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Age-matched comparison reveals early electrocardiography and echocardiography changes in dystrophin-deficient dog

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Abstract

The absence of dystrophin in the heart leads to Duchenne cardiomyopathy. Dystrophin-deficient dogs represent a critical model to translate novel therapies developed in mice to humans. Unfortunately, little is known about cardiophysiology changes in these dogs. We performed prospective electrocardiographic and echocardiographic examinations at 3, 6 and 12 months of age in four normal and three affected dogs obtained from the same litter. Affected dogs showed growth retardation and serum creatine kinase elevation. Necropsy confirmed cardiac dystrophin deficiency and histopathology. *Q*/*R* ratio elevation and diastolic left ventricular (LV) internal diameter reduction were the most consistent findings in affected dogs at all ages. At 6 and 12 months, dystrophic dogs also showed significant reduction of PR intervals, LV end diastolic/systolic volumes and systolic LV internal diameters. Epicardial and endocardial slope times were significantly reduced in affected dogs at 12 months. These results establish the baseline for evaluating experimental therapies in the future.

Keywords

Dystrophin; Duchenne muscular dystrophy; Cardiomyopathy canine; Dog; Electrocardiography; ECG; Echocardiography; Golden retriever muscular dystrophy (GRMD); Labrador muscular dystrophy; Large animal model; Heart

1. Introduction

The dystrophin gene is one of the largest genes in the genome. It encodes a 427 kD sub-sarcolemmal protein called dystrophin. Dystrophin plays an essential role in protecting the muscle cell from contraction-induced injury [1]. Thousands of different mutations have been reported in human patients [2–4]. Many abolish dystrophin expression in both skeletal and cardiac muscles. Occasionally, mutations are found to selectively eliminate dystrophin expression in the myocardium. The major consequence of dystrophin deficiency in the heart

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is Duchenne cardiomyopathy, a life threatening disease [5,6]. Affected patients often show an abnormal electrocardiogram (ECG). Hemodynamic alterations become apparent as the disease progresses. It is generally believed that end-stage patients develop dilated cardiomyopathy and die from heart failure [7]. Currently, there is no cure [8,9].

Dystrophin-deficiency also results in cardiomyopathy in mice but at a much slower pace [10–12]. Characteristic ventricular dilation is not detected in dystrophin-null mice until near the end of their life span [10]. A large animal model, such as the dog, might better meet the needs of translational research in developing novel therapies to treat this lethal disease.

Dystrophin-deficient dogs have been reported in many breeds (reviewed in [13,14]). Gene mutations have been determined in several cases. In golden retriever muscular dystrophy (GRMD) dogs, a point mutation disrupts normal splicing [15–17]. In Labrador muscular dystrophy (LMD) and corgi muscular dystrophy dogs, dystrophin expression is terminated as a consequence of repetitive DNA element insertion [14,18].

While ECG and echocardiographic changes are well established in human Duchenne cardiomyopathy patients (reviewed in [19,20]), very few studies have been performed in dystrophin-deficient dogs. Importantly, age-matched comparisons have never been conducted. To establish dystrophin-deficient dogs as a valid model for Duchenne cardiomyopathy, we performed prospective ECG and echocardiographic examinations at 3, 6 and 12 months in a cohort of four normal and three affected dogs obtained from the same litter. We observed growth (body weight)-related changes as reported before [21,22]. We also identified statistically significant differences between normal and affected dogs in some ECG and echocardiography measurements. Specifically, *Q*/*R* ratio reduction was observed in leads II, III and aVF in affected dogs at all three time points. PR interval was significantly reduced in affected dogs at 6 and 12 months of age. Affected dogs also showed a higher heart rate at every time point but the difference did not reach statistical significance. Doppler echocardiography results were less informative. However, several parameters of 2D and M-mode echocardiography showed significant changes between normal and affected dogs at the ages of 6 and 12 months. These include left atrial long axis (LAlax), left ventricular end diastolic and end systolic volumes (LVEDV and LVESV), left ventricular internal diameter at diastole and at systole (LVIDd and LVIDs) and M mode left atrial size (LAMM). LAlax and LVIDd also showed significant differences at the 3 months of age. In addition, significant differences between normal and affected dogs were observed in epicardial slope time (EpST) and endocardial slope time (EnST) by the age of 12 months.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Animal Care and Use Committee of the University of Missouri and were performed in accordance with NIH guidelines. Experimental dogs were produced by in vitro insemination using the semen from Remus, an affected LMD male, to Sequoia, a GRMD carrier at the University of Missouri. Sequoia was donated by Dr. Joe Kornegay (University of North Carolina at Chapel Hill). Remus was housed at Auburn University [18]. Semen was collected at Auburn University and shipped overnight to the University of Missouri. Vaginal inseminations were performed 4 and 6 days after the ovulatory luteinizing hormone surge as diagnosed by means of serial serum progesterone tests. A total of eight puppies were born in the litter. They include three affected dogs, four normal dogs and one GRMD carrier. Affected and normal dogs were used in this study. All dogs survived throughout the entire course of the experiment. Touta, an affected female was utilized in an invasive skeletal muscle function assay (unrelated to the current study) after the last cardiac function evaluation was finished. This dog was

