Left Ventricular Strain and Transmural Distribution of Structural Remodeling in Hypertensive Heart Disease

Tomoko Ishizu, Yoshihiro Seo, Yuri Kameda, Ryo Kawamura, Taizou Kimura, Nobutake Shimojo, Dongzhu Xu, Nobuyuki Murakoshi, Kazutaka Aonuma

Abstract—Left ventricular (LV) systolic wall strain is a new candidate for prognostic indicator of hypertensive heart failure. It remains unclear how underlying transmural structural remodeling corresponds to LV wall systolic deformation as hypertension hypertrophy progresses. We fed 68 Dahl salt-sensitive rats a high-salt (hypertensive group) or low-salt diet (control group) from 6 weeks old. At 10, 14, and 18 weeks, pressure–volume relation, transmural distribution of LV fibrosis, and myocyte hypertrophy were evaluated. LV global longitudinal and circumferential strain was measured with speckle tracking echocardiography. Emax was preserved throughout the study period, whereas τ and end-diastolic pressure–volume relation progressively deteriorated from 14 weeks (diastolic dysfunction stage). Lung weight increased significantly at 18 weeks (decompensated stage). Histological percentage area fibrosis and collagen type I/III, myocyte hypertrophy, and α-myosin heavy chain isoform increased in the subendocardial layer at 14 weeks and progressed into the midlayer at 18 weeks. Longitudinal strain progressively deteriorated in the hypertensive group versus control group at 14 weeks (hypertensive group: −17±3%, control: −27±4%; P<0.001), and circumferential strain decreased at 18 weeks (hypertensive group: −17±2%, control: −27±3%; P=0.002). After adjustment for systolic wall stress, subendocardial percentage area fibrosis was selected as the independent determinant of longitudinal strain. This study showed that LV wall strain alternations were accompanied by fibrosis and myocyte hypertrophy from subendocardium to epicardium, and longitudinal strain related significantly to subendocardial layer fibrosis. Longitudinal strain could be a surrogate of subendocardial fibrotic changes and may be useful for risk stratification of hypertensive heart failure. (Hypertension. 2014;63:00-00.) ● Online Data Supplement

Key Words: echocardiography ■ heart failure ■ hypertension ■ hypertrophy ■ myocardial contraction

Hypertension induces adverse remodeling,1,2 and heart failure develops although ejection fraction (EF) is preserved.1 Progressive remodeling consists of both an increase in the size of the cardiomyocytes and accumulation of fibrosis in the extracellular matrix. These pathological processes have been proposed to be heterogeneous across the LV wall.4 The LV myocardial layer consists of a characteristic myocardial fiber orientation in which the longitudinal fibers in the subendocardial layer gradually change to a circumferential direction in the midwall layer and revert to longitudinal in the subepicardial layer.5,6 Two-dimensional speckle tracking echocardiography allows quantitative measurements of regional deformation as strain in clinical7 and animal experimental settings.8 LV deformation as measured by longitudinal, radial, and circumferential strain is thought to be closely linked with the myofiber architecture.7 Among these, longitudinal strain has been especially focused on because of its clinical significance in patients with heart failure with hypertensive heart disease9,10 and its correlation with exercise capacity11 and prognosis.11,12 However, the underlying mechanism causing impaired longitudinal function has not been fully clarified, and there are few data on transmural histological alteration in conjunction with LV wall systolic deformation and chamber function.

The aim of this study was to investigate the following in accordance with the nonuniform structure of the LV wall: (1) transmural distribution of myocytes and extracellular remodeling in a time-course study, in which myocardial remodeling was associated with the development of hypertensive heart disease; (2) the association between myocardial strain and transmural tissue characteristics; and (3) the influence of strain on global chamber function.

Methods

Experimental Animals
In this study, 68 male Dahl salt-sensitive rats, a well-validated model of heart failure with preserved EF attributable to hypertension (DIS/
Eis; Eisai, Tokyo, Japan) were used. The control group (n=35) was fed low-salt 0.3% NaCl, and the hypertension group (HT, n=33) was fed high-salt 8% NaCl chow from 6 weeks.

