Validation of a mouse conductance system to determine LV volume: comparison to echocardiography and crystals

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Feldman, Marc D., John M. Erikson, Yi Mao, Claudia E. Korcarz, Roberto M. Lang, and Gregory L. Freeman. Feldman. Am J Physiol Heart Circ Physiol 279: H1698-H1707, 2000.-The application of left ventricular pressure-volume analysis to transgenic mice to characterize the cardiac phenotype has been problematic due to the small size of the mouse heart and the rapid heartbeat. Conductance technology has been miniaturized for the mouse and can solve this problem. However, there has been no validation of this technique. Accordingly, we performed echocardiography followed by simultaneous ultrasonic crystals, flow probe, and conductance studies in 18 CD-1 mice. Raw conductance volumes were corrected for an inhomogenous electrical field $\left(\alpha\right)$ and parallel conductance (G_{pi}) yielding a stroke volume of 14.1 \pm 3.7 µl/beat, end-diastolic volume of 20.8 \pm 6.5 µl, and end-systolic volume of 9.0 \pm 5.8 $\mu l.$ The mean conductance volumes were no different from those derived by flow probe and echocardiography but did differ from ultrasonic crystals. $G_{\rm pi}$ was determined to be 14.9 \pm 8.7 $\mu l.$ However, hypertonic saline altered dimension and pressure in the mouse left ventricle. Although G_{pi} can be determined by the hypertonic saline method, saline altered hemodynamics, questioning its validity in the mouse. Although mean measures of absolute volume may be similar among different techniques, individual values did not correlate.

conductance catheter; pressure-volume analysis; ultrasonic crystals; electrocardiography; transgenic mouse

TO BE ABLE TO DEVELOP molecular targets for gene therapy in the treatment of patients with cardiac disease, we need to determine the phenotype that results from specific genetic manipulations. Transgenic mice provide a valuable way to relate specific gene products to phenotype; unfortunately, the ability to assess cardiovascular phenotype has presented a significant impediment (7, 10, 24). Although pressure-volume analysis to examine cardiac performance in the pressure-volume plane is possible in larger animals, larger transgenic animals are not available. The application of standard pressure-volume analysis methods to mice is not possible due to the small size of the mouse heart and rapid beat. Creating technology to generate an accurate, instantaneous volume signal in the transgenic mouse and beat-by-beat pressure-volume relationships in real time as cardiac dynamics change is critical for assessment of phenotype.

Measurement of electrical conductance by an indwelling catheter (conductance technology) offers a method to generate an instantaneous left ventricular (LV) volume signal in the mouse. The electric field generated from electrodes at the apex and immediately above the left ventricle is sensed by voltage measurement in the left ventricle as it fills and ejects blood. Although this technique has been utilized by previous investigators (17, 18), it has not been previously validated. We developed a signal generator/processor and bridge amplifiers that have been modified and licensed for commercial release (Mouse Conductance System-100, Millar Instruments, Houston, TX). When combined with a miniaturized mouse conductance catheter (SPR-719, Millar Instruments), a complete commercial system is now available to generate LV pressure-volume loops. Whereas this technology offers the ability to link physiology and molecular biology, no validation studies of this commercial technology have been published to date. Accordingly, the present study was performed to validate the currently commercially available conductance system in the mouse. The purpose of this study was to 1) establish normal values to serve as a reference for future studies in genetically altered mice; 2) examine the validity of current methods in determining parallel conductance; and 3) to compare this system to alternative technologies, including echocardiography and ultrasonic crystals.

METHODS

Animal Preparation

The protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center in San Antonio and conformed with *Guidelines for the Care and Use of Laboratory Animals* (NIH Publication No. 86–23, Revised in 1985) and "Principles of Laboratory Animal Care" (published by the National Society for Medical Research). CD-1 mice (n = 18) weighing 20–30 g

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were anesthetized by administering methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) in a closed chamber, followed by the administration of urethan (1,000 mg/kg ip) and etomidate (25 mg/kg ip). Echocardiography was performed as outlined below before the chest was opened and the animal was placed on mechanical ventilation.

