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Age-Associated Changes in Hearts of Male Fischer 344/Brown Norway F1 Rats

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Abstract. Aging is associated with left ventricular hypertrophy, dilatation, and fibrosis of the heart. The Fischer 344/Brown Norway F1 (F344/BNF1) rat is recommended for age-related studies by the National Institutes on Aging because this hybrid rat lives longer and has a lower rate of pathological conditions than inbred rats. However, little is known about age-associated changes in cardiac and aortic function and structure in this model. This study evaluated age-related cardiac changes in male F344/BNF1 rats using ECHO, gross, and microscopic examinations. Rats aged 6-, 30-, and 36-mo were anesthetized and two-dimensional ECHO measurements, two-dimensional guided M-mode, Doppler M-mode, and other recordings from parasternal long- and short-axis views were obtained using a Phillips 5500 ECHO system with a 12 megahertz transducer. Hearts and aortas from sacrificed rats were evaluated grossly and microscopically. The ECHO studies revealed persistent cardiac arrhythmias (chiefly PVCs) in 72% (13/18) of 36-mo rats, 10% (1/10) of 30-mo rats, and none in 6-mo rats (0/16). Gross and microscopic studies showed left ventricular (LV) dilatation, borderline to mild hypertrophy, and areas of fibrosis that were common in 36-mo rats, less evident in 30-mo rats, and absent in 6-mo rats. Aging was associated with mild to moderate decreases of LV diastolic and systolic function. Thus, male F344/BN F1 rats demonstrated progressive age-related (a) decline in cardiac function (diastolic and systolic indices), (b) LV structural changes (chamber dimensions, volumes, and wall thicknesses), and (c) persistent arrhythmias. These changes are consistent with those in humans. The noninvasive ECHO technique offers a means to monitor serial age-related cardiac failure and therapeutic responses in the same rats over designated time intervals.

Keywords: echocardiography, aging, cardiac remodeling, cardiovascular disease

Introduction

Progressive changes in the heart, including LV hypertrophy, chamber dilatation, and fibrosis are associated with aging. Current echocardiographic (ECHO) techniques make it possible to follow age-associated changes in cardiac function and structure in a serial, noninvasive manner [1]. The Fischer 344/Brown Norway F1 (F344/BNF1) rat is recommended by the National Institutes on Aging as a model for the study of age-related pathophysiological changes, since this hybrid (Fischer 344 x Brown Norway) rat lives longer and has a lower rate of pathological conditions than inbred rats [1]. Studies have found modest declines in systolic and diastolic function in nonhybrid female F344 rats when 30-mo-old rats were compared to 6-mo-old rats. Diastolic function may decline earlier and to a greater degree than systolic function [2]. Age-associated declines in diastolic function were found

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in male F344/BNF1 rats, which may be reversible by exercise training, suggesting that such declines may not be intrinsic to aging but that other factors such as deconditioning may be contributory [2].

Previous ECHO studies showed pronounced deleterious cardiac changes in female Fischer 344 [1] or male F344/BNF1 rats [2], but the oldest rats studied were only 30-mo. In most other rat strains, male rats develop more severe age-associated cardiac decline at an earlier age than females [3,4]. To our knowledge, ECHO studies of male F344/BNF1 rats are rare and ECHO studies of rats older than 30-mo have never been reported. Probability of survival curves generated by the National Institutes on Aging show that 30-mo-old and 36-mo-old rats correspond roughly to humans in the sixth and eighth decades of life, respectively. Therefore, the purpose of our study was to gather evidence of age-associated progressive changes in indices of cardiac function and structure in male F344/BNF1 hybrid rats at 6-, 30-, and 36-mo of age.

Materials and Methods

Animals. All treatments of animals were approved by the Marshall University Animal Care Committee. Male Fischer 344/Brown Norway F1 (F344/BNF1) rats were divided into groups of 8 according to age (6-mo, 30-mo, 36-mo). The rats were housed on wood chip bedding at 23 ± 2°C in a room with 12-hr light/dark cycles.

ECHO principles. Echocardiography is a noninvasive procedure in which ultrasonic waves (frequency >20,000 cycles/sec) are emitted from a piezoelectric crystal or transducer, beamed in particular directions, and reflected back (echo) by small structures in the mm and <mm range [5,6]. The waves are beamed toward and penetrate the heart and are reflected back to the transducer as a series of echoes, which are amplified and displayed on a cathode ray tube [6]. Echocardiography is used to evaluate the position, size, and movements of cardiac valves, heart wall structures, and directional flow of blood in cardiac chambers [6].

