Cardiomyocyte hypertrophy is an early response of the heart to stress, preceding the expansion of the extracellular matrix, interstitial fibrosis, and overt clinical heart failure (HF).1,2 Clinically, cardiomyocyte hypertrophy represents a conserved, plastic, and prognostically important response to a variety of physiological (eg, exercise) and pathophysiologic (eg, hypertension, aortic stenosis) triggers.3–5 Although pathological left ventricular (LV) hypertrophy may be reversible, the presence of LV hypertrophy already confers a significantly higher risk of stroke, incident HF, and mortality5–9 and accelerates the transition to HF.10 Therefore, methods to detect and more precisely phenotype hypertrophy at the level of the cardiomyocyte may facilitate earlier detection and intervention and consequently inform preventative therapy for HF.

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In animal models, an increase in cardiomyocyte volume is an early marker of remodeling, occurring in response to mechanical stretch during the transition to HF.11–13 At a macroscopic level, increased LV thickness is a manifestation of cardiomyocyte hypertrophy and expansion of the extracellular matrix,14 both of which play an integral role in the transition from compensated cardiac hypertrophy to clinical HF.15 The lack of methods to serially quantify changes in cardiomyocyte volume in vivo in response to either therapy or varying

Quantification of Cardiomyocyte Hypertrophy by Cardiac Magnetic Resonance
Implications for Early Cardiac Remodeling

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Background—Cardiomyocyte hypertrophy is a critical precursor to the development of heart failure. Methods to phenotype cellular hypertrophy noninvasively are limited. The goal was to validate a cardiac magnetic resonance–based approach for the combined assessment of extracellular matrix expansion and cardiomyocyte hypertrophy.

Methods and Results—Two murine models of hypertension (n=18, with n=15 controls) induced by L-N^G-nitroarginine methyl ester (L-NAME) and pressure overload (n=11) from transaortic constriction (TAC) were imaged by cardiac magnetic resonance at baseline and 7 weeks after L-NAME treatment or up to 7 weeks after TAC. T1 relaxation times were measured before and after gadolinium contrast. The intracellular lifetime of water (τic), a cell size–dependent parameter, and extracellular volume fraction, a marker of interstitial fibrosis, were determined with a model for transcytollemal water exchange. Cardiomyocyte diameter and length were measured on FITC–wheat germ agglutinin–stained sections. The τic correlated strongly with histological cardiomyocyte volume-to-surface ratio (r=0.78, P<0.001) and cell volume (r=0.75, P<0.001). Histological cardiomyocyte diameters and cell volumes were higher in mice treated with L-NAME compared with controls (P<0.001). In the TAC model, cardiac magnetic resonance and histology showed cell hypertrophy at 2 weeks after TAC without significant fibrosis at this early time point. Mice exposed to TAC demonstrated a significant, longitudinal, and parallel increase in histological cell volume, volume-to-surface ratio, and τic between 2 and 7 weeks after TAC.

Conclusion—The τic measured by contrast-enhanced cardiac magnetic resonance provides a noninvasive measure of cardiomyocyte hypertrophy. Extracellular volume fraction and τic can track myocardial tissue remodeling from pressure overload. (Circulation. 2013;128:1225-1233.)

Key Words: hypertrophy ■ magnetic resonance imaging
physiological conditions has limited the value of cardiomyocyte hypertrophy as a biomarker of preclinical disease.

The goal of this study was to establish and validate a novel cardiac magnetic resonance (CMR) technique to quantify cardiomyocyte hypertrophy at a cellular level based on the concept that the lifetime of water within a cell changes with cell size or cell volume. We used 2 well-validated murine models of pressure-overload HF (hypertension and transverse aortic constriction [TAC]) to validate the technique and to establish its suitability for tracking longitudinal changes in cellular hypertrophy using CMR in vivo.