subsequently euthanized and the heart tissue was examined by immunofluorescence and histopathology.

2.2. PCR genotyping

Samples of umbilical cord (5 mm long) from the newborn puppy were prepared as we described [14]. The genotype of the GRMD mutation locus was determined by restriction enzyme digestion of the PCR products as described [23]. The wild type allele yields a 328 bp band. The mutant allele yields a 107 and a 221 bp band [23]. The details of LMD genotyping will be published elsewhere (Smith, Shin and Duan, manuscript in preparation). The normal allele yields a 500 bp band and the affected allele yields a 400 bp band.

2.3. Serum creatine kinase (CK) assay

Fresh serum was collected from the jugular vein. The CK levels were determined at the diagnostic laboratory at the veterinary medical teaching hospital, the University of Missouri.

2.4. Immunofluorescence staining and histopathology

The normal heart was from Frank, an unaffected female golden retriever we reported previously [24]. The affected heart was from Touta. Dystrophin was examined with a mouse monoclonal antibody against the C-terminal domain (Dys-2, 1:30; Novocastra, Newcastle upon Tyne, UK). Utrophin was examined with a mouse monoclonal antibody against the utrophin N-terminal domain (VP-U579, 1:20; Vector Laboratories, Burlingame, CA). Haematoxylin and eosin (HE) staining was used to reveal general histology. Fibrosis was evaluated with Masson trichrome staining. Immunostaining and histology staining was performed essentially as previously described [10,14].

2.5. Electrocardiography

To minimize inter-examiner variances [25], all ECGs were performed by a single investigator (DMF, a board certified veterinary cardiologist) with the dog genotype blinded to the investigator. Dogs were gently restrained in right lateral recumbency. ECG was obtained using a Philips Pagewriter Xli ECG machine, model #M1700A (Royal Philips Electronics, Eindhoven Area, Netherlands). ECG leads were positioned using the hexaxial and precordial lead systems [26]. Leads I, II, III, aVR, aVL, aVF, V1, V2, V3, and V10 were recorded in all dogs. Standard measurements of intervals and amplitudes of the *P*, QRS, and *T* waves were performed using lead II at a recording speed of 50 mm/s, and a calibration of 1 cm/mV. The *Q*, *R*, and *S* amplitudes and the *Q*/*R* and *R*/*S* ratios were calculated in each lead. Evaluation of heart rate, and rhythm disturbances were made from recordings obtained at 25 mm/s.

2.6. Echocardiography

Echocardiography was performed using a GE VIVID 7 ultrasound machine (GE Healthcare, Wautesha, WI) by a single investigator (DMF) without prior information of the dog genotype [25]. Probe selection was based upon image optimization and ranged from 3 to 7 mHz. Two-dimensional, M-mode, and Doppler echocardiographic examinations were performed utilizing standard views in unsedated dogs [27]. Two dimensional measurement of the left atrium (LA) was performed from a right-parasternal long-axis four chamber view just before opening of the mitral valve. The line of measurement bisected the atrium between the atrial septum and free wall [28]. Transmitral filling velocities were recorded with pulsed-wave Doppler with the sample volume placed at the tips of the open mitral valve leaflets. Mitral annular motion velocity was measured at the basilar left ventricular free wall with pulsed-wave tissue Doppler imaging using a 2 mm sampling volume. Doppler gain was minimized to optimize signal quality. Left ventricular volume measurements for

calculation of stroke volume and ejection fraction were obtained from a right-parasternal long-axis four-chamber view, using the biplane area-length (disk summation) method [29]. Left ventricular short-axis area was measured from a right-parasternal short-axis view by tracing the endocardial borders, and excluding the papillary muscles. Cardiac index was calculated as [(stroke volume × heart rate)/body surface area]. Body surface area in dogs is calculated as [$10.1 \times (\text{weight in kg})^{2/3}/100 = M^2$]. Heart rate for calculation of cardiac index was measured using the same cardiac cycles from which stroke volume was determined. Three to five consecutive cardiac cycles were measured and averaged for each variable.

