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Am J Physiol Heart Circ Physiol 274:1828-1835, 1998.

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End-systolic stress-velocity and pressure-dimension relationships by transthoracic echocardiography in mice

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Williams, Richard V., John N. Lorenz, Sandra A. Witt, David T. Hellard, Philip R. Khoury, and Thomas R. Kimball. End-systolic stress-velocity and pressure-dimension relationships by transthoracic echocardiography in mice. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1828–H1835, 1998.—The purposes of this study were to assess load-independent, end-systolic relationships in mice and compare these relationships to ejection phase indexes in assessing contractility. In 13 mice, ejection phase indexes (shortening fraction and velocity of fiber shortening) and end-systolic relationships [pressure-dimension relationship (ESPDR) and stress-velocity relationship (ESSVR)] were determined using M-mode echocardiography and simultaneous left ventricular pressure. Load was altered with phenylephrine and nitroprusside. Contractility was increased with dobutamine and decreased by induction of hypothyroidism. Ejection phase indexes increased with dobutamine infusion but were not significantly decreased with hypothyroidism. However, end-systolic relationships changed significantly with both dobutamine (γ -intercepts: ESPDR from 22 to 48 mmHg, ESSVR from 3.7 to 6.6 circ/s, $P < 0.05$) and hypothyroidism (γ -intercepts: ESPDR from 22 to 11 mmHg, ESSVR from 3.7 to 3.2 circ/s, $P < 0.05$). We conclude that end-systolic indexes can be accurately measured in the intact mouse by echocardiography with simultaneous left ventricular pressure recording and appear to be more sensitive to inotropic state than ejection phase indexes.

myocardial contractility; murine physiology

TRANSGENIC TECHNOLOGY provides extraordinary opportunities to study the effects of alterations in functional cardiac proteins in mice. The ultimate success of these genetic techniques rests on the ability to characterize the cardiac phenotype of the intact mouse where the heart exists in the context of the entire circulatory system. Traditional *in situ* approaches [e.g., aortic pressure, left ventricular pressure, and maximum rate of pressure generation (dp/dt_{max}) (17, 21, 24)] are somewhat limited because of their dependence on heart rate and loading conditions. Recently, echocardiography has been employed to evaluate cardiac function in mice *in situ* (6, 8–10, 20, 27, 28). Particularly exciting data show that echocardiographic indexes of left ventricular function reliably measure performance in genetically targeted mice (8–10, 28). Echocardiographic

measurements of left ventricular mass have also been validated with postmortem data (6). Although these efforts are extraordinary, they, too, employ indexes that are load dependent. End-systolic stress-velocity and pressure-dimension relationships are generally believed to be independent of loading conditions and, as a result, among the most powerful indexes of ventricular contractility in humans. The purposes of this study, therefore, were to develop accurate methods to assess end-systolic relationships in the mouse and to test the hypothesis that these relationships are more sensitive to changes in contractile state than traditional ejection phase indexes.

MATERIALS AND METHODS

Animals. Animals were handled according to the animal welfare regulations of the University of Cincinnati, and the experimental protocol was approved by the University of Cincinnati Institutional Animal Care and Use Committee. Thirteen adult F/VBN mice of either sex weighing 30–40 g (33 ± 3 g) were studied. Hypothyroidism, a well-described condition associated with decreased myocardial contractility as a result of a shift in the expression of the myosin heavy chain gene from the adult to the fetal isoform (22), was induced in a subset of mice ($n = 5$) by feeding the animals a diet containing 0.15% 5-propyl-2-thiouracil (PTU; Teklad Premier, Madison, WI) for a period of 4–6 wk. This is adequate, in our experience, to induce a hypothyroid state (17).

Surgical procedures. After mice were anesthetized with intraperitoneal injections of ketamine (50 μ g/g body wt) and thiobutabarbital (100 μ g/g body wt) and had their anterior chests shaved, they were placed on a thermally controlled surgical table with body temperature continually monitored via a rectal probe and maintained at 37°C. The mice were allowed to breathe spontaneously through a tracheostomy created by surgical placement of a short length of PE-90 tubing. The right femoral artery was cannulated using a custom-fashioned polyethylene tubing pulled to a small diameter from 0.25-in.-OD, thick-walled tubing, which was then connected to a CDXIII pressure transducer (COBE Cardiovascular, Arvada, CO). With the same type of tubing stretched over a flame to a diameter of ~ 200 μ m, three separate catheters were inserted through a single puncture site in the femoral vein and secured. The lumen of this tubing remains intact when stretched over a flame, and the infusion rates used in these small-caliber catheters are low (3–5 μ l/min).

These catheters were then connected to a CMA/100 microinjection pump for the infusion of experimental drugs. The left ventricle was then cannulated via the right carotid artery with a 1.8-Fr Mikro-Tip transducer (model SPR-612, Millar Instruments, Houston, TX), with continuous monitoring of the pressure waveform. The pressure waveform was calibrated electronically at each study time, and the Millar catheter was zeroed and calibrated in warm saline at the end of each experiment using a manometer. This high-fidelity transducer has a tip diameter of ~ 0.60 mm and a frequency response that is flat to 10,000 Hz and, therefore, provides accurate monitoring of the high frequency of the mouse ventricular pressure pulse. Left ventricular pressure signals were recorded and analyzed with a MacLab data acquisition system connected to a Macintosh 7100/80 computer. Raw data were acquired at a sampling rate of 1,000 samples/s. After surgery was completed, all wounds were closed using cyanoacrylate to minimize evaporative loss of fluid, and the mice were then allowed to stabilize for 30–45 min.

