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Gender Influences Cardiac Function in the *mdx* Model of Duchenne Cardiomyopathy

Brian Bostick, BS, Yongping Yue, BA, and Dongsheng Duan, PhD

Department of Molecular Microbiology and Immunology, School of Medicine, 1 Hospital Drive, Columbia, Missouri 65212, USA

Abstract

Aged *mdx* mice represent an important model for studying Duchenne cardiomyopathy. Herein we compared the cardiac phenotypes of 22-month-old male and female *mdx* mice. Surprisingly, only females displayed the characteristic cardiac dilation on pressure–volume loop analysis. Female *mdx* mice also exhibited lower contractility, larger Q waves, and higher ratios of heart weight to body weight. Our results reveal significant gender disparity in *mdx* cardiac function. Gender should be considered when using the *mdx* model for the study of Duchenne cardiomyopathy.

Keywords

animal models; cardiomyopathy; Duchenne muscular dystrophy; ECG; gender; hemodynamics; *mdx*

Duchenne muscular dystrophy (DMD) is a fatal X-linked muscle-wasting disease caused by null mutation of the dystrophin gene. The absence of dystrophin causes muscle damage, inflammation and fibrosis. Heart disease is a significant cause of morbidity and mortality in DMD.^{1,2} Current treatment for Duchenne cardiomyopathy is limited.

A critical step toward improved therapy is the development of appropriate animal models. Dystrophin-deficient *mdx* mice have been widely used as a DMD model.^{3–5} Several groups have examined *mdx* cardiac function. Although young *mdx* mice exhibit a mild cardiac phenotype, older *mdx* mice seem to display significant cardiac dysfunction.^{6–9} In particular, we have found that 20–22-month-old *mdx* mice show severe dilated cardiomyopathy (DCM) reminiscent of that seen in human patients.⁸

Animal gender has been recognized as a modulating factor for the dystrophic phenotype in skeletal muscle.^{10–12} Herein we examined whether gender affects the cardiac phenotype in aged *mdx* mice. We compared the anatomic features, cardiac fibrosis and electrocardiographic (ECG) profiles, and hemodynamic function of 22-month-old male and female *mdx* mice. The ratio of heart weight (HW) to body weight (BW) was significantly higher in aged female *mdx* mice. ECG analysis revealed larger Q waves in female *mdx* mice. Furthermore, left ventricular hemodynamic function was more severely reduced in female *mdx* mice. However, the level of cardiac fibrosis was not significantly different between genders. Our results suggest that aged *mdx* female mice display more severe cardiomyopathy.

Methods

Experimental Mice

All animal experiments were approved by local animal care and use committee and were in accordance with NIH guidelines. Age-matched C57Bl/10SnJ (BL10) and C57Bl/10ScSn-*Dmd^{mdx}/J* (*mdx*) mice were originally purchased from the Jackson Laboratory (Bar Harbor, Maine).

Hemodynamic Assay

Left ventricular hemodynamic assay was performed in a closed-chest preparation using a 1.4F Millar microtip pressure–volume catheter (SPR-839; Millar Instruments, Houston, Texas).^{8,13} Baseline hemodynamic parameters were collected using the Millar Aria-1 PV conductance system and analyzed with PVAN software (Millar Instruments).

ECG Analysis

Non-invasive 12-lead ECG was performed under isoflurane anesthesia as previously described.^{8,13,14} Signal-averaged ECG tracings were constructed from 1-min recordings using a Model MLA0112S PowerLab system running Chart ECG analysis software (version 5.5.5; AD Instruments, Colorado Springs, Colorado). The lead II tracing was used for all parameters except Q-wave amplitude, which was taken from the lead I tracing. The cardiomyopathy index is calculated as the QT interval divided by the PQ segment (QT/PQ).

Hydroxyproline Content Assay

The heart hydroxyproline content was determined in lyophilized whole mouse heart.¹³ Briefly, hydrolyzed heart lysate was oxidized with chloramine-T and then reacted with *p*-dimethylamino-benzaldehyde/perchloric acid. The absorbance at 558 nm was measured, and hydroxyproline content was determined using a standard curve.

