Measuring right ventricular function in the normal and hypertensive mouse hearts using admittance-derived pressure-volume loops

Diana M. Tabima, Timothy A. Hacker and Naomi C. Chesler

Am J Physiol Heart Circ Physiol 299:H2069-H2075, 2010. First published 8 October 2010;
doi:10.1152/ajpheart.00805.2010

Am J Physiol Heart Circ Physiol 299: H2069–H2075, 2010.
First published October 8, 2010; doi:10.1152/ajpheart.00805.2010.

Measuring right ventricular function in the normal and hypertensive mouse hearts using admittance-derived pressure-volume loops

Diana M. Tabima,[1] Timothy A. Hacker,[2] and Naomi C. Chesler[1,2]

Department of [1]Biomedical Engineering and [2]Medicine, University of Wisconsin-Madison, Madison, Wisconsin

Submitted 13 August 2010; accepted in final form 1 October 2010

Tabima DM, Hacker TA, Chesler NC. Measuring right ventricular function in the normal and hypertensive mouse hearts using admittance-derived pressure-volume loops. *Am J Physiol Heart Circ Physiol* 299: H2069–H2075, 2010. First published October 8, 2010; doi:10.1152/ajpheart.00805.2010.—Mice are a widely used animal model for investigating cardiovascular disease. Novel technologies have been used to quantify left ventricular function in this species, but techniques appropriate for determining right ventricular (RV) function are less well demonstrated. Detecting RV dysfunction is critical to assessing the progression of pulmonary vascular diseases such as pulmonary hypertension. We used an admittance catheter to measure pressure-volume loops in anesthetized, open-chested mice before and during vena cava occlusion. Mice exposed to chronic hypoxia for 10 days, which causes hypoxia-induced pulmonary hypertension (HPH), were compared with control (CTL) mice. HPH resulted in a 27.9% increase in RV mass ($P < 0.005$), a 67.5% increase in RV systolic pressure ($P < 0.005$), and a 61.2% decrease in cardiac output ($P < 0.05$). Preload recruitable stroke work (PRSW) and slope of the maximum derivative of pressure (dP/dt_{max})-end-diastolic volume (EDV) relationship increased with HPH ($P < 0.05$). Although HPH increased effective arterial elastance (E_a) over fivefold (from 2.7 ± 1.2 to 16.4 ± 2.5 mmHg/μl), only a mild increase in the ventricular end-systolic elastance (E_{es}) was observed. As a result, a dramatic decrease in the efficiency of ventricular-vascular coupling occurred (E_{es}/E_a decreased from 0.71 ± 0.27 to 0.35 ± 0.17; $P < 0.005$). Changes in cardiac reserve were evaluated by dobutamine infusion. In CTL mice, dobutamine significantly enhanced E_{es} and dP/dt_{max}-EDV but also increased E_a, causing a decrease in E_{es}/E_a. In HPH mice, slight but nonsignificant decreases in E_{es}, PRSW, dP/dt_{max}-EDV, and E_a were observed. Thus 10 days of HPH resulted in RV hypertrophy, ventricular-vascular decoupling, and a mild decrease in RV contractile reserve. This study demonstrates the feasibility of obtaining RV pressure-volume measurements in mice. These measurements provide insight into ventricular-vascular interactions healthy and diseased states.

cardiopulmonary hemodynamics; catheterization; chronic hypoxia; inotropic agents

MOUSE IS A WIDELY USED SPECIES for investigating a growing number of disease states. In particular, the availability of knockout and transgenic mice allows the molecular mechanisms of disease to be understood with ever more clarity. Cardiopulmonary status, including right ventricular function, pulmonary vascular function, and right ventricular-pulmonary vascular interactions, is an important facet of health and disease. Recently, different methods for assessing left ventricular function, systemic vascular function, and the efficiency of left ventricular-systemic vascular hemodynamic interactions in mice in situ have been described (13, 16, 17, 39, 41, 47, 53, 54, 64). However, the feasibility and utility of these techniques in the right ventricle and pulmonary vasculature of mice have not been established.

Useful and well-established parameters for assessing ventricular function include cardiac output (CO), ejection fraction (EF), end-systolic pressure-volume relations (ESPVR), end-diastolic pressure-volume relations (EDPVR), preload recruitable stroke work (PRSW), relaxation factor (τ), maximum and minimum derivative of pressure (dP/dt_{max} and dP/dt_{min}, respectively), and chamber compliance (9, 55, 56). Pulmonary vascular function is often assessed via the pulmonary vascular resistance and sometimes the pulmonary vascular impedance (6, 11, 12, 20, 28, 33, 42, 58). To understand the efficiency of ventricular interaction with the vasculature in both healthy and diseased states, the concept of ventricular-vascular coupling was developed by Sagawa and coworkers (48). In this prior work, the ventricular end-systolic elastance (E_{es}) and effective arterial elastance (E_a), each of which yields insight into the dynamic behavior of its respective system (ventricle or vasculature), were shown to provide a direct assessment of the efficiency of ventricular-vascular hemodynamic interactions via their ratio (E_{es}/E_a). The E_{es}-to-E_a ratio has been used to assess cardiovascular function in isolated hearts, intact animals, and humans (1, 8, 27).

When the ventricle and vasculature are efficiently coupled, for either the right pulmonary or left systemic interactions, minimal energy is wasted in the pulse pressure and maximal energy is transmitted in the mean pressure (39, 49). In this case, the ventricle operates at a maximum efficiency and submaximal stroke work such that $E_{es}/E_a > 0.5$. Conversely, for a poorly performing ventricle or high impedance vasculature, energy may be wasted through a variety of mechanisms; for example, overly rapid pulse wave reflections as occur with age (2, 36), increased arteriolar resistance as occurs in hypertension (39), and ventricular dilation as occurs with heart failure (39). In these cases, $E_{es}/E_a < 0.5$ and ventricular-vascular uncoupling occurs (23).