Echocardiography. After surgery, mice were placed in a custom-fashioned foam bed in a slight left lateral decubitus lie. Echocardiography was performed using a Hewlett-Packard Sonos 2500 Ultrasound System equipped with a 7.5-MHz transducer (Andover, MA), which has an axial resolution of 0.3 mm. To place the heart in the midfield of the ultrasound sector, where axial resolution is optimal, we fashioned the transducer with a latex balloon filled with warm acoustic gel such that there was a 1-cm standoff between the chest wall and the transducer face, and the depth setting of the system was set to 3 cm. A layer of warm acoustic gel was applied to the left hemithorax, and the probe was placed on the chest, carefully avoiding excessive pressure. The ventricular pressure waveform was input into the ultrasound system and superimposed directly on the echocardiographic image. Two-dimensionally guided M-mode echocardiography of the left ventricle from its short axis was recorded both on $\frac{1}{2}$ -in. S-VHS videotape and a strip chart at a speed of 100 mm/s. Satisfactory M-mode echocardiographic recordings (Fig. 1) were obtained in all mice, and collection of data at each of the study times was complete within 5 min. Long- and short-axis views of the heart were obtained with transducer orientations typical of those in the human. Excessive pressure, even that due solely to the weight of the transducer, was often associated with significant drops in ventricular pressure. Therefore, the imaging technique adopted was that of slight upward lifting of the transducer while continued contact with the chest wall was maintained.

Experimental protocol. After surgery and stabilization, echocardiographic evaluation was performed at seven study times in each mouse. All studies were performed with animals in the sedated state, and sedation was maintained with intraperitoneal thiobutobarbital ($100 \mu\text{g/g}$ body wt) as needed. Table 1 summarizes the experimental protocol. Baseline contractility was first assessed in each mouse by performing echocardiography at three different afterload states: before administration of any experimental drugs (*study period 1*), during phenylephrine administration ($40 \text{ ng}\cdot\text{g}$ body $\text{wt}^{-1}\cdot\text{min}^{-1}$) to increase afterload (*study period 2*), and during nitroprusside ($10 \text{ ng}\cdot\text{g}$ body $\text{wt}^{-1}\cdot\text{min}^{-1}$) administration to decrease afterload (*study period 3*). These infusion rates were determined from dose-response curves previously generated in our laboratory in separate groups of mice and were found to result in a 50% increase, in the case of phenylephrine, and decrease, in the case of nitroprusside, in systemic blood pressure. After this initial evaluation, drug infusion was stopped and left ventricular pressure and heart rate were allowed to return to baseline. This was confirmed by pressure tracings and echocardiographic evaluation (*study period 4*). Next, contractility was increased by administering dobutamine ($16 \text{ ng}\cdot\text{g}$ body $\text{wt}^{-1}\cdot\text{min}^{-1}$). This dose of dobutamine has been found by our lab to result in a near-maximal inotropic response in control mice (Fig. 2).

Echocardiography was performed during the three different afterload states: during dobutamine infusion alone (*study period 5*), during dobutamine and phenylephrine infusion (*study period 6*), and, after left ventricular pressure and heart rate had returned to pre-phenylephrine infusion levels, during dobutamine and nitroprusside infusion (*study period 7*).

Hemodynamic indexes. At each study time, left ventricular end-diastolic (LVEDD) and end-systolic dimension (LVESD), left ventricular end-diastolic (h_d) and end-systolic posterior wall thickness (h_s) in millimeters, end-diastolic septal thickness (s_d) in millimeters, ejection time (ET) in seconds, and R-R interval in seconds were measured from the M-mode echocardiogram. End diastole was defined as the onset portion of the upstroke of the superimposed left ventricular pressure trace. End systole was defined as the smallest left ventricular dimension.

In the human, left ventricular volume measurements are typically combined with pressure measurements to generate a pressure-volume loop. In the mouse, we believed that calculating volume from M-mode and/or two-dimensional echocardiographic measurements would add unnecessary error to our results due to the geometric assumptions of these