2.7. Statistical analysis

Statistical analysis was performed by the Biostatistics Group, Office of Research, University of Missouri using a two-factor analysis of variance with one factor (group with 2 levels, affected and non-affected) being a between subjects factor and the second factor (time or age with 3 levels, 3, 6, and 12 months) being a within subjects factor. The analysis was done using the mixed procedure in SAS v 9 (SAS Institute Inc., Cary, NC, USA) with the repeated option used to account for the dependencies inherent in having repeated measures on the same subject over time. A group by time interaction was included in the models. After comparing models using compound symmetry and auto-regressive of order 1 to model the correlation structure it was decided that the ar(1) option was a better choice. Examination of residuals from the model fit showed that the assumption of normality of the residuals was reasonable. In view of the large number of outcome variables being considered, we determined that using a significance level of 0.01 would control the false discovery rate (difference was considered significant when $P < 0.01$). If the group by time interaction term was significant then least squares means were used to determine at which times the groups may have differed.

3. Results

3.1. Generation of wild type and dystrophin-deficient dogs

To generate experimental animals, we inseminated a female GRMD carrier using the semen from an affected LMD male. The breeding yielded a litter of eight puppies consisting four wild type (Bolt, Lion, Romeo, Cougar), three affected (Tiger, Goldie and Touta) and one GRMD carrier dogs (Fig. 1 and data not shown). Wild type and affected puppies were used in this prospective study.

The male affected dog (Tiger) carried the GRMD mutation. The female affected dogs (Goldie and Touta) carried the GRMD mutation in one allele and the LMD mutation in the other allele (Fig. 1A). The CK values of affected dogs were at least 100-fold higher than those of normal dogs at birth and 1 month of age (Fig. 1B). Skeletal muscle biopsy at 6 months of age further confirmed the diagnosis of dystrophin deficiency (data not shown).

The average body weight at birth for affected puppies was 415 g (range, 410–419 g). The average body weight at birth for wild type puppies was 384 g (range, 365–418 g). There was no significant difference [14]. Postnatal growth was observed in both affected and wild type puppies until it reached a plateau at 6 months of age. However, significant growth retardation was observed in affected puppies (Fig. 1C, Table 1). The body weight of affected puppies was significantly lower than that of wild type puppies at 3, 6 and 12 months (Fig. 1C, Table 1).

Touta was euthanized after the last ECG and echocardiographic measurement for reasons unrelated to this study. As expected, immunofluorescence staining demonstrated an absence of dystrophin expression and up-regulation of utrophin in the heart (Fig. 1D). On Masson trichrome staining, we also observed intramyocardial fibrosis (Fig. 1E).

3.2. Q/R ratio increase was the most consistent ECG finding in affected dogs

It has been reported that in dogs less than 1-year-old the heart rate drops significantly when a dog ages [21,30,31]. We observed similar changes (Table 1). Further, affected dogs showed a trend toward higher heart rates at all three time points but the differences did not reach statistical significance (Table 1).

Compared to that of normal dogs, the PR interval was reduced in 3-month-old affected dogs but this difference was not statistically significant. A statistically significant decrease of the PR interval was observed in 6 and 12-month-old affected dogs compared to those of age-matched normal controls (Table 1).

The most consistent ECG change was an increase of the *Q*/*R* ratio in affected dogs. Statistically significant differences were observed in leads II, III and aVF at every time point (Table 1). In leads II, III and aVF, the mean *Q* wave was deeper in affected dogs than that of age-matched wild type dogs but it did not reach statistical significance (Table 1).

The predominant rhythm finding in affected and wild type dogs at all study time points was a sinus rhythm. Occasionally, we also noticed isolated ventricular premature contractions in the affected dogs. Besides those discussed above, changes in other leads and/or ECG parameters were not remarkable.