**Additional Methods**

The expanded Methods section in the online-only Data Supplement contains information on the hemodynamic measurements, echocardiographic analyses, tissue analyses, quantification of gene expression by real-time polymerase chain reaction and statistical methods.

**Results**

Initial measurements were obtained from all 68 rats. One control rat and 1 rat in the HT group died spontaneously at 13 weeks and did not complete the experimental protocol. The cause of death was not investigated.

**Blood Pressure, Heart Rate, and Organ Weight**

The high-salt diet induced significant and sustained elevation in blood pressure compared with that in the control group at each time point after week 8 (Figure 1). Heart rate showed no significant difference between the HT group and the age-matched control group except for that at 18 weeks (Table 1), at which time a higher heart rate was observed in the HT group than in the control group. The LV weight also progressively elevated with the increase in blood pressure, whereas weight loss occurred from 14 weeks followed by elevation in lung weight, suggesting decompensated heart failure with lung congestion.

**Echocardiographic Studies**

Measurements of standard echocardiographic parameters are shown in Table 1. The HT group showed a progressive increase in wall thickness and LV mass from 6 to 18 weeks (Table 1). Left atrial diameter increased, and E/A and E' decreased with aging in the HT group rats. In comparison, EF showed no change with time up to 16 weeks, and a slight but significant decline of EF was observed at 18 weeks in the HT group rats.

**Systolic and Diastolic Chamber Function**

Compared with the control group, the HT group showed no difference in ESPVR throughout the study period (Figure 2). It was found that τ was significantly greater from 10 weeks to 18 weeks in the HT group, and that chamber stiffness was elevated at 18 weeks.

**Transmural Stress and Fiber Angle Distribution**

Systolic wall stresses were greater in the subendocardial layer than in the subepicardial layer in both groups, and higher subendocardial wall stresses were observed in the HT group than in the corresponding layer in the control group (Figure S2 in the online-only Data Supplement). Transmural distribution of the myocardial fiber orientation was identical in the 2 groups in all study periods and was 69±19° from the horizontal plane in the subendocardium. The systolic wall stress increased successively as the HT animals grew older without affecting the myocardial fiber angles.

**Myocardial Strain Measurements**

Speckle tracking echocardiography was applied successfully to the longitudinal and short-axis views (Figure 3A). Although the 3 strain parameters did not change with time in the control group, global strain in the longitudinal direction (GLS) in the HT group showed significant impairment from week 10 and progressively deteriorated with age (Figure 3B, top, and 3C). Global strain in the radial direction (GRS) decreased from week 16 (Figure 3B, middle, and 3D), and global strain in the circumferential direction (GCS) decreased at 18 weeks in the HT group (Figure 3B, bottom, and 3E).

**Transmural Distribution of Fibrosis and Hypertrophy in Pathological Specimens**

At week 14, total burden of the percentage area fibrosis was significantly greater in the HT group than in the control group (4.9±1.4% versus 2.2±0.4%; P<0.001). The fibrosis was seen predominantly in the subendocardial layer in the HT group (Figure 4A). At 18 weeks, a significant increase in percentage area fibrosis from 0% to 75% wall depth from the subendocardium was observed (Figure 4A, bottom, and 4B, bottom). As compared with the control group, myocyte width in the HT group at week 14 was significantly greater in the subendocardial layer (Figure 5A, middle) and at week 18 was greater in the subendocardial and midwall layers (Figure 5A, bottom).

**Layer-Specific Change in Fibrosis and Hypertrophy by Real-Time Polymerase Chain Reaction Analysis**

Myocardial expression of collagen type I/III increased at 18 weeks with predominance in the subendocardial region (Figure 4C, bottom). Myocardial β-myosin heavy chain (MHC; Figure 5B) and β-MHC/α-MHC isoforms (Figure 5C) were significantly upregulated in the subendocardial and midwall layers at 14 and 18 weeks in the HT group.