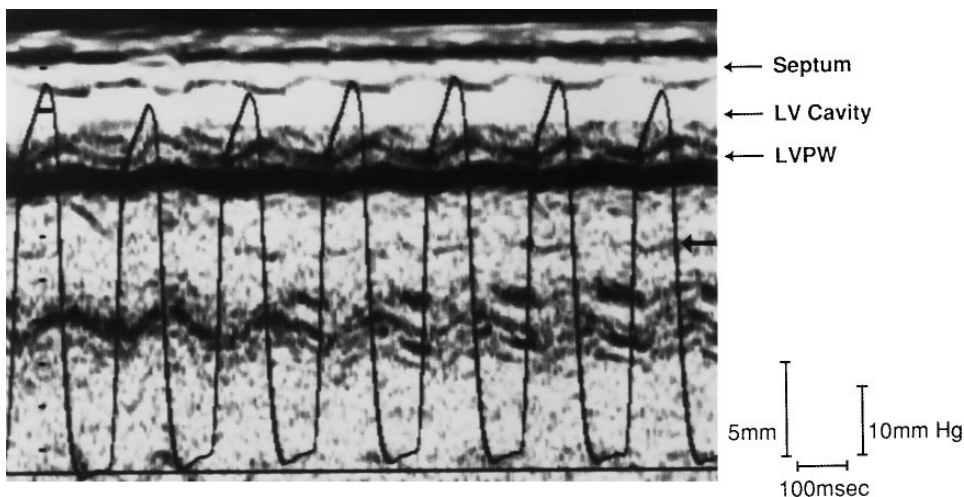


Fig. 1. Representative M-mode echocardiogram and superimposed left ventricular (LV) pressure curve (arrow) from a control mouse under baseline conditions. LVPW, LV posterior wall. Septum, interventricular septum.

Table 1. *Experimental protocol*

Time, min	Procedure
0-50	Surgery
50	Maintenance infusion; stabilization
80	<i>Study period 1</i> : 1st baseline
90	Phenylephrine infusion
95	<i>Study period 2</i> : increased afterload
100	Discontinue phenylephrine; stabilization period; start nitroprusside
110	<i>Study period 3</i> : decreased afterload
115	Discontinue nitroprusside; stabilization
125	<i>Study period 4</i> : 2nd baseline
130	Dobutamine
140	<i>Study period 5</i> : increased inotropy
145	Add phenylephrine
150	<i>Study period 6</i> : increased inotropy/increased afterload
155	Discontinue phenylephrine; stabilization period; add nitroprusside
160	<i>Study period 7</i> : increased inotropy/decreased afterload
165	Discontinue dobutamine and nitroprusside; end of study

derivatives, and, therefore, derived pressure-dimension rather than pressure-volume loops.

The ventricular pressure-dimension loop for each mouse at each study time was generated by manually digitizing the ventricular pressure waveform and septum and posterior wall (Curve Analysis, Digisonics, Houston, TX). This software generates ventricular pressure and dimension data pairs at 1-ms intervals for the entire cardiac cycle from end diastole to end diastole, with end diastole defined as the point of rapid rise in left ventricular pressure. These data pairs were then plotted graphically as (x,y) pairs in order to develop the pressure-dimension loop. Because manual tracings could introduce operator-dependent error, three consecutive cardiac cycles were measured and averaged in each mouse at each study time. These measurements were made by one of the investigators (R. V. Williams) blinded to whether a mouse had or had not received PTU. Figure 3 demonstrates examples of the loops obtained. The end-systolic pressure (P_{es} ; the pressure immediately before isovolumic relaxation) was determined from the loop.

Left ventricular mass. Left ventricular mass (4) was calculated as $LV \text{ mass (mg)} = (0.8)(1.04)[(LVEDD + h_d + s_d)^3 -$

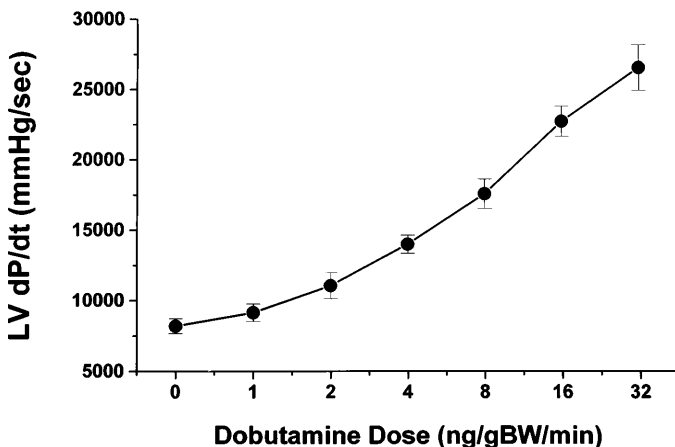


Fig. 2. Dose-response curve used to determine dobutamine dose. This curve was generated using a separate population of 8 F/VBN mice. There was a near-maximal response in first derivative of LV pressure with respect to time ($LV \text{ dP/dt}$) at an infusion rate of $16 \text{ ng} \cdot \text{g body wt}^{-1} \cdot \text{min}^{-1}$.

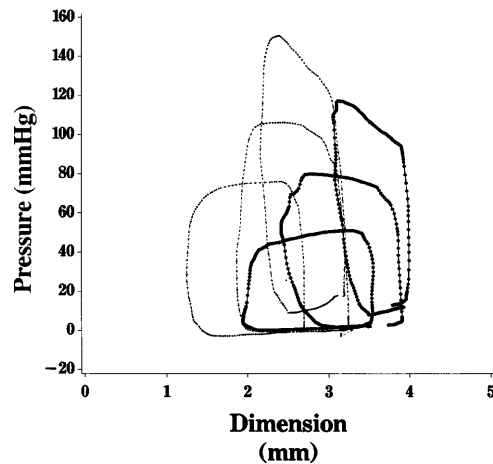


Fig. 3. Examples of pressure-dimension loops from a control mouse (thin lines) and a hypothyroid mouse (thick lines) under baseline conditions, during phenylephrine infusion (top right loops), and during nitroprusside infusion (bottom left loops) from our pilot data. Subsequent loops had more shallow slopes (see Table 3). All loops were generated by digitizing LV cavity dimension from the M-mode echocardiogram and the simultaneously recorded LV pressure trace.