3.3. Echocardiographic examination revealed consistent LVIDd reduction in dystrophin-deficient dogs

To evaluate cardiac function, we performed two-dimensional (2D), M-mode and Doppler echocardiography (Table 1). 2D examinations of affected dogs consistently showed a significantly smaller left atrial long axis (LAlax) compared to that of age-matched normal controls (Table 1). The left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volumes (LVESV) were significantly reduced in affected dogs at 6 and 12-month time points (Table 1).

The most remarkable change in M mode echocardiography was the left ventricular internal diameter in diastole (LVIDd). Affected dogs showed significant LVIDd reduction in all time points (Table 1). The left ventricular internal diameter in systole (LVIDs) and left atrial size (LAMM) were significantly reduced in affected dogs at the ages of 6 and 12 months (Table 1). Epicardial slope time (EpST) and endocardial slope time (EnST) were nominally reduced in affected dogs at 3 and 6 months of age. However, 12-month-old affected dogs showed significantly decreased EpST and EnST (Table 1).

In Doppler echocardiography, affected dogs appeared to have a lower left ventricular outflow tract velocity time integral (LVOTVTI), mitral valve *E* wave deceleration time (MADT) and mitral valve *E* wave to *A* wave ratio (ME/A ratio) (Table 1). However none of these changes were statistically significant.

4. Discussion

Dystrophin-deficient dogs are valuable tools for translational Duchenne cardiomyopathy studies. Unfortunately, they can be one of the most challenging models to work with. In some cases, less than half of affected dogs survive beyond 6 weeks of age [17]. Considering the high costs related to canine colony maintenance, investigators are often forced to depend on very few subjects in their proof-of-principle studies [24,32–36]. For this reason, establishing a set of robust functional end points is imperative.

A handful of studies have examined ECG and echocardiography changes in dystrophin-deficient dogs [35,37–41]. Unfortunately, many studies were not carefully controlled. Often puppies and adult dogs of great age variance were grouped together. For example, Moise et al. compared ECG and echocardiography recordings between 11 carrier dogs and 13 affected dogs (7 male and 6 female). The carrier dogs were used in lieu of normal dogs and were examined at the ages of 21–48-months-old. However, the age of affected dogs ranged from 2-months-old to 6-years of age [37]. The limited sample size is another issue. There is usually only one dog at each representative age [37,38,40]. Considering the dramatic influence of the age and body weight on dog cardiovascular physiology [42,43], it is not surprising that these reported findings are of limited guidance to pre-clinical translational research.

To determine whether statistically meaningful ECG and echocardiography changes could be obtained from a relatively small number of dystrophin-deficient dogs, we performed an age-matched prospective study at 3, 6 and 12 months in four wild type and three affected dogs. These time points were chosen based on a study that has examined cardiomyopathy development in GRMD dogs [44]. Valentine et al. demonstrated that there were no cardiac lesions in affected dogs at 3 months of age. Focal histological lesions were found in the heart of 6-month-old affected dogs and by 12 months of age, myocardial degeneration and fibrosis were detected in all affected dogs [44].

To limit variables we have taken several precautions. First, all experimental subjects were from the same litter with similar birth weights. Second, ECG and echocardiography were performed by the same examiner who is a board certified veterinary cardiologist. All the assays were performed with the genotype information blinded to the examiner. Third, dystrophin deficiency was confirmed in every dog by PCR genotyping, serum CK assay and skeletal muscle biopsy (Fig. 1).

Abnormal ECG recordings are seen in up to 90% of DMD patients. The most common ECG findings include sinus tachycardia, PR (or PQ) interval reduction, QT interval prolongation, and a notched QRS wave, a deep *Q* wave, a tall *R* wave and a high *R*/*S* ratio. Further, ECG changes can be detected at the early stages prior to the development of clinically evident cardiac symptoms [45–57]. In our study, we observed a trend of tachycardia and deeper *Q* waves in affected dogs. However, these differences did not reach statistical significance (Table 1). The most consistent ECG finding is the significant elevation of the *Q*/*R* ratio in affected dogs. It was observed in leads II, III and aVF at all time points (Table 1). We also observed significant PR interval reduction in affected dogs at 6 and 12 months of age (Table 1).

