Late gadolinium enhancement imaging (LGE) has become the method of choice to detect myocardial necrosis and scar with cardiac magnetic resonance (CMR), yet LGE may provide only a partial measure of fibrosis extent and burden, as it relies on a difference in signal intensity that may not exist in the case of diffuse and generalized fibrosis.

Clinical Perspective on p 141

Conditions such as nonischemic dilated, diabetic, and hypertrophic cardiomyopathies, along with hypertensive and valvular heart disease, have shown ample histopathologic evidence of diffuse fibrosis.¹⁻³ Diffuse myocardial fibrosis is an important, yet poorly characterized substrate for sudden cardiac death.⁴ It is associated with the development of heart failure in hypertensive patients⁵ and may regress under treatment.⁶

A shorter myocardial T1 after administration of an extracellular gadolinium-based contrast agent indicates extracellular matrix expansion and is associated with accumulation of connective tissue in the myocardium.⁷ Flett et al⁸ demonstrated that the extracellular volume correlates with the collagen volume fraction in patients with aortic stenosis and hypertrophic cardiomyopathy.

CMR estimation of extracellular matrix expansion relies on the indirect detection of contrast in tissue and on its effect on T1 relaxation times. T1 of tissue represents the time constant for the inversion recovery of water within the entire tissue volume.⁹

**Background**—The myocardial extracellular volume fraction (MECVF) has been used to detect diffuse fibrosis. Estimation of MECVF relies on quantification of the T1 relaxation time after contrast enhancement, which can be sensitive to equilibrium transcytolemmal water-exchange. We hypothesized that MECVF, quantified with a parsimonious 2-space water-exchange model, correlates positively with the connective tissue volume fraction in a rodent model of hypertensive heart disease, whereas the widely used analysis based on assuming fast transcytolemmal water-exchange could result in a significant underestimate of MECVF.

**Methods and Results**—No nitro-L-arginine-methyl-ester (L-NAME) or placebo was administered to 22 and 15 wild-type mice, respectively. MECVF was measured at baseline and 7-week follow-up by pre- and postcontrast T1 cardiac magnetic resonance imaging at 4.7 T, using a 2-space water-exchange model. Connective tissue volume fraction was quantified, using Masson trichrome stain. L-NAME induced hypertrophy (weight-indexed left-ventricular mass 2.2±0.3 versus 4.1±0.4 μg/g, P<0.001), and increased connective tissue volume fraction (8.6%±1.5 versus 2.58%±0.6, P<0.001), were compared with controls. MECVF was higher in L-NAME-treated animals (0.43±0.09 versus 0.26±0.03, P<0.001), and correlated with connective tissue volume fraction and weight-indexed left-ventricular mass (r=0.842 and r=0.737, respectively, both P<0.0001). Neglecting transcytolemmal water-exchange caused a significant underestimate of MECVF changes. Ten patients with history of hypertension had significantly higher MECVF (0.446±0.063) compared with healthy controls (0.307±0.030, P<0.001).

**Conclusions**—Cardiac magnetic resonance allowed detection of myocardial extracellular matrix expansion in a mouse model and in patients with a history of hypertension. Accounting for the effects of transcytolemmal water-exchange can result in a substantial difference of MECVF, compared with assuming fast transcytolemmal water-exchange. (Circ Cardiovasc Imaging. 2013;6:134-141.)

**Key Words:** cardiac magnetic resonance ■ extracellular volume fraction ■ gadolinium-DTPA ■ mice ■ myocardial fibrosis ■ No nitro-L-arginine-methyl-ester ■ transcytolemmal ■ water-exchange

L Late gadolinium enhancement imaging (LGE) has become the method of choice to detect myocardial necrosis and scar with cardiac magnetic resonance (CMR), yet LGE may provide only a partial measure of fibrosis extent and burden, as it relies on a difference in signal intensity that may not exist in the case of diffuse and generalized fibrosis.