($LVEDD$)³/(1,000). Although we did not confirm our assessment of left ventricular mass with postmortem weights, previous investigators have shown good correlation between echocardiographically determined LV mass and LV weights (6).

Preejection phase indexes. The maximal positive first derivative of left ventricular pressure (dP/dt_{max}) was determined in control and hypothyroid mice under baseline conditions and during dobutamine infusion. dP/dt was instantaneously calculated on-line by the MacLab software. For this calculation the MacLab software samples the data at 2,000 samples/s and writes the data at 1,000 samples/s.

Ejection phase indexes. Left ventricular performance was assessed by two ejection phase indexes: shortening fraction [$SF = (LVEDD - LVESD)/LVEDD$], which is proportional to ejection fraction, and mean velocity of circumferential fiber shortening ($V_{cf} = SF/ET$, where ET is ejection time in s). The units of V_{cf} are defined as circumferences per second (circ/s). The index serves as an estimate of the myocardial fiber shortening on the basis of changes in chamber dimension occurring over time. Shortening fraction and V_{cf} reflect overall left ventricular systolic performance and are well accepted noninvasive indexes used in clinical research (23).

V_{cf} is independent of changes in preload; however, because ET is required for calculation, V_{cf} is dependent on heart rate. Therefore, the ET is standardized to a heart rate of 60 beats/min by dividing it by the square root of the R-R interval according to Bazett's formula (1). This is analogous to the well-accepted practice of standardizing the Q-T interval from an electrocardiogram to a heart rate of 60 beats/min by dividing by the square root of the R-R interval. Just as with this correction, in which the heart rate-corrected Q-T interval continues to have units of seconds, so too does the heart rate-corrected ET (ET_c) is then used to obtain the heart rate-corrected V_{cf} ($V_{cf,c} = SF/ET_c$).

Like all ejection phase indexes, these indexes are dependent not only on left ventricular contractility but also on the loading conditions (preload and afterload in the case of shortening fraction and afterload in the case of $V_{cf,c}$). Therefore, indexes that measure loading conditions and contractility more directly were also calculated.

Left ventricular afterload. Left ventricular afterload was measured by calculation of end-systolic wall stress (WS) by previously described methods (2)

$$WS = [(1.35)(LVESD)(P_{es})]/(4)(h_s)[1 + (h_s/LVESD)]$$

End-systolic wall stress has been found to be a more reliable index of left ventricular afterload than systemic vascular resistance (14).

Left ventricular contractility. Contractility was assessed by examining both the end-systolic pressure-dimension and stress-velocity relationships. The end-systolic pressure-volume (7) relationship is a powerful index of left ventricular contractility because it has been shown to be independent of changes in loading conditions. The pressure-volume relationship is analogous to the length-tension relationship in isolated muscle experiments, which is determined using isometric afterload contractions (5). The end-systolic stress-velocity relationship is also a sensitive index of contractility because it is independent of preload, normalized for heart rate, and incorporates an index of afterload, i.e., end-systolic wall stress (2). The stress-velocity relationship is analogous to the force-velocity relationship in isolated muscle experiments, which is determined using isotonic afterload contractions (5). These load-independent indexes are particularly useful in assessing conditions associated with decreased afterload and vascular tone. Specifically, the pressure-dimension relationship is shifted upward and to the left with positive inotropic changes (increased slope and/or increased y -intercept), whereas positive inotropic changes result in a parallel shift upward and to the right in the stress-velocity relationship (increased y -intercept, no change in slope). These indexes have previously been assessed in mice only to a limited extent (12).

In this study the end-systolic pressure-dimension and stress-velocity relationships were determined by generating a family of curves in individual animals during different loading conditions and different inotropic states. Phenylephrine, an α -agonist with potent vasoconstrictor properties, was used to increase afterload. Sodium nitroprusside, a direct, endothelium-dependent vasodilator, was used to decrease afterload. Dobutamine was used to increase contractile state.

Pressure and dimension data pairs at end systole were obtained from the pressure-dimension loops. Contractility was assessed by determining the regression line for the pooled data from the three end-systolic pressure-dimension data pairs generated 1) before phenylephrine and nitroprusside, 2) during phenylephrine infusion to increase afterload, and 3) during nitroprusside infusion to decrease afterload. Contractile state during dobutamine was assessed by determining the regression line for the pooled data from the three end-systolic data pairs generated 1) during dobutamine infusion alone, 2) during dobutamine and phenylephrine infusion, and 3) during dobutamine and nitroprusside infusion. From each of these regression lines, slope and y -intercept were determined. An increase in contractility may affect this relationship in one of two ways (18, 25). First, increased contractility is evident by a parallel shift in the relationship upward and to the left, resulting in an increase in the y -intercept without a change in slope. Alternatively, increased contractility can result in the relationship shifting upward and to the left in a nonparallel fashion, resulting in an increase in both y -intercept and slope. In either case, the end-systolic pressure is higher for any given end-systolic dimension.