Very little is known about the cardiac structural changes underlying abnormal ECG findings in DMD patients. Several hypotheses have been proposed including loss of myofilaments, regional ischemia, myofiber degeneration and myocardial fibrosis [45,46,49,55,58]. However, except for the link between heart Purkinje fiber vacuolar degeneration and the deep *Q* wave, none of the other hypotheses have been confirmed experimentally [58]. Despite the observed ECG abnormalities, two retrospective studies suggest that ECG changes may have limited prognostic value in DMD patients [56,57]. Interestingly. ECG amelioration has been observed after various experimental therapies in the mouse model of DMD [10,11,59–61]. It is currently not clear whether ECG monitoring can be used to reflect the effect of therapeutic interventions in human patients and dystrophin-deficient dogs.

The echocardiographic profile has also been established for Duchenne cardiomyopathy (reviewed in [19,20]). Typical changes may include abnormal regional wall motion, hyperechoic lesions, chamber dilation and systolic/diastolic dysfunction (reviewed in

[19,20]). Here, we also observed several statistically significant changes in 2D and M mode echocardiography (Table 1). Of particular interest is LVIDd and LVIDs. LVIDd was significantly reduced in every time point in affected dogs and LVIDs was significantly reduced in 6 and 12-month-old affected dogs (Table 1). A recent retrospective study of 367 dogs suggests that the left ventricular diameter may represent a reliable prognostic indicator [62]. It is conceivable that LVIDd and LVIDs may represent some of the most informative parameters in evaluating cardiomyopathy progression in dystrophic dogs. It has been suggested that the progression of DMD heart disease undergoes a hypertrophic stage before ventricular dilation [63,64]. Consistent with this notion, we also detected significant left ventricular volume reduction at both systole and diastole in affected dogs at 6 and 12 months of age (Table 1). The reduction in left atrial size (LAlax by 2D and LAMM by M mode) and LA/Ao ratio also appeared as consistent findings in affected dogs, especially at the ages of 6 and 12 months (Table 1). Other significant changes in echocardiography included a significant reduction of EpST and EdST in 12-month-old affected dogs. Future studies are needed to further confirm these observations and understand their clinical implication.

Studies from one group suggest that tissue Doppler analysis of ventricular myocardial velocity may represent a sensitive index to detect early abnormalities in affected dogs [40,41]. However, we have not been able to obtain a consistent change in any Doppler parameters (Table 1). The reason behind the difference between our results and that of Chetboul et al. is not clear but it may relate to the sample size and/or dog age.

We would also like to point out several potential limitations of our study. First, the number of experimental subjects was relatively low although it is quite typical in the field of canine muscular dystrophy research [24,32–36]. It is very likely that a much larger experimental size may be needed to reach a statistically meaningful result in some ECG and echocardiographic parameters. Second, all wild type dogs used in this study were male. However, only one affected dog was male and the other two were female. Due to the difficulties in obtaining affected dogs [17], many studies have mixed male and female affected dogs in the same group [35,37]. Whether the gender affects the progression of cardiomyopathy in affected dogs remains to be determined. Nevertheless, we have recently found that gender influences the cardiac phenotype in dystrophin-deficient mice [65]. Caution should be taken when interpreting data derived from a male/female mixed group. Third, the affected dogs were obtained by crossing a LMD male with a GRMD female carrier. We demonstrated that dystrophin expression was eliminated in puppies carrying both mutations (Fig. 1D and data not shown). Since Duchenne cardiomyopathy occurs in all humans irrespective of the race, we feel that the mixed genetic background may be more representative of human patients. However, we cannot exclude the possibility that different dystrophin-null mutations may affect the cardiac phenotype.

Traditionally, dystrophin-deficient mice are the most commonly used animal models in Duchenne muscular dystrophy research. Because of the translational gap often encountered between mice and humans and also because of the phenotypic resemblance and the body size similarity between canines and humans, many investigators have begun to use dystrophin-deficient dogs in their study. Our study here has revealed several early electrocardiographic and echocardiographic changes in dystrophin-deficient dogs. These findings will be very useful in gauging therapeutic effect of novel gene/cell/pharmacological therapies in dystrophin-deficient (especially in young adult) dogs.

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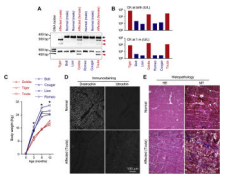

Fig. 1.

