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EXPERIMENTAL STUDIES

Cardiac Morphology and Function in Senescent Rats: Gender-Related Differences

DANIEL E. FORMAN, MD, FACC,*†‡ ANTONIO CITTADINI, MD,§ GOHAR AZHAR, MD,*§
PAMELA S. DOUGLAS, MD, FACC,§ JEANNE Y. WEI, MD, PHD, FACC*§

Boston, Massachusetts and Providence, Rhode Island

Objectives. **We sought to better understand the effects of aging and gender on left ventricular (LV) structure and function.**

Background. **Cardiovascular disease in older persons is associated with increased mortality and morbidity. The influence of gender on age-related cardiac changes is incompletely characterized.**

Methods. **We studied 34 senescent, male and female, normotensive Fischer rats with transthoracic Doppler echocardiography and morphometric and histopathologic analyses.**

Results. **Male rats were larger (396 ± 31 g vs. 282 ± 35 g), and LV mass in males was greater (1.04 ± 0.22 g vs. 0.67 ± 0.13 g). However, wall and chamber dimensions normalized to body weight revealed proportionately thicker anterior and posterior walls in females. Relative wall thickness ratio (2[Diastolic posterior wall thickness]/Diastolic LV internal chamber diameter) was greater in females, but abnormal fractional shortening and diastolic filling (E/A ratio) patterns were more common in males. Significant mitral regurgitation (MR) was sevenfold more common among males (88% vs. 12%, $p < 0.001$). Histopathologic analysis showed that the cardiac myocytes were larger, and there was greater LV fibrosis in males (both $p < 0.001$).**

Conclusions. **Gender-related morphologic and functional differences are important to consider in cardiovascular assessment. Very old rats show significant gender differences in LV size and function. Male rat hearts are larger, thinner and more fibrotic and have indexes of diminished performance. The high prevalence of MR in male rats may play a crucial role in these gender differences.**

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Several studies have investigated *age-related* differences in cardiovascular performance (1–5), but the impact of gender on such age-associated cardiovascular changes remains incompletely delineated. Similarly, other studies have investigated *gender-related* differences in cardiovascular morphology (6,7) and function (8–13) in young adults. The impact of aging on these gender differences in the absence of disease is not completely established. This question may be important because gender-related differences in cardiovascular aging may help to explain in part the greater longevity of women and of females of most of the mammalian species.

In the current study, we hypothesized that patterns of cardiovascular aging are influenced by gender in adult rats. We studied older male and female normotensive Fischer-344 rats to delineate possible gender-related differences in cardiac morphology and function. We used in vivo echocardiography and histopathologic analysis to characterize heart morphology and function in the animals.

We found significant gender differences in cardiac size and function. These differences may explain in part the differential susceptibility to hemodynamic compromise in response to abrupt cardiovascular stress.

Methods

Animals studied. Senescent Fischer-344 rats (from Harlan-Sprague-Dawley colony of the National Institute of Aging) were studied. A total of 22 males (mean age 25 ± 1 months) and 22 females (mean age 24 ± 3 months) were included in the study. All animals were apparently healthy and free of clinical signs of short- or long-term illness. Each animal underwent a physical examination and was determined to be free of congestive heart failure and of gross tumors before inclusion in the study.

Systolic blood pressures were obtained on the tails using caudal plethysmography (14). Briefly, the animals had their blood pressures measured with a small occlusion cuff (15 mm in length) placed at the base of the tail, which was connected to an inflation cuff and pressure and pulse transducers. After resting quietly for at least 30 min in a plastic restraining case that was placed on a slightly warmed (39 ± 1°C) electrical heating pad, each animal underwent at least three blood pressure measurements. All animals were normotensive (range

From the *Division on Aging, Harvard Medical School, Boston, Massachusetts; †Department of Medicine, Brown University Medical School and ‡Division of Cardiology, The Miriam and Rhode Island Hospitals, Providence, Rhode Island; and §Department of Medicine, Beth Israel Hospital, Boston, Massachusetts. This study was supported in part by Health and Human Services Grants AG00294, AG08812, AG00251 and AG10829, National Institutes of Health, Bethesda, Maryland.

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Address for correspondence: Dr. Daniel E. Forman, Division of Cardiology, Miriam Hospital, 164 Summit Avenue, Providence, Rhode Island 02906. E-mail: dforman@lifespan.org.