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A shorter myocardial T1 after administration of an extracellular gadolinium-based contrast agent indicates extracellular matrix expansion and is associated with accumulation of connective tissue in the myocardium.⁷ Flett et al⁸ demonstrated that the extracellular volume correlates with the collagen volume fraction in patients with aortic stenosis and hypertrophic cardiomyopathy.

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space, not only the space permeated by contrast. In structures where contrast agents are confined to subspaces, while water diffuses between all the subspaces, T1 depends on the rate at which water exchanges between the spaces with and without contrast, e.g., across the cytolemmal barrier between extra- and intracellular spaces. Previous CMR studies of the myocardial extracellular volume fraction (MECFV) in aortic stenosis and hypertrophic cardiomyopathy, idiopathic dilated cardiomyopathy, congenital heart disease, and healthy volunteers were all based on assuming fast transcytosomal water-exchange, which predicts a linear relationship between the relaxation rates, R1 (=1/T1), in myocardium and blood, before and after contrast enhancement. When the myocardial relaxation times are sufficiently reduced by contrast administration, the relationship between R1 in myocardial tissue and R1 in blood will change from linear to sublinear. Continuing to assume, that after contrast administration the transcytosomal water-exchange remains fast, can result in a significant underestimate of MECVF. By measuring myocardial R1 after consecutive contrast injections, one can detect the deviation from a linear relationship for R1 in myocardium and blood, and determine the underestimate of MECVF, resulting from the assumption of fast transcytosomal water-exchange.

We hypothesized that MECVF, quantified with a parsimonious 2-space water-exchange (2SX) model, correlates positively with the connective tissue volume fraction (CTVF) in a rodent model of hypertensive heart disease, induced by chronic inhibition of NO biosynthesis with N-ω-arginine-methyl-ester (L-NAME), whereas the widely used analysis based on assuming fast exchange (FX) across the cytolemmal barrier could result in a loss of sensitivity to MECVF expansion. The same CMR approach was also tested in hypertensive patients without overt signs of myocardial hypertrophy, and volunteers, to determine whether MECVF could be used as an early marker of adverse extracellular matrix remodeling.

Methods

Animal Experimental Groups

Thirty-seven male wild-type mice (mean body-weight 37.6±2.5 g, range 30–40 g, Taconic, Germantown, NY) were randomly assigned to 1 of 2 experimental groups: (1) Placebo-treated (control group; n=22) with L-NAME in the drinking water (3 mg/mL; Sigma) for 7 weeks. Animals were kept under standard conditions and had normal food and water ad libitum. Noninvasive tail blood pressures were obtained at baseline and weekly after treatment started, using a volume–pressure recording tail-cuff technique (CODA-1, Kent Scientific, Torrington, CT). CMR was performed at baseline and after 7 weeks of treatment. Retro-orbital blood sampling was performed immediately after each CMR study for hema
tocrit determination (i-STAT, Abbott, IL). Animals were killed after the second CMR study, and the hearts were excised for histological analysis. The study protocol and animal care conformed with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). The study protocol was approved by the Standing Committee on Animal Care and Use at Harvard University.

Histological Analysis

Heart tissues were fixed with buffered 10% formalin solution (Fisher Scientific, Pittsburgh, PA). Short-axis cuts (≥1 mm thickness) of formalin fixed tissue were processed, embedded in paraffin, and stained with standard hematoxylin, eosin, and Masson trichrome. All sections were scanned with ScanScope scanners (Aperio Technologies, Inc; Vista, CA). Whole-slide images were downsampled to a resolution of 1.0 μm/pixel. Pixels stained in blue with Masson trichrome were identified for quantification of CTVF, using a semiautomatic pixel color intensity algorithm.

Patient Population

CMR was performed on 8 consecutive patients referred clinically with a diagnosis of hypertension (HTN) and evidence of hypertensive heart disease defined as increased wall thickness or left atrial enlargement, confirmed by CMR. Patients were also required to have normal left-ventricular (LV) systolic function ≥50%, no LGE and no significant valvular heart disease. All CMR studies were clinically requested and indicated, and no patient received gadolinium for the sole purpose of the study. Our institutional review board approved this study for review of patient’s records. In addition, CMR data from 12 healthy volunteers using identical imaging parameters were obtained as control group. Healthy volunteers had signed informed consent for the institutional review board-approved research protocol before undergoing CMR.

