Exercise-Induced Left Ventricular Systolic Dysfunction in Women Heterozygous for Dystrophinopathy

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Background: Mutations in the X-linked gene encoding dystrophin cause skeletal and cardiac muscle diseases in men. Female "carriers" also can develop overt disease. The purpose of this study was to ascertain the prevalence of cardiac contractile abnormalities in dystrophinopathy carriers.

Methods: Twenty-four dystrophinopathy heterozygotes and 24 normal women each underwent standard exercise stress echocardiography.

Results: Heterozygotes demonstrated mildly lower left ventricular ejection fractions (LVEFs) at rest compared with controls (0.56 ± 0.10 vs 0.62 ± 0.07, $P = .02$). After exercise, the mean LVEF fell to 0.53 ± 0.14 in heterozygotes but rose to 0.73 ± 0.07 in controls ($P < .001$). Twenty-one of 24 dystrophinopathy heterozygotes demonstrated ≥1 of the following: abnormal resting LVEF, abnormal LVEF response to exercise, or exercise-induced wall motion abnormality.

Conclusions: Women heterozygous for dystrophinopathy demonstrate significant left ventricular systolic dysfunction, which is unmasked by exercise. This finding has mechanistic implications for both inherited and acquired cardiac disease states. (J Am Soc Echocardiogr 2010;23:848-53.)

Keywords: Cardiomyopathy, Women, Exercise, Heart failure, Echocardiography

In men, mutations in the X-linked gene encoding dystrophin, a component of muscle cytoskeleton, cause muscular dystrophy,[1] cardiomyopathy,[2] or both. Acquired abnormality of the cellular localization of dystrophin is a component of cardiomyopathy due to enteroviral myocarditis,[3] congenital heart disease,[4] and end-stage ischemic congestive heart failure.[5]

Women heterozygous for a disease-associated dystrophin allele are also susceptible to the development of heart failure,[6] and there is uncertainty about the optimal strategy for identifying individuals at risk.[7] Hemodynamic stress, applied prior to the onset of overt cardiomyopathy, causes myocardial injury in homozygous dystrophin-deficient mice.[8] We hypothesized that exercise stress would similarly unmask left ventricular dysfunction in asymptomatic women heterozygous for a disease-associated dystrophin mutation.

METHODS

Subject Recruitment

The University of Iowa's institutional review board approved all procedures. Dystrophinopathy heterozygotes were recruited from the Pediatric Neuromuscular Diseases Clinic at the University of Iowa, on the basis of their familial relationship to a boy with Duchenne or Becker muscular dystrophy. Dystrophin status was confirmed by deoxyribonucleic acid testing or through position in a pedigree. Potential subjects were excluded if they had any of the following risk factors for coronary artery disease: family history of myocardial infarction prior to 50 years of age, tobacco use, hypertension, diabetes, and hyperlipidemia. Potential subjects underwent directed medical histories and physical examinations and were excluded if they had any of the following potential symptoms or signs of cardiac or muscle disease: chest pain or dyspnea on exertion, orthopnea, unexplained syncope or symptomatic palpitations, peripheral edema, muscle pain or weakness, systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, jugular venous pressure > 8 cm H_2O, carotid bruit, pulmonary crackles or wheeze, cardiac murmur > 1/6 in severity, cardiac gallop, hepatomegaly, or the use of medications known to affect cardiovascular function. A total of 50 potential subjects were offered participation. Thirteen declined, and 13 others had ≥1 exclusion criteria upon initial screening. The remaining 24 asymptomatic women heterozygous for dystrophinopathy mutations provided written informed consent. All

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Abbreviation

LVEF = Left ventricular ejection fraction

24 participating subjects described their lifestyles as "active"; none was a competitive athlete or participant in a structured physical training program.

Normal subjects (n = 24) included sibling control subjects (n = 5) recruited on the basis of their familial relationships to participating dystrophinopathy carriers. Each had undergone genetic testing and was known to lack the familial dystrophin mutation. An additional group of normal volunteers (n = 19) was recruited from the community at large via word of mouth and posted notices, with recruitment targeted to the following attributes: female gender; age similar to dystrophinopathy heterozygotes; and absence of history, symptoms, and risk factors of cardiovascular disease. Exclusion criteria were the same as for dystrophinopathy heterozygotes. All 24 normal subjects described their activity levels as "active"; none was a competitive athlete or participant in a structured physical training program, and all provided written informed consent.