Statistical Analysis

Data are presented as mean ± standard error of mean. Data were analyzed by one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc analysis using statistical software (SPSS, Chicago, Illinois). $P < 0.05$ was considered statistically significant.

Results

Cardiac function in aged *mdx* mice was significantly worse than in normal BL10 mice.^{8,13} However, different patterns were observed between male and female *mdx* mice. Although there was no obvious pressure–volume (PV) loop shift in males (Fig. 1A), the characteristic rightward shift of the PV loops was observed in females (Fig. 1B).

Detailed analysis of systolic and diastolic function showed worse hemodynamic function in female *mdx* mice. Specifically, females had significantly higher end-systolic and end-diastolic volumes. The maximum pressure, maximal rate of left ventricular pressure development (dP/dt_{\max}), and the minimal rate of left ventricular pressure fall (dP/dt_{\min}) were significantly lower in females (Fig. 1C). Interestingly, there was no difference in the time constant of left ventricular isovolumetric pressure decay (τ) between males and females (Fig. 1C).

Ejection fraction was significantly decreased in both male and female *mdx* mice. However, females fared worse with a much greater reduction. Nevertheless, there was no significant difference between males and females in stroke volume and cardiac output (Fig. 1C, bottom panel).

We also compared the ECG tracings of 22-month-old male and female *mdx* mice. Both males and females had significantly increased heart rate (HR) compared with normal BL10 mice but showed no difference between genders (Fig. 1D). Analysis of the PR interval, QRS duration, and corrected QT interval (QTc) revealed the same pattern. There were significant changes in *mdx* mice compared with normal, but there was no gender disparity (Fig. 1D).

Only the lead I Q amplitude (Q_{amp}) revealed a significant gender difference. Both male and female *mdx* mice Q waves were greater than normal. However, females had significantly larger Q waves than males (Fig. 1D). There was no gender difference in Q-wave amplitudes of normal mice. Analysis of the cardiomyopathy index showed significant increases for both male and female *mdx* mice, but no gender difference (Fig. 1D).

We also compared the HW/BW ratio and the cardiac hydroxyproline content. Although both genders of *mdx* mice had significantly increased HW/BW ratios, females had a much greater increase (Fig. 1E). The cardiac hydroxyproline content was significantly elevated in *mdx* mice, but there was no difference between males and females (Fig. 1F).

Discussion

Mdx mice represent a well-established model for the study of DMD skeletal muscle disease.² However, until recently the *mdx* mouse has not been considered a useful model to investigate Duchenne cardiomyopathy. Early studies of young *mdx* mice found minimal cardiac dysfunction.^{6,7,15} Recent investigations on aged *mdx* mice have renewed interest in this model for the study of Duchenne cardiomyopathy.^{8,13,16} To further characterize the aged *mdx* mouse model for DMD heart disease, we compared cardiac function between aged male and female *mdx* mice.

Our findings suggest that gender may significantly affect the *mdx* cardiac phenotype. Specifically, the classic rightward shift of DCM was found only in females. Females also showed more severe reductions in systolic and diastolic parameters, larger Q waves in their ECG tracings, and a significantly greater increase in HW/BW ratio (Fig. 1).

Gender differences have been increasingly recognized as a critical factor in the incidence and severity of heart disease.^{17,18} Typically, heart disease is more severe in males than in females. The prevailing hypothesis suggests that estrogen may play a protective role.¹⁹ However, there are also instances in which females show more severe heart disease than males.^{20–22} In patients with diabetes, women have more severe heart disease and an increased risk of DCM and heart failure.²¹ Studies in calmodulin-induced diabetic mice have revealed more severe cardiac dysfunction in females.²²

The mechanisms behind these findings are not completely understood. One intriguing hypothesis may relate to renin–angiotensin system (RAS) alterations. In the case of diabetic cardiomyopathy, it has been shown that females are more sensitive to RAS perturbations.^{23,24} Interestingly, angiotensin receptor mRNA is elevated in the *mdx* heart.²⁵ Angiotensin-converting enzyme (ACE) inhibitors also improve *mdx* hemodynamic profiles.²⁶ In addition, ACE inhibition has been shown to be effective in treating Duchenne cardiomyopathy.²⁷ These findings suggest the RAS may play a role in dystrophic cardiomyopathy. Future studies are needed to clarify the underlying mechanisms.

