenge. It requires a technique that is easily available to the molecular biologist and accurate enough to detect minimal changes.

Recently, magnetic resonance imaging (MRI) has emerged as a highly accurate and quantitative tool for the evaluation of cardiac function.8 However, the application of MRI to study cardiac function in the mouse has been limited to very high-field magnets with imaging gradients that are not widely available.9 In addition, studies evaluating the accuracy and reproducibility of MRI in the quantification of systolic function in the mouse model are lacking. Recently, we developed and validated a highly accurate and reproducible MRI technique at 1.5 T for estimating left ventricular (LV) mass in a transgenic mouse model of cardiac hypertrophy.10 In the present study, we asked (1) Does MRI provide a reproducible assessment of cardiac output in mice that correlates with invasive measurements obtained by thermodilution? (2) What is the time course of LV remodeling in transgenic mice with heart failure in the setting of overexpression of TNF-α?
Methods

Study Population
Two groups of mice were studied. The first group consisted of 15 C57BL/6JXJL/J mice (7 transgenic and 8 wild-type). The second group consisted of 23 C57BL/6JXJL/J mice (15 transgenic and 8 littermate controls). The transgenic mouse model of heart failure was produced as previously described. From the initial founders, 2 lineages with different levels of transgene expression were created. Cardiac TNF levels were 4 times greater in lineage 1 than in lineage 2. Lineage 1 develops severe, rapidly progressive heart failure with near 100% of mortality by the fourth month of life and lineage 2 has only moderate impairment of cardiac function with survival up to 9 to 12 months. Transgenic animals were otherwise phenotypically normal at birth and remained so until the onset of heart failure. All animals were used in accordance with the guidelines of the University of Texas Southwestern Medical Center Animal Care and Research Advisory Committee and in compliance with the rules governing animal use, as published by the National Institutes of Health.

Study Design
Two separate studies were performed. In protocol 1, noninvasive MRI and invasive thermodilution measurements of cardiac output and stroke volume were compared in a cross-sectional study of 15 mice (3 from lineage 1, 4 from lineage 2, 8 wild-type controls). After surgery, MRI was performed followed by thermodilution measurements.

In protocol 2, 23 mice (7 from lineage 1, 8 from lineage 2, and 8 littermate controls) had serial MRI studies for evaluation of LV function. All mice were imaged at 4 to 6 weeks of age and every 4 weeks thereafter. Animals were weighed before MRI and anesthetized with a combination of Telazol (a 1:1 combination of tiletamine HCl and zolazepam HCl; 7.5 mg/kg IM) and xylazine (20 mg/kg IM) with atropine (0.5 mg/kg SC) to prevent excessive tracheal secretion. Mice were placed on a heating pad (temperature 35 to 37°C), and a PE-10 catheter was inserted into the right external jugular vein and advanced to the right atrium. A thermocouple in a 1F catheter (outer diameter 0.49 mm) was inserted into the right carotid artery and advanced to the aortic arch. The thermocouple was attached to a cardiac output computer (Cardiotherm 500, Columbus Instruments), and the venous catheter was attached to an automatic injector (Micro-Injector 400, Columbus Instruments) primed with 0.9% NaCl at 15°C. Probe placement was checked by a test injection. The mice were allowed to stabilize for 15 to 20 minutes and then were taken to the MRI scanner.

Immediately after MRI, the mouse was taken out of the scanner and thermodilution measurements were performed. Cardiac output was measured by monitoring the change in the temperature of the blood in the aortic arch after venous injection of cold (15°C) saline (18 to 25 μL). Dilution curves were recorded on a chart recorder, and cardiac output values were displayed automatically by the computer. For each mouse, 4 to 6 cardiac output measurements were performed at intervals of 1 minute. On completion of the experiment, the mice were euthanized with sodium pentobarbital. Venous catheter and thermocouple placements were verified after each study by postmortem examination.