Stress Testing

All stress echocardiographic procedures were performed using the Bruce protocol[9] and were personally supervised by one of the authors (R.M.W., R.E.K., or K.S.S.), who was blinded with respect to subjects' dystrophin status.

Echocardiography

Resting two-dimensional echocardiograms were obtained in standard parasternal long-axis and short-axis views and apical 2-chamber and 4-chamber views, using a Sonos 5500 or 7500 sonograph (Philips Medical Systems, Andover, MA) fitted with a 3-MHz sector-array probe. Image acquisition was resumed a few seconds after the cessation of exercise. For each echocardiographic image plane, the earliest technically acceptable cine clip was saved and used for subsequent offline quantitative analysis. Technically adequate images were acquired in all subjects, without need for echocardiographic contrast administration.

A subset of 10 dystrophinopathy heterozygotes underwent the assessment of left ventricular diastolic function at rest. Pulse-wave Doppler interrogation of mitral inflow was achieved by placing depth gates in the left ventricle, just beneath the mitral valve, in the apical 4-chamber view. Tissue Doppler acquisition was performed with regions of interest in the septal and lateral aspects, respectively, of the mitral annulus in the apical 4-chamber view.

Echocardiographic Analysis

Image analysis was conducted in blinded fashion with respect to subject dystrophin status and exercise time. Resting left ventricular end-diastolic chamber dimension and wall thickness were measured using electronic calipers at the level of the chordae tendineae in a parasternal long-axis view, using the leading-edge convention. The left atrial anteroposterior dimension was assessed at the time corresponding to ventricular end-systole. Rest and exercise ejection fractions were calculated from apical 4-chamber and 2-chamber views, using the biplane method of discs.[10] End-diastolic and end-systolic silhouettes were identified by a single author (J.K.J.), who was blinded with respect to genotype. Regional systolic function was evaluated using the standard 17-segment model.[11,12] A new wall motion abnormality was deemed present when ≥1 myocardial segment demonstrated systolic function worse by ≥1 grade than the majority of left ventricular segments, during exercise only. Regional function was evaluated by consensus of 3 authors (R.M.W., R.E.K., and P.D.L.), blinded with respect to genotype. Diastolic function was computed offline. Peak early mitral inflow (E) and peak inflow during atrial contraction (A) were recorded and expressed as a ratio (E/A). Peak early diastolic mitral annular excursion was measured for septal and lateral locations and was averaged (E′).

Table 1 Morphometric and echocardiographic data

Variable	Normal subjects	Patients with heterozygous dystrophinopathy	*P*
Age (y)	41 ± 10	39 ± 8	.45
Height (m)	1.66 ± 0.05	1.64 ± 0.09	.34
Body mass (kg)	70.7 ± 15.4	81.0 ± 18.2	.04
Body mass index (kg/m^2)	25.6 ± 4.5	30.4 ± 7.4	.008
Left atrial dimension (cm)	3.5 ± 0.4	3.7 ± 0.5	.13
LV end-diastolic dimension (cm)	4.7 ± 0.4	4.8 ± 0.5	.45
LV end-systolic dimension (cm)	3.0 ± 0.4	3.4 ± 0.6	.009
LV fractional shortening	0.36 ± 0.07	0.30 ± 0.09	.013
Posterior wall thickness (cm)	0.8 ± 0.2	0.8 ± 0.1	1
Septal wall thickness (cm)	0.8 ± 0.1	0.8 ± 0.1	1
LV ejection fraction	0.62 ± 0.07	0.56 ± 0.10	.02

LV, Left ventricular.

Statistical Analysis

Group data are reported as mean ± SD. Comparisons between groups using continuous quantitative variables used analysis of variance. Comparisons between groups of the frequency of occurrence of a binary variable (presence or absence of a finding) were performed using comparison of proportions.[13] Statistical significance was deemed present for *P* values < .05.