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Abbreviations and Acronyms

ASE = American Society for Echocardiography
CO = cardiac output
E/A = ratio of early transmitral flow velocity to atrial flow velocity
LV = left ventricular
MR = mitral regurgitation
RWT = relative wall thickness

110 to 140 mm Hg), consistent with previous animal studies showing stable, normotensive blood pressures during adult aging in Fischer-344 rats (14–16).

Echocardiography. Echocardiography was used to assess left ventricular (LV) function using previously validated two-dimensional, M-mode and Doppler echocardiographic techniques (17,18). Briefly, the animals were first weighed and then anesthetized with ketamine hydrochloride (50 mg/kg body weight) and xylazine (10 mg/kg) intraperitoneally. Their chests were shaved and electrocardiographic leads attached, and they were placed prone on a specially designed apparatus. Echocardiographic images were obtained using a commercially available echocardiographic system with a 7.5-MHz, phased array transducer (Hewlett Packard) placed against the chest wall from below.

A two-dimensional, short-axis view of the LV was obtained at the level of the papillary muscles. Anterior and posterior end-diastolic and end-systolic wall thicknesses and LV internal dimensions were measured according to modified American Society for Echocardiography (ASE) standards (posterior wall leading-edge to leading-edge and anterior wall trailing-edge to trailing-edge) from at least five consecutive cardiac cycles on the M-mode tracings (19) and analyzed by a single blinded observer with a Tomtec off-line analysis system. Cardiac dimensions were normalized to body weight (15,20). When comparing between age groups, one usually normalizes to tibial length (1,15,18). Within the same age range, however, normalization to body weight is sufficient (15).

Pulsed wave Doppler spectra of mitral inflow from the apical four-chamber view were used to assess LV diastolic flow characteristics. Sample volumes were placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximal and the flow pattern was laminar. Doppler spectra were recorded at a paper speed of 100 mm/s and analyzed off-line with the Tomtec work station. Doppler measurements, including peak early (E) and late (A) mitral valve inflow velocities, filling ratio (E/A), ejection fraction deceleration slope, isovolumetric relaxation time and aortic flow–velocity integrals, were determined from at least five consecutive cardiac cycles. Color flow Doppler echocardiography was used to identify mitral regurgitation (MR) in the apical four-chamber view. Mitral regurgitation was quantified as mild, moderate or severe according to ASE guidelines (21). In addition to the E/A ratios, a variety of indexes were calculated based on echocardiographic data.

Left ventricular mass, relative wall thickness (RWT), fractional shortening, posterior wall thickening and cardiac output (CO) were calculated using standard, validated formulas (22–26). A subset of 10 animals (five females and five males) was blotted dry and weighed before being fixed in formalin. There was no significant difference between the measured heart weights (male 1.07 ± 0.13 g, female 0.69 ± 0.08 g) and the calculated LV mass, as demonstrated previously (18). Cardiac output was calculated using the formula CO = Aortic VTI × (π[LV outflow diameter/2]^2) × Heart rate, where VTI is the measured systolic velocity–time integral (26). Tibial length was determined in 10 animals (five males and five females). The heart weight was normalized to tibial length (15,27,28) and was found to be similar for males and females.

Histopathologic methods. On completion of echocardiographic measurements, the animals were deeply anesthetized. The thorax of each animal was opened quickly under anesthesia. The hearts were immediately removed, rinsed in normal saline and fixed in buffered formalin. The atria were trimmed from the ventricles, and the LV septal walls were isolated. Three to four septal specimens per heart were obtained at the level of the mid-septum. Morphologic analysis was performed on the septum because it has more landmarks, which allow us to closely compare similar areas between animals. In addition, the septa in hearts are also better vascularized, so that theoretically these myocytes will have had a better chance of successful aging and will perhaps have undergone less ischemia. In a subset of 10 animals (five males and five females), the hearts were fixed in end-diastole by using potassium chloride infusion at a constant perfusion pressure (27,29,30). The thorax was opened and the heart was removed, rinsed and fixed.

Samples were fixed in formalin and embedded in paraffin. Slides were stained with hematoxylin-eosin for measurement of muscle fiber diameter and Masson trichrome for assessment of interstitial fibrosis. Because the fixation method may cause a small degree of cell shrinkage, we were particularly careful to use consistent methodology and checked to ensure that the cell and interstitial sizes and proportions in all the heart specimens were equivalent.