Clinical, Electrocardiographic Data

Clinical history was collected at the time of CMR. Electrocardiograms closest in time to CMR were reviewed. LV filling pressure was assessed by tissue Doppler imaging using the ratio (≥15) of the peak velocity of the mitral E wave to the peak velocity of the E’ wave of the basal lateral, and septal wall (mitral anulus). A decreasing peak velocity of the E’ wave at the mitral anulus by tissue Doppler imaging was also used as an indicator of the severity of diastolic dysfunction.

CMR Imaging Protocols

Mice

Animals were imaged supine under isoflurane anesthesia (induction 4%–5%; maintenance 1%–2.5% in oxygen from a precision vaporizer) in a 4.7-T MRI system (BioSpec 47/40, Bruker BioSpin, Billerica, MA). Mice were placed in a special cradle, ECG electrodes fixed to front and back paws, using electrode gel to optimize electric contact. For ventricular size and function, cine gradient-echo images were acquired (repetition time [TR]=5.9 ms; echo time [TE]=2.2 ms; temporal resolution =20–30 ms; in-plane spatial resolution 100–120 × 180–210 μm; 1 mm thick) in multiple parallel short-axis. Gadolinium diethylenetriamine pent-acetic acid (DTPA, Magnevist, Berlex, Wayne, NJ) was injected subcutaneously in multiple steps up to a cumulative dose of 0.5 mmol/kg. T1 was measured precontrast, and after each of 4 to 5 contrast injections with a modified Look-Locker technique, no earlier than 6 minutes after each contrast administration, using the following parameters: TR =2.5 ms; TE =1.8 ms; flip angle =15°, in-plane resolution 190 μm, slice thickness 1 mm; repetition time per segment: 22 ms; number of averages: 6 (precontrast), or 4 (postcontrast). An adiabatic hyperbolic secant pulse was applied for nonslice-selective magnetization inversion before each Look-Locker magnetization-recovery read-out. The accuracy of the T1 measurements was tested in gadolinium-doped phantoms, against the standard inversion recovery spin-echo technique.

Patients

Patients were studied supine position in a 3.0-T MRI system (Tim Trio, Siemens Medical Systems, Malvern, PA). For LV size and function, a cine steady-state free-precession sequence was used (TR =3.4 ms; TE =1.2 ms; temporal resolution ≈40–50 ms; in-plane spatial resolution =1.5–1.8 × 1.8–2.1 mm; slice=8 mm; no gaps) in multiple
parallel short-axis. An inversion recovery-prepared fast gradient-echo sequence, triggered every other heartbeat, was used to assess for LGE in all short-axis locations, matching those for cine imaging 10 minutes after a cumulative dose of 0.15 mmol/kg of gadolinium DTPA (Magnevist, Berlex, Wayne, NJ). T1 measurements were performed with a modified Look-Locker sequence with a nonslice-selective adiabatic inversion pulse, followed by segmented gradient-echo acquisition for 17 cardiac phases/times after inversion (T1’s), spread over 1 to 2 cardiac cycles (temporal resolution 105 ms precontrast, and 54 ms postcontrast, slice thickness 8 mm, TR >3 R-wave to R-wave (RR) intervals precontrast, and 2 RR intervals postcontrast). The Look-Locker sequence was repeated in the same mid LV short-axis slice, once before, and 3 additional times after the injection of gadolinium, starting at 4 minutes after injection and spanning a 30-minute period. Acquisition time with breath-holding was <20 s/slice.

Quantification of Global and Segmental MECVF

For Look-Locker images, the endo- and epicardial borders of the LV were manually drawn (QMass MR 7.1 software, Medis, The Netherlands). The signal intensity versus time curves for each segment and the blood pool were used to determine segmental T1 by multiplying each of the segmental T1 by the partition coefficient for gadolinium, starting at 4 minutes after injection and spanning a 30-minute period. Acquisition time with breath-holding was <20 s/slice.