Characterization of the experimental subjects. (A) PCR genotyping. Top panel, GRMD allele genotyping. The normal allele yields a 328 bp band and the affected allele yields two bands (107 and 221 bp). Bottom panel, LMD allele genotyping. The normal allele yields a 500 bp band and the affected allel yields a 400 bp band. Arrow, normal allele; arrowhead, affected allele. (B) Serum creatine kinase (CK) level at birth (top panel) and 1 month of age (bottom panel). (C) Body weight changes. Values at the 0 month are body weights at birth. Asterisk, significantly higher than that of affected puppies at the same age. (D) Representative images of immunofluorescence staining. Top panels, serial heart sections from a normal dog; bottom panels, serial heart sections from Touta, an affected dog. Left panels, dystrophin immunostaining; Right panels, utrophin immunostaining. (E) Representative photomicrographs of histology staining from serial heart sections. Top panels, normal; bottom panels, Touta (affected); Left panels, HE staining; right panels, Masson trichrome (MT) staining. Fibrotic tissues are in blue color.

Table 1

ECG and echocardiography characterization.

		3-m-old		6-m-old		12-m-old	
		Normal (mean ± SEM)	**Affected (mean ± SEM)**	**Normal (mean ± SEM)**	**Affected (mean ± SEM)**	**Normal (mean ± SEM)**	**Affected (mean ± SEM)**
Body weight (kg)		15.1 ± 0.6	9.9 ± 0.6*	25.6 ± 1.1	15.3 ± 0.2*	26.3 ± 1.3	19.1 ± 0.8*
ECG leads							
II	Heart rate (bpm)	144.8 ± 2.3	154.0 ± 9.0	127.0 ± 5.5	145.7 ± 6.4	84.8 ± 4.6	111.7 ± 7.4
	PR interval (ms)	85.0 ± 5.0	76.7 ± 3.3	105.0 ± 5.0	80.0 ± 0.0*	127.5 ± 7.5	90.0 ± 5.8*
	Q wave (mV)	0.5 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	1.5 ± 0.1	0.9 ± 0.3	1.5 ± 0.3
	Q/R ratio	0.2 ± 0.0	0.4 ± 0.1*	0.3 ± 0.0	0.7 ± 0.0*	0.3 ± 0.1	0.6 ± 0.1*
III	*Q* wave (mV)	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.1
	Q/R ratio	0.2 ± 0.1	1.0 ± 0.3*	0.2 ± 0.0	0.8 ± 0.3*	0.3 ± 0.0	0.7 ± 0.1*
aVF	*Q* wave (mV)	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.3 ± 0.2
	Q/R ratio	0.2 ± 0.1	0.6 ± 0.1*	0.3 ± 0.0	0.9 ± 0.2*	0.4 ± 0.0	0.7 ± 0.0*
Echocardiography							
2D	LAlax (cm)	2.8 ± 0.0	2.5 ± 0.1*	3.6 ± 0.1	3.0 ± 0.1*	4.0 ± 0.1	3.4 ± 0.1*
	LVEDV (ml)	28.1 ± 0.6	19.2 ± 2.4	47.3 ± 1.6	30.9 ± 3.5*	57.8 ± 4.2	32.6 ± 4.5*
	LVESV (ml)	9.1 ± 0.3	6.9 ± 1.2	18.6 ± 1.1	8.9 ± 1.6*	22.6 ± 1.1	10.4 ± 2.0*
	LVEF (%)	67.8 ± 0.7	64.1 ± 3.2	60.6 ± 2.5	71.6 ± 2.4*	60.6 ± 1.3	67.7 ± 5.2
	SV (ml)	19.1 ± 0.5	12.2 ± 1.4	28.7 ± 1.7	22.0 ± 1.9	35.2 ± 3.1	22.2 ± 3.8
M-mode	CI (l/min m^2)	4.5 ± 0.1	4.0 ± 0.5	4.2 ± 0.3	5.6 ± 0.4	3.3 ± 0.3	3.9 ± 0.8
	FS (%)	36.9 ± 2.5	35.0 ± 1.2	33.7 ± 0.5	39.3 ± 2.6	32.3 ± 1.7	38.8 ± 6.5
	IVSd (cm)	0.7 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	1.0 ± 0.1	0.9 ± 0.1
	IVSs (cm)	1.1 ± 0.1	0.9 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.4 ± 0.0	1.3 ± 0.2
	LVIDd (cm)	3.0 ± 0.0	2.5 ± 0.2*	3.6 ± 0.1	2.9 ± 0.1*	4.1 ± 0.2	3.2 ± 0.1*
	LVIDs (cm)	1.9 ± 0.1	1.7 ± 0.1	2.4 ± 0.1	1.8 ± 0.1*	2.8 ± 0.1	2.0 ± 0.2*
	LVPWd (cm)	0.7 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.9 ± 0.0	1.0 ± 0.1
	LVPWs (cm)	1.1 ± 0.1	0.9 ± 0.0	1.2 ± 0.0	1.0 ± 0.0	1.4 ± 0.1	1.3 ± 0.1