Cross-sectional muscle fiber diameter and area were determined quantitatively by direct measurement using a Sony coiled coupled device camera mounted on a Macintosh Centris 650 computer-interfaced Zeiss Universal microscope. Sections of the septum were magnified ×40 and 8 to 12 fields per animal were examined. Only muscle fibers that included nuclear profile were measured. The microscope field image was transferred to the computer, where specialized software (courtesy of P. Goldman-Rakic) facilitated measurement of cell perimeter and diameter. At least 100 myocyte diameters were measured per animal, and the data were averaged. The average myocyte diameters were similar between the hearts that were fixed in potassium chloride and the other hearts within each gender, and the standard deviations of the measurements were also similar between the genders.

Quantitative determination of interstitial tissue as an index of fibrosis was performed using the point-counting system (28).

Table 1. Age and Body Weight of Senescent Rats

	Male Rats	Female Rats	p Value
Age (mo)	25 ± 1	24 ± 3	NS
Body weight (g)	396 ± 31	282 ± 34	<0.001
Heart rate (beats/min)	247 ± 54	235 ± 33	NS
Systolic blood pressure (mm Hg)	123 ± 14	127 ± 17	NS
Tibial length (mm)	4.79 ± 0.08	3.64 ± 0.06	<0.01

Data are presented as mean value ± SD. Although male rats were significantly heavier and larger than female rats, age, heart rate and systolic blood pressure were similar in male and female animals.

The Zeiss microscope/Macintosh computer integrated system described previously was used to determine fibrosis (31). A grid was applied to the computer monitor providing 100 intersection points superimposed on the image of muscle cells and interstitial tissue. The intersections overlying nonmuscular tissue were counted and designated as interstitial tissue and taken as an index of fibrosis. The number of nonmuscle areas (from a possible 100 intersections) was expressed as the percent of interstitium of the tissue contained within the grid. Ten fields were examined at 10 sample sites for a total of 100 fields examined for each animal.

Data analysis and statistics. The results (mean ± SD) were analyzed for gender differences. Statistical methods were completed using the Student two-tailed *t* test to compare the data between males and females. A value $p < 0.05$ was considered to be statistically significant.

Results

Thirty-four senescent Fischer-344 rats were studied (17 males with a mean age of 25 ± 1 months and 17 females with a mean age of 24 ± 3 months). The rest heart rates and blood pressures were similar in males and females (Table 1). Body weights and tibial lengths were greater in males.

Although the LV mass and heart weight were larger in male rats than in female rats (Table 2), the LV mass normalized for body weight and the heart weight normalized for tibial length were not different between males and females. In fact, the posterior and septal wall thicknesses normalized to body weights were actually greater in the females (Table 2). The RWT normalized to body weight in females was also significantly greater than that in males, suggesting that the LV walls were thicker and the LV chambers were smaller in old female rats than in old male rats.

Moderate to severe MR was far more prevalent among the male rats (88% vs. 12% in females, $p < 0.001$). Fractional shortening and posterior wall thickening were substantially lower in male compared with female hearts (Table 3), consistent with reduced systolic function in the senescent males.

The LV inflow patterns were also different between males and females. Data showed relatively increased ejection fraction deceleration slopes and increased E/A ratios in the ventricles of males compared with those of age-matched females.

Histopathologic analysis showed a significantly higher percentage of interstitial fibrosis in the ventricular septa of males compared with females (Table 4).

Table 2. Left Ventricular Dimensions

	Male Rats	Female Rats	p Value
LV mass (g)	1.04 ± 0.22	0.67 ± 0.13	0.01
LV mass/BW ($\times 10^{-3}$)	2.6 ± 0.6	2.3 ± 0.4	NS
HW/TL (g/mm)	0.22 ± 0.07	0.19 ± 0.06	NS
PW (mm)	1.9 ± 0.2	1.7 ± 0.2	0.01
PW/BW (mm/kg)	4.7 ± 0.7	6.0 ± 0.9	<0.001
AW (mm)	1.7 ± 0.2	1.6 ± 0.2	0.06
AW/BW (mm/kg)	4.3 ± 0.7	5.6 ± 0.8	<0.001
LVID (mm)	7.7 ± 0.7	6.4 ± 0.6	<0.001
LVID/BW (mm/kg)	19.6 ± 1.7	22.5 ± 3.2	0.003
RWT(n)	1.3 ± 0.2	1.9 ± 0.3	<0.001

Data are presented as mean value ± SD. Anterior and posterior end-diastolic wall thicknesses and left ventricular internal dimensions were measured from five consecutive cardiac cycles on the M-mode tracings using the two-dimensional short-axis view of the left ventricle at level of the papillary muscles. Left ventricular (LV) mass = $1.04 \times [(\text{LVIDd} + \text{PW} + \text{AW})^3 - \text{LVIDd}^3]$, where 1.04 is the specific gravity of the muscle; PW = posterior wall thickness in diastole; AW = anterior wall thickness in diastole; and LVIDd = left ventricular internal chamber diameter in diastole. HW = heart weight; PW/BW, AW/BW, LVID/BW and LV mass/BW are wall measurements and calculated ventricular mass normalized to body weight (BW); RWT(n) (relative wall thickness normalized to body weight) = 2 (PW)/LVID; TL = tibial length.