Magnetic Resonance Imaging
The imaging sequences were the same for protocols 1 and 2. MRI was performed with the use of a 1.5-T scanner (Philips NT). The mouse was positioned supine on a Petri dish, and ECG leads were attached to both front and hind paws. The leads from the standard clinical ECG probe were attached to a home-built amplifier to amplify ECG signal for detection by the clinical gating software on the magnetic resonance system. A surface coil (4 x 8 cm) was placed over the animal’s chest and used for imaging. Multislice, multiphase, cine-MRI was performed as previously described. Four or five slices perpendicular to the long axis were obtained for each heart, spanning apex to base. The slice thickness was 1.5 mm, with a 0.2 mm gap between slices. The matrix was 256 x 256, with a field of view of 50 mm (yielding voxel sizes of 0.19 x 0.19 x 1.5 mm), flip angle of 30 degrees, repetition time of 39 ms, and echo time of 14 ms.

Data Analysis

Image Analysis
The frame with the largest chamber dimensions was used as the end-diastolic image and the smallest as end-systolic image. For LV volume determinations, the endocardial border was identified by hand and volumes calculated by summation.

Cardiac Output Measurement by Thermodilution
Mice studied in protocol 1 were weighed and then anesthetized with an intraperitoneal injection of Avertin (2.5% tribromoethanol and 0.8% 2-methyl, 2-butanol in water). The end point for premature death or 6 months of follow-up was the death of the last mouse in each lineage. LV volume determinations, the endocardial border was identified by hand and volumes calculated by summation.

Cardiac Output by Thermodilution was determined as the average of the measurements obtained in each mouse. Stroke volume was calculated as (SV = CO/HR), where HR was the average heart rate during the scan. LV mass was estimated as described previously.

Thermodilution Analysis
Cardiac output by thermodilution was determined as the average of the measurements obtained in each mouse. Stroke volume was calculated as (SV = CO/HR), where HR was the average heart rate during the thermodilution measurements.

Statistics
Data are expressed as mean ± SD.

The cardiac output and stroke volume measurements obtained by thermodilution were compared with those measured by MRI with a 2-variable linear regression analysis. A Bland-Altman analysis was performed to examine the agreement between the thermodilution and MRI measurements methods.

Interobserver, intraobserver, and interstudy variability were calculated as previously described. LV mass, volumes, and ejection fraction from transgenic and control mice were assessed for differences at each time point with a 2-tailed, unpaired t test. The changes in LV mass, volumes, and ejection fraction over time for each group were analyzed with a random-effects ANOVA test. A Bonferroni adjustment was used to account for multiple comparisons.

Results

Protocol 1: Thermodynamics and MRI Measurements of Cardiac Output and Stroke Volume
The mean age of the 15 mice was 5.1 ± 0.5 months (range 1 to 10 months) and mean body weight was 29.9 ± 4.9 g (range 19.8 to 38 g). The mean heart rate was 356 ± 42 bpm (range 260 to 420 bpm) during MRI and 361 ± 44 bpm (range 260 to 420 bpm) during the thermodynamics measurements (P = NS).

Cardiac output by thermodilution ranged from 9.3 to 19.2 mL/min (mean 13.4 ± 1.7 mL/min) and by MRI 10.8 to 17.5 mL/min (mean 13.5 ± 1.5 mL/min). Stroke volume by thermodynamisation ranged from 22 to 58 μL (mean 38 ± 8 μL) and by MRI ranged from 26 to 58 μL (mean 39 ± 8 μL). Figure 1 shows a typical thermodynamics curve obtained during the
study. As shown in Figure 2, there was a good correlation between thermodilution and MRI measurements of cardiac output \((r = 0.84)\) and stroke volume \((r = 0.94)\). Bland-Altman analysis also demonstrated good agreement between thermodilution and MRI measurements of cardiac output and stroke volume. The limits of agreement (defined as \(\pm 2 \text{SD} \) from the mean difference) between the measurements obtained with the 2 methods are shown in Figure 3.

**Protocol 2: Serial Evaluation of LV Systolic Function in Transgenic Mice and Controls by MRI**

MRI was performed in 23 mice at the first month of life and every 4 weeks thereafter. There was excellent reproducibility of magnetic resonance measurements with low intraobserver \((3 \pm 1\%)\) and interobserver \((7 \pm 6\%)\) variability. The interstudy variability for LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), and LV ejection fraction (LVEF) was \(6.1 \pm 3.6\%, 5.2 \pm 3.2\%, \) and \(3 \pm 2.6\%\), respectively.

Figure 4 shows short-axis views in diastole and systole in transgenic mice from lineages 1 and 2 and a littermate control at age 1 month and 3 months. Mean body weight, heart rate, stroke volume, and cardiac output for all mice are detailed in Table 1. The average values for LV volumes, LVEF, and LV mass at each time point, in both lineages and littermate controls, are given in Table 2.