		3-m-old		6-m-old		12-m-old	
		Normal (mean ± SEM)	**Affected (mean ± SEM)**	**Normal (mean ± SEM)**	**Affected (mean ± SEM)**	**Normal (mean ± SEM)**	**Affected (mean ± SEM)**
	LA/Ao MM	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	0.8 ± 0.0*	0.9 ± 0.0	0.8 ± 0.0
	LAMM(cm)	2.0 ± 0.1	1.7 ± 0.1	2.3 ± 0.1	1.6 ± 0.1*	2.4 ± 0.1	1.9 ± 0.2*
	EpSL (cm)	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
	EpST (ms)	185.0 ± 6.5	186.7 ± 3.3	200.0 ± 7.1	190.0 ± 10.0	230.0 ± 10.8	160.0 ± 10.0*
	EpSV (cm/s)	2.7 ± 0.3	2.1 ± 0.2	3.1 ± 0.3	2.6 ± 0.4	2.3 ± 0.3	3.2 ± 0.7
	EdSL (cm)	0.7 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	0.8 ± 0.1
	EdST (ms)	190.0 ± 10.8	180.0 ± 5.8	185.0 ± 2.9	183.3 ± 12.0	227.5 ± 8.5	156.7 ± 8.8*
	EdSV (cm/s)	3.9 ± 0.3	3.7 ± 0.5	4.9 ± 0.4	4.1 ± 0.5	4.1 ± 0.7	5.3 ± 0.7
Doppler	LVOTVmax (cm)	1.2 ± 0.0	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.0	1.1 ± 0.1
	LVOTVTI (cm)	13.7 ± 0.6	12.6 ± 1.1	15.3 ± 0.7	12.7 ± 0.8	16.2 ± 0.8	12.3 ± 1.9
	MVDS (m/s^2)	2.7 ± 0.5	2.9 ± 0.3	3.3 ± 0.4	3.0 ± 0.2	2.0 ± 0.0	2.2 ± 0.1
	MVDT (ms)	50.5 ± 7.3	41.0 ± 3.6	48.9 ± 4.0	45.8 ± 5.7	83.8 ± 4.9	68.2 ± 5.6
	MEV (m/s)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
	MAV (m/s)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	ME/A ratio	2.1 ± 0.2	1.8 ± 0.1	2.0 ± 0.3	1.7 ± 0.1	2.6 ± 0.2	2.1 ± 0.2

Abbreviations used in 2D echocardiography: LAlax, left atrial long axis; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVEF, left ventricular ejection fraction; SV, stroke volume.

Abbreviations used in M-mode echocardiography: CI, cardiac index; FS, fraction shortening; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior in diastole; LVPWs, left ventricular posterior in systole; LA/Ao MM, left atrial to aortic M mode ratio; LAMM, left atrial M mode size; EpSL, epicardial slope length; EpST, epicardial slope time; EpSV, epicardial slope volume; EdSL, endocardial slope length; EdST, endocardial slope time; EdSV, endocardial slope volume.

Abbreviations used in Doppler echocardiography: LVOTVmax, left ventricular outflow tract velocity maximum; LVOTVTI, left ventricular outflow tract velocity time integral; MVDS, mitral valve *E* wave deceleration slop; MVDT, mitral valve *E* wave deceleration time; MEV, mitral valve *E* wave inflow velocity; MAV, mitral valve *A* wave inflow velocity; ME/A ratio, mitral valve *E* wave to *A* wave ratio.

* Significantly different from that of the normal controls at the same age group ($p < 0.01$).