This concept and mathematical formulation of ventricular-vascular coupling efficiency has been used to assess right ventricular-pulmonary vascular interactions in acute pulmonary hypertension (29, 45), endotoxic shock (14, 26, 27), acute hemodilution (25, 46), and acute vasodilation (45, 59) in pigs and dogs and after the Fontan procedure in children (43). However, to our knowledge, no measurements of right-sided ventricular-vascular coupling have been performed in mice. Therefore, one goal of this study was to demonstrate the feasibility of assessing cardiopulmonary status—including ventricular-vascular coupling efficiency—in mice in situ using admittance catheterization techniques. To achieve this goal, we used an admittance catheter to measure right ventricular pressure and volume simultaneously and instantaneously in healthy

Address for reprint requests and other correspondence: N. C. Chesler, Univ. of Wisconsin at Madison, 2146 Engineering Centers Bldg., 1550 Engineering Dr., Madison, WI 53706-1609 (e-mail: chesler@engr.wisc.edu).

and hypertensive mice. We also investigated the effects of dobutamine infusion and the ability of our techniques to detect changes in cardiopulmonary status with dobutamine in healthy and hypertensive mice. Demonstration of techniques for measuring right ventricular function and ventricular-vascular coupling efficiency in mice will enable the future exploration of molecular-level mechanisms, using transgenic and knockout mice, in cardiopulmonary diseases such as pulmonary arterial hypertension.

METHODS

Animal handling. Fifteen male C57BL6/J mice 10–12 weeks old, with a body weight of 25.5 ± 1.6 g were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were exposed to 10 days of normobaric hypoxia. Hypoxia was created in an environmentally controlled chamber in which nitrogen was mixed with room air until an oxygen concentration of 10% was reached; oxygen levels were measured with a sensor in the chamber (Servoflo, Lexington, MA) that controlled a relay valve on the nitrogen gas inflow line via a custom-built closed loop control system. The chamber was opened for 10–20 min three times per week to clean cages and replenish food and water. Control mice were housed in room air. All mice were exposed to a 12-h:12-h light-dark cycle. All procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Anesthesia, ventilation and ventricular exposure. Mice were anesthetized with an interperitoneal injection of urethane solution (1 mg/g body weight), intubated, and placed on a ventilator (Harvard Apparatus, Holliston, MA) using a tidal volume of ~225 μl and respiratory rate of ~200 breaths/min. They were then placed supine on a heated pad to maintain body temperature at 38° to 39°C. A ventral midline skin incision was made from the lower mandible inferior to the xiphoid process. The thoracic cavity was entered through the sternum. The chest wall and lungs were carefully retracted to expose the right ventricle. Hydroxyethylstarch (6%; 2 mg/g body weight) was injected intravenously to restore vascular volumes as previously reported (41, 44).

Instrumentation and hemodynamic measurements. To measure systemic pressure, the right carotid was cannulated with a 1.2 F catheter-tip pressure transducer (Scisense, London, Ontario, Canada) and advanced into the ascending aorta.

Subsequently, the apex of the right ventricle was localized and a 1.2 F admittance pressure-volume catheter (Scisense) was introduced using a 20-gauge needle. The admittance catheter calibration was performed by measuring admittance magnitude and phase in saline solutions of known conductivities. The conductivity values were chosen to cover the range of expected effective conductivities for blood and muscle (1,000 to 10,000 μS/cm) (44). After instrumentation was established and initial pressure-volume measurements were obtained, the inferior vena cava was isolated and briefly occluded to obtain alterations in venous return for determination of end-systolic and end-diastolic pressure relations. The vena cava occlusion was limited to a few seconds in duration to avoid reflex responses. The magnitude and phase of the electrical admittance as well as the right ventricular pressure were continuously recorded at 1,000 Hz and analyzed on commercially available software (Notocord Systems, Croissy Sur Seine, France).

Cardiac reserve. To examine cardiac reserve, we infused dobutamine at 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ through the jugular vein. Pressure and volume data were obtained in an initial state during brief vena caval occlusion at least 10 min after the dobutamine infusion.

After all measurements were complete, a sample of blood was extracted to measure the hematocrit (Hct). The right ventricular free wall was removed and weighed as was the left ventricle (LV) plus septum (S). The right ventricular to (LV + S) ratio was calculated as an index of right ventricular hypertrophy. Right and left atria were also removed and weighed.

Hemodynamic data analysis. The signals of pressure and volume were visually checked for quality and recorded for later analysis. For each baseline or experimental condition, at least 10 consecutive cardiac cycles free of extrasystolic beats were selected and used for the analysis. Standard hemodynamic variables (heart rate, systolic pressure, and diastolic pressure), right ventricular function parameters (CO, EF, τ, chamber compliance), and E_a were calculated from the hemodynamic data.

Contractility was quantified in three ways: as the slope of the ESPVR (E_{es}), PRSW, and the slope of the dP/dt_{max}-end-diastolic volume (EDV) relationship. E_{es} gives insight into the dynamic behavior of the ventricle (49), PRSW is useful because it is chamber size and load independent (22), and the dP/dt_{max}-EDV relation is used to evaluate load-independent right ventricular contractile performance in vivo. In the left ventricle, the dP/dt_{max}-EDV relation is considered a more sensitive parameter of contractility than E_{es} or PRSW (30).

Finally, ventricular-vascular coupling efficiency was calculated as E_{es}/E_a.

Statistical analysis. The significances of the overall changes in the hemodynamic parameters with 10 days of chronic hypoxia and with dobutamine were assessed using a two-way ANOVA ($P < 0.05$). When the ANOVA reached statistical significance, Tukey multiple comparisons were used for post hoc analysis. Data were considered significant for P values less than 0.05. Data are presented in terms of means ± 1 SD. Statistical analysis was performed using R software (Foundation for Statistical Computing, version 2.6.2).