The wavelength of an ultrasonic pulse is calculated using the formula $\lambda = V/F$ = velocity of pulse in tissue/frequency of the pulse. Pulse velocity in tissue is approximately 1,500 m/sec (1,500,000 mm/sec) [6-8] so $\lambda = 1{,}500{,}000/12{,}000{,}000 = 0.125$ mm with a 12 MHz transducer. ECHO resolution is the smallest distance between two points at which the points can be distinguished as separate [6]. Resolution = wavelength x pulse length, and our pulse length was 1 sec. Therefore, we were able to measure cardiac wall thicknesses of approximately 0.125 mm or greater.

ECHO sensitivity is the ability of the system to image small targets located at specific depths in an attenuative medium [6]. It is determined by the transducer transmitting efficiency x the transducer receiving efficiency of the reflected pulse (echo). System efficiency is determined by transducer beam geometry, frequency spectrum, and energy conversion efficiency. The typical transducer used in ECHOs of adult patients is about 2.25-MHz and has an efficiency of about 4.4% [6]. 3.5-MHz transducers are typically used in younger children and 5-MHz transducers in infants and neonates. In our rats, a 12-MHz transducer was used and the beam travels a much shorter distance in rat hearts than in the case of human hearts, so that the system efficiency and ECHO sensitivity should be considerably greater.

Echocardiographic procedures. Rats aged 6-, 30-, and 36-mo were anesthetized with a ketamine (100 mg/ml)/xylazine (20 mg/ml) mixture and ventral thoraxes were shaved and covered with ultrasonic transmission gel. Two-dimensional ECHO measurements, two-dimensional guided M-mode, Doppler M-mode, and other recordings from parasternal long- and short-axis views were obtained using a Phillips 5500 ECHO system with a 12 MHz transducer. Two-dimensional measurements were used to image cardiac structures in the parasternal long- and short-axis views. The echocardiographic views were then used to position the M-mode echocardiographic line. In the long-axis procedures, the probe was oriented toward the base of the heart projecting toward the apex (x-axis) with depth along the y-axis, thus allowing pulse wave Doppler evaluation of valvular blood flow velocities. In the short-axis procedures, the probe was oriented toward the left ventricle and across the heart for evaluation of wall structure, which was utilized in the calculation of ejection fraction and fractional shortening during systole. M-mode displays were analyzed by a digital echocardiographic analysis system.

Six measurements were selected for each assessment of cardiac structure and function. The structural parameters included diastolic (IVSd) and systolic (IVSs) left ventricular septal thickness, diastolic (LVIDd) and systolic (LVIDs) left ventricular internal dimension, diastolic (LVPWd) and systolic (LVPWs) left ventricular posterior wall thickness, and right ventricular diastolic internal dimension (RV). Functional measurements included ejection fraction (EF), left ventricular fractional shortening during systole (FS), maximal aortic (AVmax), pulmonary (PVmax), mitral (MVmax), and tricuspid (TVmax) valvular blood flow velocity.

Additional echocardiographic measurements included mitral valve deceleration (decel), left ventricular mass during diastole (LVMd), LV mass during systole (LVMs), thickness ratio of the interventricular septum to LV posterior wall (IVS/PW), end-systolic volume (ESV), LV interventricular % thickness (%IVS thick), LV posterior wall thickness (%PW thick), peak velocity of the A wave (Amax), and peak velocity of the E wave (Emax). ESV, %IVS thickening, and %PW thickening were used to evaluate systolic function. Mitral valve deceleration, Emax, A max, and E:A ratio were used to evaluate diastolic function. LVmass and IVS/PW can be used to evaluate both systolic and diastolic function. In addition to direct measurements of cardiac mass, echocardiographic measurements were utilized to calculate left ventricular mass

(LVM) as previously described [8] using the equation: LVM (g) = 1.04 (LVIDd + LVIVSd + LVPWd)^3 – (LVIDd)^3.

Gross and histopathological studies. Body weights and heart weights were obtained and compared. Formalin-fixed hearts were serially sectioned and submitted for tissue processing and staining with hematoxylin and eosin (H & E), Masson's trichrome, Mallory PTAH stain for demonstration of cardiac muscle striations (Poly Scientific R & D Corp., Bay Shore, NY), and a hematoxylin-basic fuchsin stain for detection of infarction (Poly Scientific R & D Corp.). With the hematoxylin-basic fuchsin stain, normal heart fibers take up the basic fuchsin stain and are stained red, while ischemic myocardium takes up hematoxylin and basic fuchsin and is stained brown. Left ventricular and septal wall thicknesses from serially sectioned (4 mm) formalin-fixed hearts (n = 6) were measured postmortem using calipers, and compared to cardiac ventricular and septal wall thicknesses obtained from ECHO studies.