Methods

Murine Model of Hypertensive Heart Disease via \( L-N_\text{-Nitro-L-arginine methyl ester} \)

To validate the intracellular lifetime of water (\( \tau \)) as a surrogate marker of cell size, we first studied mice treated with \( L-N^\text{-Nitro-L-arginine methyl ester} \) (L-NAM), a well-described model in which myocardial fibrosis, hypertrophy, and failure occur simultaneously.\(^{16,17} \) Thirty-three 8-week-old, male, wild-type mice (mean body weight, 37.4±2.3 g; Taconic, Germantown, NY) were randomly assigned to 1 of 2 experimental groups: placebo (control group; \( n=15 \); tap water alone for 7 weeks) or L-NAM–treated (L-NAM group; \( n=18 \); L-NAM, 3 mg/kg in drinking water; Sigma, St. Louis, MO) groups for 7 weeks. Animals were kept under standard conditions and had normal food and water ad libitum. Noninvasive blood pressures were obtained at baseline and weekly after treatment started with a volume-pressure-recording tail-cuff technique (CODA-1;Kent Scientific, Torrington, CT).\(^{13} \) Mice in the control (placebo) and L-NAM groups were imaged at baseline and after 7 weeks of treatment (placebo versus L-NAM) with a 4.7-T magnetic resonance imaging (MRI) system (Bruker Biospin MRI, Billerica, MA). Blood samples were collected by retro-orbital puncture immediately after each CMR study for blood hematocrit determination (i-STAT; Abbott Point-of-Care, Princeton, NJ). Mice treated with L-NAM or placebo were euthanized after the second CMR study. Hearts were excised and fixed in formalin solution for histological analysis.

Murine Model of Pressure Overload by TAC

To study longitudinal changes in cellular hypertrophy and fibrosis under pressure overload, we selected the TAC model in which early changes in tissue structure and LV mass are dominated by cardiomyocyte hypertrophy and a later buildup of interstitial fibrosis under pressure overload, we selected the TAC model in which early changes in tissue structure and LV mass are dominated by cardiomyocyte hypertrophy and a later buildup of interstitial fibrosis, cardiomyocyte hypertrophy, and failure occur simultaneously.\(^{16,17} \) Thirty-three 8-week-old, male, wild-type mice (mean body weight, 37.4±2.3 g; Taconic, Germantown, NY) were randomly assigned to 1 of 2 experimental groups: placebo (control group; \( n=15 \); tap water alone for 7 weeks) or L-NAM–treated (L-NAM group; \( n=18 \); L-NAM, 3 mg/kg in drinking water; Sigma, St. Louis, MO) groups for 7 weeks. Animals were kept under standard conditions and had normal food and water ad libitum. Noninvasive blood pressures were obtained at baseline and weekly after treatment started with a volume-pressure-recording tail-cuff technique (CODA-1;Kent Scientific, Torrington, CT).\(^{13} \) Mice in the control (placebo) and L-NAM groups were imaged at baseline and after 7 weeks of treatment (placebo versus L-NAM) with a 4.7-T magnetic resonance imaging (MRI) system (Bruker Biospin MRI, Billerica, MA). Blood samples were collected by retro-orbital puncture immediately after each CMR study for blood hematocrit determination (i-STAT; Abbott Point-of-Care, Princeton, NJ). Mice treated with L-NAM or placebo were euthanized after the second CMR study. Hearts were excised and fixed in formalin solution for histological analysis.

Histopathological Analysis

Approximately 1-mm-thick, short-axis myocardial sections of the mouse hearts were fixed with buffered 10% formalin solution (Fisher Scientific, Pittsburgh, PA) and embedded in paraffin. To quantify cardiomyocyte size, sections were stained with FITC–wheat germ agglutinin to delineate the cell membrane.\(^{20} \) All sections were scanned with ScanScope scanners (Aperio Technologies, Inc, Vista, CA), and whole-slide images were sampled to a final resolution of 1.0 \( \mu \text{m} \) per pixel. Measurements of minor cell diameter (\( D_{\text{min}} \)) and major cell diameter (\( D_{\text{maj}} \)), equivalent to the cardiomyocyte length, were obtained by image analysis of FITC–wheat germ agglutinin–stained sections. From a midventricular short-axis slice, we measured \( D_{\text{maj}} \) and \( D_{\text{min}} \). The midventricular short-axis level was confirmed on the basis of the location and orientation of papillary muscles. Ten measurements of \( D_{\text{min}} \) were made in each of the anterior, septal, lateral, and inferior wall sections of the LV in fields with longitudinally oriented cardiomyocytes. Only cells with well-defined cell membranes and visible cell nuclei at midwall depth were selected. For \( D_{\text{maj}} \), we selected cardiomyocytes with a short-axis orientation at all transmural depths. Criteria for the short axis of the cardiomyocytes were a circular shape and the visibility of the cell nucleus. Cardiomyocyte volume and surface area were calculated assuming a cell shape in the form of a prolate ellipsoid\(^{21–24} \) using the median \( D_{\text{min}} \) and \( D_{\text{maj}} \) (see Figure 1). Connective tissue volume fraction, the histological equivalent of extracellular volume fraction (ECV) from CMR,\(^{25–27} \) was quantified on sections stained with Masson trichrome stain using a semiautomatic pixel color intensity algorithm in the Aperio Spectrum software to quantify pixels stained in blue.