RESULTS

Study Population

Age and body morphometric data for women with heterozygous dystrophinopathy are compared with those for normal women in Table 1. Age was similar in the two groups, but dystrophinopathy heterozygotes had higher body mass and body mass indexes.

Resting echocardiographic data are shown in Table 1. There were no significant differences between dystrophinopathy heterozygotes and normal subjects with respect to left atrial size, left ventricular end-diastolic dimension, or end-diastolic wall thickness. However, the dystrophinopathy heterozygotes demonstrated higher mean end-systolic left ventricular internal dimension, consequently lower fractional shortening, and lower left ventricular ejection fractions compared with normal controls. Five of the 24 dystrophinopathy heterozygotes had left ventricular ejection fractions < 0.48, which was >2 SDs below the mean for the normal group, whereas all 24 in the normal group had ejection fractions within 2 SDs of the mean (normal group range, 0.48-0.75). Linear regression analysis

Table 2 Diastolic function in heterozygous dystrophinopathy (n = 10)

Variable	Value
E (cm/s)	73 ± 12 (50-90)
A (cm/s)	62 ± 11 (51-75)
E/A	1.2 ± 0.2 (0.9-1.6)
E′ (cm/s)	12.9 ± 2.8 (8-17)
E/E′	5.9 ± 1.4 (2.9-8.0)

Data are expressed as mean ± SD (range).

Table 3 Exercise test data

Variable	Normal subjects	Patients with heterozygous dystrophinopathy	*P*
Resting heart rate (beats/min)	75 ± 13	81 ± 15	.15
Peak heart rate (beats/min)	170 ± 13	168 ± 15	.62
% maximum predicted heart rate	95 ± 6	93 ± 10	.41
Resting systolic blood pressure (mm Hg)	119 ± 9	120 ± 17	.8
Peak systolic blood pressure (mm Hg)	169 ± 21	158 ± 21	.08
Peak heart rate × blood pressure (mm Hg/min)	28,350 ± 3393	26,434 ± 4137	.09
Exercise time (s)	615 ± 144	460 ± 140	<.001
Exercise left ventricular ejection fraction	0.73 ± .07	0.53 ± 0.14	<.001
Change in ejection fraction	0.11 ± 0.05	−0.03 ± 0.15	<.001

Data are expressed as mean ± SD.

demonstrated no correlation between resting ejection fraction and body mass index ($r^2 = 0.001$, $P = .86$).

The subset of 10 dystrophinopathy heterozygotes who underwent assessments of resting left ventricular diastolic function did not differ from the group as a whole with respect to age (40 ± 6 years), left ventricular ejection fraction (0.49 ± 0.10), end-diastolic dimension (4.5 ± 0.4 cm), or wall thickness (0.8 ± 0.10 cm). Results for this subset are shown in Table 2. Only 1 subject had an E/A ratio < 1.0, and no subjects had E/E′ ratios > 8.0.

Response to Exercise

Heart rate and blood pressure at rest and during peak exercise were similar between dystrophinopathy heterozygotes and normal subjects. However, dystrophinopathy heterozygotes had significantly lower exercise times compared with normal subjects. Linear regression analysis indicated that exercise time was negatively correlated with body mass index in dystrophinopathy heterozygotes ($r^2 = 0.43$, $P = .001$; Table 3).

Ejection fraction response to exercise was markedly abnormal in the heterozygous dystrophinopathy group. Whereas exercise increased left ventricular ejection fractions in all 24 normal subjects (range, +0.02 to +0.22), dystrophinopathy heterozygotes, as a group, demonstrated decreased ejection fractions (range, −0.46 to +0.22) ($P < .001$). Ejection fraction data for individuals are shown in Figure 1. Thirteen of 24 individual dystrophinopathy heterozygotes, including 11 with normal resting ejection fractions, exhibited decreases in ejection fractions with exercise, a distinctly abnormal response. Linear regression analysis demonstrated no significant correlation between ejection fraction response to exercise and body mass index among dystrophinopathy heterozygotes ($r^2 = 0.03$, $P = .45$). Neither resting ejection fraction nor the ejection fraction response to exercise correlated well with exercise time in dystrophinopathy heterozygotes ($P = .48$ and $P = .71$, respectively).