RESULTS

The average body weight of the hypoxia-induced pulmonary hypertension (HPH) group at the end of the hypoxia exposure was not significantly different from the control (CTL) group (26.2 ± 1.3 vs. 24.9 ± 2.0 g). However, right ventricular free wall weight increased with HPH, whereas right atrial, left atrial, and left ventricular free wall and septum weights were unaffected (Table 1).

Right ventricular hemodynamic analysis. Figure 1 shows typical pressure-volume traces initially and during preload reduction by vena cava occlusion for CTL and HPH mice. The largest, right-most pressure-volume loop represents the initial condition before occlusion. Note that the pressures and volumes in the CTL group are in the physiological range for a mouse: mean pressure, 10–20 mmHg; stroke volume, 14–26 μl (17, 50, 51, 65).

As evidenced in Fig. 1, the slope of the ESPVR shifted leftward and became steeper in HPH mice. Measurements of right ventricular function and right ventricular-pulmonary vas-

Table 1. *Body weight and heart chamber weight ratios for CTL and HPH mice*

Weights	CTL	HPH
BW, g	26.2 ± 1.3	24.9 ± 2.0
RV/(LV + S), mg/mg	0.24 ± 0.05	0.31 ± 0.03*
RA/BW, mg/mg	0.12 ± 0.02	0.11 ± 0.03
RV/BW, mg/mg	0.76 ± 0.11	1.02 ± 0.12†
LA/BW, mg/mg	0.12 ± 0.01	0.12 ± 0.05
(LV + S)/BW, mg/mg	3.18 ± 0.31	3.22 ± 0.28

Values are means ± SD; $n = 8$ for control (CTL) and $n = 7$ for hypoxia-induced pulmonary hypertension (HPH) group. BW, body weight; RV, right ventricular; LV, left ventricular; S, septum; RA, right atrial; LA, left atrial. *$P < 0.005$; †$P < 0.0005$ vs. CTL.

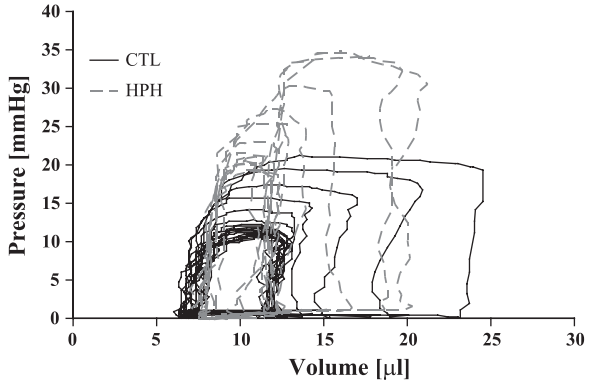

Fig. 1. Representative pressure-volume loops measured using an admittance catheter in control (CTL) and hypoxia-induced pulmonary hypertension (HPH) mouse right ventricles in situ. Data were obtained during alteration of preload by occlusion of the inferior vena cava.

cular coupling efficiency at baseline in CTL and HPH mice are summarized in Table 2.

Ten days of hypoxia caused an average 61% decrease in CO, 55% decrease in EF, 63% decrease in compliance (all $P < 0.05$), and an increase in contractility as measured by the dP/dt_{max}-EDV relation (by 110%; $P < 0.005$) and PRSW (by 62%; $P < 0.0005$). E_{es} increased somewhat but not significantly ($P = 0.1$; Table 2). Although all three contractility parameters increased with HPH, the dP/dt_{max}-EDV relation was the most sensitive, as reported in a previous study on left ventricular function (21). E_a also increased significantly with HPH ($P < 0.0005$). The mild increase in E_{es}, in conjunction with the dramatic increase in E_a, led to a significant decrease in E_{es}/E_a, from 0.71 ± 0.27 to 0.35 ± 0.17 ($P < 0.005$; Table 2).

Table 2. *Hemodynamic parameters and indexes of systolic and diastolic function derived from right ventricular pressure-volume relationships in CTL and HPH mice*

	CTL	HPH
Parameter		
Heart rate, beats/min	611 ± 31	636 ± 31
Aortic systolic pressure, mmHg	78 ± 7	72 ± 8
Aortic diastolic pressure, mmHg	38 ± 7	39 ± 7
Hematocrit, -	51 ± 7	72 ± 4‡
RV-systolic pressure, mmHg	27 ± 3	45 ± 17*
RV-diastolic pressure, mmHg	1.4 ± 0.9	2.7 ± 1.4*
End-diastolic volume, μl	26 ± 7	27 ± 9
Ejection time, mm/s	43 ± 1	38 ± 2†
Stroke work, mmHg μl	386 ± 76	926 ± 265‡
Cardiac output, ml/min	8.5 ± 2.3	5.2 ± 2.6*
Chamber compliance, μl/mmHg	0.68 ± 0.21	0.43 ± 0.13*
Systolic indexes		
Ejection fraction, %	51 ± 11	28 ± 13*
dP/dt_{max}, mmHg/s	2,522 ± 660	3,164 ± 826
dP/dt_{max}- end-diastolic volume, $mmHg \cdot s^{-1} \cdot \mu l^{-1}$	84 ± 17	177 ± 93†
E_{es}, mmHg/μl	1.8 ± 0.5	2.4 ± 0.2
Preload recruitable stoke work, mmHg	20.9 ± 5.6	33.9 ± 5.9‡
Diastolic indexes		
dP/dt_{min}, mmHg/s	−1,971 ± 499	−3,009 ± 1,120
Relaxation factor τ, ms	5.3 ± 0.9	6.1 ± 2.7
Pulmonary vascular indexes		
E_a, mmHg/μl	2.7 ± 1.2	16.4 ± 2.5‡
Coupling efficiency		
E_{es}/E_a, -	0.71 ± 0.27	0.35 ± 0.17†

Values are means ± SD; $n = 8$ for control (CTL) and $n = 7$ for HPH group. dP/dt_{max} and dP/dt_{min}, maximum and minimum derivative of pressure; E_{es}, ventricular end-systolic elastance; E_a, effective arterial elastance. *$P < 0.05$, †$P < 0.005$, ‡$P < 0.0005$ vs. CTL.