**Relation Between Strain and Pathological Findings**

Longitudinal strain correlated significantly with subendocardial (Figure 6A) and midlayer percentage area fibrosis.
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There was a significant negative relation between wall stress and strains (Figure 6B), as was expected from the findings of original studies of the stress–strain relation. Therefore, adjustment by corresponding layer-specific wall stress was performed (Table 2). After we adjusted for stress, subendocardial percentage area fibrosis, but not subendocardial myocyte width, was selected as the independent determinant of GLS. Midwall and epicardial layer percentage area fibrosis and myocardial

**Table 1. Heart Rate and Standard Echocardiographic Measurements**

<table>
<thead>
<tr>
<th>Groups</th>
<th>HR, bpm</th>
<th>LWT, mm</th>
<th>LVDd, mm</th>
<th>RWT</th>
<th>LV mass, mg</th>
<th>LAD, mm</th>
<th>E/A</th>
<th>E’, mm/sec</th>
<th>EF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-6 W</td>
<td>452±36</td>
<td>1.3±0.1</td>
<td>6.3±0.4</td>
<td>0.48±0.07</td>
<td>382±57</td>
<td>3.9±0.2</td>
<td>1.5±0.3</td>
<td>55.2±7.5</td>
<td>73.5±5.6</td>
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<td>HT-6 W</td>
<td>453±43</td>
<td>1.4±0.2</td>
<td>6.2±0.6</td>
<td>0.54±0.05*</td>
<td>486±89</td>
<td>3.9±0.4</td>
<td>1.5±0.2</td>
<td>44.9±12.0</td>
<td>73.8±7.0</td>
</tr>
<tr>
<td>Control-8 W</td>
<td>396±49</td>
<td>1.6±0.1</td>
<td>6.7±0.7</td>
<td>0.47±0.07</td>
<td>617±60</td>
<td>3.6±0.6</td>
<td>1.6±0.2</td>
<td>54.2±5.3</td>
<td>77.3±5.1</td>
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<tr>
<td>HT-8 W</td>
<td>391±59</td>
<td>1.8±0.2*</td>
<td>6.6±0.7</td>
<td>0.56±0.11*</td>
<td>608±101</td>
<td>3.5±0.4</td>
<td>1.6±0.3</td>
<td>41.8±13.8</td>
<td>79.4±6.3</td>
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<tr>
<td>Control-10 W</td>
<td>389±37</td>
<td>1.7±0.2</td>
<td>6.8±0.6</td>
<td>0.53±0.07</td>
<td>673±96</td>
<td>3.4±0.3</td>
<td>1.5±0.3</td>
<td>46.5±12.6</td>
<td>76.0±4.7</td>
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<tr>
<td>HT-10 W</td>
<td>391±32</td>
<td>2.1±0.2*</td>
<td>7.0±0.5</td>
<td>0.61±0.07*</td>
<td>816±112*</td>
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<td>1.3±0.2</td>
<td>44.7±6.6</td>
<td>76.7±7.7</td>
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<td>Control-12 W</td>
<td>395±29</td>
<td>1.8±0.2</td>
<td>6.9±0.6</td>
<td>0.52±0.07</td>
<td>884±68</td>
<td>3.5±0.6</td>
<td>1.5±0.3</td>
<td>44.5±5.1</td>
<td>78.7±4.9</td>
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<tr>
<td>HT-12 W</td>
<td>401±34</td>
<td>2.4±0.3*</td>
<td>6.6±0.7</td>
<td>0.76±0.09*</td>
<td>1082±83*</td>
<td>4.0±0.5*</td>
<td>1.3±0.2*</td>
<td>48.1±16.8</td>
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<td>Control-14 W</td>
<td>388±40</td>
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<td>0.52±0.07</td>
<td>825±208</td>
<td>3.6±0.8</td>
<td>1.6±0.2</td>
<td>46.4±7.8</td>
<td>75.8±6.4</td>
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<tr>
<td>HT-14 W</td>
<td>398±52</td>
<td>2.5±0.3*</td>
<td>6.9±0.5</td>
<td>0.75±0.10*</td>
<td>1176±102*</td>
<td>5.1±0.6*</td>
<td>1.2±0.1*</td>
<td>48.1±5.4</td>
<td>74.8±8.0</td>
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<td>Control-16 W</td>
<td>381±36</td>
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<td>7.1±0.2</td>
<td>0.60±0.07</td>
<td>885±79</td>
<td>3.8±0.2</td>
<td>1.3±0.2</td>
<td>50.9±4.9</td>
<td>75.4±4.9</td>
</tr>
<tr>
<td>HT-16 W</td>
<td>431±31</td>
<td>2.4±0.2*</td>
<td>7.7±0.3*</td>
<td>0.69±0.06</td>
<td>1309±280*</td>
<td>5.1±0.6*</td>
<td>1.1±0.1*</td>
<td>34.8±7.1*</td>
<td>73.3±6.9</td>
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<tr>
<td>Control-18 W</td>
<td>385±24</td>
<td>1.8±0.1</td>
<td>7.1±0.5</td>
<td>0.56±0.04</td>
<td>894±69</td>
<td>3.8±0.5</td>
<td>1.7±0.4</td>
<td>50.4±3.1</td>
<td>75.6±5.1</td>
</tr>
<tr>
<td>HT-18 W</td>
<td>420±26*</td>
<td>2.6±0.2*</td>
<td>7.3±0.3*</td>
<td>0.75±0.05*</td>
<td>1232±171*</td>
<td>5.5±0.3*</td>
<td>1.0±0.1*</td>
<td>36.5±7.0*</td>
<td>65.5±5.4*</td>
</tr>
</tbody>
</table>