Regional Left Ventricular Function

Thirteen of 24 dystrophinopathy heterozygotes developed new exercise-induced wall motion abnormalities in ≥1 segment (range, 0-4 per subject), including 5 of 8 subjects who had normal resting ejection fractions and who also had increased global ejection fractions with exercise. Twenty-one new regional wall motion abnormalities were identified in those 13 subjects and were preferentially located in the mid (n = 13) or apical (n = 8) third of the left ventricle but did not demonstrate a preference for any circumferential region (eg, anterior vs inferior). Only 1 of 24 control subjects developed a new wall motion abnormality with exercise ($P = .003$ for the proportion vs dystrophinopathy heterozygotes). Still-frame images from a dystrophinopathy heterozygote, depicting exercise-induced worsening of inferior wall motion, are shown in Figure 2. Moving images can be viewed in Videos 1 and 2. (View video clips online).

Overall Prevalence of Abnormal Findings on Stress Echocardiography

Five of 24 dystrophinopathy heterozygotes had resting left ventricular ejection fractions < 0.48 and were considered abnormal. Of the remaining 19, 11 had decreased ejection fractions with exercise, an abnormal result. Of the remaining 8 study subjects, 5 had new regional wall motion abnormalities with exercise. Thus, 21 of 24 dystrophinopathy heterozygotes demonstrated ≥1 abnormal finding by echocardiography, for an overall prevalence of 88%, whereas only 1 of 24 normal subjects demonstrated any abnormality, for a prevalence of 4% ($P < .001$ for the comparison of proportions).

Electrocardiography

There was no evidence of left ventricular hypertrophy, conduction delay, or sustained arrhythmia at rest or during exercise in either group. Three of 24 dystrophinopathy heterozygotes and no controls met electrocardiographic criteria for exercise-induced "ischemia": >1-mm horizontal or down-sloping ST-segment depression in ≥2 contiguous leads[9] ($P = .49$).

DISCUSSION

The most important finding of this study is that dystrophinopathy heterozygotes, as a group, demonstrate impaired left ventricular systolic function at rest and/or with exercise. Individually, ≥1 indicator of global or regional systolic dysfunction was present in 88% of dystrophinopathy heterozygotes.

As the genetic basis for inherited cardiomyopathy becomes better understood,[14] physicians are challenged by the need to identify

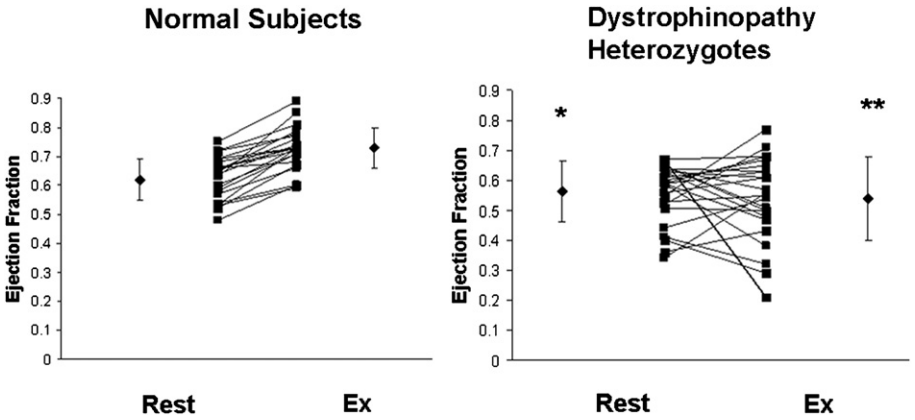

Figure 1 Left ventricular ejection fraction. *(Left)* Ejection fraction at rest and with exercise (Ex) in normal subjects. *(Right)* Data for dystrophinopathy heterozygotes. Group data are expressed as mean ± SD. *P = .02 versus normal; **P < .001 versus normal.

