Published in final edited form as:

Pediatr Cardiol. 2011 February ; 32(2): 145–153. doi:10.1007/s00246-010-9833-3.

A Juvenile Murine Heart Failure Model of Pressure Overload

Kristopher M. Cumbermack, Jun Cheng, Yibing Nong, William T. Mahle, Ronald W. Joyner, William L. Border, Mary B. Wagner, Derek A. Fyfe, Traci Leong, and Yanggan Wang

Department of Pediatrics, Children's Healthcare of Atlanta and Sibley Heart Center, Emory School of Medicine, Atlanta, GA 30322, USA

Abstract

Persistent pressure overload can cause cardiac hypertrophy and progressive heart failure (HF). The authors developed a pressure-overload HF model of juvenile mice to study the cardiac response to pressure overload that may be applicable to clinical processes in children. Severe thoracic aortic banding (sTAB) was performed using a 28-gauge needle for 40 juvenile (age, 3 weeks) and 47 adult (age, 6 weeks) C57BL/6 male mice. To monitor the structural and functional changes, M-mode echocardiography was performed for conscious mice that had undergone sTAB and sham operation. Cardiac hypertrophy, dilation, and HF occurred in both juvenile and adult mice after sTAB. Compared with adults, juvenile HF is characterized by greater impairment of ventricular contractility and less hypertrophy. In addition, juvenile mice had significantly higher rates of survival than adult mice during the early postoperative weeks. Consistent with clinical HF seen in children, juvenile banded mice demonstrated a lower growth rate than either adult banded mice or juvenile control mice that had sham operations. The authors first developed a juvenile murine model of pressure-overload HF. Learning the unique characteristics of pressure-overload HF in juveniles should aid in understanding age-specific pathologic changes for HF development in children.

Keywords

Echocardiography; Heart failure; Hypertrophy; Murine

The burden of cardiovascular disease remains high in the United States, where heart failure (HF) alone accounts for up to 15 million office visits and 6.5 million hospital days per year. In 2005, expenditures linked to HF approached \$28 billion [11,13]. Many patients, such as those with hypertension, aortic stenosis, and familial hypertrophic cardiomyopathy, often first undergo a stage of cardiac hypertrophy before the development of HF [9,12,23].

Cardiac hypertrophy is a well-documented response of the ventricular myocardium to a hemodynamic stress, such as pressure overload [4,8,10,11,30]. The Framingham Heart Study has shown that the prevalence of left ventricular hypertrophy (LVH) in adults approaches 21% and that LVH significantly increases the risk for HF, ventricular arrhythmias, and sudden cardiac death [11,14,16]. However, the mechanisms underlying hypertrophy development and the transition to HF remain elusive.

Given their relatively low cost, the breadth of their transgenic possibilities, and their genetic similarity to humans, mice have become a common animal model for the study of cardiovascular disease. Thoracic aortic banding (TAB) has been used as a powerful tool to

study cardiac hypertrophy and failure in adult rodent species [1,6,20,25]. However, the cardiac response to pressure overload in the juvenile has not been well characterized. Given the known differences between children and adult patients, an understanding of the juvenile response to pressure overload and the transition to HF is critical to the development of efficient treatment for these morbid diseases in children.

Advances in technology currently allow precise ultrasound-based imaging of small animal species, including newborn and even fetal mice [2,24]. Several studies have demonstrated the feasibility of echocardiography for mice exposed to a diversity of hemodynamic and genetic alterations [3,5,15,17].

Using murine echocardiography, Tanaka et al. [27] detected increased left ventricular fractional shortening (FS) in mice with dobutamine treatment, increased end-diastolic dimensions in mice with volume overload (arteriovenous fistula), and increased wall thickness in mice with pressure overload (aortic banding). They also demonstrated excellent reproducible inter- and intra-rater variability with M-mode analysis. Murine echocardiography enables proper assessment of the cardiac structural and functional changes that occur in mice in response to a variety of pathologic stressors, including those resulting in cardiac hypertrophy and failure.

This study investigated the structural and functional cardiac responses to aortic banding in juvenile mice to better understand the pathophysiologic processes that occur in the development of hypertrophy and HF due to pressure overload. We hypothesized that inherent characteristics of juvenile murine myocardium lead to a distinct response in cardiac structure and function compared with that of adult mice exposed to pressure overload.