Respiration was controlled through a tracheostomy cannula, and the animals were mechanically ventilated with room air at 60 breaths/min using a rodent ventilator (Harvard Apparatus model 680, South Natick, MA). The chest was entered via an anterior thoracotomy, and the descending thoracic aorta was dissected free above the level of the diaphragm. A small animal blood flowmeter (T 106, Transonic Systems, Ithaca, NY) was placed around the descending aorta. An apical stab was made with a 30-gauge needle, and the miniaturized mouse conductance catheter was advanced retrograde into the left ventricle along the cardiac long axis with the proximal electrode just within the myocardial wall of the LV apex. A pair of 1.0-mm piezoelectric crystals (Sonometrics, London, ON, Canada) were attached to the anterior and posterior walls of the left ventricle using cyanoacrylate adhesive (Vetbond, 3M Animal Care Products, St. Paul, MN). To determine parallel conductance with the hypertonic saline technique of Bann et al. (3), a 30-gauge needle was inserted into the right ventricle and left in place for the duration of the protocol. The needle was attached to a 4-mm length of polyethylene tubing-10 tubing connected to a 1-ml syringe containing 1.5% saline. At the termination of the experiment, the heart was removed from the chest cavity. The right ventricle, aorta, left atrium, mitral apparatus, and other structures were dissected away from the left ventricle. The heart was blotted, and the wet weight was determined (Electronic Balance).

Conductance System

A 1.4-Fr miniaturized pressure-conductance catheter (SPR-719, Millar Instruments, Houston, TX) was used in these studies. The catheter has four platinum electrodes, each 0.25 mm in length. Interelectrode spacing is 0.5, 4.5, and 0.5 mm between *electrodes 1* and 2, 2 and 3, and 3 and 4, respectively. A constant excitation current (17 µA root mean square) and single frequency (10 kHz) were applied to the two outermost electrodes (electrodes 1 and 4) using a custom signal generator/processor and bridge amplifiers developed by us and subsequently modified (MCS-100, Millar Instruments). The results of the conductance studies in this paper also apply to the currently marketed ARIA Pressure Volume Conductance System (Millar Instruments) because the electronics for the first generation MCS-100 and the second generation ARIA are similar. The two inner electrodes (electrodes 2 and 3) were used to measure the instantaneous conductance signal. A high-fidelity pressure transducer was mounted between *electrodes 2* and 3 to measure ventricular pressure (frequency response to 10 kHz).

The theory behind the determination of volume using the conductance catheter in larger animals has been described in detail elsewhere (3, 5). Briefly, the tetrapolar catheter generates an intraventricular electric field between the outer electrodes. The potential differences between the inner electrode pair are measured continuously and divided into current to yield instantaneous conductance. The time-varying ventricular volume $[V_i(t)]$ is calculated as

$$V_{i}(t) = (1/\alpha)(\rho L^{2})[G_{i}(t) - G_{pi}]$$
(1)

where α is a volume calibration factor, ρ is the blood resistivity, *L* is the distance between the sensing electrodes (*elec*-

System Calibration

The signal processor provides an inverted analog voltage output that when multiplied by the current is equal to the conductance detected by the measurement electrodes. A calibration procedure was developed to convert this conductance output (μ S) into volume (μ l) for each catheter and signal generator/processor. Six cylindrical holes ranging in size from 2- to 5-mm diameter and 15-mm deep hole were drilled in a Plexiglas block. The holes were filled with heparinized mouse blood at 25°C. The miniaturized mouse conductance catheter was suspended in the center of the hole, and the output conductance was digitally acquired and measured. Assuming a homogenous electrical field and no $G_{\rm pi}$, Eq. 1 simplifies to

$$\mathbf{V}_{\mathbf{i}}(t) = (\rho L^2) G_{\mathbf{i}}(t) \tag{2}$$

$$G_{\rm i}(t) = A/\rho L \tag{3}$$

where A is the cross-sectional area of the cyclinder. Regression of the calculated volume against the corresponding conductance volume was determined. The regression line (volume = $0.031 \times \text{conductance} - 13.678$, with $r^2 = 0.9905$, P < 0.0001) was used to convert measured conductance to volume.

We also calculated $G_{\rm pi}$ to convert the conductance signal to true volume according to Eq. 1. For this purpose, the hypertonic saline technique described by Baan et al. (3) was modified for the mouse. A 0.02-ml bolus of hypertonic saline (1.5%) was injected into the right ventricle as described above. As the saline entered the left ventricle following transit through the lungs, it transiently increased blood conductivity. This theoretically results in an increase in ventricular conductance and an apparent increase in end-systolic (ESV) and end-diastolic volume (EDV) without a true change in LV volume. ESV and EDV are related during saline washin

$$\mathbf{ESV} = m \cdot \mathbf{EDV} + b \tag{4}$$

where *m* is the slope and *b* is the intercept of the regression line. The intercept between the linear regression of EDV and ESV and the line of identity (i.e., ESV = EDV) is $G_{\rm pi}$. This is based on the assumption that when the conductivity of blood is zero, all current is conducted through surrounding structures. $G_{\rm pi}$ can be solved as follows

$$G_{\rm pi} = b/(1-m) \tag{5}$$

Effect of Hypertonic Saline On Hemodynamics

We also tested the assumption that hypertonic saline bolus injection would not have an effect on LV hemodynamics (3). LV anterior to posterior short-axis dimension, LV end-systolic and end-diastolic pressures, and LV peak rate of pressure development (+dP/dt) and peak -dP/dt were evaluated via digital ultrasonic crystals (Sonometrics) and micromanometer catheter (SPR-719, Millar Instruments), respectively. Data from immediately before the change in LV conductivity to the peak change in conductivity as detected from the conductance catheter were compared.