Contractility was also assessed by determining the stress-velocity relationship (i.e., the V_{cf} -wall stress data pairs) at end systole. Wall stress can be measured at any point in the

cardiac cycle. However, wall stress at end systole is particularly powerful because it represents the pressure that the left ventricle can no longer overcome, thereby prohibiting further shortening (i.e., the true afterload). Because V_{cf} is independent of preload and can be corrected for heart rate (V_{cf}), and end-systolic wall stress is a powerful index of afterload, the relationship of V_{cf} to end-systolic wall stress is an index of contractility. When contractility is enhanced, the V_{cf} at a given wall stress (or afterload) is greater, resulting in a shift of this relationship upward and to the right in a parallel fashion. Conversely, when contractility is depressed, the V_{cf} at a given wall stress (or afterload) is decreased, resulting in a shift of this relationship downward and to the left in a parallel fashion. Contractility was quantitatively assessed by determining the regression line for the pooled data from the stress-velocity data pairs generated during the three loading conditions before and during dobutamine infusion.

Statistical analysis. All data are shown as means \pm SD. All statistical analyses were performed using SAS software (Cary, NC). A one-way analysis of variance (ANOVA) with repeated measures was used to determine differences in variables in the same mouse between conditions (e.g., baseline vs. dobutamine). A one-way between-groups ANOVA was used to determine differences in variables between different mice (e.g., control vs. hypothyroid mice). Covariance analyses on the end-systolic pressure-dimension and stress-velocity relationships were performed using General Linear Models Procedure to determine differences between conditions and mice. Values were considered statistically significant at a $P < 0.05$ probability level.

Previous investigators have demonstrated low inter- and intraobserver variability in echocardiographically determined chamber sizes, wall thickness, and fractional shortening in mice (8, 28). For assessment of intra- and interobserver variability in our laboratory, the raw data from a subset of eight mice were measured by two different investigators (T. R. Kimball and R. V. Williams) and by the same investigator on two different occasions 1 wk apart (T. R. Kimball). Linear regression analysis was used to determine correlation coefficients for intra- and interobserver variability.

RESULTS

Intra- and interobserver variability. Intra- and interobserver variabilities were low for all echocardiographic indexes. The regression coefficients between measurements made by the same investigator at two different times (i.e., intraobserver variability) ranged from 0.84 to 1.0, depending on the index. The correlation coefficients for end-systolic pressure, end-systolic dimension, end-systolic wall stress, and V_{cf} ranged from 0.94 to 1.0. The regression coefficients for measurements made by two different investigators were equally good and ranged from 0.83 to 0.99 for echocardiographic indexes and from 0.83 to 0.99 for end-systolic pressure, end-systolic dimension, end-systolic wall stress, and V_{cf} .

Effects of dobutamine. Dobutamine infusion resulted in significant increases in the traditional ejection phase and isovolumic indexes of left ventricular function in the control mice. Both the shortening fraction and V_{cf} increased significantly with dobutamine infusion (Table 2). The dP/dt_{max} also increased significantly during dobutamine infusion ($7,362 \pm 1,899$ to $17,420 \pm 4,096$ mmHg/s, $P < 0.001$).

Table 2. Baseline echocardiographic measurements

	Control Mice		Hypothyroid Mice	
	Baseline	Dobutamine	Baseline	Dobutamine
Body wt, g	34 ± 4		33 ± 2	
Heart rate, beats/min	369 ± 69	493 ± 77*	235 ± 25*	301 ± 33†‡
LV ejection time, ms	65 ± 7	47 ± 6*	93 ± 17*	81 ± 14†
LV end-diastolic dimension, mm	3.7 ± 0.3	2.9 ± 0.5*	3.9 ± 0.6	3.6 ± 0.4†
LV end-systolic dimension, mm	2.2 ± 0.4	1.1 ± 0.3*	2.4 ± 0.7	1.6 ± 0.6†
LV posterior wall thickness, mm	1.0 ± 0.2		1.0 ± 0.2	
Septal thickness, mm	0.8 ± 0.1		0.8 ± 0.1	
LV mass, mg	79 ± 19		80 ± 17	
LV shortening fraction, %	42 ± 10	62 ± 7*	40 ± 9	56 ± 13
V_{cfc} , circ/s	2.6 ± 0.6	4.6 ± 0.6*	2.2 ± 0.8	3.2 ± 1.0†
LV end-systolic wall stress, g/cm ²	93 ± 34	62 ± 19*	83 ± 45	77 ± 26
dP/dt, mmHg/s	7,362 ± 1,899	17,420 ± 4,096*	3,773 ± 811*	8,319 ± 2,209†‡

Values are means ± SD; $n = 8$ control and 5 hypothyroid mice. LV, left ventricle; V_{cfc} , heart rate-corrected velocity of circumferential fiber shortening [in circumferences per second (circ/s)]; dP/dt, maximum rate of pressure generation. Significant differences: * $P < 0.05$ vs. control mice at baseline; † $P < 0.05$ vs. control mice during dobutamine infusion; ‡ $P < 0.05$ vs. hypothyroid mice at baseline.