Methods

Animal Selection

For this study, 126 C57BL/6 male mice were randomly divided into five experimental groups. The first group consisted of 3-week-old mice that did not undergo intervention. These mice were serially evaluated to determine normal growth and development (body and heart). The remaining mice were stratified into juvenile and adult groups that underwent severe thoracic aortic banding (sTAB) at 3 and 6 weeks of age, respectively. Small matching sham groups for each age category also were created (Fig. 1).

sTAB Procedure

The mice were anesthetized with ketamine (100 mg/kg, administered intraperitoneally [IP]) plus xylazine (5 mg/kg, IP). Adequate sedation was determined by a lack of toe-pinch reflex [28].

A 3-mm left-sided thoracotomy was created at the second intercostal space, and the transverse aortic arch was ligated (7-0 Prolene) between the innominate and left common carotid arteries with an overlying 28-gauge needle. The needle then was removed, leaving a discrete region of stenosis. Successful banding was confirmed with visual confirmation of differential carotid pulsatility.

The chest was closed, and the left-sided pneumothorax was evacuated by a syringe. During this surgical procedure, the chest was closed in 1–2 min. No intubation or mechanical ventilation was required. The procedure resulted in an estimated reduction in the aortic arch diameter from ~1.7 to 0.36 mm in 6-week-old mice and from 1.4 to 0.36 mm in 3-week-old mice, as measured by echocardiography. The control mice for each group underwent sham

operations at equivalent time points with the same process of anesthesia and chest opening but no aortic banding.

About 10% of the mice died of oversedation or bleeding before aortic banding. The first 24-h mortality rate was 15.4% for juvenile mice and 14.6% for adult banded mice. No death occurred among the juvenile or adult mice that underwent the sham operation.

Echocardiography

The VisualSonics Vevo 770 ultrasound system with a 30-MHz mechanical probe was used to obtain two-dimensional images and M-mode tracings in conscious mice before surgical intervention, then each week (± 2 days) after surgery for six consecutive weeks. If mouse heart rates were beyond the 500- to 800-bpm range, the mice were allowed to rest and recover before they were reimaged again at a later time point.

From the parasternal short-axis position, M-mode tracings of the left ventricle (LV) were obtained at the level of the papillary muscles. Wall thicknesses and cavitory dimensions at both end-systole and end-diastole were measured by offline analysis (Fig. 2). An increase in LV wall thickness was used as a marker of LV hypertrophy. Ejection fraction (EF), FS, and LV mass (LVM) were automatically calculated by the VEVO 770 integrated software system based on the measurements of LV internal dimension at end-diastole (LVIDd), LV internal dimension at end-systole (LVIDs), and LV anterior and posterior wall thicknesses at end-diastole (LVAWd and LVPWd, respectively). The following equations were implemented: $FS (\%) = (LVIDd - LVIDs)/LVIDd$, $EF (\%) = [(LVIDd^3 - LVIDs^3)/LVIDd^3] \times 100$, and $LVM (g) = 1.053 [(LVIDd + LVAWd + LVPWd)^3 - (LVIDd)^3]$. Two-dimensional images from the parasternal long axis also were recorded for measurement of the aortic valve annulus and ascending aorta outer diameter at end-systole to evaluate the degree of aortic constriction after banding with a 28-gauge (~ 0.36 mm) needle (Fig. 2).

Morphometric Analysis

One to three representative mice were selected to be sacrificed at each postoperative week from the control, juvenile banded, and adult banded mice. One to two mice from the juvenile and adult groups that had the sham operation (sham mice) were selected to be sacrificed every other week. The mice were sacrificed with IP pentobarbital. Lungs, liver, and heart were removed to obtain weights. Heart weight included both atria and ventricles. The weight of the LV included the weight of the myocardium after removal of the atria and the right ventricular free wall. Increased heart weight/body weight ratios and increased lung weight/body weight ratios were used as markers of cardiac hypertrophy and HF, respectively.

Statistical Analysis

The Mann–Whitney *U* test was used to compare two groups with respect to a continuous end point, such as LVIDd. A longitudinal model using generalized estimated equations was performed to adjust for within-group correlation while comparing the means over all time points. Kaplan–Meier analysis was implemented to analyze survival. The mean coefficient of variance was used to calculate intraobserver variability. All *P* values less than 0.05 were considered statistically significant.

Use of Vertebrate Animals

All experiments using live vertebrates were performed in accordance with protocols approved by Emory's Institutional Animal Care and Use Committee.

Statement of Responsibility

The authors had full access to the data and take full responsibility for its integrity. All the authors have read the manuscript and agree to it as written.