The myocyte diameters were also larger in the hearts of males compared with those of females (Table 4).

Discussion

The current study showed that compared with the hearts of old female rats, the hearts of old male rats demonstrated indexes of impaired systolic performance, with an increased

Table 3. Left Ventricular Systolic and Diastolic Functional Measures

	Male Rats	Female Rats	p Value
FS (%)	54 ± 8	60 ± 11	0.02
PW thickening (mm/s)	8.0 ± 2.1	9.2 ± 2.8	0.15
CO (ml/min)	68 ± 14	63 ± 11	0.15
CO/BW (ml/kg body weight per min)	172 ± 45	233 ± 54	0.003
E/A	5.6 ± 2.6	1.6 ± 0.82	<0.001
EF deceleration (m/s)	14.9 ± 5.9	9.7 ± 1.6	0.003
IVRT (m/s)	29.4 ± 7.4	32.4 ± 5.6	0.03

Data are presented as mean value ± SD. Systolic indexes include fractional shortening (FS) = (LVIDd − LVIDs)/LVIDd, where LVIDd = internal diameter of the left ventricle in diastole; and LVIDs = internal diameter of the left ventricle in systole. Posterior wall (PW) thickening = (LVPWd − LVPWs)/LVPWd, where LVPWs = left ventricle posterior wall thickness in systole; and LVPWd = posterior wall thickness in diastole. Cardiac output (CO) = Aortic VTI × (π[LV outflow diameter/2]^2) × Heart rate, where the aortic flow velocity–time integral (VTI) is a calculated value. CO/BW = cardiac output normalized to body weight. Diastolic indexes are based on Doppler measurements, including peak early (E) to late (A) mitral valve inflow filling velocities (E/A ratio); ejection fraction (EF) deceleration slope; and isovolumetric relaxation time (IVRT).

Table 4. Histopathologic Findings of the Ventricular Septa

	Male Rats	Female Rats	p Value
Myocyte diameter (μm)	31.2 ± 3.3	29.3 ± 2.7	<0.001
LVIF (%)	26.8 ± 11.9	17.5 ± 7.7	<0.001

Data are presented as mean value ± SD. Myocyte diameters were only measured on muscle fibers that included a nuclear profile. At least 100 myocytes per animal were measured and averaged. Diameters of myocytes in males were significantly larger than those in females. Percent left ventricular interstitial fibrosis (LVIF) was measured using a grid applied to the computer monitor. The number of nonmuscle areas (from a possible 100 intersections) was expressed as the percent interstitium of the tissue contained within the grid. Ten fields were examined at 10 sample sites in each animal. Left ventricular interstitial fibrosis was significantly greater in male rats.

prevalence of MR. Histopathologic analysis showed increased cardiac interstitial tissue and larger myocyte diameters in the males.

The gender-related differences of cardiac function in older male and female hearts may indicate intrinsic functional differences with superior female ventricular performance. However, this assumption was apparently not supported by a previous study by Schaible and Scheuer (11), which showed better LV function in male rats compared with female rats. Alternatively, the higher prevalence of MR in the male rats may be a key factor contributing to the observed morphologic and functional gender differences. However, Schaible and Scheuer studied younger animals (18 months). Also, their study included no assessment of valvular function. It is unclear whether MR was present in the younger rats that were used in that study.

Mitral regurgitation is common with advanced aging because progressive myxomatous changes in valve integrity are compounded by mitral valve annular calcification, ischemia and dysrhythmias, all of which can exacerbate regurgitant flow (32). Moreover, MR tends to progressively worsen with time. The LV gradually dilates to accommodate the MR-related fluid overload state (to achieve a greater mechanical work advantage through the Starling mechanism), with increasing distortion of mitral valve morphology and escalating valvular insufficiency (33).