Cine CMR Assessment

Manually traced epicardial and endocardial borders of matching short-axis cine locations at end systole and end diastole were used to determine the LV ejection fraction, LV end-diastolic volume index, LV end-systolic volume index, and LV myocardial mass (end diastole only). Left atrial area were traced in 4-chamber and 2-chamber views in human subjects to calculate the biplane left atrial volume, which was indexed to body surface area.

Statistical Analyses

Continuous data were expressed as mean±SD. Continuous variables were compared between different groups using t tests. Paired t test was used to compare continuous measurements at baseline and follow-up within a group. Bland-Altman analysis was used to compare 2 methods (eg, for estimating MECVF; or measuring R1), and obtain the 5% and 95% limits of agreement. Multivariate linear regression analysis was performed to assess the association of the difference of MECVF values from 2SX and FX models, with the maximum R1 in blood and the LV mass index. A log-likelihood ratio test was used to compare the fits with the 2SX and FX models. The likelihood ratio test was used for statistical significance by assuming that it is asymptotically χ² distributed with degrees of freedom equal to the difference in adjustable parameters between models.

Dichotomous data were compared using a Fisher exact test when possible, and with a χ² test otherwise. Spearman rank correlation was used to assess statistical dependence between variables. All analyses were performed with SAS 9.2 (SAS Institute, Inc, Cary, NC) and R (version 2.13.1, R Foundation for Statistical Computing, Vienna, Austria, 2011, URL: http://www.R-project.org).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the article as written.

Results

Animal Hemodynamic Data

Table 1 summarizes the hemodynamic and left-ventricular parameters for the L-NAME and control groups. At baseline, control, and L-NAME groups did not show any significant differences in body-weight, blood pressure, heart rate, and CMR-derived data (Table 1).17–19 The chronic administration of L-NAME was associated with a significant increase in the mean blood pressure.17–19

<table>
<thead>
<tr>
<th>Table 1. Baseline and Follow-Up Characteristics and CMR Data</th>
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<tbody>
<tr>
<td><strong>Characteristics</strong></td>
</tr>
<tr>
<td>Body-weight, g</td>
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<tr>
<td>HR, bpm</td>
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<tr>
<td>Mean-BP, mm Hg</td>
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<tr>
<td>LVEF, %</td>
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<tr>
<td>LVEDV, μL</td>
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<tr>
<td>LVESV, μL</td>
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<tr>
<td>LV mass, μg</td>
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<tr>
<td>LV mass index to body-weight, μg/g</td>
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<tr>
<td>MECVF</td>
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</table>

BP indicates blood pressure; CMR, cardiac magnetic resonance; HR, heart rate; LV, left ventricular; LVEDV, left-ventricular end-diastolic volume; LVESV, left-ventricular end-systolic volume; and MECVF, the myocardial extracellular volume fraction.

*Control vs L-NAME mice, unpaired t test.
†P<0.05; ‡P<0.01; §P<0.005; ‖P<0.001, baseline vs 7-week mice paired by group (control and L-NAME), paired t test.
Histological and CMR Markers of Fibrosis