Results

In the initial validity study, the mean HR (672 ± 49 bpm), LVAWd (0.9 ± 0.1), LVIDd (3.1 ± 0.2 mm) and FS (0.55 ± 0.05) values were similar to those reported previously for conscious C57BL/6 adult mice [21,26]. The intraobserver variability for this early validation study was 7.9% for LVIDd, 11.9% for total wall thickness (TWTd = LVAWd + LVPWd), and, 7.9% for FS.

The normal growth and development data for the control C57BL/6 mice in terms of echocardiography and morphometry are summarized in Table 1. The ventricular function remained constant, whereas heart size and body weight experienced normal growth.

After sTAB surgery, a minimum 5 days of recovery were required for imaging of conscious mice without causing opening of chest wounds. Thus, banded mice were imaged starting on postoperative days 6 to 8. However, aortic arch flow velocities in anesthetized mice were measured immediately before and after sTAB.

The sTAB procedure induced an increase in blood flow velocity in the ascending aorta before the restriction site of $22.3 \pm 2.8\%$ for the adult mice ($n = 10$) and $26.9 \pm 7.9\%$ for the juvenile mice ($n = 10$; $P > 0.05$). These results suggest that sTAB caused a similar hemodynamic change by pressure load to the heart in the adult and juvenile mice. In contrast, changes in blood flow velocities across the restriction site were $62.4 \pm 8.2\%$ for the adult mice and $42.3 \pm 5.1\%$ for the juvenile mice ($P < 0.05$), indicating a relatively smaller blood flow restriction for the juvenile mice (a smaller effect on descending aorta flow).

Compared with the developmental changes seen in the control juvenile mice, the sTAB mice manifested dramatic ventricular dilation, mild hypertrophy, and severe ventricular dysfunction from postoperative week (POW) 1, continuing through POW 6 (Tables 2, 3). Representative M-mode images illustrating ventricular dilation and dysfunction that occurred in the juvenile banded mice are shown in Fig. 2. In addition, the juvenile mice showed significant deficiencies in growth rates after aortic banding (Tables 2, 3). Heart weight/body weight and lung/body weight ratios (mg/g) dramatically increased in the banded mice compared with the control and sham mice in both the juveniles and the adults, similar to the findings in the previous investigations [17] (Fig. 3). These changes are reflective of the hypertrophic response in the LV to the increase in afterload with aortic banding and the accumulation of pulmonary edema as HF develops.

For parameters affected by normal growth, including wall thickness, cavity size, and body weight, Z-score analysis was used. The Z-scores were calculated based on age-matched data from the control group. Longitudinal models found no significant difference in the degree of ventricular dilation between the adult and juvenile banded mice. Interestingly, compared with the adults, the juvenile banded mice manifested less LV wall thickening, with a reduction in TWTd Z-score by 1.59 (z statistic, -4.65 ; $P < 0.0001$). As anticipated, ventricular function was equivalent at baseline between the juveniles and adults. However, left ventricular FS was decreased in both the juvenile and adult mice after banding, with a more significant reduction in the juveniles at POWs 2 and 3 ($P < 0.01$, Fig. 4). The body weight Z-scores for the adults were greater than for the juveniles at POW 2 (-0.73 vs. -2.49 ; $P = 0.006$) and at POW 3 (-1.00 vs. -2.51 ; $P = 0.031$). In addition, the juvenile mice had a higher survival rate after aortic banding in the first three postoperative weeks, but the

overall survival rate did not reach statistical significance after Kaplan–Meier survival analysis ($P = 0.14$; Fig. 5).

Discussion

Recent progress in basic and translational research has greatly advanced our understanding of ventricular hypertrophy development and the transition to HF in adults. However, the same is not true for children, for whom health care providers often must extrapolate from adult studies to determine treatment strategies.

The mouse, whose genome shares a surprising amount of similarity with that of humans, serves as an ideal model for studying the development of organ function [19]. This study aimed to expand our understanding of the normal developmental myocardial changes in a commonly used mouse strain (C57BL/6) and to establish the unique response to pressure overload in juvenile murine hearts, thereby establishing an experimental database for a murine pressure-overload HF model.

Tiemann et al. [29] provided useful developmental data for sedated C57BL/6 mice over a 580-day period in terms of blood pressure levels as well as electrocardiographic, echocardiographic, and morphometric parameters. In the current study, our observations of the developmental changes are focused on a 6-week period, with more frequent interval evaluation and a wider breadth of echocardiographic parameters. This carefully selected time scale likely reflects the developmental period from juvenile to adult.

More importantly, all our measurements were conducted with conscious C57BL/6 mice. Echocardiography with conscious mice removes the known confounding effect of anesthesia on body temperature, heart rate, and cardiac function [22,31]. This first established data set on conscious mice for the developmental period from juvenile to adult may serve as a useful reference for future studies of murine cardiac development and may have potential implications for the translational application to the pediatric population.