Statistical methods. Results are given as mean ± SD. Group comparisons between ECHO functional and structural parameters, morphologic indices, and the incidence of cardiac arrhythmias data with aging were evaluated by ANOVA with the appropriate post hoc test as needed; $p < 0.05$ was chosen as the criterion for significance.

Results

Body weights and heart weights are compared in Table 1. These weight comparisons were consistent with the ECHO, gross, and microscopic indications of cardiac hypertrophy seen in this study. Rat weights increased with age between ages 6- and 30-mo; heart weights increased at a greater rate so that heart weight/total body weight ratios increased with age.

ECHO results. Echocardiographic evaluation of the 6-, 30-mo, and 36-mo-old male F344/BNF1 rats showed minor changes in several structural features of the left ventricle (Table 2). The diastolic (LVIDd) and systolic (LVIDs) left ventricular (LV) internal dimensions were increased in 30-mo and 36-mo rats when compared to 6-mo rats. Diastolic (LVPWd) and systolic (LVPWs) LV posterior wall thicknesses were increased ($p < 0.05$) in 30-mo, but not in 36-mo hearts. There were no significant differences in the structural parameters of 30-mo vs 36-mo-old rats, indicating that the development of these structural changes occurs by the time the rats are 30-mo-old (Table 2).

Mild, progressive decrements in resting LV systolic function were suggested by increases in LV end-systolic volume (ESV), which was most dramatic in the 36-mo-old rats (Table 4). There were no other statistically different systolic functional values (EF, FS, and valvular flow velocities) in 6-, 30-, and 36-mo-old rats (Table 3). Mild, progressive diastolic dysfunction was suggested by increases in E:A ratios, which were most severe in 36-mo-old rats (Table 4).

Table 4 presents additional ECHO evaluation of cardiac parameters in male rats at the different ages. Two distinct phases of the transmitral flow velocity profile can be readily identified (E wave and A wave), which represent the flow velocities of the early filling phase and the atrial contraction phase, respectively [9]. These waves usually appear as well-defined triangles and the slopes of the triangle sides define the acceleration (upslope) and deceleration (downslope) of the wave. The height of each triangle represents the peak velocity (Emax and Amax) [9]. The increased E:A ratio seen especially in the 36-mo-old rats suggests impaired (slow) left ventricular (LV) relaxation, possibly with moderately decreased left ventricular compliance [10]. E-wave and A-wave peak velocities were not statistically different from those of 6-mo-old rats. It is probable that any effects of impaired (slow) early LV relaxation, if it exists, were offset by increased left atrial pressures, so that early diastolic transmitral pressure gradients and mitral valve flow velocity patterns remained normal or returned to normal (pseudonormalization), although significant abnormalities of diastolic function may be present [11]. Therefore, it is possible in these rats that there is impaired LV relaxation, mild to moderate decrease in LV compliance, and mild to moderate decrease in LV filling pressures. Increased diastolic and systolic LV masses are apparent in 30- and 36-mo, compared to 6-mo rats, consistent with the other indications of ventricular hypertrophy.

Cardiac arrhythmias. ECHO evaluations revealed frequent arrhythmias, chiefly PVCs, in 36-mo-old rats (13/18), which were less frequent in 30-mo-old rats (1/10) and absent in 6-mo-old-rats (0/16) ($p < 0.005$ vs 36-mo-old-rats by Chi-square test).