At follow-up, the CTVF was significantly higher in the L-NAME group compared with placebo-treated mice (8.6%±1.5 versus 2.58%±0.6, $P<0.001$), as shown in Figure 1. This was mirrored with CMR by an increased MECVF in the L-NAME-treated animals, compared with controls (0.43±0.09 versus 0.26±0.03, $P<0.001$). The myocardial R1 showed a noticeable sublinear dependence on R1 in blood, as exemplified for an L-NAME treated mouse in Figure 2A. The 2SX model resulted in better fits to the data ($P<0.05$ for likelihood ratio test), compared with the linear FX model, in 77% of the L-NAME cases, and in 35% of the cases with placebo or before L-NAME treatment. Figure 2B shows that MECVF from the 2SX model correlated with the connective tissue fraction ($\rho=0.737\ P<0.0001$), whereas MECVF from FX model did not correlate with CTVF ($P=0.44$). Bland-Altman analysis showed that the FX model fit can cause a significant underestimate MECVF expansion (mean difference: –0.068; 5% and 95% limits of agreement: –0.051 to –0.085). The percent bias (difference/mean in %) to underestimate MECVF with the FX model became larger with extracellular matrix expansion, LV hypertrophy, and increased with the range of R1 in blood, covered by the measurements ($P<0.001$). This was tested statistically with a multivariable linear regression model for the % MECVF difference, with the mean MECVF, the maximum R1 in blood ($P<0.002$), and LV mass index ($P<0.002$) included as predictors. A separate analysis shows that estimates of MECVF obtained with FX model over a low, restricted R1 range (R1 in blood $<2$ s$^{-1}$) agree with the 2SX model within –0.003 (5% and 95% limits of agreement: 0.076–0.07). All further results for MECVF refer to the MECVF obtained with the 2SX model over the full range covered of R1 measurements. The mean MECVF at baseline did not differ between L-NAME and control mice (0.2645±0.046 versus 0.269±0.034, $P=0.719$, Figure 3A).

Seven studies with 2 consecutive T1 measurements without intermediate contrast injection were used to estimate the half life ($t_{1/2}$) for Gd clearance after subcutaneous injections. The range of times between the 2 T1 measurements averaged 21 minutes (range: 7–33 minutes.). The $t_{1/2}$ was 70±16 minutes (mean±SD)

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**Figure 1.** Representative examples of myocardial tissue stained with Masson trichrome in a midlevel myocardial slice from the control group (A) and the L-NAME group (B) shows a visually clear difference of blue-colored, connective tissue. Connective tissue fraction, shown in (C), and defined as the number of pixels with a bluish hue, divided by the total number of myocardial pixels in the slice, was significantly different between controls and L-NAME treated mice.

**Figure 2.** A, The relation between myocardial R1 and blood pool R1 was fit with a 2-space 1H exchange model (2SX), to account for transcytotic water-exchange (solid black line). Assuming fast water-exchange (FX) predicts a linear relationship between myocardial R1 and blood R1. For lower R1 values (gray dashed line) this gives reasonable agreement, but results in an 80% underestimate of MECVF (0.38 vs 0.21) if the FX model is used over the entire R1 range (black dashed line). B, MECVF correlated significantly with the connective tissue fraction obtained from myocardial slices stained with Masson trichrome stain. C, No significant correlation could be observed when the R1 relationship was analyzed with the fast 1H exchange assumption. MECVF indicates myocardial extracellular volume fraction.
after subcutaneous injections of contrast, sufficiently long to allow for contrast equilibrium between blood and tissue.

R1 measurements at 4.7 T with the Look-Locker technique were compared against a standard IR-prepared spin-echo technique in 10 gadolinium-doped phantoms with R1 ranging from 0.35 to 8.4 s\(^{-1}\). The mean difference (R1 from IR cine – R1 from IR-SE) was –0.01±0.09 s\(^{-1}\), with the 5% and 95% limits of agreement obtained by Bland-Altman analysis at –0.19 s\(^{-1}\) and 0.16 s\(^{-1}\). For 3T (Siemens Trio), the mean difference of R1 in 10 phantoms, using the Look-Locker and IR-spin-echo techniques was –0.093 s\(^{-1}\) (Bland-Altman 5% and 95% limits of agreement: –0.44 to 0.27), with the mean R1 covering a range from 0.795 to 6.7 s\(^{-1}\).

Morphological and Functional Changes Associated with Chronic L-NAME treatment

Treatment with L-NAME for 7 weeks was associated with a significant increase in cardiac mass (92.6±12.4 versus 162.9±19.4 μg, P<0.001) and reduction in LV ejection fraction (58.7±2.9% versus 51.4±8.2%, P=0.003, Figure 3B and 3C). At follow-up, MECVF correlated significantly with mean arterial pressure, LV ejection fraction, and LV mass indexed with body-weight (Figure 4). Similarly, CTVF correlated with mean arterial pressure (ρ= 0.71; P<0.001), LV ejection fraction (ρ= −0.4; P=0.03), and LV mass index (ρ= −0.52; P<0.004).