Transverse aortic banding with a 28-gauge needle causes a significant increase in afterload to the LV. The literature, primarily for adult models, consistently demonstrated that pressure overload results in ventricular wall thickening. From a physiologic standpoint, wall thickening can serve as a compensatory response to pressure overload via the attenuation of wall stress [10]. However, debate remains regarding this historical dogma of hypertrophy being a compensatory mechanism. In addition, hypertrophy can result from a variety of mechanisms and myocardial insults, many of which are not fully understood. The Framingham Heart Study, an epidemiologic study with adults, has shown that the presence of LV hypertrophy alone significantly increases the patient's risk for ventricular arrhythmias, HF, and sudden cardiac death [11,14,16].

In an elegant study by Esposito et al. [7], two distinct transgenic murine models (TgGqI and *Dbh*^{-/-}) designed to attenuate the hypertrophic response to pressure overload were shown to have preserved LV function and structure after aortic banding, whereas wild type mice experienced ventricular dilation and ventricular dysfunction. This finding occurred despite measurement of a twofold increase in wall stress in the transgenic models, whereas the wall stress had normalized in the hypertrophied wild type mice, suggesting that hypertrophy with normalization of wall stress does not necessarily prevent dilation or preserve cardiac function [7].

Interestingly, in our study, juvenile mice experienced a limited hypertrophic response compared with the adult group, whereas cavity size and ventricular function were not preserved. The differential response in wall thickening and ventricular function seen in our

study despite a similar degree of aortic constriction (74% for adult vs. 79% for juvenile mice), combined with the observations from the Esposito study, indicate that cardiac hypertrophy does not consistently produce or protect ventricular dilation and dysfunction. These observations strongly suggest a yet undiscovered divergent response to pressure overload on the molecular level that warrants further investigation.

To our knowledge, this is the first report to describe the successful aortic banding of mice as young as 3 weeks to investigate the myocardial effects of pressure overload. Despite their small size, less than half the weight of their adult counterparts, the juvenile mice tolerated the surgical procedure very well with low mortality (~83% survival overall at POW 1). With adjustments in handling due to their small size and an inclination for the development of bradycardia, excellent two-dimensional images with clear M-mode tracings were attainable with these juvenile mice.

Despite no significant difference in overall survival according to Kaplan–Meier analysis, the juvenile mice yielded significantly higher rates of survival than the adult mice for the first 3 postoperative weeks. This divergent time course in survival may be critical in the timing for HF treatment, suggesting that earlier intervention for juveniles may be indicated.

Previous studies with adult mice consistently demonstrated that transverse aortic banding causes wall thickening, dilation, and ventricular dysfunction [17,18]. Our results exhibited similar responses to increased afterload in both juvenile and adult mice. However, after correction for normal growth and development (via the use of Z-scores), the juvenile myocardial profile of pressure overload consisted of ventricular dilation and a limited degree of ventricular wall thickening. The blunted wall thickening leads to an increase in wall stress with exposure to elevated afterload. Augmented wall stress causes the ventricle to dilate and leads to ventricular dysfunction. A more robust hypertrophic response in the adults seemed to limit the progressive decline in ventricular function that the juvenile mice experienced, suggesting a protective role of the hypertrophic response during the time course of this study.

Consistent with the classic constellation of signs seen in children with HF, the banded juvenile mice experienced a significant decrease in their growth rates compared with the control, sham, and adult groups. This effect is likely related in some degree to metabolic demands exceeding energy supply in the setting of HF but also may reflect alterations in molecular signaling involved in growth and development. Paralleling alterations seen in human HF adds further implication to the translational utility of this juvenile murine model in terms of therapeutic interventions and molecular investigations.

Study Limitations

By excluding bradycardic mice with heart rates lower than 500, we may have omitted the sickest mice with HF because HF mice were less tolerant of being held for imaging. As a consequence, we are likely underestimating the extent of dilation and ventricular dysfunction of banded juvenile mice because more juvenile mice met exclusion heart rate criteria than adult mice, consistent with their higher rate of severe HF development overall. This seems to strengthen the differential response to aortic constriction. That is, the differences likely would have been greater if the sickest mice had not been excluded.