Table 3. *Hemodynamic variables, right ventricular function parameters, and ventricular-vascular coupling measurements in CTL mice during dobutamine infusion*

	Baseline	Dobutamine
Parameter		
Heart rate, beats/min	611 ± 31	589 ± 56
Aortic systolic pressure, mmHg	78 ± 7	66 ± 7
Aortic diastolic pressure, mmHg	38 ± 7	24 ± 4
RV-systolic pressure, mmHg	27 ± 3	22 ± 11
RV-diastolic pressure, mmHg	1.4 ± 0.9	1.1 ± 0.8
End-systolic RV pressure, mmHg	25 ± 3	26 ± 2
End-diastolic volume, μl	26 ± 7	24 ± 6
Stroke work, mmHg μl	386 ± 76	234 ± 91
Cardiac output, ml/min	8.5 ± 2.3	6.0 ± 1.0
Chamber compliance, μl/mmHg	0.68 ± 0.21	0.25 ± 0.15
Systolic indexes		
Ejection fraction, %	51 ± 11	51 ± 11
dP/dt_{max}, mmHg/s	2,522 ± 660	2,375 ± 288
dP/dt_{max}-end-diastolic volume, $mmHg \cdot s^{-1} \cdot \mu l^{-1}$	84 ± 17	116 ± 46*
Ventricular end-systolic elastance, mmHg/μl	1.8 ± 0.5	2.8 ± 0.5*
Preload recruitable stoke work, mmHg	20.9 ± 5.6	23.6 ± 4.1
Diastolic indexes		
dP/dt_{min}, mmHg/s	−1,971 ± 499	−1,839 ± 216
Relaxation factor τ, ms	5.3 ± 0.9	6.2 ± 1.2
Pulmonary vascular indexes		
Effective arterial elastance, mmHg/μl	2.7 ± 1.2	4.4 ± 1.8†
Coupling efficiency		
E_{es}/E_a, -	0.71 ± 0.27	0.41 ± 0.11*

Values are means ± SD; $n = 8$ for CTL group and $n = 5$ during dobutamine infusion. *$P < 0.05$; †$P < 0.0005$ vs. baseline.

Dobutamine-stimulated right ventricular hemodynamics. To examine cardiac reserve, we administered dobutamine. Heart rate did not increase in either CTL (Table 3) or HPH (Table 4) mice. No alteration in systemic blood pressure was observed in response to dobutamine for either group.

In the CTL group, the administration of dobutamine tended to decrease CO and compliance, increased E_{es} and dP/dt_{max}-EDV ($P < 0.05$), and tended to increase PRSW. E_a increased dramatically with dobutamine ($P < 0.0005$) and, as a consequence, E_{es}/E_a decreased significantly ($P < 0.05$).

Administration of dobutamine in the HPH group increased CO ($P < 0.05$) and tended to decrease compliance, contractility (E_{es}, PRSW, and dP/dt_{max}-EDV), E_a and ventricular-vascular coupling (E_{es}/E_a) (Table 4). However, only the increase in CO was significant.

In many respects, the response of the right ventricle to dobutamine infusion was similar between groups. End-systolic right-ventricular pressure, end-diastolic volume, relaxation factor τ, and EF remained near baseline values in both groups.

Table 4. *Hemodynamic variables, right ventricular function parameters, and ventricular-vascular coupling measurements in mice exposed to 10 days of chronic hypoxia (HPH) during dobutamine infusion*

	Baseline	Dobutamine
Parameter		
Heart rate, beats/min	636 ± 31	654 ± 42
Aortic systolic pressure, mmHg	72 ± 8	71 ± 9
Aortic diastolic pressure, mmHg	39 ± 7	37 ± 6
RV-systolic pressure, mmHg	45 ± 17	41 ± 10
RV-diastolic pressure, mmHg	2.7 ± 1.4	2.4 ± 1.4
End-diastolic volume, μl	27 ± 9	24 ± 6
Stroke work, mmHg μl	926 ± 265	531 ± 240†
Cardiac output, ml/min	5.2 ± 2.6	8.4 ± 2.1*
Chamber compliance, μl/mmHg	0.43 ± 0.13	0.29 ± 0.22
Systolic indexes		
Ejection fraction, %	28 ± 13	39 ± 22
dP/dt_{max}, mmHg/s	3,164 ± 826	3,308 ± 615
dP/dt_{max}-end-diastolic volume, $mmHg \cdot s^{-1} \cdot \mu l^{-1}$	177 ± 93	179 ± 71
Ventricular end-systolic elastance, mmHg/μl	2.4 ± 0.2	2.0 ± 0.7
Preload recruitable stoke work, mmHg	33.9 ± 5.9	28.1 ± 3.6
Diastolic indexes		
dP/dt_{min}, mmHg/s	−3,009 ± 1,120	−2,997 ± 825
Relaxation factor τ, ms	6.1 ± 2.7	6.4 ± 2.9
Pulmonary vascular indexes		
Effective arterial elastance, mmHg/μl	16.4 ± 2.5	14.4 ± 3.1
Coupling efficiency		
E_{es}/E_a, -	0.35 ± 0.17	0.20 ± 0.12

Values are means ± SD; $n = 7$ for HPH group and $n = 7$ during dobutamine infusion. *$P < 0.05$; †$P < 0.005$ vs. baseline.