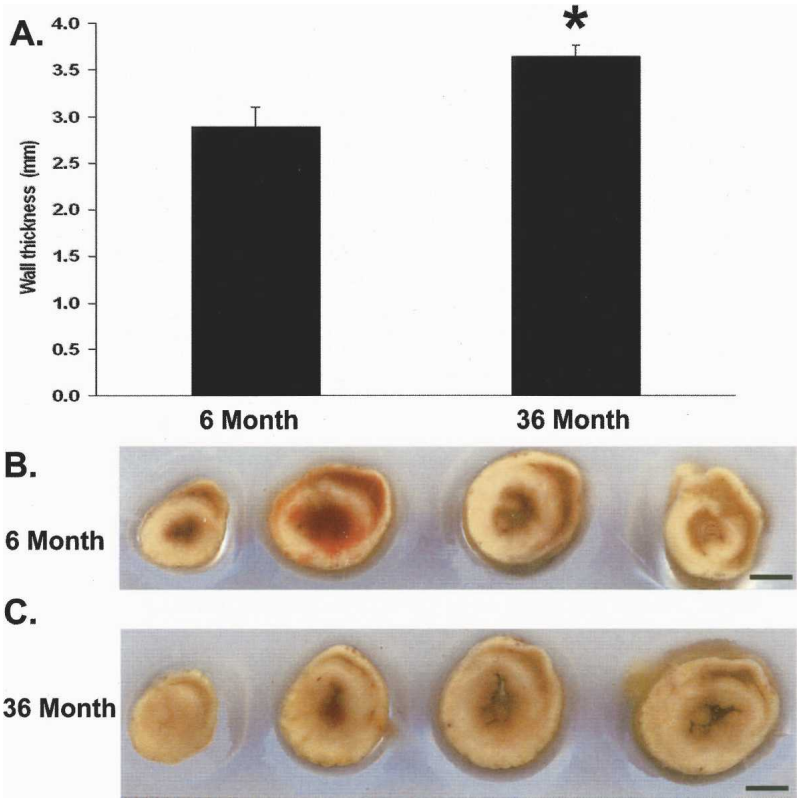

Fig. 1. (Panel A): Left ventricular wall thickness is increased with aging. Ventricular and septum wall thicknesses were measured postmortem from serially sectioned (4 mm) formalin-fixed hearts (n = 6; *p <0.05). Panels B and C show representative serial cross-sections (4 mm) of 6- and 36-mo-old hearts, respectively. Bar = 5 mm.

Table 1. Total body weight (BW) and heart weight (HW) in male F344/BN F1 rats at 6-, 30-, and 36-mo of age. Results are expresed as g or HW/BW ratio (means ± SD; 8 rats/group).

Group	Body wt (g)	Heart wt (g)	HW/BW ratio (%)
6-mo	406.0 ±16.5	0.96 ±0.08	0.237 ±0.015
30-mo	565.0* ±40.4	1.81* ±0.18	0.320* ±0.025
36-mo	500.0* ±17.5	1.66* ±0.15	0.327* ±0.021

* $p < 0.05$ vs corresponding value for 6-mo group.

Gross and microscopic findings. Gross and microscopic evaluations confirmed cardiac hypertrophy and chamber dilation, especially ventricular, in 36-mo rat hearts. Masson's trichrome staining demonstrated extensive subendocardial (Fig. 2C) and interstitial fibrosis (Fig. 2D) in many areas of the left ventricle in 36-mo rats (fibrotic infiltration), but these findings were absent in 6-mo rats (Fig. 2A, 2B). Figs. 2C and 2D also show loss of ventricular cardiomyocytes in 36-mo old rat hearts. Mallory's special cardiac PTAH stain showed prominent, uniform myocardial cross striations in 6-mo rat hearts (Fig. 2E), which were lacking or greatly decreased in 36-mo rat hearts (Fig. 2F).

ECHO results vs gross measurements of cardiac ventricular and septal walls. Ultrasound imaging captures dynamic, real-time images that can be analyzed to obtain quantitative structural and