Although both groups had severe afterload imposed on the LV with aortic banding, the degree of constriction may not have been equivalent because the aortas were larger in the adult mice than in the juvenile mice. However, after making echocardiographic measurements of the ascending aorta from the parasternal long-axis view, we demonstrated that the degrees of constriction were 79% for adults and 74% for juveniles, which are very

similar. In line with this, sTAB induced an increase in blood flow velocity in the ascending aorta before the restriction site similarly in adult and juvenile mice, indicating a similar hemodynamic change by pressure load to the hearts of the adult and juvenile mice. However, given potential maturational differences in ventricular compliance, this may result in different LV wall stress between juveniles and adults.

Perspective

This study established the first data set on the cardiac structural and functional development of conscious mice extending from the juvenile period into adulthood. In addition, we successfully generated a pressure-overload HF model in the juvenile mouse. This study showed distinct myocardial alterations in juvenile mice after pressure overload, which likely reflects inherent differences in the developing myocardium. Juvenile HF from pressure overload was characterized by ventricular dilation, attenuated wall thickening, and progressive ventricular dysfunction.

These findings hopefully will spur further investigation, leading to a better understanding of pressure-overload physiology. This knowledge may then be clinically applied to improve treatment strategies for children with a variety of related congenital and acquired disorders involving cardiac hypertrophy, dilation, and ventricular dysfunction.

Acknowledgments

This work was supported by grants awarded to Yanggan Wang from the NIH (R21HL-088168, R01HL-083271), American Health Assistant Foundation (H2007-019), and Emory University (seed grant 280263, pilot grant 33059), as well as support from Children's Healthcare of Atlanta. Yibing Nong is a fellow also supported by Beijing New Star Project of Science and Technology (2008A058).

References

1. Boluyt MO, Robinson KG, Meredith AL, Sen S, Lakatta EG, Crow MT, Brooks WW, Conrad CH, Bing OH. Heart failure after long-term supralvalvular aortic constriction in rats. *Am J Hypertens*. 2005; 18:202–212. [PubMed: 15752948]
2. Bose AK, Mathewson JW, Anderson BE, Andrews AM, Martin GA, Benjamin PM, Grossfeld PD. Initial experience with high-frequency ultrasound for the newborn C57BL mouse. *Echocardiography*. 2007; 24:412–419. [PubMed: 17381652]
3. Broberg CS, Pantely GA, Barber BJ, Mack GK, Lee K, Thigpen T, Davis LE, Sahn D, Hohimer AR. Validation of the myocardial performance index by echocardiography in mice: a noninvasive measure of left ventricular function. *J Am Soc Echocardiogr*. 2003; 16:814–823. [PubMed: 12878990]
4. Chambers J, Takeda S, Rimington H, Lambert-Hammill M, Shetty C, Wierzbicki A. Determinants of left ventricular mass in aortic stenosis. *J Heart Valve Dis*. 2004; 13:873–880. [PubMed: 15597576]
5. Collins KA, Korcarz CE, Lang RM. Use of echocardiography for the phenotypic assessment of genetically altered mice. *Physiol Genomics*. 2003; 13:227–239. [PubMed: 12746467]
6. Ding B, Price RL, Goldsmith EC, Borg TK, Yan X, Douglas PS, Weinberg EO, Bartunek J, Thielen T, Didenko VV, Lorell BH. Left ventricular hypertrophy in ascending aortic stenosis mice: anoinis and the progression to early failure. *Circulation*. 2000; 101:2854–2862. [PubMed: 10859293]
7. Esposito G, Rapacciuolo A, Naga Prasad SV, Takaoka H, Thomas SA, Koch WJ, Rockman HA. Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. *Circulation*. 2002; 105:85–92. [PubMed: 11772881]
8. Friehs I, Barillas R, Vasilyev NV, Roy N, McGowan FX, Del Nido PJ. Vascular endothelial growth factor prevents apoptosis and preserves contractile function in hypertrophied infant heart. *Circulation*. 2006; 114:I290–I295. [PubMed: 16820588]