Cardiac MRI

Mice were first anesthetized with isoflurane (induction, 4%–5%; maintenance, 1%–2.5% in oxygen from a precision vaporizer) in an induction chamber and then positioned supine on a water-heated bed in the MRI scanner. CMR images were acquired with ECG and respiratory gating (model 1025 L; SAI, Stony Brook, NY). For LV size and function, short-axis cine gradient-echo images were obtained with full LV coverage (repetition time, 5.9 milliseconds; echo time, 2.2 milliseconds; temporal resolution, 20–30 milliseconds; in-plane spatial resolution, 100–120×180–210 \( \mu \text{m} \); slice thickness, 1 mm; no gap). Manually traced epicardial and endocardial contours at end systole and end diastole were used to determine the LV end-diastolic volume, LV end-systolic volume, and LV myocardial mass using the Simpson rule and LV ejection fraction.

Gadolinium diethylenetriaminepentaacetic acid (Magnevist, Berlex, Wayne, NJ) was injected subcutaneously in multiple steps up to a cumulative dose of 0.5 mmol/kg. Myocardial T1 was measured in a mid-LV slice once before contrast and at least 4 times after contrast using a Look-Locker technique no earlier than 6 minutes after contrast administration, as described previously\(^{28} \) (repetition time, 2.5 milliseconds; echo time, 1.8 milliseconds; flip angle, 10°; in-plane resolution, 190 μm; slice thickness, 1 mm). Each Look-Locker acquisition was made \( \approx 6 \) to 8 minutes after a subcutaneous injection of contrast. As a result of the slow clearance of contrast with the subcutaneous injections (\( t_{\text{1/2}} = 70 \text{ minutes} \)),\(^{28} \) this amounted to an essentially stepwise increase in R1 in blood over the course of the MRI study (<2 hours).

For 6 myocardial segments and the blood pool, signal intensity was plotted versus time after inversion. T1 values were obtained by nonlinear least-squares fitting of the curves of signal intensity versus time after inversion to an analytic expression for the magnitude of the signal measured during the inversion recovery\(^{29} \) and correction for the radiofrequency pulse effects on the inversion recovery.\(^{24} \) The reciprocal of T1 (R1=1/T1) was used to plot the myocardial R1 against the R1 in the blood pool.

Determination of \( \tau_{\text{ic}} \)

Water molecules exchange mostly by diffusion between the interstitial (extracellular) and intracellular spaces. The average \( \tau_{\text{ic}} \) depends on the mean time for diffusion to the cell membrane (Figure 1).
The intracellular lifetime ($\tau_{ic}$) of a water molecule undergoing diffusional motion within the cell is proportional to the volume-to-surface ratio (V/S), a measure of cell size. Cardiomyocytes have an elongated shape with the ratio of the major to minor cell diameter on the order of 4:1. The myocardial T1 after administration of gadolinium contrast were used to determine $\tau_{ic}$. The myocardial ECV and $\tau_{ic}$ were determined by fitting the model to the observed T1 data using a nonlinear regression approach (2-space water-exchange model of equilibrium transcytodal exchange conditions prevail. The initial linear part of the myocardial T1 curve without a direct dependence on the T1 of blood when the exchange of water between intracellular and extracellular spaces starts to constrain the rate of relaxation in the intracellular space.