Eventually, the intrinsic ventricular adaptations to volume overload may lead to systolic and diastolic functional decline (27,28,33). The chamber enlargement that is necessary to accommodate the volume overload of MR depends on myocyte hypertrophy and side to side cellular slippage. This growth stimulus in senescent myocytes may exceed cellular synthesizing capacity and perhaps in some cases lead to cell death. Ventricular mechanical impairment is exacerbated by increasing interstitial fibrosis (28,29,32), which evolves as myocytes die, particularly in the LV where volume and pressure stresses are greatest. Thus, although ventricular dilation is physiologically advantageous in terms of maintaining stroke volume in the presence of a regurgitant lesion, increases in diastolic stress, progressive myocyte cell death, fibrosis and ventricular stiffening are concomitant effects that are ultimately detrimental to ventricular performance (16,32–34).

The ventricular filling indices in male rats are also influenced by the *direct* effects of MR. Regurgitant flow increases atrial pressures as well as rapid filling volume, resulting in increased early atrial to ventricular flow velocities (E wave). These volume and pressure effects of MR confound the assessment of ventricular function based on echocardiographic variables (21–23,35). Given the high prevalence of MR in male hearts in the present study, the systolic indices, as measured, are likely *underestimates* of the degree of LV dysfunction. Mitral regurgitation typically results in enhanced LV systolic performance because of reduced afterload.

The finding of relatively larger myocyte diameters in the old male compared with old female ventricles is consistent with the notion of greater growth stimulation in response to MR (35). The histopathologic finding of increased interstitial fibrosis in males compared with females may help to explain in part the differences in systolic ventricular functional variables that were detected by echocardiography. The increased fibrotic composition of the male hearts may contribute to the diminished systolic performance (27,29,36). Similarly, fibrotic, nondistensible ventricular chambers would also reduce ventricular diastolic filling potential. Age-associated differences in mitochondrial function or bioenergetics, or both, could be further exacerbated by these structural changes (37,38).

The strong correlation between MR and gender in very old Fischer rats has not been documented previously. This new finding probably relates to our innovative use of in vivo echocardiography to assess the animals' ventricular function. It is not entirely clear from our data why the prevalence of MR would be so much greater among male than among female animals. One possible explanation is that cardiac ischemia may be greater among the males. Mitral regurgitation itself is often a symptom of ischemia when papillary muscle function becomes impaired, resulting in functional valvular abnormalities. Coronary ischemia may be more common in males than in females, perhaps as a result of differences in arteriosclerosis, coronary flow or dilatory reserve, or a combination of these (39,40). It is possible that coronary flow reserve may be diminished in male rats as a result of their greater ventricular mass and greater ventricular fibrosis compared with female rats. In addition, the gender difference could also be hormonally mediated, because estrogen is known to augment endothelial-mediated vasoresponsiveness, resulting in increased coronary flow reserve (40).

When comparing the heart weights of rats between different age groups, tibial length has been shown to be more constant than body weight, and therefore may be a preferred normalization variable for between-age comparisons (15,27,41). However, in animals within a given age group, we have previously demonstrated high correlations between normalization for tibial length and normalization for body weight (15,27,28,42). In our current study comparing male versus female animals within an age group, we found that the results were similar when we used either body weight or tibial length for normalization. Although the old male rats had greater body weights, heart weights and tibial lengths than the old female rats, the

gender effect became insignificant when heart weights were normalized for either body weight or tibial length.

One potential methodologic concern might be the possible regional heterogeneity of myocyte cell morphology in the rat hearts (30). We consistently analyzed the myocytes that were located in similar areas of the LV septum to minimize possible differences based on regional distribution. Our data are generally consistent with those reported in other animal (29) and human studies (43), with similar gender and aging effects.

Previous studies in animals and humans showed mild cardiac hypertrophy to be common with aging, partially in response to age-related vascular changes with increased afterload resistance (1,5,15,28,32,34,44). These age-associated morphologic changes may be exacerbated by the effects of hypertension (44,45) or exercise training (46–48), but they may also occur in normotensive adults, presumably because of vascular impedance changes with age that are independent of blood pressure (32,49). The age-associated increase in LV mass has been shown to be greater in women than in men, and LV mass increases more in hypertensive women than in hypertensive men (50–53). These findings suggest that there is a gender difference in the myocardial hypertrophic response to increased afterload.

Conclusions. We have shown in the current study that gender affects cardiovascular aging. Mitral regurgitation probably contributes to these observed gender differences in ventricular adaptation and may play a substantial role in cardiovascular changes often attributed to aging alone. We now face the question of whether age-associated cardiovascular changes relate to stresses that are potentially modifiable (43,54–56). The possible benefits of aggressive afterload reduction or similar interventions to modify aging patterns, and thereby increase cardiac function and longevity, merit further investigation.

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