Pilot Data From a Cohort of Hypertensive Patients

Patients with treated HTN tended (P=0.136) to be older than healthy volunteers (Table 2). There was no substantial difference in systolic and diastolic blood pressure between volunteers and HTN patients (Table 2). Among HTN patients, 6 (60%) took a calcium channel blocker, 5 (50%) took a β-blocker, 5 (50%) took an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker, and 4 (40%) took a diuretic. LV hypertrophy on ECG was present in 3 (30%) patients, suggesting that increased MECVF can occur without common signs of hypertensive heart disease. Echocardiograms were available in 8 HTN patients. Among them, the mean E/E’ ratio was 11.6±2.1 at the basal lateral wall and 16.6±4.8 at the basal septal wall, indicating high LV filling pressure. By CMR, LV volumes, mass, and function were not substantially different between HTN patients and volunteers (Table 2). Biplane left atrial volume index was substantially higher in HTN patients than in volunteers (Table 2). Half of the patients had thickened LV walls (≥12 mm in men and ≥10 mm in women). 20 The dependence of the myocardial R1 on R1 of blood showed a similar sublinear dependence (Figure 5) using a standard clinical contrast dosage (≤0.2 mmol/kg). The deviation from the linear FX model had similar effects, as observed in the experimental studies. The MECVF for all patients and volunteers correlated significantly with left atrial volume (ρ=0.58, P=0.008).

Discussion

CMR showed significant MECVF expansion as a result of arterial HTN from inhibition of NO synthesis by chronic administration of L-NAME. The expansion of the extracellular matrix, and myocardial hypertrophy, can exacerbate the
bias to underestimate of MECVF if the effect of transcytlemmal water-exchange on myocardial T1 is neglected. With the use of a parsimonious 2SX model, the estimate of MECVF resulted in a good correlation with histological measurements of the connective tissue fraction, using Masson trichrome staining. Using this model, we found significant extracellular matrix expansion in patients with treated hypertension, undetected by LV mass index, or LGE imaging. Increased MECVF correlates strongly with the left atrial volume index in patients, which supports the pathophysiologically plausible role of diffuse fibrosis in ventricular stiffening.

The CMR-derived MECVF was previously validated against collagen volume fraction determined by picrosirius red staining.8,21 Picrosirius red is a relatively sensitive way to identify collagens in dense fibrosis, particularly type I collagen; the characteristic red stain under polarized light provides a strong signal, and fibril orientation is easily demonstrated. However, with lesser amounts of extracellular fibrosis, or at earlier stages of deposition when glycosaminoglycans, proteoglycans, fibronectin, and type III collagen predominate, picrosirius is an insensitive method to assess matrix content. Thus, we have used Masson trichrome to highlight and quantify all elements of the extracellular matrix, including the noncollagenous components. Myocardium lends itself well to the Masson stain because the usual matrix content is low. There is a distinct colorimetric difference between myocytes and fibrosis.

Transcytlemmal water-exchange plays an important role in determining myocardial T1 when an extracellular contrast agent creates a difference of R1 rates between intra- and extracellular spaces on the order of the transcytlemmal water-exchange rate. A parsimonious 2SX model was previously worked out in detail and validated.11,16 The potential benefits of using this model for estimating MECVF was not considered before. The 2SX model predicts a sublinear dependence of R1 in tissue on R1 in blood (plasma), and the degree of curvature is determined by the intracellular lifetime of water, and the R1 range in blood (plasma) covered by the T1 measurements. The intracellular lifetime reflects a tissue property that is stable with homeostasis, and there is no evidence that it changes with gadolinium contrast administration.

