Conductance catheter-based assessment of arterial input impedance, arterial function, and ventricular-vascular interaction in mice

Patrick Segers,1 Dimitrios Georgakopoulos,2 Marina Afanasyeva,3 Hunter C. Champion,2 Daniel P. Judge,2 Hunity D. Millar,4 Pascal Verdonck,1 David A. Kass,2 Nikos Stergiopulos,5 and Nico Westerhof6

1Hydraulics Laboratory, Institute Biomedical Technology, Ghent University, Gent, Belgium; Division of Cardiology, 2Department of Medicine, and 3Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland; 4Millar Instruments, Houston, Texas; 5Laboratory of Hemodynamics and Cardiovascular Technology, Swiss Federal Institute of Technology (École Polytechnique Fédérale de Lausanne), Lausanne, Switzerland; and 6Laboratory for Physiology, ICaR-VU, Vrije Universiteit Medical Center, Amsterdam, Netherlands

Submitted 13 May 2004; accepted in final form 19 October 2004

Segers, Patrick, Dimitrios Georgakopoulos, Marina Afanasyeva, Hunter C. Champion, Daniel P. Judge, Hunity D. Millar, Pascal Verdonck, David A. Kass, Nikos Stergiopulos, and Nico Westerhof. 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; doi:10.1152/ajpheart.00414.2004.—Global assessment of both cardiac and arterial function is important for a meaningful interpretation of pathophysiologic changes in animal models of cardiovascular disease. We simultaneously acquired left ventricular (LV) and aortic pressure and LV volume (VLV) in 17 open-chest anesthetized mice (26.7 ± 3.2g) during steady-state (BL) and caval vein occlusion (CVO) using a 1.4-Fr dual-pressure conductance catheter and in a subgroup of eight animals during aortic occlusion (AOO). Aortic flow was obtained from numerical differentiation of VLV. AOO increased input impedance (Zin) for the first two harmonics, increased characteristic impedance (0.025 ± 0.007 to 0.040 ± 0.011 mmHg μl-1 s-1, P < 0.05), and shifted the minimum in Zin from the third to the sixth harmonic. For all conditions, the Zin could be well represented by a four-element windkessel model. The augmentation index increased from 116.7 ± 7.8% to 145.9 ± 19.5% (P < 0.01) as well as estimated pulse-wave velocity (3.50 ± 0.94 to 5.95 ± 1.62 m/s, P < 0.05) and arterial elastance (Ea, 4.46 ± 1.62 to 6.02 ± 1.43 mmHg μl/P < 0.01). AOO altered the maximal slope (Emax) and intercept (V0) of the end-systolic pressure-volume relation (ESPVR) measured during transient loading conditions (31, 32). Over the past few years, miniaturized conductance catheters have been successfully applied in the mouse to assess cardiac function (Emax) in wild-type and genetically modified mice (7, 21). It has been demonstrated that, after normalization for heart rate, the change of ventricular elastance over the cardiac cycle in the human and the mouse are similar, indicating similarities in the intrinsic contraction patterns of both species (7). From these measurements, one can also assess vascular function indexed by effective arterial elastance (Ea), which can be approximated as the ratio of end-systolic pressure and stroke volume (SV) (10). This index represents a lumped model of the vascular system and is useful to study heart-arterial coupling in terms of the Eal/Emax framework.

Arterial Zin, derived from simultaneously measured aortic pressure (Pao) and flow, however, remains the most complete description of global arterial function (14). In recent work, Reddy et al. (20) measured aortic Zin in the mouse using Doppler ultrasound for flow velocity measurement, whereas pressure was measured invasively using human intracoronary pressure sensor. They demonstrated that the mouse input impedance spectrum is similar to that in other mammals and exhibits the same changes with aging as reported in humans (14, 16). The technology, however, does not allow for simultaneous assessment of cardiac parameters.