E/A indicates transmitral early to late diastolic flow velocity ratio; E’, septal mitral annulus velocity at early diastole; EF, ejection fraction; HR, heart rate; HT, hypertension; LAD, left atrial dimension; LV, left ventricular; LWT, left ventricular wall thickness; LVDd, left ventricular end-diastolic diameter; RWT, relative wall thickness; and W, weeks. *P<0.05 vs age-matched control.

(R=0.59, P=0.004 and R=0.51, P=0.0018, respectively), and all-layer myocyte width (R=0.61, P=0.001), and by corresponding layer-specific wall stress was performed (Table 2). After we adjusted for stress, subendocardial percentage area fibrosis, but not subendocardial myocyte width, was selected as the independent determinant of GLS. Midwall and epicardial layer percentage area fibrosis and myocardial

**Figure 2.** Left ventricular (LV) chamber function in rats with hypertension (HT). Top shows representative pressure–volume waveforms during manipulations (compression of the inferior vena cava) to decrease preload performed at 10, 14, and 18 weeks. Emax was not significantly different throughout the experiments [bottom, left], whereas relaxation time constant τ elongated from the early period [bottom, right], and chamber stiffness elevated significantly at 18 weeks [bottom, middle]. EDPVR indicates end-diastolic pressure–volume relation; and ESPVR, end-systolic pressure–volume relation. *P<0.05 vs control group.
cell width were revealed to be the significant determinants of GRS, and total and subendocardial myocardial hypertrophy, but not the degree of collagen deposition, significantly related to GCS.

Relation Between Strain and Chamber Function

Although GLS showed no relation with Emax, there was a significant relation between GLS and chamber stiffness (Figure 6C) and $\tau$ ($R=0.58$, $P<0.001$). GRS showed a negative

Figure 4. Distribution of transmural fibrosis in rats with hypertension (HT). Areas of fibrosis (stained in blue) were predominant in the subendocardial layer at 14 weeks and propagated into the midlayer by 18 weeks in the HT group (A). The percentage area fibrosis (B) was significantly greater in the subendocardial layer at 14 weeks and subendocardium to midmyocardium at 18 weeks in the HT group. Collagen I/III ratio (C) was increased significantly in the subendocardial layer at 18 weeks in the HT group. $^*P<0.05$ vs control group.
Myocardial Strain
Transmural Fibrosis Distribution and Myocardial Strain