Calculations

Conductance-derived pressure-volume (P/V) data were analyzed with customized software (PVAN). End-systolic pressure and volume were defined at the point of maximal P/V ratio, and end-diastolic pressure and volume were defined at the peak of the R wave on the electyrocardiogram. Stroke volume (SV) was defined as EDV – ESV, and end-systolic elastance was determined from end-systolic pressure-volume relationships using the method of Suga et al. (35) to identify the point of maximal P/V ratio using an iterative algorithm, where $P_{es} = E_{es}(V_{es} - V_o)$, where P_{es} is end-systolic pressure, E_{es} is end-systolic elastance, V_{es} is end-systolic volume, and V_o is the volume intercept.

Echocardiography

Equipment. A SONOS 5500 echocardiographic system (Hewlett-Packard, Andover, MA) was used to image the mouse hearts. Two-dimensional guided M mode imaging data were acquired at 1,000 lines/s using a 12-MHz phased array pediatric transducer operating at a frame rate of 70 Hz. The scrolling memory function built into the machine was used to select the best quality images for the M mode data. All data were recorded on commercial VHS video tape and printed with a Sony video printer (Sony, Japan) on 7.5 × 10 cm prints.

Imaging technique. Just before echocardiography, each animal was anesthetized as outlined above. The anterior chest wall was shaved, and the mouse was then secured to a custom-made water bed in a shallow left lateral decubitus position to facilitate ultrasound imaging. The animal was imaged in the parasternal short-axis plane through the anterior chest. The finger of a latex glove was filled with acoustic coupling gel (Aquasonic 100, Parker, Orange, NJ). The glove tip was centrifuged at 2,000 g to remove air bubbles contained within the gel and then placed over the tip of the transducer. This improved image quality by providing a 1.0- to 1.5-cm standoff between the transducer and the chest wall.

Electrocardiographic electrode clips were placed on the limbs for recording heart rate and timing intracardiac events using the single-channel electrocardiogram available on the echo machine. After two-dimensional imaging, a single M mode line was directed across the midventricular short-axis view perpendicular to the anterior and posterior walls. Position was verified using the two-dimensional image updated every few seconds. Analysis was performed on-line using the built-in analysis package.

Data Analysis

All measurements were made using leading-edge conventions specified by the American Society of Echocardiography for clinical use in humans (41). Systole was defined as the smallest cavity diameter and diastole as the largest. LV volume was calculated using a prolate ellipse model assuming that the major axis was twice the minor axis. The rationale and limitations of this method have been reviewed and well validated in humans without regional wall motion abnormalities (9).

Determination of Corrected SV

The conductance catheter creates an inhomogenous electrical field and therefore underestimates SV (44). The conductance signal has to be multiplied by a correction factor (ratio of true SV to conductance SV). To determine true SV, a small animal blood flowmeter (T 106, Transonics Systems) was placed around the descending thoracic aorta immediately above the level of the diaphragm. It could not be placed on the ascending aorta for technical reasons. To correct for the SV not passing through the ascending aorta, we determined the percentage of SV in the descending thoracic aorta as outlined below.

A total of five CD-1 mice were used to determine true SV. This method has been previously described in part (12). A pediatric short focal length 12-MHz transducer (SONOS 5500, Hewlett-Packard) was used for cardiac imaging. SV was determined in both the ascending (sinotubular junction) and descending aorta (at the left subclavian artery level). SV was determined noninvasively from pulsed wave aortic Doppler recordings (gate length 0.06 cm without angle correction) and two-dimensional measurements of the diameter of the proximal ascending and descending aorta. All echocardiographic data were obtained from the suprasternal approach.

SV was determined by multiplying the flow velocity integral of the pulsed-Doppler recording of each beat by the respective aortic cross-sectional area (CSA). CSA was calculated assuming a circular orifice with the following formula: $CSA = \Pi \times (Aod)^2/2$, where Aod is the aortic outer diameter. Cardiac output was then calculated as the product of SV and heart rate.