In many disease states, alterations in both cardiac and vascular properties occur to maintain blood pressure and flow, thereby making it difficult to independently assess relative changes in each system (23). Therefore, simultaneous acquisition of all components of cardiovascular function is a prerequisite for a meaningful interpretation of individual changes. In this study, we use a high-fidelity 1.4-Fr custom-made, dual-pressure sensor mouse conductance system to simultaneously assess Paw and intraventricular pressure and volume. Given the fact that aortic flow can be estimated as the derivative of ventricular volume during ejection (with the assumption of intact mitral valve function), it is possible to simultaneously acquire cardiac function and arterial Zin using a single catheter.

CARDIOVASCULAR RESEARCH is increasingly being performed in small rodents, such as the mouse or the rat. In particular, studies in the mouse allow for specific genetic alterations in either the heart or vascular system. The backbone of this type of fundamental research is the quantitative assessment of both cardiac and arterial function to provide the link between cardiovascular genotyping and phenotyping. Currently, the gold standard for the quantification of cardiac function remains the maximal slope (Emax) and intercept (V0) of the end-systolic pressure-volume relation (ESPVR) measured during transient loading conditions (31, 32).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Demonstrating the feasibility of this technique of $Z_{in}$ measurement is the first goal of the study. 

$Z_{in}$ is a mathematically complex property that encompasses mechanical characteristics of the cardiovascular system and the effects of wave propagation and reflection. Its interpretation may be facilitated by the use of lumped parameter “windkessel” models (39). These models consist of a limited number of parameters [such as total systemic vascular resistance, aortic characteristic impedance ($Z_0$), and total arterial compliance], which represent biophysical characteristics of the arterial system. By matching measured impedance to the impedance of the model, the $Z_{in}$ is translated in terms of model parameter values that correspond to physiological properties, thereby providing a convenient way to describe the arterial system. The second goal of this study is therefore to fit the measured data to a four-element windkessel model (27) and to provide reference values for the lumped model parameters in the mouse. In the past, we have also done research on methods to assess total arterial compliance, i.e., the area method, the ratio of SV to pulse pressure (PP), and their relation to the PP method (24, 26). We make use of the available data sets to apply these methods and verify whether our findings in large animals also apply to the mouse.

MATERIALS AND METHODS

Experimental protocol. This study was performed in 17 anesthetized open-chest mice of either sex weighing 26.7 ± 3.2 g and consisting of C57BL/6, 129SVEV, BALB/c, and BL6/129 strains. Animal treatment and care were provided in accordance with institutional guidelines, and the protocol was approved by the Animal Care and Use Committee of the Johns Hopkins University. Anesthesia was initiated with methoxyflurane inhalation followed by intraperitoneal injection of urethane (750 mg/kg) and etomidate (1–2 mg/kg) dissolved in normal saline. Supplemental intraperitoneal anesthesia (one-fifth dose) was provided if needed so that the animals remained unresponsive to tail pinch by forceps as assessed by changes in heart rate and blood pressure. A heating pad was placed underneath the animal, and the temperature was set to 37.5°C. The animals were intubated with a blunt 19-gauge needle via a tracheotomy and were ventilated with a custom-designed constant-pressure ventilator with 100% oxygen at 120 breaths/min and a tidal volume of 200 μL. The left external jugular vein was exposed by blunt dissection under topical lidocaine anesthesia, and a 31-gauge needle was inserted directly into the lumen. Fluid supplementation was achieved using 12.5% human albumin infused at 20 μL/min for 5 min.