In the present study, longitudinal wall deformation was affected by the midcardial to epicardial layer, mainly by the subendocardial fibrosis and collagen subtype. Radial strain was affected by the midcardial layer to epicardial layer fibrosis that extended from the subendocardium. Therefore, transmurality of collagen deposition might be associated with longitudinal strain early, and with radial strain late, in the disease course. The impairment in LV contractility has been thought to occur mainly because of cardiac myocyte injury, and a definitive cause–effect relation between fibrosis and systolic function has not been established. Recently, several reports have assumed that abnormalities in the extracellular matrix impair organ contractile function even if myocyte contractility is preserved.13,14 The extracellular collagen network, which maintains the alignment of myocytes with each other and provides for the transmission of force, generated by myocyte contraction, to the ventricular chamber,13 becomes fused and thickened in the interconnecting layer in the hypertensive heart.15 Fibrosis in the present study accumulated predominantly in the subendocardial layer, where the myocardial fiber angle is longitudinally oriented. In addition, maximum shearing occurs in the subendocardium, in comparison with that in other layers, by sliding of myocardial laminae relative to each other.16 This suggests that fibrosis itself could directly inhibit the rearrangement of myocardial sheets in each layer and may result in systolic wall deformation abnormality.

On the molecular level, collagen I/III was elevated in subendocardium in the HT group. Elevated collagen I/III ratio also has been reported to result in chamber stiffness,17 suggesting established fibrosis.18 In several clinical investigations, longitudinal systolic dysfunction was associated with adverse clinical outcome.19–20 Thus, the results of this study suggest that measurement of GLS allows assessment of the degree of fibrotic changes and use of this information for prognostic risk stratification in hypertensive heart failure.

Myocyte Hypertrophy Gradient Across the Ventricular Wall and Strain

Myocyte width increased predominantly in the subendocardial to midlayer because concentric hypertrophic remodeling progressed. Also, the myosin phenotype in the subendocardial and midwall differed from that in the subepicardial layer and corresponding layer in the control group. Expression of the isoform of MHC mRNA shifted from the α-MHC isoform to the fetal myosin isoform β-MHC and also increased in the subendocardium. To our knowledge, there has been no previous detailed research reporting such a heterogeneous distribution of myocyte hypertrophy across the LV wall in the hypertensive heart. These transmural gradients of myocyte width and contractile protein isoforms were significant although less prominent than the fibrosis with apparent subendocardial accumulation. These results support the concept that fibrosis and myocyte growth basically occur independently of each other,13 and myocyte hypertrophy may be strongly affected by systemic neurohormonal factors. Myocyte hypertrophy is an adaptive response to pressure overload that accompanies an adaptive response to pressure overload that accompanies an

Discussion

This study presents a comprehensive set of functional and histological data gathered within the LV wall during the full course of disease development and demonstrated the following features: (1) heterogeneous transmural distribution in quality and quantity of fibrosis and myocyte hypertrophy, with a significant histological and molecular burden in the subendocardium; (2) progressive impairment of longitudinal shortening occurred parallel to the subendocardial stiff fibrosis; and (3) chamber diastolic dysfunction was characteristic of the LV with impaired longitudinal strain.

Transmural Fibrosis Distribution and Myocardial Strain

In the present study, longitudinal wall deformation was affected mainly by the subendocardial fibrosis and collagen subtype alternation present in the subendocardial layer. Furthermore, radial strain was affected by the midcardial to epicardial layer.

Figure 5. Transmural myocyte width and fetal isoform of contractile protein in rats with hypertension (HT). The myocyte width (A) was significantly greater in the subendocardial layer at 14 weeks and subendocardium to midmyocardium by 18 weeks. β-myosin heavy chain (MHC) messenger RNA (B) significantly increased in the subendocardial and midwall at 14 and 18 weeks as compared with the control group, β-MHC/α-MHC (C) was also increased significantly in the subendocardial to midlayer at 14 and 18 weeks in the HT group. *P<0.05 vs control group.

Figure 6. Relations of global longitudinal strain (GLS) to endocardial percentage area fibrosis, endocardial wall stress, and chamber stiffness. GLS showed significant positive relations with endocardial percentage area fibrosis (A), endocardial wall stress (B), and chamber stiffness derived from the end-diastolic pressure–volume relation (C).
systolic myocardial strain after adjustment for systolic wall stress in the present study.