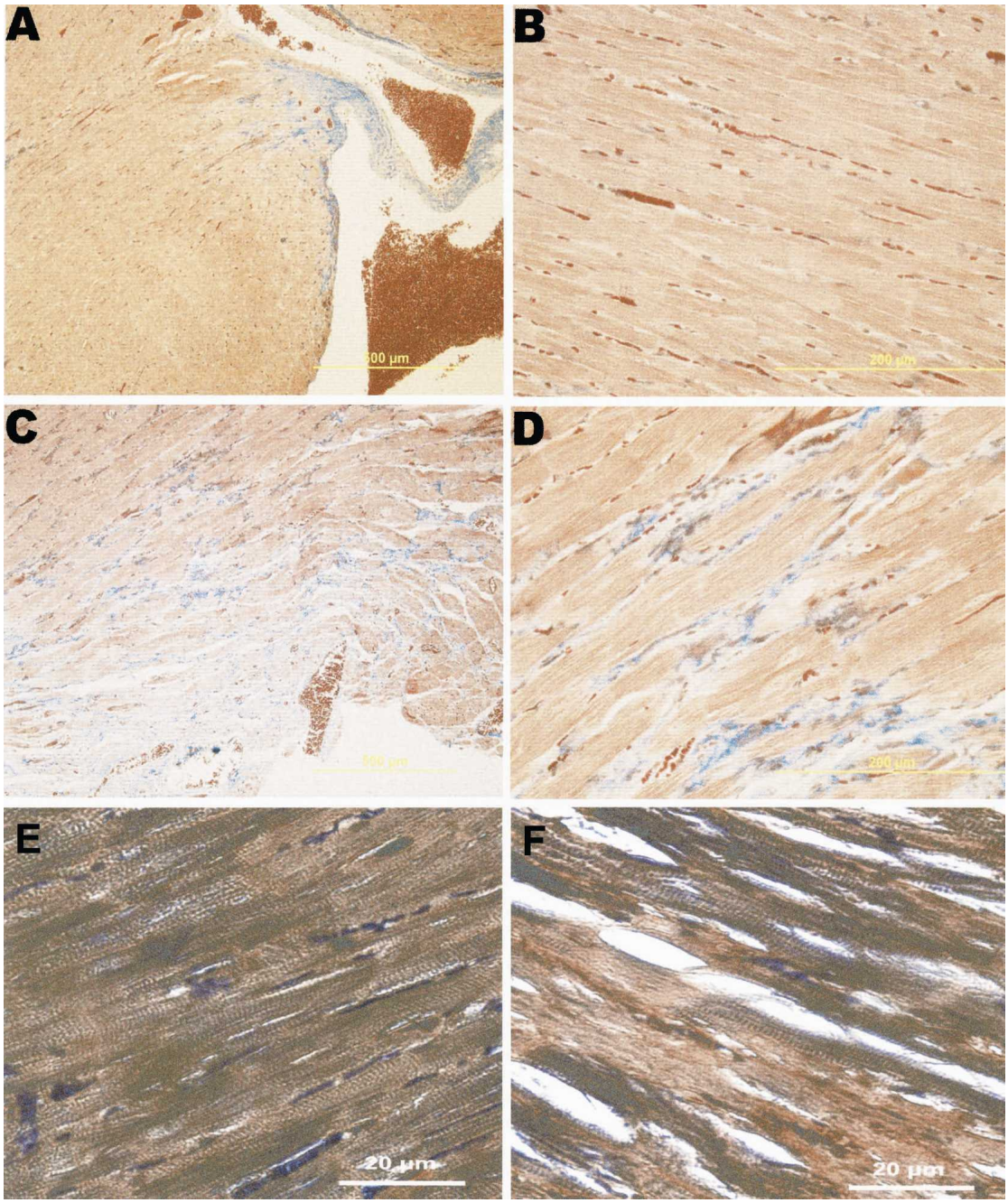

Fig. 2. Panel A (Masson's trichrome stain, 100x) and Panel B (Masson's trichrome stain, 400x): 6-mo-old rat heart shows absence of subendocardial fibrosis (A) and absence of interstitial fibrosis in the mid-portion of the left ventricle (B).

Panel C (Masson's trichrome stain, 100x) and Panel D (Masson's trichrome stain, 400x): 36-mo-old rat heart shows subendothelial (C) and interstitial fibrosis (D).

Panel E (Mallory's PTAH stain, 600x) and Panel F (Mallory's PTAH stain, 600x): 6-mo-old rat heart shows presence of uniform myocardial cross striations (E), while the 36-mo-old rat heart shows patchy loss of cross striations (F).

Table 2. Echocardiographic evaluation of cardiac structural parameters in male F344/BN F1 rats of different ages. Results are reported as cm or g (mean ± SD; 8 rats/group).

Group	N	LVIVSd (cm)	LVIVSs (cm)	LVIDd (cm)	LVIDs (cm)	LVPWd (cm)	LVPWs (cm)	RVIDd (cm)	LVM** (g)
6-mo	8	0.10 ±0.01	0.17 ±0.04	0.65 ±0.07	0.38 ±0.04	0.16 ±0.01	0.23 ±0.02	0.09 ±0.02	0.518 ±0.119
30-mo	8	0.09 ±0.02	0.20 ±0.03	0.86* ±0.10	0.50* ±0.06	0.20* ±0.02	0.29* ±0.02	0.10 ±0.03	0.923* ±0.284
36-mo	8	0.09 ±0.02	0.19 ±0.03	0.86* ±0.12	0.54* ±0.08	0.18 ±0.03	0.28 ±0.05	0.09 ±0.02	0.859 ±0.347

*p <0.05 (30- or 36-mo versus 6-mo).
** Calculated from equation.

Table 3. Echocardiographic evaluation of cardiac functional parameters in male F344/BN F1 rats of different ages. Results are reported as % or cm/sec (mean ± SD; 8 rats/group).