The chest was entered through an anterior thoracotomy by visualization under a dissecting microscope, and a small apical stab was made at the left ventricular (LV) apex, leaving the pericardium as intact as possible. A custom-made, four-electrode conductance catheter with a dual-pressure sensor (Millar Instruments, Houston, TX) was then advanced retrogradely into the LV along the cardiac longitudinal axis with the distal tip (containing a pressure sensor) in the aortic root and the proximal electrode just within the endocardial wall of the LV apex. With the catheter fixed in place, the animal was turned over on its side. Care was taken to maintain catheter position by online visualization of the shape and position of the pressure-volume (PV) loops. A limited lateral thoracotomy was performed, and the descending aorta was dissected free from the spinal column just above the level of the diaphragm. A flow probe (1RB, Transonic; Ithaca, NY) was placed around the thoracic aorta and filled with conducting gel, and the measured flow served for calibration of the conductance catheter (gain factor $\alpha$) using regressions of SV from thoracic flow and SV from conductance catheter on a beat-by-beat basis during inferior vena cava (IVC) occlusion for each mouse. This provides an estimate of the gain over a large loading range instead of a single estimate at steady state (7). Hypertonic saline infusion was performed for the assessment of parallel conductance ($V_c$) by bolus injection of 5–10 μL 35% saline.

Data were measured during steady-state conditions (baseline, BL) and during transient preload decline, achieved by manual compression of the IVC (VCO). Data were also recorded in a subgroup of eight animals during transient afterload increase, achieved by progressive occlusion of the aorta (AAO). To facilitate this manipulation, a 32-gauge stainless steel wire was deployed in the shape of an “L” and slipped underneath the esophagus as support when the thoracic aorta was manually compressed just above the diaphragm, which progressively occluded the aorta until complete occlusion was obtained. All signals were digitized at 2 kHz and stored to disk for subsequent analysis.

Hemodynamic analysis. The VCO procedure yielded 10–20 successive cardiac cycles over the ensuing 2 s. We approximated the ESPVR as linear, fitting a linear regression line through the end-systolic points of typically 15–25 cycles, selected from the moment that LV pressure (P_{LV}) started to decline until achievement of a new steady state. An iterative method programmed in Matlab (The Mathworks; Natick, MA) was used to identify the end-systolic points, where elastance $E(t)$ was first calculated as $P_{LV}/(V_{LV} - V_0)$ (where $V_{LV}$ is LV volume) with an initial value for $V_0 = 0$. The points in the PV plane corresponding to maximal $E(t)$ for each cycle were identified as the end-systolic points. Linear regression analysis on these points yielded a first estimate of $E_{max}$ and a new estimate of $V_0$. This procedure was repeated with the resulting $V_0$ until successive values for $V_0$ (and $E_{max}$) did not differ by >0.1%, typically within three to four iterations. We also assessed the slope and intercept of the ESPVR during transient aortic occlusion in the subgroup of eight animals. The period with transient data was shorter than in VCO and yielded 5 to 10 cycles in transient conditions.

Steady-state measurements, obtained during a 1- to 2-s data sequence, contained 10–20 cycles and were averaged to construct a representative steady-state beat with $P_{ao}$, $P_{LV}$, and $V_{LV}$ tracings. Aortic flow ($Q_{ao}$) was obtained as the time derivative (d$V_{LV}$/dt) of $V_{LV}$ during the ventricular ejection period: $Q_{ao} = -dV_{LV}/dt$, as illustrated in Fig. 1. Because the numerical differentiation of $V_{LV}$ may introduce noise into the $Q_{ao}$ signal, ventricular volume data were first filtered using a Savitsky-Golay filter of the third-order and 15-sample frame width (Matlab, The Mathworks), which implies that smoothing is obtained by fitting a third-order polynomial over a 15-sample point window (1 cardiac cycle typically contains 200 samples) sliding over the data. We chose this type of filter because it is better in preserving the pertinent high-frequency components of a signal than more traditional low-pass filters (17). The Savitsky-Golay filter does not have a clear cut-off frequency above which the signal is filtered, but the power spectra demonstrated that the additional smoothing effect of the filter (on top of smoothing effect of averaging the data) was only apparent for frequencies above 200 Hz. When applied by itself to the raw data with the parameters used, the Savitsky-Golay filter reduced the power of the frequencies above 100 Hz.