**Ventricular Wall Systolic Dysfunction and Chamber Diastolic Impairment**

GLS did not correlate with systolic chamber elastance, but it did correlate significantly with diastolic hemodynamics. Myocardial hypertrophy and increased fibrosis may play important roles in maintaining both systolic and diastolic chamber stiffness in the presence of chronic pressure overload.23 Especially, excessive amounts of myocardial stiff collagen are the major determinant of passive chamber stiffness in the hypertensive heart.24 In view of the fact that chamber deformation starts from the longitudinal direction at early diastole,5 it is reasonable to assume that a slow relaxation rate is cross-related to longitudinal dysfunction. In addition, the increase in the slow β-MHC isoform in longitudinally oriented fibers in the subendocardial layer may cause retardation of chamber relaxation.

**Limitations**

In the present study, transmural wall stress was estimated based on a previously reported calculation; however, the measurement of systolic wall stress in the epicardial layer was fundamentally difficult because the epicardial layer was attached to the pericardium and thorax. We did not investigate segmental differences of hypertrophy or fibrosis, as well as layer-specific myocyte or microvascular function was not assessed. Although many factors were evaluated, the causal relations of the factors were not fully demonstrated. The pathogenesis of increased subendocardial fibrosis has not been assessed; however, increased subendocardial wall stress, oxidative stress,25 and the renin–aldosterone system have been reported to be promising candidates as causative factors of subendocardial fibrosis.26–28 Finally, interventions to observe the effects of therapy on myocyte hypertrophy or fibrosis were not performed. All of these issues are relevant and should be the subject of further studies.

**Perspectives**

In summary, a rat model of hypertensive heart failure with preserved LVEF was characterized by the progressive deterioration of longitudinal contraction from the early stage, followed by wall thickening abnormality, and then impairment of circumferential shortening at the decompensated stage. Pathophysiological assessment of transmural distribution of the abnormality showed that elevation of wall stress, accumulation of percentage area fibrosis with stiff collagen-subtype, and myocardial cell hypertrophy with increased α-MHC isoform in the subendocardial longitudinally oriented myocyte layer occurred at the early stage of heart failure. As disease progressed, both collagen deposition and myocardial hypertrophy extended to the midwall circumferentially oriented myocyte layer, and finally to the subepicardial longitudinally oriented myocyte layer. After adjustment for the corresponding layer wall stress, longitudinal strain impairment was found to relate significantly to the level of subendocardial fibrosis, and furthermore, decreased radial strain related to midepicardial and subepicardial fibrosis. Longitudinal systolic impairment was accompanied by delayed relaxation and elevated end-diastolic chamber stiffness. From the standpoint that the extracellular collagen deposition plays the major role in the pathophysiology of hypertensive heart failure in terms of progression, global longitudinal strain could be a noninvasive functional surrogate marker of subendocardial fibrosis and could be a useful guide to the risk of developing future heart failure from hypertensive heart disease by an individual patient.

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**Disclosures**

None.

**References**

Myocardial Strain and Hypertensive Heart Fibrosis

Novelty and Significance

What Is New?

- Global longitudinal strain was significantly associated with accumulation of stiff fibrosis subendocardially in hypertensive heart failure.
- Abnormality of global longitudinal strain was associated with chamber diastolic stiffness.

What Is Relevant?

- Global longitudinal strain could be a useful functional surrogate of subendocardial fibrosis in clinical practice.

Summary

Whether left ventricular longitudinal contraction abnormality could relate to the myocardial remodeling is unknown. Here, we report that in a model of hypertensive heart failure with preserved ejection fraction, global longitudinal strain progressively deteriorates with subendocardial fibrosis and chamber diastolic stiffness developing. Global longitudinal strain could be a noninvasive surrogate marker of subendocardial fibrosis in hypertensive heart disease.
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Left Ventricular Strain and Transmural Distribution of Structural Remodeling in Hypertensive Heart Disease

Running title: Myocardial Strain and Hypertensive Heart Fibrosis

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Expanded Materials and Methods

Experimental Animals
Animal experiments were carried out in a humane manner after we received approval for this study from the Institutional Animal Experiments Committee of the University of Tsukuba and were in accordance with the Regulation for Animal Experiments in our university. In this study, male Dahl salt-sensitive (DSS) rats, a well-validated animal model of heart failure with preserved EF due to hypertension (DIS/Eis; Eisai, Tokyo, Japan) were used. The control group was fed low-salt 0.3% NaCl, and the hypertension group was fed high-salt 8% NaCl chow from 6 weeks. Blood pressure measurement by a tail cuff system (BP-98A; Softron, Tokyo, Japan) was performed every 2 weeks from 6 weeks of age.