9. Frohlich ED, Apstein C, Chobanian AV, Devereux RB, Dustan HP, Dzau V, Fauad-Tarazi F, Horan MJ, Marcus M, Massie B. The heart in hypertension. *N Engl J Med*. 1992; 327:998–1008. [PubMed: 1518549]
10. Grossman W, McLaurin LP, Moos SP, Stefadouros M, Young DT. Wall thickness and diastolic properties of the left ventricle. *Circulation*. 1974; 49:129–135. [PubMed: 4271709]
11. Haider AW, Larson MG, Benjamin EJ, Levy D. Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. *J Am Coll Cardiol*. 1998; 32:1454–1459. [PubMed: 9809962]
12. Hein S, Arnon E, Kostin S, Schonburg M, Elsasser A, Polyakova V, Bauer EP, Klovekorn WP, Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation*. 2003; 107:984–991. [PubMed: 12600911]
13. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, Jessup M, Konstam MA, Mancini DM, Michl K, Oates JA, Rahko PS, Silver MA, Stevenson LW, Yancy CW. Focused update incorporated into the ACC/AHA 2005 Guidelines for the diagnosis and management of heart failure in adults: A report of the American College of Cardiology Foundation/ American Heart Association Task Force on Practice Guidelines Developed in Collaboration with the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol*. 2009; 53:e1–e90. [PubMed: 19358937]
14. Kannel WB. Incidence and epidemiology of heart failure. *Heart Fail Rev*. 2000; 5:167–173. [PubMed: 16228142]
15. Leatherbury L, Yu Q, Chatterjee B, Walker DL, Yu Z, Tian X, Lo CW. A novel mouse model of X-linked cardiac hypertrophy. *Am J Physiol Heart Circ Physiol*. 2008; 294:H2701–H2711. [PubMed: 18424640]
16. Levy D, Savage DD, Garrison RJ, Anderson KM, Kannel WB, Castelli WP. Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study. *Am J Cardiol*. 1987; 59:956–960. [PubMed: 2952002]
17. Liao Y, Ishikura F, Beppu S, Asakura M, Takashima S, Asanuma H, Sanada S, Kim J, Ogita H, Kuzuya T, Node K, Kitakaze M, Hori M. Echocardiographic assessment of left ventricular hypertrophy and function in aortic-banded mice: necropsy validation. *Am J Physiol Heart Circ Physiol*. 2002; 282:H1703–H1708. [PubMed: 11959634]
18. Lu Z, Xu X, Hu X, Zhu G, Zhang P, van Deel ED, French JP, Fassett JT, Oury TD, Bache RJ, Chen Y. Extracellular superoxide dismutase deficiency exacerbates pressure overload-induced left ventricular hypertrophy and dysfunction. *Hypertension*. 2008; 51:19–25. [PubMed: 17998475]
19. Pennacchio LA. Insights from human/mouse genome comparisons. *Mamm Genome*. 2003; 14:429–436. [PubMed: 12925891]
20. Roos KP, Jordan MC, Fishbein MC, Ritter MR, Friedlander M, Chang HC, Rahgozar P, Han T, Garcia AJ, Maclellan WR, Ross RS, Philipson KD. Hypertrophy and heart failure in mice overexpressing the cardiac sodium-calcium exchanger. *J Card Fail*. 2007; 13:318–329. [PubMed: 17517353]
21. Rottman JN, Ni G, Brown M. Echocardiographic evaluation of ventricular function in mice. *Echocardiography*. 2007; 24:83–89. [PubMed: 17214630]
22. Rottman JN, Ni G, Khoo M, Wang Z, Zhang W, Anderson ME, Madu EC. Temporal changes in ventricular function assessed echocardiographically in conscious and anesthetized mice. *J Am Soc Echocardiogr*. 2003; 16:1150–1157. [PubMed: 14608286]
23. Spirito P, Maron BJ, Bonow RO, Epstein SE. Occurrence and significance of progressive left ventricular wall thinning and relative cavity dilatation in hypertrophic cardiomyopathy. *Am J Cardiol*. 1987; 60:123–129. [PubMed: 3604925]
24. Spurney CF, Leatherbury L, Lo CW. High-frequency ultrasound database profiling growth, development, and cardiovascular function in C57BL/6J mouse fetuses. *J Am Soc Echocardiogr*. 2004; 17:893–900. [PubMed: 15282495]
25. Stansfield WE, Rojas M, Corn D, Willis M, Patterson C, Smyth SS, Selzman CH. Characterization of a model to independently study regression of ventricular hypertrophy. *J Surg Res*. 2007; 142:387–393. [PubMed: 17574596]

26. Syed F, Diwan A, Hahn HS. Murine echocardiography: a practical approach for phenotyping genetically manipulated and surgically modeled mice. *J Am Soc Echocardiogr.* 2005; 18:982–990. [PubMed: 16153531]
27. Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, Ross J Jr. Transthoracic echocardiography in models of cardiac disease in the mouse. *Circulation.* 1996; 94:1109–1117. [PubMed: 8790053]
28. Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. *Physiol Genomics.* 2004; 16:349–360. [PubMed: 14679301]
29. Tiemann K, Weyer D, Djoufack PC, Ghanem A, Lewalter T, Dreiner U, Meyer R, Grohe C, Fink KB. Increasing myocardial contraction and blood pressure in C57BL/6 mice during early postnatal development. *Am J Physiol Heart Circ Physiol.* 2003; 284:H464–H474. [PubMed: 12414444]
30. Wisenbaugh T, Allen P, Cooper G, Holzgreffe H, Beller G, Carabello B. Contractile function, myosin ATPase activity, and isozymes in the hypertrophied pig left ventricle after a chronic progressive pressure overload. *Circ Res.* 1983; 53:332–341. [PubMed: 6224606]
31. Yang XP, Liu YH, Rhaleb NE, Kurihara N, Kim HE, Carretero OA. Echocardiographic assessment of cardiac function in conscious and anesthetized mice. *Am J Physiol.* 1999; 277:H1967–H1974. [PubMed: 10564153]