The R1 for myocardial tissue and blood data were fit with a 2-space water-exchange model of equilibrium transcytodal exchange conditions developed by Landis and colleagues.29 The myocardial ECV and $\tau_{ic}$ are adjustable parameters of the model, determined by fitting the model to the observed R1 data using a nonlinear orthogonal distance regression algorithm (http://www.netlib.org/odrpack/). The measured blood hematocrit was a fixed parameter. All R1 measurements, a minimum of 5, were used for fitting to the model and determining ECV and $\tau_{ic}$.

An increase in $\tau_{ic}$, for example, as a result of increasing cell dimensions, increases the curvature in the relation between the R1 in myocardial tissue and the R1 of blood, which provides the basis for detecting cardiomyocyte size changes. This is illustrated in Figure 1. Expansion of the myocardial interstitial space affects primarily the slope of the initially linear myocardial R1 curve without a direct effect on its curvature at higher R1 values. In the low R1 range, the relation between R1 in tissue and blood is linear because conditions of fast (transcytodal) exchange conditions prevail. The initial linear slope agrees with the widely used formula for calculating ECV from the change of R1 in tissue divided by the corresponding change of R1 in blood.26,31

Statistical Analyses

Continuous data were expressed as mean±SD. Continuous variables were compared between groups of animals (eg, L-NAME versus placebo) by an independent t test applied at each time point (baseline and 7 weeks). A paired t test was used to compare measurements at baseline and follow-up within groups. Correlations were assessed by the Pearson product-moment correlation coefficient. For the TAC model, longitudinal changes in CMR parameters, including $\tau_{ic}$ and ECV, were analyzed with linear mixed-effects regression models (package lme4: http://lme4.r-forge.r-project.org/), with time since TAC as the single predictor in each model, and including a random intercept component for each animal. The $P$ values for the fixed effects in the linear mixed-effects models were calculated by Markov chain Monte Carlo sampling (function pvals.fnc in the languageR package; http://lib.stat.cmu.edu/R/CRAN/).

Standard regression analysis was used to test the association of each histological parameter with the time since TAC. Analyses were performed with SAS 9.3 (SAS Institute, Cary, NC) or R (version 2.15.1, The R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/).

Results

L-NAME and TAC Induce Significant LV Hypertrophy

At baseline, the control and L-NAME groups did not show any significant differences in body weight, blood pressure, heart rate, or CMR indexes of LV structure and function (Table 1). Consistent with prior reports,16-18 long-term administration of L-NAME led to a significant increase in mean blood pressure (88±7 mm Hg at baseline to 127±6 mm Hg at follow-up; $P<0.001$). After 7 weeks of treatment, the L-NAME– and placebo-treated mice showed significant differences. LV mass...
increased in the L-NAME group (164±22 versus 96±14 mg for placebo group; *P<0.001; *P<0.001 when indexed to body weight), and systolic function decreased (LV ejection fraction, 61±3% versus 50±8%; *P<0.001).

Table 2 summarizes the changes in cardiac structure and function between weeks 2 and 7 after TAC. TAC led to significant longitudinal changes for LV volumes, mass, function, ECV, and τ c. Weight-indexed LV mass was significantly higher after TAC (*P<0.001 for unpaired comparisons) and LV ejection fraction was lower (*P<0.001 when indexed to body weight) compared with controls. The histological cardiomyocyte volume was significantly higher with longer exposure to TAC (57±7.4×10⁴ μm³ at 2 weeks after TAC versus 73±2.5×10⁴ μm³ for 7 weeks; *P=0.006 for unpaired t test), driven mostly by changes in D min. The histological measurements showed that cardiomyocyte diameter (D maj), cardiomyocyte volume, and V/S were each significantly associated with time after TAC (Table 2).

The histological measurements of mice with TAC are summarized in Table 2. The mice exposed to TAC had a significantly higher D maj compared with controls, with D maj reaching values similar to those in L-NAME–treated mice.