The less than linear increase of the myocardial R1 as a function of R1 in blood results in a systematic underestimate of MECVF, if the data are analyzed with a linear FX model (Figures 2 and 5). The bias introduced by assuming FX was observed to increase significantly with MECVF and intracellular lifetime, and also depended on the range of R1 values in blood. The better fit with the 2SX model was most apparent in the L-NAME-treated mice, which probably

### Table 2. Clinical and CMR Characteristics of Human Subjects Cohort

<table>
<thead>
<tr>
<th></th>
<th>Hypertension</th>
<th>Normal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>66.2±16.3</td>
<td>56.3±5.2</td>
<td>0.136</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89.6±30.4</td>
<td>76.4±17.9</td>
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</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.047±0.198</td>
<td>1.874±0.358</td>
<td>0.241</td>
</tr>
<tr>
<td>Female</td>
<td>2 (25%)</td>
<td>7 (58%)</td>
<td>0.310</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>133±10.7</td>
<td>125.1±10.5</td>
<td>0.118</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>70±13.4</td>
<td>78.1±7.1</td>
<td>0.162</td>
</tr>
<tr>
<td>NYHA class ≥ II</td>
<td>5 (62.5%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDV index, mL/m²</td>
<td>72.5±17.6</td>
<td>67.6±14.0</td>
<td>0.521</td>
</tr>
<tr>
<td>LVESV index, mL/m²</td>
<td>67.0±19.4</td>
<td>25.7±7.5</td>
<td>0.487</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>62.1±8.9</td>
<td>62.3±5.2</td>
<td>0.746</td>
</tr>
<tr>
<td>LV mass index, g/m²</td>
<td>49.9±12.1</td>
<td>45.1±6.8</td>
<td>0.331</td>
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<tr>
<td>Biplane LA volume, mL/m²</td>
<td>32.61±20.12</td>
<td>12.98±5.35</td>
<td>0.028</td>
</tr>
<tr>
<td>Myocardial extracellular volume fraction (2SX model)</td>
<td>0.446±0.063</td>
<td>0.307±0.030</td>
<td>0.0002</td>
</tr>
<tr>
<td>Myocardial extracellular volume fraction (FX model)</td>
<td>0.332±0.045*</td>
<td>0.263±0.027*</td>
<td>0.004</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; LV, left ventricular; LVEDV, left-ventricular end-diastolic volume; LVEF, left-ventricular ejection fraction; LVESV, left-ventricular end-systolic volume; and NYHA, New York Heart Association.

*P<0.01 for paired t-test of 2-site water-exchange model (2SX) and against standard fast exchange model (FX).

Figure 5. In an 82-year-old male with a history of hypertension, and no evidence of ischemic heart disease, myocardial R1 vs blood R1 was fit with a 2-space 1H exchange model (2SX), shown as solid line. Similarly to Figure 2, the myocardial R1 initially increases linearly (shown as gray dashed line, extrapolated to larger R1 values), and then develops a convex shape as the rate of transcytlemmal moves away from the fast exchange condition. If all R1 data points are included for a fit with a linear model, one obtains a 67% lower value for myocardial extracellular volume fraction (MECVF) (0.18), compared with the analysis with the 2SX model (0.54).
reflects the substantial development of hypertrophy and interstitial fibrosis, compared with controls. The values for the transcytolemmal (first-order) water-exchange rate constants (median = 19 Hz, interquartile range = 9–24 Hz) obtained in this study with the 2-space model of Landis et al. are within the range reported for myocardium in previous studies.

The values of MECVF for mice in this study fall within the normal range measured in healthy human volunteers. MECVF and cardiomyocyte size or volume (= 24 × 10^9 μm^3 in young adult male humans) and 25 × 10^9 μm^3 in mice) are expected to be relatively similar across species, although they are affected by age and sex.

The hypertensive patients were selected to include only those with LV mass within the sex-specific normal range, but the MECVF was significantly higher than in controls. A higher than normal LV mass is currently an accepted sign of hypertensive heart disease, if other causes of hypertrophy can be excluded. The CMR measurements may suggest that expansion of the extracellular space could precede a significant increase of LV mass. The latter may in fact represent the combined effect of extracellular matrix expansion and cell hypertrophy. Drug therapies that can achieve regression of LV hypertrophy, such as angiotensin-converting enzyme inhibitors, may benefit from an earliest possible application. MECVF could become an early marker of adverse myocardial remodeling.