Echocardiographic Studies
Echocardiographic studies were performed every 2 weeks. The echocardiographic method has been described elsewhere. Briefly, rats were first anesthetized using isoflurane adjusted to a dose to maintain the heart rate at 300 to 350 beats per minute. Cardiac image sequences were acquired with a Vevo2100 (VisualSonics Inc., Toronto, Canada) using a 13-24-MHz linear transducer (MS-250). The standard 2D echocardiographic studies included parasternal long-axis and a short-axis view to obtain wall thickness (WT) and left ventricular (LV) diameters. LV ejection fraction and LV mass were estimated by tracing epicardial and endocardial borders of long-axis images.

Speckle Tracking Echocardiography-Derived Strain
Strain analysis was performed as previously described. The parasternal long- and short-axis images were analyzed with 2D speckle tracking software (Vevostrain™ Analysis; VisualSonics Inc.). Global strain was quantified in the longitudinal (GLS), radial (GRS), and circumferential directions (GCS).

Strain analyses were conducted by trained investigators on all animals using a speckle tracking algorithm provided by VisualSonics. The investigator manually traced the endocardial and epicardial borders, and the data were then processed for strain measurement. The quality of tracking throughout each cine loop was verified, and the data was then processed in a frame-by-frame manner for strain measurement. Each image of the LV myocardium was divided into 6 standard anatomic segments throughout the cardiac cycle in a long- or short-axis view. If there was dropout of 2 or more myocardial segments in all acquired loops, the tracing was excluded from speckle tracking analysis. Acceptable image quality of fundamental echocardiography for speckle tracking analyses was obtained in 96% of the animals.

All strain data were measured over 3 heart beats and averaged. Intra-observer reproducibility for longitudinal strain was 6.7%, circumferential strain 7.1%, and radial strain 7.6%.

Calculation of Systolic Transmural Wall Stress
Because myocardial strain is affected considerably by wall stress, distribution of transmural wall stress was calculated from the derivations of Mirsky:

\[ \text{Systolic wall stress (dynes/mm}^2\text{)} = \]
ESP(Ds/2)^3 \cdot (1+(Ds/2+Ts)^3/2((Ds/2)+[(Ts \cdot I_o]/I_o))]^3/[(Ds/2+Ts)^3-(Ds/2)^3],

where ESP is end-systolic pressure, Ds is systolic chamber diameter, Ts is systolic wall thickness, I_o is the number of incremental layers from the endocardial surface of the wall to the epicardium, and I_i is the total number of incremental layers in the ventricular wall. ESP was estimated as 0.98 times the mean arterial pressure plus 11 mmHg. In our preliminary studies of wall stress calculations in 15 samples, end-systolic pressure by this formula correlated well with arterial pressure at the point of the dicrotic notch obtained from cardiac catheterization examinations (R=0.95, P<0.001). The bias between P and arterial pressure at the point of the dicrotic notch was 0.5 mm Hg by the Bland-Altman method, and the 95% confidence interval was −7.6 to 8.4 mm Hg. Thus, mean arterial pressure is feasible for the estimation of end-systolic arterial pressure.

Hemodynamic Measurements
We obtained the slope of the LV end-systolic and end-diastolic pressure-volume relation (ESPVR, EDPVR) by gradual inferior vena cava occlusion with the use of a conductance catheter technique. The animals were anesthetized with isoflurane. A 2F microtip pressure-volume (PV) catheter (SPR-838; Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the left ventricle. The signals were continuously recorded using a PV conductance system (MPVS-300; Millar Instruments). All PV loop data were analyzed using a cardiac PV analysis program (LabCart v7; Millar Instruments). The relaxation time constant (tau), was calculated by the method of Weiss et al. Hemodynamic parameters were calculated and corrected according to in vitro and in vivo volume calibrations. The slope of the ESPVR was calculated as the load-independent index of LV chamber contractility: P = Emax(Vmax – V0). The EDPVR data were fit to a nonlinear equation \[ P = \beta \times e^{(\kappa \times V)} \] and the slope \( \kappa \) as a reliable index of ventricular diastolic stiffness. After the hemodynamic measurements were made, diastolic arrest was induced by perfusing the heart with a 1 mol/L KCl solution, and the heart and lungs were weighed.