### Table 1. Baseline and Follow-Up Hemodynamic and CMR Characteristics of Control and L-NAME–Treated Mice

<table>
<thead>
<tr>
<th>Characteristics (Hemodynamic and CMR Data)</th>
<th>Baseline</th>
<th>Control (n=15)</th>
<th>L-NAME (n=18)</th>
<th>PValue*</th>
<th>Control (n=13)</th>
<th>L-NAME (n=17)</th>
<th>PValue*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>37.5±2.7</td>
<td>37.7±2.4</td>
<td>0.865</td>
<td>44.8±4.5†</td>
<td>40.0±3.2†</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>492±108</td>
<td>500±74</td>
<td>0.827</td>
<td>403±94</td>
<td>474±70</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>92±7.3</td>
<td>88±7.4</td>
<td>0.209</td>
<td>91±8</td>
<td>126.7±6.2†</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>58±5.9</td>
<td>58±3.0</td>
<td>0.629</td>
<td>60.8±3.1</td>
<td>50.1±7.7†</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LVESV, μL</td>
<td>130±32</td>
<td>147±32</td>
<td>0.187</td>
<td>111±26</td>
<td>123±37†</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>55±14</td>
<td>61±14</td>
<td>0.255</td>
<td>42±11†</td>
<td>61±20</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>LV mass indexed to body weight, mg/g</td>
<td>97±17</td>
<td>94±12</td>
<td>0.615</td>
<td>96±14</td>
<td>164±22†</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Myocardial extracellular volume fraction (MRI)</td>
<td>0.26±0.02</td>
<td>0.27±0.03</td>
<td>0.677</td>
<td>0.25±0.03</td>
<td>0.42±0.08†</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Intracellular lifetime of water, 1/s</td>
<td>0.15±0.07</td>
<td>0.17±0.05</td>
<td>0.791</td>
<td>0.19±0.07</td>
<td>0.44±0.12†</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Connective tissue fraction (histology), %</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>2.6±0.6</td>
<td>8.5±1.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>D maj, μm</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>84.2±3.6</td>
<td>90.4±5.1</td>
<td>0.0012</td>
<td></td>
</tr>
<tr>
<td>D min, μm</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>19.8±0.9</td>
<td>26.2±1.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>D maj/D min</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>4.26±0.22</td>
<td>3.46±0.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cardiomyocyte volume by histology, ×10⁴ μm³</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>42.3±6.0</td>
<td>78.1±12.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cell surface area, ×10⁴ μm²</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>1.70±0.14</td>
<td>2.41±0.26</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Volume-to-surface ratio, μm</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>24.7±1.4</td>
<td>32.2±1.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*P values for unpaired t test of control versus L-NAME mice.
†P<0.05 for paired t test of mice at baseline versus 7 weeks (control and L-NAME).

### Histological Assessment of Cardiomyocyte Hypertrophy

We measured the major (D maj) and minor (D min) cardiomyocyte dimensions and calculated cardiomyocyte volume, assuming a cell shape in the form of prolate ellipsoid (Figure 2). L-NAME induced a significant but relatively modest change in D maj (P<0.001 versus placebo) averaging 7% and a mean 32% change for D min (P<0.001 versus placebo; Table 1). The cardiomyocyte volume was 84% higher in mice treated with L-NAME compared with placebo (78±13×10⁴ μm³ for L-NAME–treated mice and 42±6×10⁴ μm³ for placebo; *P<0.001). V/S was also higher in L-NAME– versus placebo-treated mice (Table 1).

**Interstitial Fibrosis and Extracellular Space Expansion in the L-NAME and TAC Models**

After 7 weeks of L-NAME treatment, the histological connective tissue fraction was significantly higher than in placebo-treated mice (8.5±1.6% versus 2.6±0.6%; *P<0.001). In parallel, the myocardial ECV measured by CMR was also significantly higher for L-NAME compared with placebo (0.42±0.08 versus 0.25±0.03; *P<0.001). Although mice exposed to TAC showed a significant increase in LV
mass, they did not exhibit as marked an increase in histological connective tissue fraction (2.3±0.1% for TAC at 7 weeks versus 8.5±1.6% for L-NAME treated; P<0.001) or myocardial ECV (0.30±0.04 for TAC at 7 weeks versus 0.42±0.08 for L-NAME; P=0.01) by 7 weeks (Table 2 and Figure 3).