The aged *mdx* mouse represents a promising model for developing novel therapies tailored to the heart. Our results suggest that aged female *mdx* mice may exhibit a more severe cardiac phenotype. Gender should be carefully considered when using this model for studying Duchenne cardiomyopathy.

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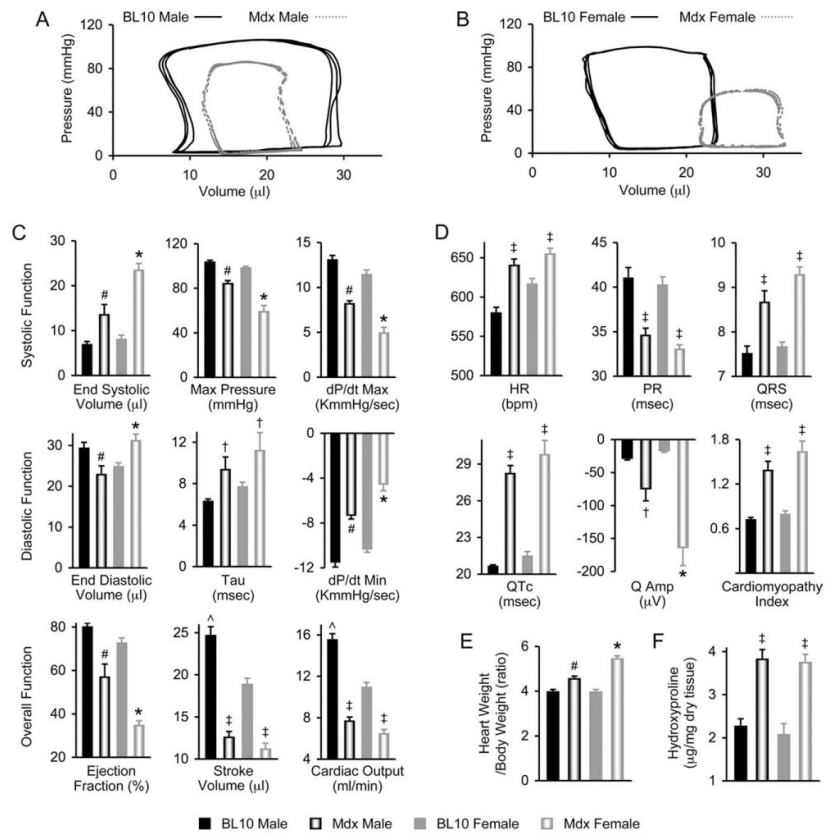
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Abbreviations

ACE	angiotensin-converting enzyme
ANOVA	analysis of variance
BW	body weight
bpm	beats per minute
<i>mdx</i>	C57Bl/10SnJ, BL10; C57Bl/10scSn-Dmd ^{mdx} /J
DCM	dilated cardiomyopathy
DMD	Duchenne muscular dystrophy
ECG	electrocardiography
dP/dt_{\max}	maximal rate of left ventricular pressure development
dP/dt_{\min}	minimal rate of left ventricular pressure development

**FIGURE 1.**

Heart disease in 22-month-old *mdx* mice is more severe in female mice than in male mice. (A) Representative PV loops from male BL10 and *mdx* mice. (B) Representative PV loops from female BL10 and *mdx* mice. (C) Baseline hemodynamic profiles ($N = 15$ for BL10 males, 16 for *mdx* males, 16 for BL10 females, 13 for *mdx* females). (D) ECG profiles ($N = 13$ for BL10 males, 11 for *mdx* males, 11 for BL10 females, 12 for *mdx* females). (E) Ratios of heart weight to body weight ($N = 24$ for BL10 males, 35 for *mdx* males, 24 for BL10 females, 44 for *mdx* females). (F) Hydroxyproline content ($N = 7$ for BL10 males, 18 for *mdx* males, 6 for BL10 females, 16 for *mdx* females). **mdx* female significantly different from all other groups; #*mdx* male significantly different from all other groups; †significantly different from gender-matched group by *t*-test analysis; ^BL10 male significantly greater than BL10 female; ‡*mdx* groups are significantly different from BL10 groups by ANOVA analysis, but not from each other.