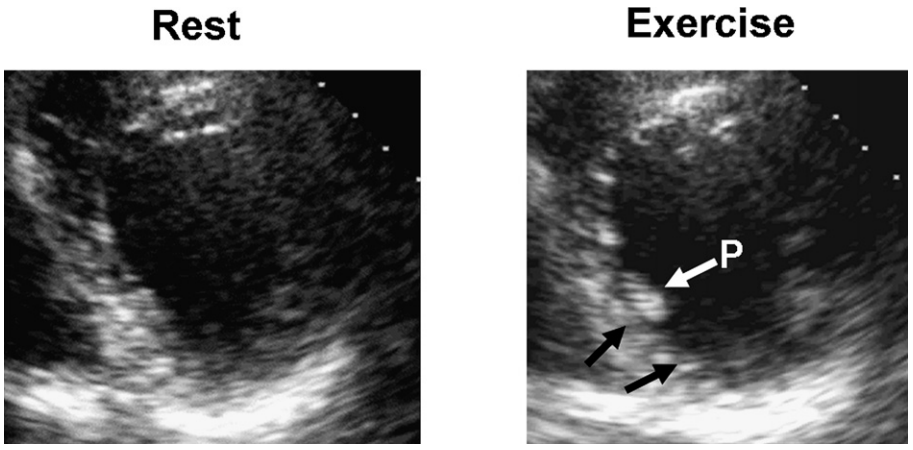

Figure 2 Exercise echocardiography. End-systolic frames obtained at rest *(left)* and after exercise *(right)* in a dystrophinopathy heterozygote, in the parasternal short-axis view. *Dark arrows* indicate mid inferior myocardium, underlying the posteromedial papillary muscle (P), which demonstrates worse systolic function after exercise (see Video 1; view video clip online).

persons at risk to optimize the timing of therapeutic interventions aimed at avoiding overt end-stage heart failure. Screening may consist of routine clinical examination only or may also include electrocardiography and possibly routine two-dimensional echocardiography. However, this approach can suffer from limited sensitivity.[15]

It is known that some women heterozygous for dystrophinopathy progress to overt syndromic cardiomyopathy, but they probably represent a minority. Indeed, Holloway et al[16] reported several instances of early cardiopulmonary death (at ages 21 and 41 years) using retrospective review of death certificates from "carriers" of Duchenne or Becker muscular dystrophy. However, actuarial analysis did not identify an effect on longevity in the cohort as a whole in that study, raising the question of whether systematic cardiac surveillance is warranted for dystrophinopathy carriers.

The findings and recommendations by Holloway et al[16] appear to be at odds with American Heart Association and American College of Cardiology guidelines, which identify asymptomatic patients with "structural heart disease," including abnormal ejection fraction, as having stage B heart failure.[17] Increased attention to risk factor modification and, in selected cases, therapeutic intervention are recommended for that group.[17] For example, patients with asymptomatic left ventricular dysfunction have been shown to be less likely to progress to overt heart failure when they are treated with angiotensin-converting enzyme inhibitors.[18]

Exercise time was poorly correlated with either resting or exercise ejection fraction in this study, a finding in keeping with prior studies that showed similarly poor correlations between ejection fraction and functional status in patients undergoing evaluation for heart failure[19,20] and those with coronary artery disease.[21]

There was no significant correlation between resting left ventricular ejection fraction and the ejection fraction response to exercise in dystrophinopathy heterozygotes. Some women with impaired resting ejection fractions were able to increase their ejection fractions with exercise. This finding has been reported previously in patients with "idiopathic" cardiomyopathy.[22,23]

Exercise caused new regional wall motion abnormalities in 13 of 24 dystrophinopathy heterozygotes, yet 5 of those subjects actually had increased ejection fractions with exercise. This finding is reminiscent of findings from studies in coronary artery disease, in which patients with (only) single-vessel disease, as a group, tended to have increased ejection fractions with exercise, despite the appearance of regional wall motion abnormalities.[24]

Our findings challenge the designation of women heterozygous for X-linked dystrophin mutations as "carriers," because they are prone to

develop cardiac functional abnormalities with a high prevalence. The term "carrier" denotes heterozygosity for a recessive gene. However, for X-linked genes such as dystrophin, each somatic cell inactivates one allele.[25] Thus, a heterozygous woman will express only mutant dystrophin protein in some of her somatic cells, and only the normal allele in the remainder. Our findings indicate that mechanical dysfunction occurs in a sufficient proportion of cardiac myocytes to result in whole-organ systolic dysfunction at rest in a minority of dystrophinopathy heterozygotes, and that latent dysfunction is unmasked by exercise in the majority.