Table 2. Hemodynamic and CMR Characteristics of TAC Mice at 2 and 7 Weeks After TAC

<table>
<thead>
<tr>
<th>Hemodynamics and CMR Data</th>
<th>At 2 wk After TAC (14±1 d after TAC)</th>
<th>At 7 wk After TAC (46 d after TAC)</th>
<th>P Value for Effect of Time After TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>25.4±1.6</td>
<td>29.8±0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>37±6</td>
<td>29±6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LVEDV, μL</td>
<td>93±24</td>
<td>79±18</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LVESV, μL</td>
<td>59±20</td>
<td>57±13</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>121±12</td>
<td>145±9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LV mass index (body weight), mg/g</td>
<td>4.7±0.3</td>
<td>4.9±0.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Myocardial extracellular volume fraction</td>
<td>0.25±0.03</td>
<td>0.30±0.04</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Intracellular lifetime of water, 1/s</td>
<td>0.22±0.05</td>
<td>0.49±0.18</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Histological measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>(D_{maj}), μm</td>
<td>87.1±1.1</td>
<td>88.5±1.0</td>
<td>0.155</td>
</tr>
<tr>
<td>(D_{min}), μm</td>
<td>22.8±1.4</td>
<td>25.9±0.9</td>
<td>0.002</td>
</tr>
<tr>
<td>(D_{maj}/D_{min})</td>
<td>3.83±0.24</td>
<td>3.43±0.13</td>
<td>0.008</td>
</tr>
<tr>
<td>Connective tissue fraction (histology)</td>
<td>0.023±0.007</td>
<td>0.032±0.009</td>
<td>0.0012</td>
</tr>
<tr>
<td>Cardiomyocyte volume by histology, (×10^4) μm³</td>
<td>56.7±7.4</td>
<td>73.4±5.1</td>
<td>0.0025</td>
</tr>
<tr>
<td>Volume-to-surface ratio, μm</td>
<td>28.3±1.6</td>
<td>31.8±1.0</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

The P value refers to the coefficient for the linear trend of the displayed CMR or histological index over time after TAC. For CMR measures, this involves repeated measurements for 11 mice at 2 time points, and a linear mixed-effects model was used for each variable. For histology, the effect of time after TAC was tested by standard linear regression because it involved only 1 measurement per mouse (Mice necessarily had to be euthanized for histological assessment). CMR indicates cardiac magnetic resonance; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; and TAC, transverse aortic constriction.

**Figure 2.** Cellular hypertrophy and interstitial fibrosis in L-NAME and transaortic constriction (TAC) mice. Short-axis, midlevel left ventricular sections of cardiac tissue from mice treated with placebo (A), L-NAME (B), and TAC (C). L-NAME and TAC are representative tissues after 7 weeks of exposure. Panels were stained with FITC-conjugated wheat germ agglutinin to delineate cell membranes and scanned at a resolution equivalent to 1.0 μm per pixel to measure minor and major cell diameters in 15 fields within 4 myocardial segments. A and B are representative illustrations of larger short-axis diameters in the L-NAME and TAC groups relative to controls. Adjacent short-axis sections from the same mice stained with Masson trichrome stains (D–F) illustrate the absence of interstitial fibrosis in the control group and higher levels of interstitial fibrosis (blue) in L-NAME vs TAC mice.
as a Marker of Cardiomyocyte Hypertrophy

The \( \tau_{ic} \) determined by CMR was significantly higher after 7 weeks of L-NAME compared with placebo treatment (0.19±0.07 versus 0.44±0.12; \( P<0.001 \); Table 1 and Figure 3). In the TAC group, \( \tau_{ic} \) increased significantly between 2 and 7 weeks after exposure to TAC (Table 2 and Figure 4). The rate of change in \( \tau_{ic} \) with time after TAC surgery was estimated to be 0.0581 s/wk (\( P<0.002 \)) with the use of a linear mixed-effects model for the repeated measurements of \( \tau_{ic} \) ranging from 2 to 7 weeks after TAC. There was no significant difference in \( \tau_{ic} \) in mice after 7 weeks of L-NAME treatment and mice after 7 weeks of TAC (\( P=0.58 \)).

