Gender-Related Differences in Myocyte Remodeling in Progression to Heart Failure

Tetsutaro Tamura, Suleman Said, A. Martin Gerdes

Abstract—Gender-related differences responsible for the better prognosis of females with heart failure have not been clearly established. To address this issue, we investigated potential gender-related differences in myocyte remodeling in spontaneously hypertensive heart failure rats. Echocardiograms and myocyte growth were compared between males and females at compensated (2, 4, and 6 months) and decompensated (18 months in males and 24 months in females) stages of cardiac hypertrophy. Although left ventricular diastolic dimensions did not differ significantly between failing male and female rats, fractional shortening declined significantly only in failing males. Myocyte cross-sectional area did not change after 4 months of age in both genders, which is likely to be responsible for the absence of a change in left ventricular wall thickness during the progression to heart failure. Myocyte volume and cross-sectional area were significantly larger in males than females at 2, 4, and 6 months of age, although there were no significant differences at the failing stage. Reduced adaptive hypertrophic reserve was observed in males, which is likely to contribute to the higher morbidity and mortality of males with chronic heart failure. (Hypertension. 1999;33:676-680.)

Key Words: heart failure ■ remodeling, ventricular ■ myocytes

Heart failure is one of the major causes of death in the United States, with 4 to 5 million individuals living in the United States estimated to have chronic heart failure. Irrespective of the underlying cause, in end-stage heart failure, chamber dilation and an increased ratio of chamber diameter to wall thickness are common characteristics. Hypertension is still one of the major underlying diseases leading to heart failure. A thickened wall with normal ventricular chamber diameter is found at the compensated stage of hypertension, followed by chamber dilation and an increase in the ratio of chamber diameter to wall thickness in the decompensated stage. It has also been reported that males are more prone to heart failure caused by hypertension than females.1,2 According to the Statistical Abstract of the United States,3 139% more males than females died of heart disease between the ages of 45 to 64 years; however, after the age of 65, the number of females who died of heart disease exceeded that of males by 22%.3 Since the prognosis of heart failure is much worse in males than females, it is important to identify potential gender-related differences responsible for the better prognosis in females. The purpose of the present study was to explore potential gender-related differences in myocyte remodeling in spontaneously hypertensive heart failure (SHHF) rats. In this genetic model of hypertension and heart failure, the progression to failure is accelerated in males. It has already been demonstrated that myocyte remodeling in female SHHF rats is similar to that observed in humans with hypertension progressing to failure.4 It is not known, however, whether this adverse myocyte remodeling is accelerated in male SHHF rats or whether the pattern of myocyte remodeling is similar to that observed in humans with hypertension and heart failure.

Methods

Experimental Animals
In the present study, lean male SHHF rats at 2, 4, 6, and 18 months of age and lean female SHHF rats at 2, 4, 6, and 24 months of age were obtained from Genetic Models Inc (Indianapolis, Ind). All procedures were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for animals.

Myocyte Isolation
Animals were anesthetized with an intramuscular injection of ketamine HCl (30 mg/kg) and xylazine (5 mg/kg). Heparin (3000 U/kg, Sigma Chemical Co) was also injected intraperitoneally. The hearts were quickly removed, trimmed of excessive tissue, and weighed. The procedure for isolating myocytes has been described previously.5 Briefly, hearts were perfused in a retrograde manner on a Langendorff apparatus with Joklik’s medium followed by Joklik’s medium with collagenase (Worthington Biochemicals). Left ventricular (LV) tissue was minced in Joklik’s medium followed by Joklik’s medium with collagenase (Worthington Biochemicals). Left ventricular (LV) tissue was minced in Joklik’s medium and filtered through nylon mesh (250 μm). Myocytes were fixed immediately in 1.5% glutaraldehyde in 80 mmol/L phosphate buffer. The isolated cell suspensions were centrifuged through a 4% Ficoll gradient to remove capillaries, blood cells, and unwanted debris.

Myocyte Morphometry
Myocyte volume was measured with a Channelizer (model Z2, Coulter Corp). Myocyte length, defined as the longest length parallel
to the longitudinal axis of the myocyte, was measured in 50 cells from each sample using a Video Analysis System (Jandel Scientific). Myocyte cross-sectional area was calculated from the ratio of cell volume to cell length. Thus, calculated cross-sectional area represents average values along the entire length of the myocyte.