In addition, the end-systolic stress-velocity and pressure-dimension relationships also changed significantly during dobutamine infusion (Table 3). All mice showed the expected shifts in the pressure-dimension and stress-shortening relationships, although the magnitude of the change was variable. For clarity in presentation, the data from the individual animals were pooled (Figs. 4 and 5). The end-systolic pressure-dimension relationship shifted significantly upward and to the left (Fig. 4A). The end-systolic stress-velocity relationship shifted upward and to the right with a significant increase in both the slope and y -intercept compared with baseline (Fig. 4B). These changes during dobutamine were also apparent, but to a lesser extent, in the hypothyroid mice (Table 3).

Effects of hypothyroidism. Hypothyroidism resulted in a significantly lower heart rate (Table 2) and dP/dt_{max} (3,773 ± 811 vs. 7,362 ± 1,899 mmHg/s, $P < 0.001$). Importantly, there were no significant differences in shortening fraction or V_{cfc} between the control and hypothyroid mice (Table 2). However, when end-systolic relationships were assessed, significant differences in contractility were apparent between control and hypothyroid mice (Table 3). Hypothyroidism resulted in a shift of the end-systolic pressure-dimension relationship downward and to the right compared with control mice (Fig. 5A). In addition, the stress-velocity relationship was shifted significantly downward and to

the left without a significant change in the slope (Fig. 5B). These changes are indicative of a decreased contractile state. Therefore, with hypothyroidism, ejection phase indexes did not detect a difference in contractile state, whereas end-systolic indexes were able to detect a difference between control and hypothyroid mice.

DISCUSSION

The significant findings in this study are that the end-systolic pressure-dimension and stress-velocity relationships can be reliably measured in the mouse and are sensitive indexes of murine myocardial contractile state. When control and hypothyroid mice are compared, our data indicate that these end-systolic relationships are more sensitive than the traditional ejection phase indexes (shortening fraction and velocity of circumferential fiber shortening) in detecting more subtle changes in contractility. These findings are important because recent advances in gene targeting and transgenic techniques allowing the creation of murine models of cardiovascular disease have necessitated the development of methods to reliably evaluate left ventricular contractility *in vivo*.

Echocardiography in mice. Echocardiography has recently emerged as a useful tool in the evaluation of mice used in biomedical research (6, 8, 19, 20, 28). For example, Gardin et al. (6) and Manning et al. (20) have used transthoracic echocardiography to reliably evaluate left ventricular mass in intact mice. In addition, echocardiographic assessment of transgenic mice overexpressing the *H-ras* gene (resulting in myocardial hypertrophy) has demonstrated increased septal and posterior wall thickness compared with control animals (28). Manning et al. (19) have also shown in a recent case report that transthoracic echocardiography may be sensitive enough to detect wall motion abnormalities in a mouse after myocardial infarction. The left ventricular chamber dimension and wall thickness results in the current study are similar to findings in these previous investigations. In addition, the left ventricular fractional shortening and velocity of circumferential fiber shortening in our control mice were also similar to

Table 3. Contractility measurements

	Control Mice		Hypothyroid Mice	
	Baseline	Dobutamine	Baseline	Dobutamine
ESPDR				
Slope, mmHg/mm	33	36	27	31
y -Intercept, mmHg	22	48*	11*	40†‡
ESSVR				
Slope, circ·cm ² ·g ⁻¹ ·s ⁻¹	-0.009	-0.028*	-0.013	-0.019
y -Intercept, circ/s	3.7	6.6*	3.2*	4.8†‡

ESPDR, end-systolic pressure-dimension relationship; ESSVR, end-systolic stress-velocity relationship. Significant differences: * $P < 0.05$ vs. control mice at baseline; † $P < 0.05$ vs. control mice during dobutamine infusion; ‡ $P < 0.05$ vs. hypothyroid mice at baseline.