When all \( \tau_{ic} \) values from CMR are pooled with time-matched histological data, \( \tau_{ic} \) demonstrated a strong positive association with cardiomyocyte V/S (\( r=0.78, P<0.001 \); Figure 5). The correlation of \( \tau_{ic} \) with cell volume was \( r=0.75 (P<0.001) \); with median minor cell diameter, \( r=0.79 (P<0.001) \); and with median major cell diameter, \( r=0.43 (P=0.006) \). \( \tau_{ic} \) also demonstrated an inverse association with LV ejection fraction (\( r=-0.36, P=0.002 \)).

Discussion

This study validates a novel T1-based CMR technique to detect and quantify changes in cardiomyocyte hypertrophy. Serial measurements of \( \tau_{ic} \) and myocardial ECV allowed the noninvasive identification of distinct but complementary aspects of myocardial remodeling at the cellular level. Specifically, \( \tau_{ic} \) was strongly associated with the histological V/S, a measure of the characteristic cell size, and minor cell diameter. Not unexpectedly, the correlation of \( \tau_{ic} \) with the major cell diameter was much weaker; this parameter was also not well suited to differentiate between normal and hypertrophied cardiomyocytes on histology. Our results suggest that the development of interstitial fibrosis and cardiomyocyte hypertrophy can be temporally distinct and followed up noninvasively. This is the first demonstration of the ability to track cardiomyocyte hypertrophy in vivo noninvasively. It could be used in conjunction with more established applications of T1 mapping by CMR to facilitate earlier detection of pathological hypertrophy and to assess myocardial remodeling in response to therapeutic interventions.

Postcontrast T1 relaxation time measurements have been used in both animals\(^{27,34} \) and patients\(^{35} \) with hypertension as an index of pathological diffuse interstitial expansion. In aortic stenosis, the value of 1 minus myocardial ECV fraction was reported as an index of cell volume fraction\(^{36} \); it represents a combination of cell volume and density rather than a direct measure of cellular hypertrophy. Our results suggest that myocardial interstitial expansion (eg, myocardial ECV) and cardiomyocyte hypertrophy represent distinct characteristics of tissue structure, which can be measured serially by CMR. In mice after TAC, we demonstrate that cardiomyocyte hypertrophy may precede interstitial fibrosis, underlining the distinction between ECV and \( \tau_{ic} \).

From a biological perspective, increases in cardiac mass can result from interstitial matrix expansion (eg, fibrosis or aberrant protein deposition) or increases in cardiomyocyte volume.\(^{37} \) A differentiation between physiological and pathological hypertrophy may therefore require characterization of not only wall thickness and LV mass but, more specifically, interstitial matrix expansion and cardiomyocyte cell size. In states of pathological hypertrophy in response to pressure overload, an increase in cardiomyocyte cell size has been considered an early and conserved hallmark, putatively occurring before the onset of irreversible myocardial fibrosis and subsequent ventricular dysfunction, remodeling, and HF.\(^{11-13} \)

In fact, mechanical stress has been associated with activation of a profibrotic, prohypertrophic genetic program that may reinforce subsequent HF.\(^{38-41} \) In turn, increases in interstitial fibrosis appear to mark the transition from compensated cellular and organ-level pathological hypertrophy to HF.\(^{11,42} \) In patients with LV hypertrophy at risk for HF, alterations in the balance of collagen metabolism (as reflected by increases in matrix metalloproteinases and procollagen fragments) identify patients with clinical HF.\(^{44-48} \) Early intervention before the development of overt fibrosis and myocardial dysfunction may ameliorate the progression to overt HF.\(^{47} \)

The proposed method has potential limitations. It is assumed that the cytolemmal permeability coefficient remains constant with the development of cell hypertrophy. Ischemic conditions could alter cell membrane permeability and decrease active, ATP-dependent water transport across the

![Figure 3](http://circ.ahajournals.org/) Intracellular lifetime of water (\( \tau_{ic} \)) and extracellular volume fraction in \( L-\text{N}^\text{G} \)-nitroarginine methyl ester (L-NAME) mice. **A**, The \( \tau_{ic} \) increased significantly in mice treated with L-NAME and was significantly higher than in placebo-treated controls. **B**, In mice exposed for 7 weeks to L-NAME, the extracellular volume fraction also increased significantly compared with baseline and was significantly higher than in placebo-treated controls. F/U indicates follow-up.
However, it is unlikely that ischemia was a confounding factor in this study because of the absence of apparent infarction by late gadolinium enhancement. Other cardiac resident cell types (eg, fibroblasts) may bias or impair the detection of cardiomyocyte hypertrophy, but in viable myocardium, the volume fraction of connective tissue and the percentage of fibroblast volume are relatively small compared with the cardiomyocyte volume.