The methodology for the measurements and postprocessing were essentially similar for the studies in mice and humans. The differences relate mostly to requirements for higher spatial and temporal resolution in mice compared with humans. For the mouse studies, the acquisition of the k-space segments was kept to <20 ms to avoid motion artifacts. Subcutaneous contrast injections for the mice resulted in a sufficiently slow blood clearance to assure equilibrium conditions. Importantly, accounting for the effects of water-exchange had a significant effect for both the mouse and human studies, even though the latter were based on a clinically acceptable gadolinium contrast dosage of 0.15 mmol/kg.

Limitations
The use of MECVF as a marker of fibrosis assumes that a pathological expansion of the extracellular matrix is coupled with a build-up of connective tissue, which was quantified in this study by Masson trichrome stain. Although a correlation between extracellular space expansion and CTVF is not unexpected, the characteristics of such a relationship can also be affected by the density of connective tissue in the extracellular space. The effect of connective tissue density on MECVF measurements remains unclear at this point.

A further limitation is the absence of any measurements of diastolic function indices in the mouse model. For the mice, we note that there is a significant association between systolic function and MECVF.

Our observation of a significant underestimation of MECVF, when based on the FX assumption of water exchange applies mostly to protocols where R1 in the blood after contrast administration is relatively high. For R1 in blood < 2.0 s^-1 the percent underestimation of MECVF with the FX assumption is, in our experience, generally < 5%, but may vary by presence and degree of hypertrophy. In this study, the maximum R1 in blood in each mouse averaged 5 s^-1. The findings from the comparison of MECVF obtained with the 2SX and the FX models are dependent on the contrast dosages, and the cumulative effect of contrast injections on R1 in the blood.

Conclusion
MECVF, quantified by CMR T1 measurements, detects myocardial extracellular matrix expansion, a marker of interstitial fibrosis, in a mouse model of hypertensive heart disease. The analysis of the myocardial T1 data with the assumption that transcytolemmal exchange remains in the fast exchange limit after contrast administration can result in a significant underestimation of MECVF. Underestimates of MECVF attributed to the FX assumption depend on the degree of LV hypertrophy and the maximum T1 in the blood pool. A generalization of the model for determination of MECVF may allow a more sensitive detection of diffuse fibrosis in patients with hypertension, compared with healthy controls.

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Disclosures
Dr Jerosch-Herold is listed as coinventor on a pending patent application related to detection of diffuse fibrosis by MRI.

References


**CLINICAL PERSPECTIVE**

Myocardial T1 measurements or mapping with cardiac magnetic resonance has been gaining increased attention as a valuable complement to late gadolinium enhancement imaging to assess pathological changes in the myocardium in the absence of late gadolinium enhancement. Diffuse fibrosis (and collagen deposition) is associated in several myocardial pathologies with an expansion of myocardial extracellular space. T1 measurements performed before and after injection of an extravascular contrast agent have been used to quantify the extracellular volume (ECV) fraction in myocardial tissue. As the expansion of the extracellular space with the build-up of diffuse fibrosis can be relatively small (∼10%), it is important to evaluate the limits of current approaches and determine the conditions for an accurate determination of ECV. We show that the estimation of ECV can be sensitive to water-exchange across cell-membranes, and more so when the ECV is expanded, and in the presence of ventricular hypertrophy, using a mouse model of hypertensive heart disease. Neglecting this effect, or assuming that water exchange is sufficiently fast even when contrast uptake creates large T1 differences between intra- and extracellular spaces leads to underestimation of ECV. The potential clinical relevance is tested in a small cohort of patients with hypertension. The findings of this study could help to better define conditions when relatively straightforward T1 measurements can give accurate estimates of ECV in patients.
Role of Transcytosemmal Water-Exchange in Magnetic Resonance Measurements of Diffuse Myocardial Fibrosis in Hypertensive Heart Disease


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