DISCUSSION

This study demonstrates that high fidelity, instantaneous, and simultaneous measurements of pressure and volume in the right ventricle of mice are possible and that the resulting pressure-volume data yield significant insight into changes in right ventricular and ventricular-vascular function with disease.

To our knowledge, no other studies have simultaneously measured beat-by-beat pressure and volume in the mouse right ventricle. However, other studies have measured right ventricular pressures and time-averaged CO. Right ventricular pressures in this study were similar to several prior studies (~25 mmHg) (17, 37, 38, 50, 51, 65) but higher than others (~18 mmHg) (32, 61), which may be due to the significantly slower heart rates in those studies compared with the present study. CO measured from the right ventricle in this study was similar to CO measured previously in the right ventricular by thermodilution (10) and estimated by transesophageal echocardiography (51), as well as left ventricular output measured by a variety of techniques (3, 4, 15, 18, 53). End-diastolic volumes were also similar to those measured by echocardiography (51), but much lower than that found by Rockman et al. (47) using X-ray contrast microangiography. However, the Rockman study infused a contrast agent, which may have increased venous return, and in that study the heart rate was significantly slower, both of which likely affected the measured end-diastolic volumes.

Right ventricular contractility has not been previously measured in a mouse model. Other measures of right ventricular function in mice do exist in the literature. For example, the surrogate measure for contractility, dP/dt_{max}, measured in the right ventricle in this study (2,522 ± 660 mmHg/s, in CTL) was higher than that measured by Otto et al. (736 ± 100 mmHg/s) (40). Lower right ventricular pressures and heart rate obtained by Otto et al. (40) may account for this difference. Ejection fraction in our study was similar to that estimated by echocardiography (51) and by X-ray contrast microangiography (47). Measures of contractility (E_{es}, PRSW) by the gold standard of pressure-volume analysis have not been published for the mouse right ventricle, but are lower than values for the mouse left ventricle (18, 21, 41, 53, 63); this difference is likely due to the lower pressure in the right ventricle.

Values of effective arterial elastance E_a for the pulmonary circulation in mice have not been published. E_a incorporates the principal elements of total ventricular afterload, including peripheral resistance, arterial compliance, and characteristic impedance (24, 35, 53). For either the systemic or the pulmonary circulation, E_a can be calculated as the peak ventricular pressure divided by the stroke volume (48, 49). The value of E_a for the pulmonary circulation found here is approximately fourfold lower than the value of E_a for the systemic vasculature of mice published previously (13, 41, 53, 62). Given that the peak ventricular pressure in the right ventricle is approximately fourfold less than that in the left ventricle for the same CO, this result is good validation of the values obtained here.

In contrast with E_a, E_{es}/E_a is comparable in the two sides of the heart (53). In the CTL mice assessed here at baseline, the ventricle operated at a maximum efficiency and submaximal stroke work such that E_{es}/E_a was greater than 0.5 on average. This value is remarkably lower than values reported in other species (7, 60) but similar to normal E_{es}/E_a values reported for left ventricular-aortic coupling in mice (13, 44).

Chronic hypoxia increased right ventricular systolic pressure, contractility and effective arterial elastance, and decreased ejection fraction, CO, and chamber compliance. Systolic dysfunction was also observed in the HPH group, characterized by an increase in chamber contractility indexes, such as the dP/dt_{max}-EDV relation and PRSW. This increase in myocardial contraction was also evident by the increase in stroke work found with unchanged end-diastolic volume.

The right ventricle can increase its contractility through homeometric autoregulation and in theory accommodate a doubling of its afterload before it becomes uncoupled and decreases stroke volume (19). However, in our study, the increase in contractility, increase in effective arterial elastance, and decrease in chamber compliance caused the ventricle to uncouple from the vasculature, likely indicating that the right ventricle in mice exposed to 10 days of chronic hypoxia does not have enough reserve to compensate for the increased load.

Dobutamine infusion had unexpected effects in this study. The dosage regimen of 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was chosen because it is within the dosage range (2.5–10 $\mu g \cdot kg^{-1} \cdot min^{-1}$) most commonly used in experimental (5, 52) and clinical pulmonary hypertension (34). In CTL mice, dobutamine caused a significant decoupling through an increase in contractility and larger increase in effective arterial elastance. The augmentation of

contractility was expected but the increase in E_a was not. Given that dobutamine has been shown to cause peripheral pulmonary vasodilation (42), we expected a drop in peak right ventricular pressure with no change in stroke volume; i.e., we expected a decrease in E_a. The increase in E_a suggests that either peak pressure increased for the same stroke volume or stroke volume decreased for the same peak pressure. In this case, peak right ventricular pressure was not increased by dobutamine but stroke volume did decrease, although the variability was high. Dobutamine infusion at 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ did not improve coupling in either group.

It has previously been reported that the effects of dobutamine are highly dose- and flow dependent (5, 42). As a consequence, the effects of this dose of dobutamine are likely different in CTL and HPH conditions. Our results with dobutamine infusion also may be limited by the fact that it did not produce the anticipated increase in heart rate in either group. Nevertheless, the infusion of dobutamine provided a second state for each group at which right ventricular-pulmonary vascular coupling could be assessed with the admittance catheter technology described here. In each group at least some changes were evident; thus the effect of this inotropic agent on cardiopulmonary status could be quantified.

The importance of mice as animal models for disease cannot be understated; the mapping of the mouse genome has enabled the development of knockout and transgenic mice with which the molecular mechanisms of disease are being understood with ever more clarity. With technological advances speeding the miniaturization of physiological instrumentation, more sophisticated measurements in mice are increasingly possible. In this study, we demonstrated the use of admittance catheter technology to assess the right ventricular and cardiopulmonary status of healthy and hypertensive mice before and after dobutamine infusion. The parameters measured were in agreement with prior studies, for those cases in which prior measurements in mice were available, and compatible with comparable measures for the left ventricle and systemic vasculature.