Others have reported variable prevalence of resting cardiac abnormalities in dystrophinopathy heterozygotes. In an Italian cohort, contractile impairment was seen in about 11%, although subtler "preclinical" findings were present in 84%.[6] In an unblinded registry report from the Netherlands, 5% of dystrophinopathy heterozygotes had "dilated cardiomyopathy," and an additional 18% had left ventricular dilation without detectable contractile dysfunction.[26] The present study reports resting systolic dysfunction in 21% of dystrophinopathy heterozygotes, which is somewhat more frequent than prior reports. The apparent difference is possibly more striking when it is realized that our study cohort excluded symptomatic women. It is possible that the respective cohorts are genetically different, with respect to actual dystrophinopathy genotype or other genetic covariates.

A number of mechanisms could explain the unmasking of cardiac functional impairment by exercise. Dystrophin is a component of the dystrophin-glycoprotein complex, a cytoskeletal assembly that links sarcomeres to the extracellular matrix. Disruption of this complex theoretically could cause ineffective cardiac muscle shortening. Prior to the development of overt cardiomyopathy, dystrophin-deficient mice demonstrate afterload intolerance.[8] In the present study, systolic blood pressure rose by about 32%, which may have been sufficient to unmask afterload intolerance in dystrophinopathy heterozygotes.

Mice with disruption of δ-sarcoglycan or γ-sarcoglycan, also components of the dystrophin-glycoprotein complex, develop exercise-induced coronary vasospasm and consequent myocardial ischemia.[27,28] Boys with Duchenne muscular dystrophy develop exercise-induced skeletal muscle ischemia as a consequence of unopposed sympathetic vasoconstriction, arising from dystrophinopathy-induced uncoupling of neuronal nitric oxide synthase from sarcolemma.[29] Thus, ischemia due to exercise-induced microvascular dysfunction could explain the high prevalence of cardiac dysfunction in dystrophinopathy heterozygotes in our study. These women may thus comprise a subset of "syndrome X": myocardial ischemia in the absence of epicardial coronary stenosis.[30]

Although we demonstrated cardiac function abnormalities in the dystrophinopathy heterozygote group as a whole, there was considerable variation among individuals. Genetic variables may influence cardiac function. The pattern of X inactivation can be skewed in favor of either the normal or the mutant allele.[31] Women who have a higher percentage of dystrophin-deficient cardiomyocytes may be more prone to cardiac dysfunction.

Limitations

The sample size of this study was relatively small and was restricted to a single time point for each subject. Our study included only active asymptomatic women and thus probably tended to minimize the functional impact of the dystrophin mutation.

In most cases, data for this study were gathered using a standard clinical exercise stress echocardiographic protocol. Diastolic function was assessed only in a minority subset of dystrophinopathy heterozygotes, which did not differ from the group as a whole with respect to left ventricular size, wall thickness, or resting systolic function. The subset demonstrated no clear evidence of diastolic dysfunction at rest, although one subject had an E/A ratio of 0.9. However, recent studies using more sophisticated analysis of ventricular torsion and/or strain rate have uncovered abnormalities in other forms of inherited cardiomyopathy.[32-34] Further studies will be needed to determine whether dystrophinopathy heterozygotes demonstrate similar abnormalities.

CONCLUSION

Women heterozygous for a disease-associated dystrophin mutation have a high prevalence of left ventricular systolic dysfunction, which can be unmasked by exercise testing. Exercise testing may prove useful in identifying cardiac abnormalities in subjects with this and other genotypes. Long-term longitudinal studies are needed to further clarify the prognostic importance of this finding.

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