There was a significant increase in LVEDV and in LVESV in both lineage 1 and lineage 2 compared with controls \((P<0.0001\) for each variable) over time. LVEF was significantly lower in both lineages 1 and 2 compared with controls \((P<0.0001)\). LV mass was significantly increased in lineage 1 but not in lineage 2 when compared with controls \((P<0.0001\) versus \(P=0.18)\). There were no significant differences in stroke volume over time in lineage 1 and lineage 2 versus control mice. However, there was a significant decrease in cardiac output in mice from lineage 1 compared with controls \((P<0.02)\). Figure 5 illustrates the progression of LV volumes and ejection fraction for transgenic and wild-type mice.

There was a significant increase in LV mass and end-systolic volume over time in lineage 1 as compared with lineage 2 \((P<0.0001\) for each variable). There was also a decrease in ejection fraction over time in lineage 1 as compared with lineage 2 \((P<0.0008)\).

**Discussion**

The results of this study indicate that (1) there is a good correlation between MRI and the thermodilution method in measuring cardiac output and stroke volume, (2) MRI is an accurate technique with low interstudy variability and thus well suited for longitudinal studies of cardiac size and function; and (3) in the TNF-\(\alpha\) transgenic model of heart failure, the rate of progression and severity of LV dysfunction are dependent on the degree of TNF-\(\alpha\) overexpression.

**Noninvasive Assessment of LV Systolic Function in Mice**

Serial reliable measurements of LV function to noninvasively track cardiac function would be useful in evaluating the development of heart disease in transgenic mouse models. Echocardiography has been used to evaluate LV function in mice.\(^{13,14}\) However, these studies have not been validated against an independent measurement. Further, the quantification of LV volumes by 2-dimensional echocardiography is generally based on geometric models of the left ventricle.\(^{15}\) With congestive heart failure geometry may change, limiting the applicability of a specific model. Although Doppler techniques have been applied with some success to evaluate LV function in mice,\(^{16}\) Doppler measurements of cardiac output and stroke volume are highly dependent on accurate measurements of aortic diameter and also require that the Doppler beam is oriented parallel to flow.

MRI has proven to be a highly accurate and reproducible technique\(^{8}\) for evaluation of ventricular function and myocardial mass both in animal studies\(^{17}\) and in humans.\(^{18,19}\) MRI provides a volumetric, 3-dimensional evaluation of the LV and does not rely on geometric assumptions. Recently, using

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**Figure 1.** Typical thermodilution curves obtained in 1 wild-type mouse. In this mouse, heart rate was 360 bpm, blood temperature was 34.6°C, injectate temperature was 15°C, and injectate volume was 23 \(\mu\)L.

**Figure 2.** Linear regression analysis. Scatterplots for 15 mice showing correlation between MRI and thermodilution for cardiac output (left) and stroke volume (right).
a widely available clinical MRI system (1.5 T), we developed an MRI method to estimate LV mass that was validated by necropsy. Our present study indicates that this approach can be extended to the quantification of LV volumes and ejection fraction in mice.

Independent Confirmation of MRI by Thermodilution Method

One problem in performing in vivo validation of MRI measurements of LV volumes in mice is determining an appropriate reference standard. Cardiac output measurements may be obtained in mice with open-chest surgical techniques by placement of a flow probe on the ascending aorta. However, the surgery is difficult and results in significantly depressed hemodynamics and force-frequency relations when compared with closed-chest preparations.

Although microsphere techniques have been used to assess cardiovascular hemodynamics in conscious mice, studies with microspheres are technically demanding and permit only a limited number of measurements of cardiac output. This method requires a significant blood withdrawal, leading to eventual hemodynamic degradation and preventing an adequate comparison with the cardiac output measurements obtained from MRI.

The indicator-dilution method has been used as a reference for cardiac output measurements in humans, large animal models, and more recently in mice. The thermodilution method, in which a bolus of chilled water is the indicator, has been applied widely in patients and animal models including rats.

Several features of thermodilution make it a reasonable approach for use in the setting of MRI. First, the surgery can be performed quickly (15 to 20 minutes) with minimal blood loss. Second, the venous catheter and thermoprobe can be inserted before the MRI and the mouse stabilized so that measurements by MRI and thermodilution can be done under similar hemodynamic conditions. Third, the measurements can be repeated as required without adverse effects.