Group	N	EF (%)	FS (%)	AVmax (cm/sec)	PVmax cm/sec)	MVmax (cm/sec)	TVmax (cm/sec)
6-mo	8	77.9 ± 5.7	41.6 ± 5.4	54.0 ± 8.9	77.0 ±17.5	41.2 ± 5.0	46.0. ±12.3
30-mo	8	77.1 ±7.2	41.6 ±6.5	63.6 ±20.2	98.4 ±17.1	53.9 ±10.0	49.0 ±9.5
36-mo	8	74.5 ±9.7	39.9 ±8.3	55.8 ±12.6	95.9 ±23.2	54.3 ±14.5	47.9 ±6.4

Table 4. Echocardiographic evaluation of cardiac parameters in male F344/BN F1 rats of different ages. Results are reported as m, sec, g, or % (mean ± SD, 8 rats/group).

Group	Decel (sec)	LVMd (g)	LVMs (g)	IVS/PW (ratio)	ESV	IVS thick(%)	PW thick(%)	Emax	Amax	E:A
6-mo	0.06 ±0.01	1.00 0.09	0.98 ±0.10	0.61 ±0.10	0.14 ±0.04	90 ±34	45 ±20	51 ±7	29 ±7	1.7 ±0.2
30-mo	0.06 ±0.01	1.35* ±0.23	1.28* ±0.13	0.54 ±0.11	0.31* ±0.10	99 ±28	58 ±10	54 ±91	26 ±5	2.1 ±0.3
36-mo	0.06 ±0.01	1.26* ±0.15	1.24* ±0.14	0.53 0.10	0.38* ±0.15	128 ±40	52 ±10	59 ±13	26 ±5	2.6* ±0.4

*p < 0.05 (30- or 36-mo versus 6-mo).

Table 5. Ventricular and septal wall thicknesses in male F344/BNF11 rats at 6- or 36-mo (mm, mean ± SD, n = 6).

Group	RV wall thickness	LV wall thickness	Septal thickness
6-mo	0.69 ±0.17	2.89 ±0.48	2.90 ±0.21
36-mo	0.86 ±0.16	3.65 ± 0.27	2.66 ±0.41

functional information. Ultrasound imaging has the advantage of being noninvasive. M-mode echocardiograms have been used to assess LV structure and function in rats [12,13] and mice [14]. Nonetheless, M-mode-derived volume and mass calculations may be susceptible to error because they are based on a single measurement from one point through the ventricle and do not include ventricular length measurement [15]. To examine this possibility, we examined age-associated changes in left ventricular thickness postmortem (Table 5; Fig. 1). Compared to ECHO-determined values, direct measurement of left ventricular thickness was higher by 17.8% and 22.2% in 6- and 36-mo rats, respectively ($p < 0.05$). Nonetheless, both procedures yielded similar age-associated differences (+20%) in left ventricular wall thickness (Tables 4 , 5; Fig. 1). Conceivably, the observed differences in magnitude between the two methods may be due to tissue swelling after formalin fixation [16].

Discussion

Cardiovascular disease (CVD) is responsible for >30% of deaths worldwide and heart failure is the leading cause of death in persons >65 years old [10,17]. The occurrence, severity, and prognosis of CVD may reflect age-associated changes in cardiovascular structure and function [10].

Fischer 344/Brown Norway F1 (F344/BNF1) rats were chosen for this study since this hybrid rat lives longer and has a lower rate of pathological conditions than inbred rats. Previous ECHO studies demonstrated pronounced deleterious cardiac changes in F344/BN rats, but to our knowledge ECHO studies of male F344/BNF1 rats older than 30-mo have not been reported. In most other rat strains, male rats develop more severe age-associated cardiac decline at an earlier age than female rats [3,4]. Therefore, male rats were chosen for this study and included those age 6-mo (adult), 30-mo (aged), and 36-mo (very aged). Probability of survival curves generated by the National Institutes on Aging indicate that the aged and very aged rats used corresponded roughly to humans in their sixth, and eighth decades of life, respectively.