Digital Ultrasonic (Crystal) Measurement System

A pair of 1.0-mm piezoelectric crystals (Sonometrics) attached to the anterior and posterior walls of the left ventricle provided an instantaneous, short-axis, epicardial diameter. Intraventricular volume was derived from this signal by first converting it to an outer volume of the LV (LV_o) by means of the standard formula for a prolate ellipse, assuming that the major axis is twice the minor axis

$$LV_o = \frac{4}{3} \pi \left(\frac{2LVD_o}{2}\right) \left(\frac{LVD_o}{2}\right) \left(\frac{LVD_o}{2}\right) \tag{6}$$

The inner volume of the LV (LV_i) was defined by subtracting the volume of the myocardium, derived from the postmortem mass of the LV corrected for density, from the LV_o (19, 28, 29, 32). LV_i could thus be derived for any point in the cardiac cycle, allowing quantification of LVEDV_i and LVESV_i, allowing calculation of ejection fraction and other functional parameters.

Statistics

Separate analyses were performed for SV, EDV, and ESV using repeated measures analysis of variance (ANOVA), followed by pair-wise Newman-Keuls multiple comparisons. Pearson correlation coefficients were used to compare LV weight and $G_{\rm pi}$ determined with the hypertonic saline method. All computations were performed using SAS (version 6.11, SAS Institute, Cary, NC).

RESULTS

In five CD-1 mice examined by echocardiography and descending aortic flow probe, we assessed the fraction of SV distributed to the descending aorta. In these mice, $72.5 \pm 4.8\%$ of the SV ejected into the ascending thoracic aorta was detected in the desending thoracic aorta. In subsequent studies, we used this value to correct for total SV when flow probe data were used to calibrate conductance data. Thus the SV-corrected data shown in Table 1 are adjusted to reflect total cardiac output.

Table 1 is a summary of hemodynamic and conductance parameters for 13 mice studied. The baseline

 Table 1. Conductance parameters

Mouse No.	HR	LVESP	SV Flow Probe	SV Corrected	SV Raw Cond	α	$G_{ m pi}$	EDV Raw Cond	ESV Raw Cond	EDV Corrected	ESV Corrected	SV Corrected
1	428	60	9.7	13.4	5.3	2.5	12.3	25.4	22.2	31.3	21.9	14.4
2	426	51	9.6	13.2	10.6	1.2	2.5	17.2	7.1	17.8	5.4	12.8
3	384	54	13.6	18.8	11.7	1.6	30.1	39.6	30.6	23.7	8.8	18.3
4	417	110	9.3	12.8	7.2	1.8	10.8	27.5	20.6	32.6	18	16.4
5	432	53	11.6	16.0	15	1.1	28	48.1	36.4	23	13.5	12.4
6	371	53	10.5	14.5	13.7	1.1	9.3	25.9	14.1	20.1	6	15.3
7	507	58	7.1	9.8	7.6	1.3	8.6	20.1	13.3	17.6	4.5	13.6
8	370	44	9.3	12.8	14.4	0.9	9.1	25.8	12.4	14.1	2.8	11.7
9	535	53	5.9	8.1	9.5	0.9	21	28.2	23.6	8.6	5.1	5.1
10	471	60	14	19.3	7.6	2.5	25.9	32	24.4	22.9	9.5	20.5
11	404	50	9.2	12.7	12.7	1	7.4	25.2	13.9	21.8	11.2	11.6
12	427	55	10	13.8	12	1.2	17	27.7	17.8	21.4	7.2	15.5
13	365	47	11.7	16.1	9.3	1.7	12.2	21.1	15.1	15.1	3.2	15.6
Mean	426	57	10.1	13.9	10.5	1.4	14.9	28	19.3	20.8	9	14.1
$\pm{ m SD}$	52	16	2.3	3.1	3	0.5	8.7	8.2	8.1	6.5	5.8	3.7

HR, heart rate (beats/min); LVESP, left ventricular end-systolic pressure (mmHg); SV, stroke volume (μ l/beat); SV Corrected, SV flow probe normalized for the 27.5% of blood lost to the head and arm blood vessel before arriving to the flow probe; Cond, data derived with the conductance catheter; α , the ratio of SV corrected to SV cond; G_{pi} , parallel conductance (in μ l) determined with hypertonic saline; EDV, end-diastolic volume (μ l); ESV, end-systolic volume (μ l).

heart rate was 426 \pm 52 beats/min. The LV end-systolic pressure for our open-chest mice was 57 \pm 16 mmHg. The descending thoracic aorta flow was 10.1 \pm 2.3 µl/beat. Proper determination of α requires ascending aorta flow. Therefore, the flow probe data were normalized by 72.5% as described above. The corrected SV determined from the flow probe was 13.9 \pm 3.1 µl/beat.