An important application of these findings is to the poorly understood disease of pulmonary arterial hypertension, which is defined as a mean pulmonary arterial pressure of more than 25 mmHg at rest; a pulmonary capillary wedge pressure, left atrial pressure, or left ventricular end-diastolic pressure less than or equal to 15 mmHg; and a pulmonary vascular resistance greater than 3 Wood units (31). Pulmonary arterial hypertension is a serious disease with a poor prognosis. Understanding and predicting right ventricular failure is critical to managing this disease and developing more effective treatment strategies. Our ability to measure ventricular function and ventricular-vascular coupling in mice will enable these techniques to be used with transgenic and knockout mice to assess the role of genetic factors in right ventricular failure secondary to pulmonary arterial hypertension and to assess the effects of conventional or novel treatment strategies in this species.

ACKNOWLEDGMENTS

We thank Gouqing Song for performing in vivo hemodynamic measurements.

GRANTS

The present study was supported in part by DNP-Fulbright-Colciencias program and Universidad de los Andes-Colombia (to D. M. Tabima) and National Institutes of Health Grant R01HL-086939 (to N. C. Chesler).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **Asanoi H, Sasayama S, Kameyama T.** Ventriculoarterial coupling in normal and failing heart in humans. *Circ Res* 65: 2: 483–493, 1989.
2. **Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF.** Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 68: 50–58, 1983.
3. **Barbee RW, Perry BD, Re RN, Murgo JP.** Microsphere and dilution techniques for the determination of blood flows and volumes in conscious mice. *Am J Physiol Regul Integr Comp Physiol* 263: R728–R733, 1992.
4. **Barbee RW, Perry BD, Re RN, Murgo JP, Field LJ.** Hemodynamics in transgenic mice with overexpression of atrial natriuretic factor. *Circ Res* 74: 747–751, 1994.
5. **Bradford KK, Deb B, Pearl RG.** Combination therapy with inhaled nitric oxide and intravenous dobutamine during pulmonary hypertension in the rabbit. *J Cardiovasc Pharmacol* 36: 146–151, 2000.
6. **Brimioulle S, Maggiorini M, Stephanazzi J, Vermeulen F, Lejeune P, Naeije R.** Effects of low flow on pulmonary vascular flow-pressure curves and pulmonary vascular impedance. *Cardiovasc Res* 42: 183–192, 1999.
7. **Brimioulle S, Wauthy P, Ewalenko P, Rondelet B, Vermeulen F, Kerbaul F, Naeije R.** Single-beat estimation of right ventricular end-systolic pressure-volume relationship. *Am J Physiol Heart Circ Physiol* 284: H1625–H1630, 2003.
8. **Burkhoff D, Sagawa K.** Ventricular efficiency predicted by an analytical model. *Am J Physiol Regul Integr Comp Physiol* 250: R1021–R1027, 1986.
9. **Carabello BA.** Evolution of the study of left ventricular function: everything old is new again. *Circulation* 105: 2701–2703, 2002.
10. **Champion HC, Villnave DJ, Tower A, Kadowitz PJ, Hyman AL.** A novel right-heart catheterization technique for in vivo measurement of vascular responses in lungs of intact mice. *Am J Physiol Heart Circ Physiol* 278: H8–H15, 2000.
11. **Chesler NC, Argiento P, Vanderpool R, D'Alto M, Naeije R.** How to measure peripheral pulmonary vascular mechanics. *Conf Proc IEEE Eng Med Biol Soc* 2009: 173–176, 2009.
12. **Chesler NC, Roldan A, Vanderpool RR, Naeije R.** How to measure pulmonary vascular and right ventricular function. *Conf Proc IEEE Eng Med Biol Soc* 2009: 177–180, 2009.
13. **Clark JE, Kottam A, Motterlini R, Marber MS.** Measuring left ventricular function in the normal, infarcted and CORM-3-preconditioned mouse heart using complex admittance-derived pressure volume loops. *J Pharmacol Toxicol Methods* 59: 94–99, 2009.
14. **D'Orio V, Lambermont B, Detry O, Kolh P, Potty P, Gerard P, Marcelle R.** Pulmonary impedance and right ventricular-vascular coupling in endotoxin shock. *Cardiovasc Res* 38: 375–382, 1998.
15. **Feldman MD, Erikson JM, Mao Y, Korcarz CE, Lang RM, Freeman GL.** Validation of a mouse conductance system to determine LV volume: comparison to echocardiography and crystals. *Am J Physiol Heart Circ Physiol* 279: H1698–H1707, 2000.
16. **Frenneaux M, Williams L.** Ventricular-arterial and ventricular-ventricular interactions and their relevance to diastolic filling. *Prog Cardiovasc Dis* 49: 252–262, 2007.
17. **Fujita M, Mason RJ, Cool C, Shannon JM, Hara N, Fagan KA.** Pulmonary hypertension in TNF-alpha-overexpressing mice is associated with decreased VEGF gene expression. *J Appl Physiol* 93: 2162–2170, 2002.
18. **Georgakopoulos D, Mitzner WA, Chen CH, Byrne BJ, Millar HD, Hare JM, Kass DA.** In vivo murine left ventricular pressure-volume relations by miniaturized conductance micromanometry. *Am J Physiol Heart Circ Physiol* 274: H1416–H1422, 1998.
19. **Handoko ML, de Man FS, Happe CM, Schalij I, Musters RJ, Westerhof N, Postmus PE, Paulus WJ, van der Laarse WJ, Vonk-Noordegraaf A.** Opposite effects of training in rats with stable and progressive pulmonary hypertension. *Circulation* 120: 42–49, 2009.
20. **Hunter KS, Lee PF, Lanning CJ, Ivy DD, Kirby KS, Claussen LR, Chan KC, Shandas R.** Pulmonary vascular input impedance is a combined