In other rat strains and stocks (conventional and specific-pathogen free), progressive myocardial degeneration and fibrosis develop with aging [3,4,18-22]. The incidence of lesions is greater and their onset is earlier in males than females [3,4]. Typical microscopic findings consist of myocardial atrophy, degeneration, necrosis, condensation fibrosis of the stroma, and an inflammatory mononuclear infiltrate consisting of lymphocytes, macrophages, and Anitschkow cells [3,4,19,20,22]. The papillary muscles and their attachment sites in the wall of the left ventricle are the most frequently involved areas, but the heart base, interventricular septum, papillary muscle attachments in the right ventricle, apex of the heart, and areas adjacent to the coronary arteries may be lesion sites [1,17,18]. The incidence of lesions in both sexes may be as high as 60-80%, with the greatest increase after l8-mo of age [3,20]. Most myocardial fibers do not demonstrate cross-striations in very aged rats [3]. In addition, aged rats have increased heart size relative to weight as a result of left ventricular hypertrophy. Aged rats have few or no major signs of cardiac insufficiency or decreases in cardiac performance [3,20], but they may show minor electrocardiogram alterations [3,23].

Additional age-associated cardiac lesions reported in rats include intracardiac thrombi in the left and right atria and left ventricle [3,4,20], valvular endocardiosis (thickening of the heart valves by myxomatous connective tissue) [3,20], endocardial/subendocardial proliferative lesions (most common in the left ventricle) [3,24], cardiac fibromas [3,25], and chronic auriculitis [3,20].

Previous ECHO studies. Boluyt et al [26] conducted the first noninvasive echocardiographic assessment of systolic and diastolic function in aging female Fischer F344 rats. Female Fischer F344 rats have been used as models to examine age-associated changes in the extracellular matrix of the heart [27-29] and in age-related cardiac responses to pressure-overload hypertrophy [26,30]. The rat age extremes were 4- to 30-mo, designed to include ages that exhibit significant differences in (a) collagen content and cross-linking [27-29] and (b) expression of myosin isoforms [31]. The conclusions from these studies [1,26] include: (a) progressive, mild age-

associated decrements in multiple aspects of resting LV systolic function, as evidenced by declines in LV ejection fraction, FS, and velocity of circumferential fiber shortening, which appeared to accelerate after 22-mo of age, and (b) mild diastolic dysfunction in selected parameters such as increased isovolumic relaxation time and decreased tissue Doppler peak E waves at the septal annulus and at the lateral annulus of the mitral valve, which became readily evident only after 22-mo of age. Evaluation of chamber and wall dimensions revealed a pattern of dilatation of the LV between 13- and 22-mo and thickening of the walls between 22- and 30-mo of age. These first studies [1,26] used Fischer 344 rats but did not use F344/BNF1 rats or rats >30-mo of age.

Systolic function is relatively well preserved, but diastolic function declines steadily with advancing years after age 30 in humans, even in the absence of CV disease, which may explain the increased incidence of diastolic heart failure with advancing age [2,32-34]. Brenner et al. [2] used 6- and 24-mo-old male Fischer 344/Brown Norway F1 (F344/BNF1) rats to determine whether exercise training reverses age-associated declines in diastolic function. They chose male F344/BNF1 rats since the hybrid rats have longer life spans and lower rates of the pathological conditions associated with inbreeding. Three aspects of diastolic function were investigated: (a) LV filling in vivo, (b) LV passive compliance, and (c) the degree of ischemia-induced LV stiffening. LV filling was impaired early in diastole in aged hearts but systolic function was not compromised, which corresponds to results seen in humans with aging. Exercise improved LV filling in the rats and has been reported to improve resting diastolic function in patients with cardiomyopathy and impaired relaxation [35]. Cardiac myofilament tension must be rapidly released or relaxed at the end of systole in order for adequate diastolic LV filling to occur. Age-associated declines in the degree of LV relaxation and myocardial calcium uptake are reversed by exercise training [36], which agrees with whole heart manifestation of the improved calcium uptake and relaxation results reported in isolated cardiac muscle strips from trained, aged rats [36,37]. Passive LV stiffness (stiffness constant from the LV pressure-volume relationship) was not altered with age or training, a finding consistent with the literature. Vasculature clearly stiffens with age [38] while LV compliance apparently does not in the absence of accompanying hypertension or other CV disease [39,40]. The degree and rate of ischemia-induced LV stiffening is increased in old, untrained hearts. This stiffening induces increases in LV end-diastolic pressure (EDP) and even minor decreases in EDP of 5 mm Hg can significantly affect pulmonary function [2]. The degree of impairment to ischemic tolerance was less in the 24-mo-old Fischer 344/BNF1 rats than in most other rat strains of similar ages since these rats are still presenescent, in contrast to the senescent rats used in many other studies [41-43]. Brenner et al [2] noted in the F344/BNF1 rat that ischemia-induced EDP increases may be due to subendocardial rigor [44,45] and that training may slow the rate at which rigor develops. Brenner et al [2] concluded that the increased rate and degree of ischemia-induced diastolic impairment may reflect deconditioning rather than inevitable consequences of aging.