In this group of mice, α was 1.4 \pm 0.5, and $G_{\rm pi}$ was 14.9 \pm 8.7 µl. The $G_{\rm pi}$ was large in comparison to the raw conductance EDV of 28.0 \pm 8.2 µl and ESV of 19.3 \pm 8.1 µl. When the raw conductance signals were corrected for α and $G_{\rm pi}$, the EDV was 20.8 \pm 6.5 µl, the ESV was 9.0 \pm 5.8 µl, and the SV was 14.1 \pm 3.7 µl/beat.

Increase in G_{pi} With LV Mass

Figure 1 is a plot of calculated $G_{\rm pi}$ as a function of LV mass (in mg) determined directly after the left ventricle was excised and weighed. There was a correlation between $G_{\rm pi}$ determined by the hypertonic saline method and LV mass (r = 0.74, P = 0.006).

Caval Occlusion

There were appropriate decreases in volume and dimension with occlusion of the inferior vena cava for both the conductance catheter and epicardial piezoelectric crystals. Figure 2 is example data from a single representative mouse. There are beat-by-beat decreases in simultaneous conductance volume and crystal dimension. The conductance-derived end-systolic elastance for this mouse was 10.62 mmHg/ μ l and V_o was 6.22 μ l. The crystal data are discontinuous because the magnitude of wall motion in these small hearts is near the absolute level of resolution for this instrument.

SV

Eight of thirteen mice had complete crystal data. Of the five mice that were excluded, two had negative ESV and EDV calculated from Eq. 6 in the METHODS, and three had technical errors. These mice were excluded based on inadequate crystal data. The conductance and echo data were usable. Therefore, the following ANOVA were performed on the eight mice with data common to all techniques.

SV values determined by corrected flow probe (means \pm SD = 13.9 \pm 3.1 µl/beat), conductance (14.1 \pm 3.7 µl/beat), and echocardiography (14.1 \pm 4.9 µl/beat) were not significantly different by repeated measures ANOVA (P = 0.84). A Newman-Keuls multiple comparisons procedure indicated that crystal SV



Fig. 1. A plot of calculated parallel conductance as a function of left ventricular (LV) mass (in mg) determined directly after the left ventricle was excised. There was a correlation between parallel conductance determined by the hypertonic saline method and LV weight.



Fig. 2. Beat-by-beat decreases in simultaneous conductance volume (B) and crystal dimension (A) from a single representative mouse are shown. The conductance-derived end-systolic elastance for this mouse was 10.62 mmHg/µl, and volume intercept (V_o) was 6.22 µl. The crystal data are discontinuous because the magnitude of wall motion and heart rate in these small hearts is near the level of resolution for this instrument. P_{es}, end-systolic pressure; V_{es}, end-systolic volume.

(10.6 ± 1.7 µl/beat) was significantly less than any of the other three measurements (P < 0.05). Figure 3 is a plot of SV using the conductance technique, echocardiography, and piezoelectric crystals versus SV defined by the corrected flow probe. Despite similar mean conductance, echocardiography, and flow probe-derived SV, for a given mouse there are dissimilarities between the methods examined. There is an apparent correlation for individual mouse SV between the flow probe and the conductance catheter technique, but this is due to the use of the flow probe to derive α and correct the raw conductance signal.

EDV

EDV values determined by corrected conductance $(20.8 \pm 6.5 \ \mu)$ and echocardiography $(18.2 \pm 7.5 \ \mu)$ were not significantly different (P = 0.41), but EDV defined from ultrasonic dimension gauges ($38.7 \pm 8.9 \ \mu$) were elevated compared with the other techniques (P < 0.01). Figure 4 is a plot of EDV using echocardiography and piezoelectric crystals versus EDV defined by the conductance catheter. Despite similar mean



Fig. 3. A plot of stroke volume (SV) using the conductance technique, echocardiography, and piezoelectric crystals vs. SV defined by the corrected flow probe is shown. Despite similar mean conductance, flow probe, and echocardiography-derived SV values, for a given mouse there are dissimilarities between the methods examined (thick and thin lines). There is an apparent correlation for individual mouse SV values between the flow probe and the conductance technique (medium line), but this is due to the use of the flow probe to derive inhomogenity of the electrical field and correct the raw conductance signal.

conductance and echocardiographic EDV, for a given mouse there is variability between the methods examined.

