Cardiac Characterization of mdx Mice Using High-Resolution Doppler Echocardiography

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Objectives—Duchenne muscular dystrophy is an X-linked neuromuscular disorder. The heart is traditionally involved, leading to heart failure. The mdx mouse is a natural animal model of the disease. We conducted a prospective study to analyze left ventricular (LV) function in mdx mice at different ages using high-resolution Doppler echocardiography.

Methods—Echocardiography was performed with a 30-MHz cardiac probe. Wild-type and mdx mice were scanned at 10 and 12 months. We measured the interventricular septal wall thickness, posterior wall thickness, and LV diameter in systole and diastole. The LV shortening fraction, LV ejection fraction, and LV mass were then calculated.

Results—At 10 months, the shortening fractions in mdx and wild-type mice were relatively close (29% ± 5% versus 25% ± 4%). We found a significant difference in the posterior wall thickness change (40% ± 12% in mdx versus 28% ± 10% in wild-type; P = .048). The LV mass/body weight ratio was higher in mdx than wild-type mice (3.67 ± 0.25 versus 3.39 ± 0.26; P = .05). At 12 months, the LV mass was elevated in mdx mice compared to wild-type (152 ± 16 versus 135 ± 3.7 mg; P = .04). The diastolic posterior wall thickness change was decreased in mdx mice at 12 months compared to wild-type (21% ± 7% versus 33% ± 4%; P = .01). The LV ejection fraction was not statistically different between mdx and wild-type mice (50% ± 6% versus 54% ± 2%).

Conclusions—Ten-month-old mdx mice had a significantly higher posterior wall thickness than wild-type mice, but it was not significant at 12 months. In 12-month-old mdx mice, the posterior wall thickness change was significantly lower, and the LV mass was significantly higher. These findings indicate the role of LV function in the early stages of Duchenne muscular dystrophy.

Key Words—Doppler echocardiography; Duchenne muscular dystrophy; mdx mice

Duchenne muscular dystrophy is an X-linked neuromuscular disorder. It is caused by a mutation in the dystrophin gene on chromosome Xp21.1. Dystrophin is a component of the dystrophin glycoprotein complex, which forms a link between the extracellular matrix and the cytoskeletal proteins.1 This link is thought to have a structural and mechanical function and likely plays a part in other cellular activities such as signaling pathways. Dystrophin deficiency leads to myofiber damage, inflammation, and fibrosis.1 The heart is traditionally involved in Duchenne muscular dystrophy, leading to progressive heart failure at the end of the second decade.2 The incidence of cardiomyopathy in Duchenne muscular dystrophy increases with age, affecting 30% of patients by 14 years, 50% by 18 years, and all older patients.3
Doppler echocardiography is a noninvasive procedure that allows analysis of cardiac morphologic characteristics and function. This procedure is routinely used for analysis of cardiac function in murine models of disease. The mdx mouse is an animal model of Duchenne muscular dystrophy with a milder phenotype. Because the mdx mouse has features observed in patients with Duchenne muscular dystrophy, this animal model is used for studying pathophysiologic mechanisms and therapeutics in the disease. Twenty-one-month-old mdx mice have severe heart failure that corresponds to adult end-stage Duchenne muscular dystrophy. In the early stages of the disease, the posterior wall of the left ventricle is affected. To assess left ventricular (LV) function in the early stages of the disease, we conducted a prospective study to analyze echocardiographic values of mdx mice aged 10 and 12 months using high-resolution Doppler echocardiography.

Materials and Methods

Animals
Seven C57Bl/10 (wild-type) mice (control group) and 8 mdx mice were included in our study. All mice were handled in accordance with the guidelines of the Genethon committee. Each animal was shaven from the left sternal border to the left axillary line with depilatory cream. Mice were anesthetized with isoflurane before echocardiography was performed. Initially, the mouse was placed in an induction chamber with constant inflow of 3% isoflurane mixed with oxygen; then the mouse was removed from the induction chamber and placed on a heating platform with electrocardiographic contact pads for monitoring the heart rate and temperature. The nose was placed into a nose cone with 1% isoflurane in 100% oxygen. Mice were kept on a heating pad in a supine position. Ultrasound gel was applied to the chest of the mouse before echocardiography.

Echocardiography
Echocardiography was performed with a Vevo 770 system (VisualSonics, Inc, Toronto, Ontario, Canada) and a 30-MHz cardiac probe (RMV707B). The wild-type and mdx mice were followed and scanned at 10 and 12 months. For echocardiographic recording, we optimized the sweep speed, depth, focus, and gain settings to obtain the best possible images. Two-dimensional and M-mode echocardiographic images were obtained from the long-axis view at the level of the largest LV diameter. The LV dimensions (LV end-diastolic diameter and end-systolic diameter), posterior wall thickness, and interventricular septal wall thickness were measured with the use of the leading-edge convention of the American Society of Echocardiography. The LV shortening fraction, LV ejection fraction, posterior wall thickness change, and LV mass were calculated from the above dimensions (Figure 1).