The analyses of myocardial T1 and \( \tau_{ic} \) were based on transmural myocardial signal intensity averages. Because of the transmural variation of wall stress, it is conceivable that cardiomyocyte hypertrophy varies between endocardial and epicardial layers. The spatial resolution of our T1 measurements in mice and the use of a cine technique were not suitable for investigating any transmural variation of cell hypertrophy in this study. Mice in the TAC group were not imaged before TAC surgery because of restrictions on taking mice out of the animal facility before surgery. Our results, although limited to models of pressure overload, may also be applicable to eccentric hypertrophy. In human heart samples, the cell diameter changes significantly in both concentric and eccentric hypertrophy (by 80% and 40%, respectively). Similarly, in animal models of pressure overload (concentric remodeling) and volume overload (predominantly eccentric remodeling), the cell diameter changed to a similar degree. We empirically observe that \( \tau_{ic} \) is most sensitive to changes in minor cell dimension (cell diameter), suggesting that \( \tau_{ic} \) may be able to track changes in cell size in cases of eccentric hypertrophy. These empirical observations are in agreement with the theoretical prediction that \( \tau_{ic} \) changes in proportion to the V/S.

The V/S for a cardiomyocyte with a length-to-diameter ratio of \( \approx 4:1 \) has a higher sensitivity to changes in cell diameter than changes in cell length, consistent with the stronger correlation of \( \tau_{ic} \) with \( D_{min} \) than with \( D_{maj} \). An expected limitation is a lack of sensitivity to changes in cell length, expected with the development of eccentric hypertrophy, or variations in cardiomyocyte shape such as cylindrical versus ellipsoidal. The application of \( \tau_{ic} \) to eccentric hypertrophy requires further validation.

Conclusions
This study validates a noninvasive T1-based CMR method for the assessment of cardiomyocyte hypertrophy. In models of pressure-overload HF, CMR distinguished cellular hypertrophy, characterizing the early tissue phenotype in TAC, and extracellular space expansion, a hallmark of chronic pressure overload. Ultimately, these results suggest a role for CMR as a noninvasive tool to quantify 2 critical aspects of early myocardial remodeling at the transition between compensated hypertrophy and clinical HF.

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Disclosures

None.

References

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Cardiac hypertrophy is a conserved response to myocardial stress and injury that predates the development of systolic and diastolic dysfunction. Large epidemiological studies have demonstrated the adverse prognostic impact of pathological hypertrophy on the development of stroke, heart failure, and all-cause mortality in at-risk patients with hypertension. In animal models of pressure-overload hypertrophy, there appears to be a progression through cellular hypertrophy, fibrosis, and whole-organ hypertrophy/dysfunction that marks the transition to heart failure, with cellular hypertrophy as one of the earliest myocardial responses. Although in vitro research in these animal models has elucidated pathways of cellular hypertrophy development, the ability to translate these findings in vivo in a serial fashion (to observe the coordinated development of hypertrophy, fibrosis, and dysfunction) has been limited by the availability of noninvasive metrics of cellular hypertrophy and fibrosis. In this report, we present a magnetic resonance–based technique to quantify cardiomyocyte size and interstitial fibrosis noninvasively in 2 models of pressure-overload hypertrophy in mice. This technique, based on a T1-based magnetic resonance imaging determination of the intracellular lifetime of a water molecule, provides a surrogate measure of average cardiomyocyte size and myocardial fibrosis in serial noninvasive examinations, demonstrating the sequential development of hypertrophy and fibrosis in pressure overload. Ultimately, this technique may allow the detection of early hypertrophy phenotypes in human disease for targeting more aggressive prevention and identifying individuals at high risk for progression to heart failure.
Quantification of Cardiomyocyte Hypertrophy by Cardiac Magnetic Resonance: Implications for Early Cardiac Remodeling

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