Our ECHO findings include a number of cardiac left ventricular (LV) structural changes: (a) minor, progressive diastolic and systolic LV chamber dilatation as judged by increased LV internal dimensions (LVIDd and LVIDs), (b) mild diastolic and systolic LV hypertrophy as judged by increased diastolic and systolic LV posterior wall (LVPWd and LVPWs) thicknesses and LV masses (LVMd and LVMs), (c) progressive, mild age-associated decrements in resting LV systolic function as suggested by increases in LV end-systolic volumes (ESV), and (d) mild diastolic dysfunction as evidenced by significant increases in E:A ratios, especially in 36-mo-old rats. Other parameters of systolic function (EF, FS, AVmax, PVmax, MVmax, TVmax) in 30- and 36-mo-old rats were not statistically different from those in 6-mo-old male rats, suggesting cardiac compensation for the mild systolic declines. Most LV structural and functional parameters did not differ significantly in 30- and 36-mo-old rats, which suggests that most of the age-related changes have occurred or are well underway by age 30-mo in these rats. In addition, the LV hypertrophy so apparent in the 30-mo-old rats appears to be somewhat offset by apoptosis and

loss of ventricular cardiomyocytes (Figs. 2C, 2D) with additional aging, thus counteracting overall increases in LV thickness and LV mass, between ages 30-and 36-mo (Table 2).

Our findings in aging male F344/BNF1 rats agree with the reports [1,2,45] that LV diastolic and systolic dysfunction becomes evident or begins to accelerate after age 22-mo in a similar rat strain, the aging female, nonhybrid Fischer F344 rats. The lack of sustained hypertension in aged F344/BN rats [1,2] complicates explanations for the age-associated LV chamber dilatation and hypertrophy resulting from cardiac remodeling.

The aortas were evaluated in a parallel study of the same rats as the present investigation and these results are reported in separate publications [10,46]. Aortic measurements included aortic contractility and relaxation determinations, as well as gross and microscopic examinations. These data showed that male Fischer 344/BNF1 rats have progressive, age-related declines in aortic structure and function including depressed contractility, increased aortic stiffness, aortic medial thickening, and alterations in elastin/collagen composition [10,46]. Thus, similar to humans, the aging F344/BNF1 rats exhibit age-related alterations in cardiovascular (heart and aortic) structure and function.

The investigators [10,46] also studied aortic response to increased intraluminal pressure and found: (a) a disconnect between aging and load-induced Akt- and MAPK-dependent signaling, (b) changes in the expression/basal phosphorylation and/or load-induced regulation of signaling molecules that precede age-associated changes in the mechanical properties of the aorta, and (c) changes in phosphatase activity, which may help to explain age-associated changes in aortic signaling [10,46]. These findings suggest the hypothesis that age-related alterations in aortic vessel remodeling may be due, at least in part, to activation of certain mitogen-activated protein kinase (MAPK) pathways [10,46], which could offer a partial explanation for the observed age-associated alterations in cardiac structure, function, and remodeling in the F344/BNF1 rat model.

Our present findings agree with previous reports regarding progressive cardiac changes, including LV hypertrophy, chamber dilatation, and fibrosis that appear to be associated with aging. Our findings agree with other observations [1,2] of (a) progressive, mild age-associated decrements in multiple aspects of resting LV systolic function as evidenced by declines in LV ejection fraction, FS, and velocity of circumferential fiber shortening, which appeared to accelerate after 22-mo of age, and (b) mild diastolic dysfunction in selected parameters, such as increased isovolumic relaxation time and decreased tissue Doppler peak E waves at the septal annulus and at the lateral annulus of the mitral valve, which became evident only after 22-mo of age. Chamber and wall dimensions revealed a pattern of dilatation of the LV between 13-mo and 22-mo of age and thickening of the walls between 22-mo and 30-mo of age, but with partial offsetting of the ventricular thickening after age 30-mo due to loss of ventricular cardiomyocytes.

Microscopic evaluations of 6-, 30-, and 36-mo-old rat hearts demonstrate progressive increases in the degree and extent of interstitial fibrosis with aging. Appreciable infiltrating fibrosis extending from the endocardium into the myocardium is seen in the 36-mo-old rat hearts. The age-associated functional and structural changes in these rat hearts, together with the loss of cardiac cells, arrhythmias, cardiac fibrosis, and other changes are similar to the microscopic and gross findings commonly observed in aging human hearts [47].

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