Fig. 1.

Study population includes the number of echo studies (not mice) excluded due to abnormal heart rate

Fig. 2.

a Sample M-mode measurements of a juvenile banded mouse (postoperative week [POW] 0). **b** Aortic measurements of a juvenile mouse that had a sham operation (POW 4). **c** Juvenile mouse (3 weeks old) at baseline before banding (POW 0). **d** Normal ventricular function in a juvenile mouse that had a sham operation (POW 6). **e** Moderate ventricular dysfunction in a juvenile banded mouse (POW 2). **f** Severe ventricular dysfunction in a juvenile banded mouse (POW 2)

Fig. 3.
a Mean heart weight/body weight ratios with standard error of mean *error bars* shown for each murine subgroup. **b** Mean lung weight/body weight ratios with standard error of mean *error bars* shown for each murine subgroup

Fig. 4.
Juvenile versus adult band. Change in fractional shortening percentage from baseline

Fig. 5.
Juvenile versus adult Kaplan-Meier survival curve after aortic banding

Table 1
Echocardiographic (a) and morphometric (b) parameters for control, conscious C57BL/6 mice

Parameter	3 Weeks (n = 24)	4 Weeks (n = 20)	5 Weeks (n = 19)	6 Weeks (n = 17)	7 Weeks (n = 15)	8 Weeks (n = 13)	9 Weeks (n = 11)
(a) Echocardiographic							
HR (bpm)	664 ± 45	729 ± 40	696 ± 53	714 ± 40	720 ± 26	734 ± 23	737 ± 17
TWTD (mm)	1.4 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.1
TWTs (mm)	2.5 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	3 ± 0.1	3.1 ± 0.1	3.1 ± 0.2	3 ± 0.1
LVIDd (mm)	2.6 ± 0.3	2.9 ± 0.3	3.2 ± 0.4	3.3 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	3.8 ± 0.2
LVIDs (mm)	1.1 ± 0.2	1.3 ± 0.2	1.5 ± 0.3	1.5 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	1.8 ± 0.2
FS (%)	56.2 ± 2.9	55.6 ± 4.5	54.1 ± 2.5	55.3 ± 3.6	53.0 ± 2.2	56.0 ± 3.1	52.1 ± 2.9
LVM (mg)	47.3 ± 6.4	63.5 ± 10.4	80.1 ± 9.6	87.1 ± 10.3	99.2 ± 12.0	103.8 ± 15.2	102.5 ± 7.6
AoV (mm)	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
(b) Morphometric							
BW (g)	9.4 ± 1.5	15.6 ± 1.7	19.2 ± 1.1	21.0 ± 1.5	22.7 ± 1.4	23.9 ± 1.3	24.7 ± 1.2
LV (mg)	39.3 ± 4.2	61 ± 8.5	64.5 ± 0.7	69 ± 4.2	74 ± 1.4	82.5 ± 9.2	80.2 ± 8.0
HW/BW (mg/g)	6.5 ± 0.4	5.6 ± 1.0	5.1 ± 0.2	5.5 ± 0.3	4.8 ± 0.2	5.2 ± 0.2	4.8 ± 0.2
Lung/BW (mg/g)	8.5 ± 0.8	8 ± 0.5	7.8 ± 0.2	8.1 ± 0.2	5.6 ± 0.1	5.7 ± 0.1	5.2 ± 0.1

Values are reported as mean ± standard deviation. *Note:* *n* for BW is equivalent to *n* reported in a, whereas the remainder of the morphometric data corresponds to *n* noted in each column

HR heart rate, *TWT* total wall thickness, *LVID* left ventricular internal dimension, *FS* fractional shortening, *LVM* left ventricular mass, *AoV* aortic valve annulus, *BW* body weight, *LV* left ventricle, *HW* heart weight, *s* systolic, *d* diastolic, *n* sample size