**Data Analysis**

Results are presented as mean±SD for animal data and mean±SEM for cellular data. ANOVA was used to compare data between groups. The Bonferroni test was used to examine statistically significant differences observed with the ANOVA.6

**Results**

Male SHHF rats initially exhibited symptoms of heart failure (dyspnea, cyanosis, edema, lethargy) at approximately 16 months of age, whereas female SHHF rats became symptomatic at approximately 22 months of age.

Figure 1A, 1B, and 1C show changes in body weight (BW) (A), heart weight (HW) (B), and ratio of heart weight to body weight (HW/BW) (C). Solid black bars indicate male; solid white bars, male. A, Both males and females maintained a relatively stable body mass after 6 months (M) of age, although male rats had significantly larger body mass than females at each time point examined. *P<0.01 compared with females of the same age. B, Males had significantly larger hearts than females at 2, 4, and 6 months of age. HW did not differ significantly between males and females with heart failure. *P<0.01 compared with females of the same age. C, HW/BW differed significantly only between males and females with heart failure. *P<0.01 compared with males of the same age.
The Table shows comparative echocardiographic data from male and female SHHF rats. LV end-diastolic and end-systolic diameters increased significantly between 6 months of age and the onset of failure in both males and females. There was, however, no significant difference in LV diastolic dimension between failing male and female rats, although this dimension was significantly larger in 6-month-old males versus females. LV end-systolic dimension, however, was larger in males than females in failure. As a result of these changes, fractional shortening declined in males but not in females progressing to failure. Anterior and posterior wall thicknesses did not differ significantly in either males or females with progression to failure (eg, from 6 months to the failing stage). Consequently, the ratio of chamber diameter to wall thickness increased in both males and females as they progressed to failure.

LV isolated myocyte data are shown in Figure 2. Myocyte volume was significantly larger in males than females at 2, 4, and 6 months of age. Although myocyte length tended to be larger in males at these time points, the larger cell volume in males was primarily due to larger cross-sectional areas. Myocyte cross-sectional area reached a value of approximately 350 µm² in females by 4 months of age and did not change thereafter. A similar trend was noted in males, in which myocyte cross-sectional area reached a value of approximately 400 µm² at 4 months of age and did not change with progression to failure. At the failing stage, cell volume, cell length, and cross-sectional area were not significantly different between males and females.

### Discussion

Cardiovascular disease is the leading cause of death in both men and women older than 75 years, and cardiovascular mortality is higher in men than in women at every age category.² In the present study, we investigated gender-specific differences in cardiac myocyte shape in progression to heart failure to determine whether structural features may contribute to the higher morbidity and mortality rates in males. Male and female SHHF rats were examined during compensatory hypertrophy (2, 4, and 6 months) and after the appearance of symptoms of heart failure (approximately 18 months of age for male rats and 24 months of age for female rats) to determine whether any differences in myocyte remodeling may contribute to accelerated progression to failure in males. Animals with signs of failure also displayed significant chamber dilatation and an increase in the ratio of chamber diameter to wall thickness as assessed by echocardiography.

Campbell et al⁷ reported that the capacity for myocyte hypertrophy was greater in female rats compared with males after inducing hypertension by aortic constriction in young growing rats. This result was not surprising since it was known that normal males have larger cardiac myocytes than females presumably because of the increased volume load from larger body mass.⁸ It is not known, however, whether the reduced hypertrophic reserve of myocytes from males may predispose them to earlier onset of heart failure in hypertension. In the present study, it was demonstrated that cardiac myocyte volume was significantly less in young female SHHF rats (2, 4, and 6 months old) but was not significantly different after the onset of heart failure, which occurred much earlier in males. Consequently, LV myocytes from females were able to enlarge by approximately 40% between 6 months of age and the onset of failure, while LV myocytes from males enlarged by about only 12% between 6 months and failure. This increased hypertrophic reserve likely played a major role in the 6-month delay in the progression to failure observed in females. Myocyte cross-sectional areas and lengths tended to be larger in males at 2, 4, and 6 months of age, but differences were not always significant. Myocyte cross-sectional area did not change significantly in males or females after 4 months of age, but myocyte length increased significantly between 6 months and failure in both genders. Reflecting this stabilization of myocyte cross-sectional area (eg, thickness), wall thickness did not change in males or females after 6 months of age. Additionally, it should be noted that the increase in myocyte length between 6 months and failure was associated with significant chamber dilatation in both males and females. Previous studies have demonstrated that LV myocyte cross-sectional area stabilizes at about 4 months of age in lean female SHHF rats, whereas myocyte length continues to increase until failure.⁹ Other experiments suggest that myocyte lengthening alone can account for all of the chamber dilatation in the progression to failure in lean female SHHF rats.¹⁰ The current study shows that LV myocyte remodeling occurs in a similar but accelerated manner in lean male SHHF rats progressing to failure. It is our belief that LV myocyte transverse growth becomes arrested at an early time point because the stimulus for growth in this cellular parameter, systolic wall stress, continues to rise progressively until failure.⁹