Histological Studies
Left ventricles were fixed with 6% formaldehyde. After fixation, transmural myocardial structure of mid lateral wall was evaluated as shown in Figure 1. Sequential sections parallel to the epicardium were cut by microtome at 4-µm slice thickness at a pitch of 100 µm. Pathological specimens were stained with Masson’s-trichrome or hematoxylin-eosin stain for light microscopy (BZ-8100; Keyence, Osaka, Japan), and 21 slices from endocardium to epicardium were selected for pathological analysis (Figure S1).

Fiber angle of the myocardial fibers, \( \alpha \), was determined by using the digital microscope (Figure S1). Zero degree in this reference system was defined as the circumferential direction, and ±90 degrees were defined as the longitudinal direction. Transmural distribution of the percent area of fibrosis (% area fibrosis) was calculated as the total area of fibrosis (defined as the amount of collage deposition stained with aniline blue) divided by the sum of the total tissue area.

Myocyte width was measured in approximately 100 cells at the site of a visible nucleus in each slide stained with hematoxylin and eosin.
Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)
The LV mid lateral wall was divided into endocardial, mid, and epicardial layer.
Quantitative analysis of the target mRNA expression was performed with TaqMan RT-PCR
(Life Technologies, Gaithersburg, MD) by the relative standard curve method. Total RNA
was extracted from the unfixed heart with the RNeasy® Mini Kit (QIAGEN, Hilden,
Germany). Total RNA of 1 µg was reverse transcribed and amplified in triplicate with the
Primer Script™ RT reagent Kit with gDNA Eraser (TAKARA, Shiga, Japan) and ABI
PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA),
according to the manufacturers’ instructions.

For each assay, 10 ng of the cDNA were mixed with 12.5 µL of TaqMan Universal PCR
Master Mix and 1.25 µL of TaqMan primer and probe (Life Technologies). Fold-change
analysis was based on standardizing RNA levels by correcting for 18S RNA levels in the
sample. The result for each gene was obtained from 3 independent measurements (n=4 per
group) performed in duplicate.

Statistical Analysis
Data are expressed as mean±SD. For each dependent variable, differences between groups
were compared by one-way ANOVA followed by a Fisher’s test for multiple comparisons.
If the result of ANOVA was significant, the unpaired Student 𝑡-test was used. Association of
the strain data and histological and hemodynamic findings was assessed with Pearson’s
correlation coefficient. Statistical significance was defined as a value of 𝑃<0.05. All
calculations were made with SPSS II for Windows (SPSS Inc., Chicago, IL).
References


2. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group, et al. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005;18:1440-1463.


Figures and Supporting Information

Figure S1. Histological preparation and myocardial fiber angle definition. Schematic depicts the left ventricular (LV) mid-lateral wall histological slice preparation (top). Myocardial fiber angle, $\alpha$, is positive when measured counterclockwise from the circumferential axis parallel to the mitral annulus.
Figure S2. Transmural systolic wall stress distribution and myocardial fiber angle in rats with hypertension (HT). A progressive elevation in systolic wall stress as aging progressed was noted in the subendocardial layer of the HT group (A). The myocardial fiber angle changed gradually from subendocardium to subepicardium and to horizontal in the mid-wall layer, and there were no significant differences between the HT and control groups throughout the study period (B). *P<0.05 vs control group.
**Video files**
The following video files show speckle tracking echocardiography of left ventricular short axis (SAX) and long axis (LAX) in representative rats at weeks 10, 14, and 18.
Movie S1. Speckle tracking echocardiography of short-axis view at 10 weeks of age in a hypertensive rat.
Movie S2. Speckle tracking echocardiography of long-axis view at 10 weeks of age in a hypertensive rat.
Movie S5. Speckle tracking echocardiography of short-axis view at 18 weeks of age in a hypertensive rat.