Table 2

Juvenile control versus juvenile banded mice: difference in mean Z-scores for wall thickness, dilation, and body weight (a) and mean fractional shortening (%) (b)

Outcome	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
(a) Difference in mean Z-scores ^a							
TWTd	0.97	-0.66	-1.03*	-1.13	-1.27	-0.35	-2.1
LVIDd	-0.19	-0.94*	-1.13*	-3.20**	-4.26**	-4.0**	-8.07**
BW	0.07	0.77	2.49**	2.51**	3.71**	4.38**	5.14**
(b) Mean fractional shortening (%) ^b							
n (J/C)	41/24	30/20	24/19	16/17	13/15	9/13	10/11
Juvenile (J)	56.0	34.5	29.4	23.7	21.6	21.8	18.6
Control (C)	56.2	55.35	54.1	55.3	53.0	56.0	52.1
p Value	0.89	**	**	**	**	**	**

TWT total wall thickness, LVID left ventricular internal dimension, BW body weight, d diastolic, n sample size

^aValues are reported as the difference in the mean Z-score of the juvenile control minus the juvenile banded mice

^bValues are reported as mean fractional shortening percentages

* P value < 0.01

** P value < 0.001

Table 3

Raw data for juvenile sham mice (a), juvenile banded mice (b), adult sham mice (c), and adult banded mice (d)

Parameter	Baseline (n = 5)	POW 1 (n = 6)	POW 2 (n = 5)	POW 3 (n = 3)	POW 4 (n = 3)	POW 5 (n = 2)	POW 6 (n = 2)
(a) Juvenile sham mice							
TWTd (mm)	1.3 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.9 ± 0.2	1.6 ± 0.3
LVIDd (mm)	2.7 ± 0.1	2.9 ± 0.2	3.1 ± 0.5	3.5 ± 0.1	3.6 ± 0.3	3.5 ± 0.1	3.7 ± 0.1
FS (%)	58 ± 2	57 ± 3	55 ± 4	53 ± 1	53 ± 1	57 ± 3	53 ± 2
BW (g)	10.3 ± 1.4	16.2 ± 1.5	19.5 ± 0.7	20.7 ± 0.5	21.5 ± 0.5	22.5 ± 0.01	23.7 ± 0.01
Parameter	Baseline (n = 41)	POW 1 (n = 30)	POW 2 (n = 24)	POW 3 (n = 16)	POW 4 (n = 13)	POW 5 (n = 10)	POW 6 (n = 9)
(b) Juvenile banded mice							
TWTd (mm)	1.25 ± 0.2	1.6 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.5	1.75 ± 0.3	1.7 ± 0.2
LVIDd (mm)	2.7 ± 0.3	3.2 ± 0.4	3.8 ± 0.5	4.3 ± 0.6	4.8 ± 0.7	5.0 ± 0.6	5.5 ± 0.8
FS (%)	56 ± 3	34 ± 7	29 ± 7	24 ± 7	22 ± 7	22 ± 9	19 ± 6
BW (g)	9.5 ± 1.6	14.3 ± 2.3	16.4 ± 2.5	17.2 ± 2.8	17.4 ± 3.2	18.0 ± 3.0	18.5 ± 3.1
Parameter	Baseline (n = 5)	POW 1 (n = 5)	POW 2 (n = 5)	POW 3 (n = 3)			
(c) Adult sham mice							
TWTd (mm)	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.4	1.8 ± 0.3			
LVIDd (mm)	3.0 ± 0.4	3.3 ± 0.5	3.4 ± 0.3	3.9 ± 0.7			
FS (%)	52 ± 3	55 ± 1	51 ± 4	49 ± 7			
BW (g)	21.4 ± 0.9	24.3 ± 0.6	24.8 ± 0.5	25.7 ± 0.9			
Parameter	Baseline (n = 46)	POW 1 (n = 21)	POW 2 (n = 17)	POW 3 (n = 15)			
(d) Adult banded mice							
TWTd (mm)	1.7 ± 0.2	1.9 ± 0.3	2 ± 0.3	1.9 ± 0.2			
LVIDd (mm)	2.9 ± 0.3	3.8 ± 0.6	4 ± 0.7	4.3 ± 0.6			
FS (%)	54 ± 4	35 ± 10	34 ± 10	33 ± 12			
BW (g)	20.4 ± 1.7	21.4 ± 2.3	22.9 ± 2.5	23.5 ± 2.5			

Data are shown as mean ± standard deviation. Data not analyzed for adult mice after POW 3

POW postoperative week, TWT total wall thickness, LVID left ventricular internal dimension, FS fractional shortening, BW body weight, d diastolic, n sample size