ESV

ESV values determined by corrected conductance $(9.0 \pm 5.8 \ \mu)$ and echocardiography $(4.1 \pm 5.6 \ \mu)$ were not significantly different (P = 0.17), but piezoelectric crystal ESV ($32.1 \pm 13.1 \ \mu$) was significantly elevated compared with both other procedures (P < 0.01). Figure 5 shows a plot of ESV using echocardiography and piezoelectric crystals versus ESV defined by the conductance catheter. Despite similar mean conductance



Fig. 4. Plot of end-diastolic volume (EDV) using echocardiography and piezoelectric crystals vs. EDV defined by the conductance technique is shown. Despite similar mean conductance and echocardiography EDVs, for a given mouse there is variability between the methods examined (thick and thin lines).



Fig. 5. Plot of end-systolic volume (ESV) using echocardiography and piezoelectric crystals vs. ESV defined by the conductance technique is shown. Despite similar mean conductance and echocardiography ESVs, for a given mouse there is variability between the methods examined (thick and thin lines).

and echocardiographic ESV, for a given mouse there is variability between the methods examined.

Effect of Hypertonic Saline on Hemodynamics

The use of the hypertonic saline method to determine $G_{\rm pi}$ is based on the assumption that LV blood conductivity is changing but not LV hemodynamics (8). Table 2 examines whether this assumption is true with saline washin data from LV epicardial piezoelectric crystal dimensions and LV pressures. There is a significant increase in end-diastolic and end-systolic dimension from immediately before the LV saline washin phase (baseline) to the peak of data acquisition for the determination of $G_{\rm pi}$ (saline). There is also a significant increase in LV end-systolic and end-diastolic pressures (Fig. 6) and in +dP/dt and -dP/dt over this same time.

Figure 7 is an example of simultaneous data from an individual mouse. Figure 7A demonstrates the calculation of $G_{\rm pi}$ using Eqs. 4 and 5 in the METHODS. $G_{\rm pi}$ was 12.48 µl. Figure 7B is the conductance-derived LV pressure-volume relationship during hypertonic saline washin used to calculate ESV and EDV on a beat-by-



Fig. 6. Example LV pressure recorded during bolus injection of 20 μl of hypertonic saline into the right ventricle. There is a visual increase in LV peak pressure as well as the development of a more prominent a wave at end diastole as LV end-diastolic pressure rises beat by beat.

beat basis shown in Fig. 7A. Figure 7C is the LV pressure-dimension relationship at the same time demonstrating that dimension and pressure were increasing on a beat-by-beat basis.

DISCUSSION

The present study provides reference values in mice for the miniaturized mouse conductance system. This includes reference values for leakage of the electrical field into the myocardium $(G_{\rm pi})$ and correction of the inhomogenity of the electrical field in the left ventricle (α) . Using these reference values, we have determined for the first time the absolute ESV, EDV, and SV using conductance technology in the mouse. We then examined the validity of determining $G_{\rm pi}$ with the standard hypertonic saline techniques used in larger animals (4, 27, 36-38, 40, 42), and we found that a steady-state hemodynamic condition was not maintained while the hypertonic saline traveled through the left ventricle. Given the assumptions of this approach known to be valid in larger animals (3), this provides a substantial limitation of this approach for its use in mice. Finally, we compared the absolute LV volumes obtained with

Table 2. Effect of hypertonic saline on hemodynamics

Mouse No.	EDD		ESD		LVESP		LVEDP		+ dP/dt		- dP/dt	
	Baseline	Saline										
1	3.37	3.42	3.25	3.27	92.0	94.0	7.6	8.7	4,352	4,486	-3,195	-3,339
2	3.74	3.79	3.54	3.57	60.9	62.9	6.9	7.8	4,007	4,360	-2,450	-3,036
3	3.44	3.50	3.24	3.30	53.8	61.7	2.6	3.3	4,592	5,268	-2,774	-3,201
4	4.67	4.73	4.48	4.53	56.7	64.7	7.6	8.0	4,069	4,451	-3,731	-4,217
5	4.76	4.89	4.19	4.27	61.8	69.4	1.9	3.5	4,666	4,866	-4,263	-4,552
6	3.66	3.72	3.44	3.50	46.3	54.0	4.1	5.0	2,815	3,035	-2,308	-2,640
7	3.69	3.72	3.58	3.60	56.1	66.4	4.2	6.2	5,329	5,803	-3,381	-3,998
8	4.12	4.14	3.88	3.90	47.6	49.0	5.8	6.7	3,289	3,337	-2,487	-2,519
Mean	3.93	3.99	3.70	3.74	59.4	65.3	5.1	6.2	4,140	4,451	-3,074	-3,438
$\pm \mathrm{SD}$	0.53	0.55	0.44	0.46	14.30	13.39	2.2	2.1	797	920	693	744
P value	0.0008		0.0006		0.001		0.0003		0.002		0.0008	

EDD, crystal end-diastolic dimension (mm); ESD, crystal end-systolic dimension (mm); LVEDP, LV end-diastolic pressure (mmHg); $\pm dP/dt$, peak rate of pressure development; baseline, immediately before right ventricular bolus injection of 0.02 ml of 1.5% saline; saline, peak of data acquisition for determination of parallel conductance. *P* value is from the paired *t*-test between baseline and saline.