Our results show an excellent correlation between MRI and thermodilution across a wide range of stroke volumes. In addition, the Bland-Altman analysis shows no major bias between the thermodilution and MRI methods. Furthermore, the values for cardiac output that we obtained both with MRI and thermodilution are within the ranges previously reported in mice by investigators using a variety of measurement techniques.

Serial Evaluation of LV Systolic Function in TNF-α Model of Heart Failure by MRI

Multiple clinical observations have reported an increase in the plasma levels of TNF-α in patients with congestive heart failure. One problem in performing in vivo validation of MRI measurements of LV volumes in mice is determining an appropriate reference standard. Cardiac output measurements may be obtained in mice with open-chest surgical techniques by placement of a flow probe on the ascending aorta. However, the surgery is difficult and results in significantly depressed hemodynamics and force-frequency relations when compared with closed-chest preparations.

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cardiomyopathy, and there appears to be a direct relation between disease severity and circulating levels of TNF-α. Recently we have shown that myocyte production of TNF-α is sufficient to produce severe myocarditis in mice that results in myocardial dysfunction and cardiac failure. Survival in these transgenic animals in 2 different lineages was related to the amount of TNF-α expressed in the myocardium. However, the progression of ventricular remodeling and dilatation related to the overexpression of TNF-α in the myocardium was not examined.

### TABLE 2. LV Mass, Volumes, and Ejection Fraction in Transgenic Mice and Littermate Controls

<table>
<thead>
<tr>
<th>1st Month</th>
<th>2nd Month</th>
<th>3rd Month</th>
<th>4th Month</th>
<th>5th Month</th>
<th>6th Month</th>
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<tr>
<td>Lineage 1</td>
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<tr>
<td>LV mass, mg</td>
<td>83 ± 9</td>
<td>110 ± 11</td>
<td>128 ± 12</td>
<td>165 ± 3</td>
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<tr>
<td>LV EDV, μL</td>
<td>54 ± 9.9</td>
<td>68 ± 7.8</td>
<td>87 ± 8.3</td>
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<tr>
<td>LV ESV, μL</td>
<td>22 ± 5.7</td>
<td>31 ± 6.4</td>
<td>45.7 ± 7</td>
<td>64 ± 3.5</td>
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<td>LV EF</td>
<td>0.58 ± 0.03</td>
<td>0.46 ± 0.06</td>
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<td>0.27 ± 0.01</td>
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<td>6</td>
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<tr>
<td>Lineage 2</td>
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<tr>
<td>LV mass, mg</td>
<td>86.7 ± 7</td>
<td>105 ± 12</td>
<td>109 ± 9</td>
<td>113 ± 6</td>
<td>123 ± 15</td>
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<tr>
<td>LV EDV, μL</td>
<td>45 ± 4.5</td>
<td>53 ± 5.2</td>
<td>62 ± 9.2</td>
<td>71 ± 5.9</td>
<td>78 ± 9.6</td>
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<td>LV ESV, μL</td>
<td>18 ± 1.6</td>
<td>21 ± 1.0</td>
<td>27 ± 4.5</td>
<td>36 ± 2.7</td>
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<td>LV EF</td>
<td>0.62 ± 0.03</td>
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<td>0.58 ± 0.03</td>
<td>0.49 ± 0.03</td>
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<td>Littermate controls</td>
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<tr>
<td>LV mass, mg</td>
<td>80 ± 7</td>
<td>94 ± 11</td>
<td>104 ± 9</td>
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<tr>
<td>LV EDV, μL</td>
<td>42 ± 4.5</td>
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<td>58 ± 5.4</td>
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<td>LV ESV, μL</td>
<td>13 ± 2.1</td>
<td>16 ± 2.4</td>
<td>19 ± 1.6</td>
<td>21 ± 2.6</td>
<td>21 ± 1.4</td>
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<tr>
<td>LV EF</td>
<td>0.70 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.68 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.66 ± 0.01</td>
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<td>n</td>
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LV indicates left ventricle; EDV, end-diastolic volume; ESV, end-systolic volume; and EF, ejection fraction. All values represent mean ± 1 SD.