Statistical Analysis
Data are expressed as mean ± SD. The echocardiographic parameters were compared between wild-type and mdx groups at the two time points by an unpaired Student t test. Excel statistical software (Microsoft Corporation, Redmond, WA) was used for data analysis. P < .05 was considered statistically significant.

Results
The wild-type and mdx mice were similar in body weight and heart rate at 10 months (Table 1). At 12 months, the mean heart rate was not statistically different between the mdx and wild-type mice, but body weight was statistically different between the groups (Table 2).

Table 1 shows LV measurements from M-mode echocardiography in the wild-type and mdx mice at 10 months. At 10 months, LV systolic function, evaluated by the shortening fraction and ejection fraction, was similar in the two groups. At this age, we found a significant difference in the posterior wall thickness change during end systole between the groups. The LV was not dilated at 10 months. We found a trend toward a significant difference between mdx and wild-type mice for the LV mass/body weight ratio.

Table 2 shows LV measurements from M-mode echocardiography in the wild-type and mdx mice at 12 months. At 12 months, the LV mass became elevated in the mdx mice compared to wild-type mice. The LV end-diastolic diameter was not statistically different between the mdx and wild-type mice. At 12 months, we found a significant alteration in the diastolic posterior wall thickness change in the mdx mice compared to the wild-type mice. Left ventricular systolic function (ejection fraction) was not statistically different between the mdx and wild-type mice. The LV mass/body weight ratio was higher in the mdx mice.

Discussion
In this study, we analyzed echocardiographic parameters in mdx mice, an animal model for Duchenne muscular dystrophy, a neuromuscular disorder caused by a mutation in the dystrophin gene on chromosome Xp21.1. Dystrophin is a component of the dystrophin glycoprotein complex, which links the extracellular matrix and the cytoskeletal proteins. Dystrophin deficiency leads to myofiber damage,
inflammation, and fibrosis. Heart failure is a major complication of Duchenne muscular dystrophy because of cardiomyocyte degeneration and fibrosis. The mdx mouse is an animal model of Duchenne muscular dystrophy with the dystrophin mutation. The disease phenotype is milder in mdx mice.

We found a significant alteration in the diastolic posterior wall thickness change in the mdx mice compared to the wild-type mice at 12 months. However, we did not find a significant decrease in the LV ejection fraction and LV shortening fraction in both 10- and 12-month-old mdx mice compared to wild-type mice. The posterior wall of the LV is affected in the early stages of the disease. Mori et al reported decreased peak systolic radial strain in the LV posterior wall of young patients with Duchenne muscular dystrophy. Ogata et al reported strain abnormalities in the LV posterior wall of patients with Duchenne muscular dystrophy who had a normal LV ejection fraction. The LV mass was higher in the mdx mice at 12 months. Quinlan et al reported modifications of echocardiographic parameters in 10-month-old mdx mice and a significantly different LV mass at 29 weeks in mdx mice. Spurney et al reported heart dysfunction at 9 to 10 months in mdx mice with an increase in the LV internal diameter and a decrease in the posterior wall thickness. Left ventricular abnormalities may be explained by degenerative foci, limited inflammation, moderate myocardial necrosis, and fibrosis in the mdx mouse heart. Prominent heart disease does not appear until mice are about 12 months old, and it gets worse as mice age. Myocardial fibrosis is involved in the pathophysiologic mechanisms of cardiomyopathy in mdx mice and seems to be limited by losartan.

In the mdx mouse, cardiomyopathy shares many but not all of the features of Duchenne muscular dystrophy cardiomyopathy. Our results are consistent with the milder phenotype of mdx mice. Double-deletion mice (mdx/
utrophin-deficient) have been promoted as more appropriate models for heart disease in Duchenne muscular dystrophy.\textsuperscript{15} 

This study had some limitations. Anesthetic agents may have a deleterious effect on heart function in mouse models. Isoflurane is known to decrease contractility and the heart rate in mice;\textsuperscript{16} heart rate values were lower in our study compared to previous studies,\textsuperscript{10,11} and the shortening fraction was lower in our study because of possible isoflurane effects on LV contractility. Heart disease in patients with Duchenne muscular dystrophy is a segmental cardiomyopathy.\textsuperscript{4} Because wall motion impairment is focal, it may be difficult to evaluate overall cardiac function in patients with Duchenne muscular dystrophy by M-mode echocardiography.

In conclusion, 10-month-old \textit{mdx} mice had a significantly higher posterior wall thickness than wild-type mice, but it was not significant in the 12-month-old group. In 12-month-old \textit{mdx} mice, the posterior wall thickness change was significantly lower, and the LV mass was significantly higher. These findings indicate the role of LV function in the early stages of Duchenne muscular dystrophy.

**References**