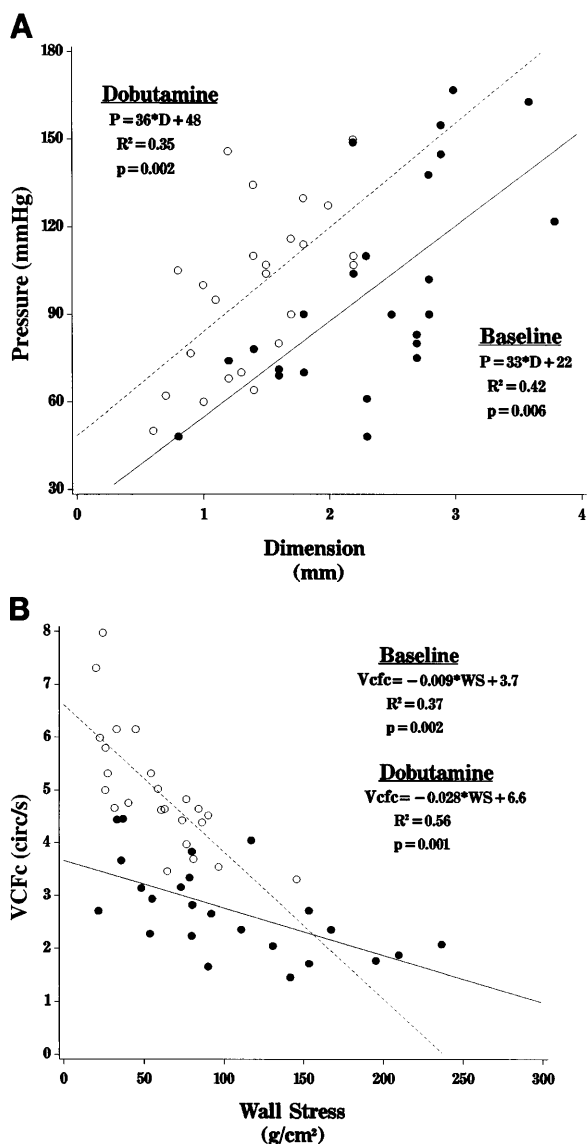


Fig. 4. Scatterplots demonstrating the end-systolic pressure-dimension (A) and stress-velocity (B) relationships for pooled data in control mice under baseline conditions (solid circles and solid line) and during dobutamine infusion (open circles and dashed line). The pressure (P)-dimension (D) relationship is shifted significantly upward and to the left with the positive inotropic intervention of dobutamine infusion. The stress-velocity relationship is shifted upward with a significant increase in both the slope and y-intercept during dobutamine infusion. WS, wall stress; V_{cfc} , heart rate-corrected velocity of circumferential fiber shortening; circ/s, circumferences per second.

these previously published findings (8, 28). Our current study extends these previous, largely morphological, data by showing that echocardiography can be used to elucidate accurate and detailed physiological data regarding myocardial contractility. With continuing improvement in transducer resolution, echocardiography in mice will become even more accurate and facile.

In vivo murine myocardial contractility. Previous investigators have largely focused on shortening fraction and velocity of circumferential fiber shortening in assessing global left ventricular systolic performance. For example, M-mode shortening fraction has been

shown to be severely depressed in transgenic mice that develop severe pulmonary edema compared with normal mice (6). Hoit et al. (8) have also demonstrated an increase in the velocity of circumferential fiber shortening in mice deficient in phospholamban, an inhibitor of the Ca^{2+} -ATPase in the cardiac sarcoplasmic reticulum; however, shortening fraction was not different between phospholamban-deficient and control mice. Mice overexpressing β_2 -receptors have been shown to demonstrate an increase in both shortening fraction and velocity of circumferential fiber shortening (28). However, because these ejection phase indexes are also dependent on loading conditions, they may not accurately reflect the ventricular contractile state and, therefore, may be potentially misleading.

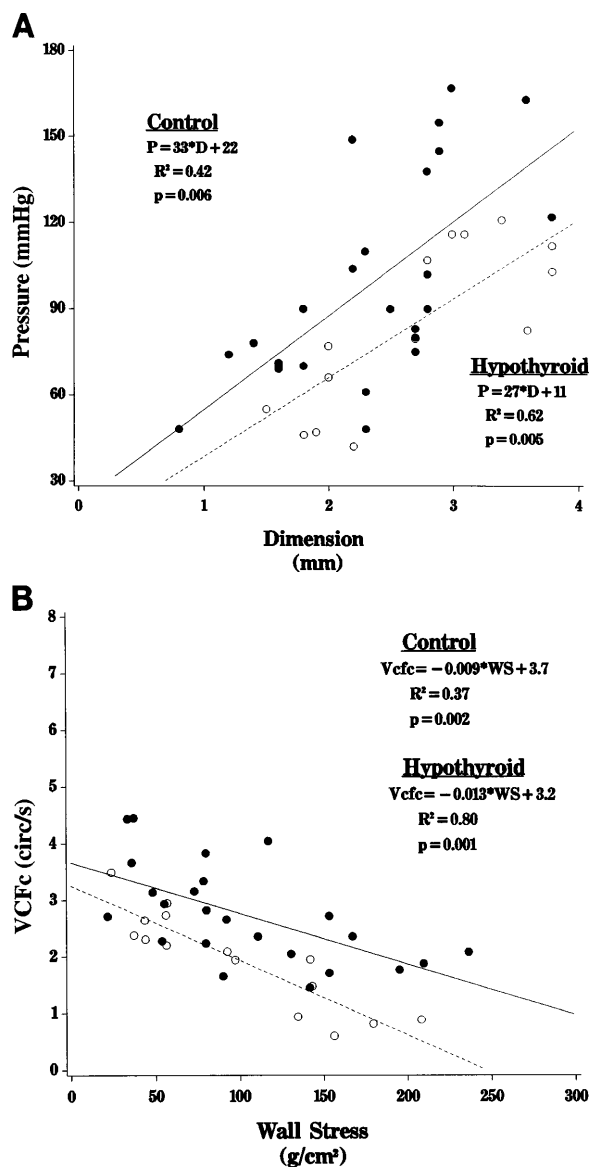


Fig. 5. Scatterplots demonstrating the end-systolic pressure-dimension (A) and stress-velocity (B) relationships for the pooled data in both control (solid circles and solid line) and hypothyroid (open circles and dashed line) mice. The pressure-dimension relationship is shifted downward and to the right, and the stress-velocity relationship is shifted downward and to the left in a parallel fashion with the negative inotropic intervention of hypothyroidism.