*P<0.02 (2-tailed t test comparing wild-type vs transgenic mice); †P<0.02 (2-tailed t test comparing lineage 1 vs lineage 2).

‡P<0.001 (2-tailed t test comparing wild-type vs transgenic mice); §P<0.001 (2-tailed t test comparing lineage 1 vs lineage 2).
The MRI assessment of LV volumes and ejection fraction over time demonstrated the development of LV dysfunction in transgenic mice when compared with littermate controls. Several lines of evidence support a causal relation between the myocardial expression of TNF and ventricular dilatation with pump dysfunction: First, the secretion of TNF-α in this mouse model begins perinatally, and our data show that a significant difference in LV ejection fraction is present in the first month of life in both lineages compared with wild-type mice. Second, the degree of overexpression of TNF in transgenic mice correlates with the severity and rate of development of disease. In mice from lineage 1, in which the amount of TNF determined in the myocardium is 4 times greater than in lineage 2, ventricular dilatation and dysfunction were more severe. In addition, the serial measurements of volumes and function in this study clearly show a progressive increase in LV volumes and deterioration in systolic function over time that is significantly different between the 2 lineages. Also, interindividually variability observed in LV volumes and ejection fraction was larger in the transgenic groups than in the littermate controls, perhaps reflecting variability of TNF-α expression within each lineage. This is not dissimilar from the clinical heterogeneity found in patients with heart failure. Our results are also consistent with a recent report describing the effects of TNF-α infusion in rats.

Third, our study demonstrated evidence of remodeling in terms of an increase in myocardial mass that reached significance at 3 months in lineage 1 but not until 6 months in lineage 2. This suggests an ongoing process that occurs earlier if the level of TNF overexpression is higher. It is known that the animals in lineage 1 develop severe myocarditis with apoptosis of cardiac myocytes and fibrosis early in their life, resulting in 50% mortality rates by the 70th day of life. It has been proposed that myocardial TNF production might mediate compensatory hypertrophy. Our data could be interpreted as indicating that a high level of TNF leads to hypertrophy or that hypertrophy develops only after a certain level of dysfunction is present with result activation of compensatory mechanisms.

Last, the progressive increase in LV volumes and LV mass seen in this study is similar to the ventricular remodeling that occurs in patients with heart failure. Importantly, it appears that the amount of TNF produced in the heart correlates well with the severity of the disease that these animals develop. This is consistent with the observation that in patients with cardiac failure, the plasmatic levels of TNF-α are directly correlated with the severity of the disease and supports the hypothesis that TNF-α may play a role in myocardial damage, ventricular dilatation, and cardiac decompensation observed in patients with congestive cardiomyopathy.

Limitations of the Study
First, the typical anesthetized mouse heart rate is ≈450 bpm, 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.

Second, a relatively limited number of slices was used, raising the possibility of partial volume effects. In our prior studies in which the myocardial wall volume was used to estimate the myocardial mass, there was good agreement with necropsy measurements. This suggests that effects of slice thickness on volume determinations are minimal.

Third, thermodilution has been successfully used to measure cardiac output in rats. However, the limitations of the thermodilution cardiac output in small animals have been well described. It has been argued that thermodilution measurements in small animals may be associated with
Development of LV Dysfunction in Transgenic Mice

greater errors than in larger animals. Heat diffusion across the wall of the vessel increases as its diameter decreases, and it is greater across a thin-walled vessel. This would lead to loss of "thermal bolus" as an indicator affecting measurements. Despite that concern, our data show good agreement between MRI and thermodilution, suggesting that a major bias is not present.

Fourth, anesthesia may affect cardiac output, and both the magnitude and direction of the effect vary with the anesthetic used. Ideally, the measurement of cardiac function in mouse models should be done in conscious animals. This may be possible with the use of restraint devices and should be examined in further work.

Conclusions

The present study demonstrates that MRI at 1.5 T, readily accessible at most medical centers, provides accurate and reproducible quantification of LV volumes and ejection fraction. Thus MRI can in 1 step measure LV mass, volumes, and ejection fraction accurately and determine ventricular geometry in a mouse model of heart failure. In addition, MRI is well suited for serial evaluation of the LV size and function in living mice and can be used to track the development of LV dilatation and failure. The comparison between normal control littermates and the 2 lineages of mice overexpressing TNF-α showed a clear progressive increase in cardiac volumes that was related to differing expression of TNF-α.

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