Fig. 7. Example simultaneous data from an individual mouse is shown during the calculation of parallel conductance with bolus injection of hypertonic saline. A: calculation of parallel conductance using Eqs. 4 and 5 in METHODS. Parallel conductance $(G_{\rm pi})$ was 12.48 μ l. B: conductance-derived LV pressure-volume relationships during hypertonic saline washin used to calculate ESV and EDV on a beat-by-beat basis shown in A. C: LV pressure-dimension relationship at the same time demonstrating that dimension and pressure were increasing on a beat-by-beat basis. Data in A and B were used previously (11a).

other independent technologies that also have the capability to generate LV pressure-volume or dimension relationships. Although similar mean results were obtained among echocardiography, the conductance technique, and the flow probe, there were poor correlations for individual mice for EDV and ESV for the conductance technique, echocardiography ($r^2 = 0.119$, $r^2 = 0.094$, respectively) and ultrasonic crystals ($r^2 = 0.028$, $r^2 = 0.066$, respectively).

The three techniques examined, the conductance technique, echocardiography, and ultrasonic crystals, were chosen because all have the capability to generate instantaneous LV pressure-volume or dimension relationships (8, 17, 18, 26, 30). Review of current published studies with mice report the use of numerous echocardiographic measures of LV function that are known to be load dependent (15, 20, 22, 31, 34, 39). More load-independent reports of stress-shortening or stress-velocity relationships (12, 21, 43) using echocardiography have been successfully applied to the in vivo mouse left ventricle. However, two-dimensional echocardiography suffers from transducers of which the typical imaging rate is 30 Hz, which is too slow for mouse hearts whose rate exceeds 5 Hz, making these studies dependent on M mode at 1,000 Hz. This prevents imaging the mouse LV long axis and the subsequent substitution of meridional for circumferential wall stress in these studies. Finally, the use of pharmacological agents to alter steady-state load (such as phenylephrine or methoxamine) may have direct myocardial effects (13), which alters the value of this technique as a way to define contractility. The use of M mode echocardiography also precludes the application of stress-shortening relationships to ventricles with wall motion abnormalities, which is not a limitation of the conductance technique.

Other investigators have applied ultrasonic epicardial crystals to generate LV pressure-dimension relationships (26, 30). This approach is limited by the size of the heart, because the LV wall thickness of the mouse is roughly the same thickness as the crystals. The result is an offset of the crystals above the epicardium. Because EDV and ESV are derived from the cube of crystal dimension minus LV mass, EDV and ESV will always be overestimated compared with the conductance and echocardiographic results as we found. In addition, the majority of LV thickening occurs on the endocardial surface (14, 33) and not the epicardial surface where the crystals are located. Therefore, epicardial crystals would be anticipated to underestimate SV compared with endocardial methods such as conductance or echocardiography, as we found. Finally, because ultrasonic crystals work by transmitting and receiving short pulses of ultrasonic energy, there is a finite limit of temporal resolution inherent in the technique. This resolution limit is most evident when absolute motion is small as is visually evident in Figs. 2 and 7, where simultaneous conductance and ultrasonic crystal data are shown. The discontinuous appearance of the crystal signal results from this phenomena and may be particularly relevant in the mouse due to its rapid heart rate. Although the use of smoothing algorithms will improve this, there will be a small loss of precision in the measurement. A similar occurrence is not evident in the conductance signal, because the voltage change occurs instantaneously, and analog-todigital conversion occurs at very high rates. Some of these limitations of ultrasonic crystals may be overcome with endocardial placement, an approach that has recently been used successfully (11).

Conductance technology seems ideally suited to generate pressure-volume relationships in the mouse left ventricle. The electrical field generated by the catheter is independent of LV chamber geometry, and therefore mice with hypertrophy or regional wall motion abnormalities can be accurately assessed. The conductance catheter is measuring an analog voltage output, which varies instantaneously with the volume of the left ventricle, and therefore provides a continuous LV volume signal that is accurate despite the rapid heart rate of the mouse. The accuracy of conductance technology in determining absolute volumes and SV has been established previously in in vivo rat (23), canine (1, 2, 5), and human hearts (3). In the current study, we extend these findings to the mouse and have shown that the mean SV for a group of eight mice, determined by the conductance method, is comparable to the mean SV defined by flow probe or echocardiography. Likewise, we found that the mean conductance EDV and ESV are comparable to echocardiography.