measure of pulmonary vascular resistance and stiffness and predicts clinical outcomes better than pulmonary vascular resistance alone in pediatric patients with pulmonary hypertension. *Am Heart J* 155: 166–174, 2008.
21. **Joho S, Ishizaka S, Sievers R, Foster E, Simpson PC, Grossman W.** Left ventricular pressure-volume relationship in conscious mice. *Am J Physiol Heart Circ Physiol* 292: H369–H377, 2007.
22. **Kass DA, Hare JM, Georgakopoulos D.** Murine cardiac function: a cautionary tail. *Circ Res* 82: 519–522, 1998.
23. **Kass DA, Kelly RP.** Ventriculo-arterial coupling: concepts, assumptions, and applications. *Ann Biomed Eng* 20: 41–62, 1992.
24. **Kelly RP, Ting CT, Yang TM, Liu CP, Maughan WL, Chang MS, Kass DA.** Effective arterial elastance as index of arterial vascular load in humans. *Circulation* 86: 513–521, 1992.
25. **Kolh P, Lambermont B, Ghuysen A, D'Orio V, Gerard P, Morimont P, Tchana-Sato V, Pierard L, Dogne JM, Limet R.** Alteration of left ventriculo-arterial coupling and mechanical efficiency during acute myocardial ischemia. *Int Angiol* 22: 148–158, 2003.
26. **Lambermont B, Delanaye P, Dogne JM, Ghuysen A, Janssen N, Dubois B, Desaive T, Kolh P, D'Orio V, Krzesinski JM.** Large-pore membrane hemofiltration increases cytokine clearance and improves right ventricular-vascular coupling during endotoxic shock in pigs. *Artif Organs* 30: 560–564, 2006.
27. **Lambermont B, Ghuysen A, Kolh P, Tchana-Sato V, Segers P, Gerard P, Morimont P, Magis D, Dogne JM, Masereel B, D'Orio V.** Effects of endotoxic shock on right ventricular systolic function and mechanical efficiency. *Cardiovasc Res* 59: 412–418, 2003.
28. **Lankhaar JW, Westerhof N, Faes TJ, Marques KM, Marcus JT, Postmus PE, Vonk-Noordegraaf A.** Quantification of right ventricular afterload in patients with and without pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 291: H1731–H1737, 2006.
29. **Leather HA, Segers P, Berends N, Vandermeersch E, Wouters PF.** Effects of vasopressin on right ventricular function in an experimental model of acute pulmonary hypertension. *Crit Care Med* 30: 2548–2552, 2002.
30. **Little WC.** The left ventricular dP/dt_{max}-end-diastolic volume relation in closed-chest dogs. *Circ Res* 56: 808–815, 1985.
31. **McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, Varga J, Harrington RA, Anderson JL, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Grines CL, Hlatky MA, Jacobs AK, Kaul S, Lichtenberg RC, Lindner JR, Moliterno DJ, Mukherjee D, Pohost GM, Rosenson RS, Schofield RS, Shubrooks SJ, Stein JH, Tracy CM, Weitz HH, Wesley DJ, ACCF/AHA.** ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. *Circulation* 119: 2250–2294, 2009.
32. **Morecroft I, Pang L, Baranowska M, Nilsen M, Loughlin L, Dempsie Y, Millet C, MacLean MR.** In vivo effects of a combined 5-HT1B receptor/SERT antagonist in experimental pulmonary hypertension. *Cardiovasc Res* 85: 593–603, 2010.
33. **Morpurgo M, Jezek V, Ostadal B.** Pulmonary input impedance or pulmonary vascular resistance? *Monaldi Arch Chest Dis* 50: 282–285, 1995.
34. **Murali S, Uretsky BF, Reddy PS, Tokarczyk TR, Betschart AR.** Reversibility of pulmonary hypertension in congestive heart failure patients evaluated for cardiac transplantation: comparative effects of various pharmacologic agents. *Am Heart J* 122: 1375–1381, 1991.
35. **Nakamoto T, Cheng CP, Santamore WP, Iizuka M.** Estimation of left ventricular elastance without altering preload or afterload in the conscious dog. *Cardiovasc Res* 27: 868–873, 1993.
36. **Nichols WW, O'Rourke MF, Avolio AP, Yaginuma T, Murgo JP, Pepine CJ, Conti CR.** Effects of age on ventricular-vascular coupling. *Am J Cardiol* 55: 1179–1184, 1985.
37. **Nikam VS, Schermuly RT, Dumitrascu R, Weissmann N, Kwapiszewska G, Morrell N, Klepetko W, Fink L, Seeger W, Voswinckel R.** Treprostinil inhibits the recruitment of bone marrow derived circulating fibrocytes in chronic hypoxic pulmonary hypertension. *Eur Respir J*. 2010 Jun 4 [Epub ahead of print].
38. **Ochoa CD, Yu L, Al-Ansari E, Hales CA, Quinn DA.** Thrombospondin-1 null mice are resistant to hypoxia-induced pulmonary hypertension. *J Cardiothorac Surg* 5: 32, 2010.
39. **O'Rourke MF, Yaginuma T, Avolio AP.** Physiological and pathophysiological implications of ventricular/vascular coupling. *Ann Biomed Eng* 12: 119–134, 1984.
40. **Otto C, Hein L, Brede M, Jahns R, Engelhardt S, Grone HJ, Schutz G.** Pulmonary hypertension and right heart failure in pituitary adenylate cyclase-activating polypeptide type I receptor-deficient mice. *Circulation* 110: 3245–3251, 2004.
41. **Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA.** Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc* 3: 1422–1434, 2008.
42. **Pagnamenta A, Fesler P, Vandinivit A, Brimioulle S, Naeije R.** Pulmonary vascular effects of dobutamine in experimental pulmonary hypertension. *Crit Care Med* 31: 1140–1146, 2003.
43. **Pekkan K, Frakes D, De Zelicourt D, Lucas CW, Parks WJ, Yoganathan AP.** Coupling pediatric ventricle assist devices to the Fontan circulation: simulations with a lumped-parameter model. *ASAIO J* 51: 618–628, 2005.
44. **Porterfield JE, Kottam AT, Raghavan K, Escobedo D, Jenkins JT, Larson ER, Trevino RJ, Valvano JW, Pearce JA, Feldman MD.** Dynamic correction for parallel conductance, GP, and gain factor, alpha, in invasive murine left ventricular volume measurements. *J Appl Physiol* 107: 1693–1703, 2009.
45. **Rex S, Missant C, Claus P, Buhre W, Wouters PF.** Effects of inhaled iloprost on right ventricular contractility, right ventriculo-vascular coupling and ventricular interdependence: a randomized placebo-controlled trial in an experimental model of acute pulmonary hypertension. *Crit Care* 12: R113, 2008.
46. **Robotham JL.** Saline volume expansion and cardiovascular physiology: novel observations, old explanations, and new questions. *Crit Care* 8: 315–318, 2004.
47. **Rockman HA, Ono S, Ross RS, Jones LR, Karimi M, Bhargava V, Ross J Jr, Chien KR.** Molecular and physiological alterations in murine ventricular dysfunction. *Proc Natl Acad Sci USA* 91: 2694–2698, 1994.
48. **Sagawa K.** The end-systolic pressure-volume relation of the ventricle: definition, modifications and clinical use. *Circulation* 63: 1223–1227, 1981.
49. **Sagawa K, Maughan L, Suga H Sunagawa K.** *Cardiac Contraction and the Pressure-Volume Relationship.* 1988.
50. **Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, Sydykov A, Lai YJ, Weissmann N, Seeger W, Grimminger F.** Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 115: 2811–2821, 2005.
51. **Scherrer-Crosbie M, Steudel W, Hunziker PR, Foster GP, Garrido L, Liel-Cohen N, Zapol WM, Picard MH.** Determination of right ventricular structure and function in normoxic and hypoxic mice: a transesophageal echocardiographic study. *Circulation* 98: 1015–1021, 1998.
52. **Schneider AJ, Groeneveld AB, Teule GJ, Nauta J, Heidendal GA, Thijs LG.** Volume expansion, dobutamine and noradrenaline for treatment of right ventricular dysfunction in porcine septic shock: a combined invasive and radionuclide study. *Circ Shock* 23: 93–106, 1987.
53. **Segers P, Georgakopoulos D, Afanasyeva M, Champion HC, Judge DP, Millar HD, Verdonck P, Kass DA, Stergiopulos N, Westerhof N.** Conductance catheter-based assessment of arterial input impedance, arterial function, and ventricular-vascular interaction in mice. *Am J Physiol Heart Circ Physiol* 288: H1157–H1164, 2005.
54. **Stegger L, Heijman E, Schafers KP, Nicolay K, Schafers MA, Strijkers GJ.** Quantification of left ventricular volumes and ejection fraction in mice using PET, compared with MRI. *J Nucl Med* 50: 132–138, 2009.
55. **Suga H, Sagawa K.** Instantaneous pressure-volume relationships and their ratio in the excised, supported canine left ventricle. *Circ Res* 35: 117–126, 1974.
56. **Suga H, Sagawa K, Shoukas AA.** Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. *Circ Res* 32: 314–322, 1973.
57. **Sunagawa K, Yamada A, Senda Y, Kikuchi Y, Nakamura M, Shibahara T, Nose Y.** Estimation of the hydromotive source pressure from ejecting beats of the left ventricle. *IEEE Trans Biomed Eng* 27: 6: 299–305, 1980.
58. **Vanderpool RR, Naeije R, Chesler NC.** Impedance in isolated mouse lungs for the determination of site of action of vasoactive agents and disease. *Ann Biomed Eng* 38: 1854–1861, 2010.
59. **Wauthy P, Abdel Kafi S, Mooi WJ, Naeije R, Brimioulle S.** Inhaled nitric oxide versus prostacyclin in chronic shunt-induced pulmonary hypertension. *J Thorac Cardiovasc Surg* 126: 1434–1441, 2003.