Hypothyroidism is a condition that has been shown to result in decreased contractility on the basis of assessment of isolated mouse hearts using retrogradely perfused Langendorff preparations and antegradely perfused working heart preparations (22). We found that although ejection phase indexes increased in control mice during dobutamine infusion, these indexes did not change with hypothyroidism. In contrast, both the end-systolic pressure-dimension and stress-velocity relationships changed significantly with hypothyroidism, suggesting that end-systolic relationships are more advantageous than traditional ejection phase indexes in assessing contractile state in the intact mouse.

Differences were also detected between the two groups when dP/dt data were compared. The dP/dt for the hypothyroid mice was significantly lower than that for control mice, and dP/dt was significantly higher in control mice during dobutamine infusion than under baseline conditions (Table 2). Although these changes are consistent with an increase in contractility, dP/dt is also dependent on heart rate and preload and requires direct measurement of left ventricular pressure. Although the current study also uses invasive left ventricular pressure measurement, echocardiographically derived end-systolic indexes can potentially be obtained with minimally invasive or noninvasive methods of assessing arterial blood pressure in the future.

A potential limitation of the technique may exist at very low wall stress (i.e., afterload) values. At very low wall stress, the relationship may become nonlinear. However, for the wall stress values encountered in our study, wall stress has been shown to be linear (2, 11). Another limitation of the technique is that it assumes that the left ventricle is a thin-walled prolated ellipse. However, despite these limitations, this index has been shown to be useful in the evaluation of contractile state in a variety of conditions in humans.

Another potential limitation of this study is the potential effects on contractility of the agents used to manipulate loading conditions. The effects of phenylephrine and sodium nitroprusside on myocardial contractility have not been systematically evaluated in the mouse. Studies using other animal models have yielded disparate results. However, Crystal and Gurevicius (3) found that nitroprusside did not affect contractility in *in situ* canine hearts. The effects of phenylephrine on myocardial contractility remain ambiguous (13, 26). These agents have been used previously to manipulate loading conditions in both animal (18) and human studies (15). Therefore, we felt the use of pharmacological agents to manipulate loading conditions in our study was acceptable and has the advantage of being technically more feasible than other methods of altering loading conditions, e.g., caval or arterial occlusion, in the small animals that were used.

Although our data showed some overlap between control and hypothyroid mice and between control mice at baseline and during dobutamine infusion, differences in these end-systolic indexes under various contractile states could be demonstrated. When control mice were studied during dobutamine infusion, we

found that the end-systolic pressure-dimension relationship was shifted upward and to the left in a parallel fashion (increased y -intercept with no significant change in slope). Previous investigators have found that the slope of the pressure-dimension, or pressure-volume, relationship may also increase with positive inotropic intervention when preload is altered by vena caval occlusion (16). However, investigators using pharmacological afterload manipulation in the conscious canine model have found that enhanced contractility results in a shift of the pressure-dimension relationship upward and to the left in a parallel fashion without a change in slope (18), similar to our results.

The end-systolic stress-velocity relationship also changed in a predictable manner with alterations in contractile state. Interestingly, the control mice demonstrated a significant increase in the slope of this relationship during dobutamine infusion. These findings may be explained by an inadequate increase in afterload with phenylephrine during simultaneous dobutamine infusion. Specifically, our experimental design may have been limited because we may have been unable to accurately assess velocity of circumferential fiber shortening at higher afterloads due to the simultaneous and antagonistic effects of the vasodilatory, β -agonist properties of dobutamine and the vasoconstrictor, α -agonist properties of phenylephrine. This would tend to skew the data to low afterloads (e.g., low wall stress), where the stress-velocity relationship may be different.

Perspectives. Both the end-systolic stress-velocity and pressure-dimension relationships are sensitive indexes of contractile state in the mouse. These indexes may be particularly useful in conditions in which it is difficult to separate the effects of changes in contractility from changes in loading conditions, such as in dilated cardiomyopathies or in lesions associated with mitral regurgitation.

Although intracardiac pressure monitoring was used in this study to evaluate left ventricular pressure, minimally invasive (e.g., femoral arterial catheterization) or noninvasive (e.g., tail-cuff pressure) monitoring of arterial pressure may allow serial assessment of end-systolic relationships in closed-chest, intact animals, and, therefore, detection of changes in ventricular contractility in murine models of cardiovascular disease over time. These methods may have advantages over isolated heart preparations, which, by their very nature, do not allow evaluation of the heart *in situ* in a living organism. The ability to assess contractility more directly in the intact animal, in which the total circulatory system is preserved, may help improve understanding of the mechanisms involved in alterations of ventricular function in a variety of transgenic murine models. Echocardiographically derived end-systolic indexes may provide a new tool in the assessment of myocardial contractility in mice.

The authors acknowledge Jeffrey Robbins for thoughtful review of this manuscript and Sheila Wheatley and Vicki Anderson for assistance in its preparation.

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Received 26 June 1997; accepted in final form 27 January 1998.

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