Whereas we found similar mean values for conductance, flow probe, and echocardiographic SVs and similar mean values for conductance and echocardiographic ESV and EDV for a given mouse, there were discrepancies between the methods examined. It is important to note that each of these methods has the capability to detect similar directional changes in instantaneous volume or dimension. Figure 2 shows simultaneous data from epicardial crystals and an intraventricular conductance catheter: as the inferior cava is occluded, both crystal dimension and conductance volume fall on a beat-by-beat basis. Therefore, each technique is internally consistent for the detection of acute hemodynamic changes. However, individual animals did not correlate, likely due to limitations inherent in each technique, which will vary from mouse to mouse. For instance, echocardiography has a variable ability to define the endocardial border. Crystals have a variable offset due to their placement on the epicardium. Conductance has a variable ability to determine parallel conductance. Because these variances operate randomly between animals, it is not surprising that specific indexes do not correlate well, despite concurrent average results. Importantly, however, each technique is consistent. The choice of which of these three methods to utilize will therefore vary with the particular study design. Echocardiography is ideal for measurement of LV thickness, morphology, and mass; crystals for segmental function; and conductance for determination of an instantaneous volume change during inferior vena cava occlusion.

The electrical field generated by the conductance catheter leaks into the LV myocardium. Therefore, the instantaneous volume signal is a combination of LV blood volume and myocardium. The portion of this signal not arising from the LV blood volume has been termed $G_{\rm pi}$. The use of bolus injection of hypertonic

saline to define $G_{\rm pi}$ has been validated by previous investigators in larger animals (4, 27, 36-38, 40, 42). Of critical importance to the derivation of G_{pi} by this method is the assumption that the saline has no significant effect on LV volume or performance (3). In larger animals the typical volume injected is 1 ml in the canine or 10 ml in the human and represents < 2%of the LV blood volume. In the mouse, the injection of $20 \mu l$ of hypertonic saline represents nearly the entire LV blood volume (Table 1), an amount that we found to cause significant hemodynamic changes in LV pressure, dP/dt, and dimension (Table 2). Previous investigators (6) have similarly shown errors in small mammals with the saline technique. Alternative techniques to quantify G_{pi} such as the use of more than one input frequency (16) need to be developed. Despite these limitations of the accuracy of the hypertonic saline technique, we were able to detect increased G_{pi} in mice with greater LV weight (Fig. 1) as would be anticipated. This has not been previously demonstrated in larger animals.

Another limitation of this study is that an open-chest preparation was used. This approach lowers blood pressure and heart rate (25) and is terminal. Such an approach raises issue with using conductance technology to study more physiological hemodynamics, as found in the conscious mouse, and with making serial studies in precious transgenic mice. One solution is the use of the carotid artery to cannulate the left ventricle. The inferior vena cava can be accessed from a subxyphoid incision. This approach offers the possibility that the mouse could survive an initial study. In addition, the use of 100% oxygen via mouse ventilators and cannulation of the internal jugular vein for fluid, colloid, or blood administration were not utilized in the present study and may have improved hemodynamics.

A final limitation is that hypertonic saline overestimated $G_{\rm pi}$ by altering hemodynamics. The injection of 20 µl of saline into the right ventricle represents a large portion of LV blood volume. This leads to significant beat-to-beat changes in EDV greater than ESV as demonstrated in Fig. 7B. This is expected because the bolus of hypertonic saline produces a sudden increase in preload without a change in contractility. To determine $G_{\rm pi}$, $V_{\rm es}$ is plotted on the ordinate and $V_{\rm ed}$ is plotted on the abscissa as shown in Fig. 7A, and $V_{\rm p}$ is determined as the intercept of the extrapolated data with the line of identity. Because $V_{\rm ed}$ is changing faster than V_{es} , violating the assumptions of Baan et al. (3), the intersection with the line of identity will be artificially increased. The result will be overestimation of V_p , and smaller calculated EDV and ESV determined with the conductance system.

In conclusion, the miniaturized mouse conductance system can generate an instantaneous volume signal for the study of cardiovascular performance in the pressure-volume plane in very small hearts. This is important because genetic alterations will allow definitive structure-function studies of the cardiovascular system. Because selected mutations may affect not only the molecular structure of cardiac myocytes, but also the vasculature, altering both contractility and loading conditions, the ability to evaluate alterations in contractility in vivo independent of simultaneous changes in loading conditions will allow a better physiological understanding of the cardiovascular phenotype of gene-altered mice.

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