60. **Wauthy P, Pagnamenta A, Vassalli F, Naeije R, Brimioulle S.** Right ventricular adaptation to pulmonary hypertension: an interspecies comparison. *Am J Physiol Heart Circ Physiol* 286: H1441–H1447, 2004.
61. **Wu X, Du L, Xu X, Tan L, Li R.** Increased nitrosoglutathione reductase activity in hypoxic pulmonary hypertension in mice. *J Pharmacol Sci* 113: 32–40, 2010.
62. **Yang B, Larson DF, Beischel J, Kelly R, Shi J, Watson RR.** Validation of conductance catheter system for quantification of murine pressure-volume loops. *J Invest Surg* 14: 341–355, 2001.
63. **Yang B, Larson DF, Watson R.** Age-related left ventricular function in the mouse: analysis based on in vivo pressure-volume relationships. *Am J Physiol Heart Circ Physiol* 277: H1906–H1913, 1999.
64. **Yuan L, Wang T, Liu F, Cohen ED, Patel VV.** An evaluation of transmitral and pulmonary venous Doppler indices for assessing murine left ventricular diastolic function. *J Am Soc Echocardiogr* 23: 887–897, 2010.
65. **Zhao L, Long L, Morrell NW, Wilkins MR.** NPR-A-deficient mice show increased susceptibility to hypoxia-induced pulmonary hypertension. *Circulation* 99: 605–607, 1999.