The cell-lengthening process was due to series addition of new sarcomeres since sarcomere length was not changed

### Table: Echocardiographic Data

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>LVDD, mm</th>
<th>LVDs, mm</th>
<th>AWd, mm</th>
<th>PWd, mm</th>
<th>AWs, mm</th>
<th>PWs, mm</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, 6 months</td>
<td>5.9±0.3</td>
<td>3.5±0.3</td>
<td>2.1±0.1</td>
<td>2.1±0.2</td>
<td>3.0±0.4</td>
<td>3.0±0.2</td>
<td>41.1±4.1</td>
</tr>
<tr>
<td>Male, 6 months</td>
<td>6.6±0.3</td>
<td>4.2±0.6</td>
<td>2.3±0.2</td>
<td>2.3±0.3</td>
<td>3.7±0.3</td>
<td>3.0±0.2</td>
<td>36.6±6.1</td>
</tr>
<tr>
<td>Female, 24 months</td>
<td>8.4±0.2*</td>
<td>4.8±0.7*</td>
<td>2.0±0.4</td>
<td>1.9±0.4</td>
<td>3.2±0.4</td>
<td>2.8±0.5</td>
<td>42.1±9.7</td>
</tr>
<tr>
<td>Male, 18 months</td>
<td>8.5±0.7*</td>
<td>6.0±0.8*</td>
<td>2.4±0.3</td>
<td>2.3±0.2</td>
<td>3.5±0.3</td>
<td>3.1±0.3</td>
<td>28.6±5.0*</td>
</tr>
</tbody>
</table>

LVDD indicates left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; AWd, diastolic anterior wall thickness; PWd, diastolic posterior wall thickness; AWs, systolic anterior wall thickness; PWs, systolic posterior wall thickness; FS, fractional shortening.

*P<0.01 compared with 6 months of the same gender.
from 2 months to the failing stage (approximately 1.90 μm, data not shown). Since cell lengthening was the major morphological change found in this study, the results indicated that heart failure resulted in a selective increase in myocyte length only in this model. It is unlikely that this cell-lengthening process was due to aging rather than heart failure caused by hypertension, because it was reported that mean LV myocyte length was 124, 124, and 126 μm in 4-, 8-, and 24-month-old female Sprague-Dawley rats, respectively.9

In summary, a dramatic increase in cell length occurred in LV myocytes during the progression to heart failure in both male and female SHHF rats, a genetic model of hypertension and heart failure. Cross-sectional area did not change after 4 months of age in both genders, which is likely to be responsible for the absence of a change in LV wall thickness during the progression to heart failure. Reduced adaptive hypertrophic reserve was observed in males, which is likely to contribute to the higher morbidity and mortality of males.

Figure 2. Changes in cell volume (A), cross-sectional area (CSA) (B), and myocyte length (CL) (C). Solid black bars indicate female; solid white bars, male. A, Myocyte volume was significantly larger in males at 2, 4, and 6 months of age. *P<0.01 compared with females of the same age. B, CSA was significantly larger in males at 2, 4, and 6 months of age. Myocyte CSA did not change significantly in both genders after 4 months of age. *P<0.01 compared with females of the same age. C, CL was significantly larger only at 2 months of age. *P<0.01 compared with females of the same age.
with chronic heart failure. At this time, it is not clear whether the observed cellular differences in SHHF rats are predominantly due to body mass or gender. Additionally, no similar data are available from patients. Examination of gender-related differences in a large population of weight-matched patients with hypertension/failure could prove informative.

Acknowledgments
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References
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