Standard hemodynamic parameters such as heart rate, systolic blood pressure (SBP), mean arterial pressure (MAP), and diastolic (DBP) arterial blood pressure were derived. SV was calculated as the area under the flow curve, and cardiac output (CO) was calculated as a product of SV and heart rate. For each cycle, ventricular end-diastolic ($P_{eda}$, $V_{eda}$) and end-systolic ($P_{es}$, $V_{es}$) pressure and volume, respectively, were derived. Effective $E_0$ was calculated as the ratio of $P_{es}$ and SV. For the VCO and AOO sequences, two to three successive beats at the end of each procedure were averaged to obtain a representative beat.

For each animal, $P_{ao}$ and $Q_{ao}$ were decomposed into a series of sinusoidal harmonics using a discrete Fourier transform (Matlab, The Mathworks), transforming them from the time domain into the frequency domain. The pressure and flow wave are thus considered as

AJP-Heart Circ Physiol • VOL 288 • MARCH 2005 • www.ajpheart.org
simultaneously present sinusoidal waves (harmonics) with frequencies that are natural multiples of the heart frequency, and the arterial system can then be studied at a specific frequency. It is common to represent these harmonics in their complex form by their amplitude (or modulus) and phase angle. For a given frequency, \( Z_{in} \) is then calculated as the ratio of the pressure and flow harmonics at that frequency, and we considered the steady (or DC component) and the first 10 harmonics. \( Z_{in} \) is also a complex number, represented in the frequency domain, with the amplitude of \( Z_{in} \) for a given harmonic frequency being the ratio of the amplitudes of the same pressure and flow harmonic frequency, and the phase angle the difference between pressure and flow phase angle of the same harmonics. The ratio of mean \( P_{ao} \) and \( Q_{ao} \) (DC component of \( Z_{in} \)) is systemic vascular resistance (\( R \)). Aortic \( Z_0 \) was estimated \( I \) in the frequency domain (\( Z_{0-FD} \)) as the average modulus of the fifth to tenth harmonic and \( 2 \) in the time domain (\( Z_{0-TD} \)) by calculating the slope to the \( Q_{ao}-P_{ao} \) relation in the early systolic upstroke, where this relation was linear (6). \( P_{ao} \) was separated into a forward (\( P_f \)) and backward (\( P_b \)) running components using the linear wave separation method (38), and the reflection coefficient was calculated as the ratio of the amplitude of \( P_b \) and \( P_f \). We also calculated the augmentation index (\( AIx \)) as a measure of arterial wave reflection intensity (16). To further allow comparison of our data with parameters commonly used in arterial function analysis, we estimated pulse-wave velocity (\( PWV \)) from \( Z_{0-FD} \) as \( PWV = Z_{0-FD}A/p \), assuming density of blood \( \rho = 1.060 \) kg/m\(^3\) and (constant) aortic cross-sectional area \( A = 1.13 \) mm\(^2\) (diameter 1.2 mm) (8). Because \( Z_0 \) represents the characteristics of the proximal aorta, the calculated PWV applies to the proximal part of the aorta.

The averaged steady-state data at baseline, VCO, and AOO were subsequently fitted to a four-element lumped parameter windkessel model consisting of total peripheral resistance, total arterial compliance (\( C \)), total inductance (\( L \)), and aortic \( Z_0 \). Fitting was performed using a Matlab nonlinear least-squares fitting algorithm (Gauss-Newton method) with default settings for convergence and tolerance criteria. Fitted \( P_{ao} \) was calculated from the response of the four-element windkessel model to the measured \( Q_{ao} \), which was applied as an input into the model (25). Simultaneous fitting of all four parameters with this routine introduced large uncertainty in the estimation of \( C \) and \( L \), with results depending on initial values. Therefore, these two parameters were fitted in an iterative way. First, \( C \) was given a fixed arbitrary starting value (0.5 \( \mu l/mmHg \)), and \( R \), \( Z_0 \), and \( L \) were assessed. Subsequently, \( R \), \( Z_0 \), and \( C \) were fitted with fixed \( L \), using the value from the first iteration. This alternating scheme was repeated until values for the four parameters no longer changed, which occurred maximally after two repetitions. Confidence intervals of 95% of the four parameters were calculated.

The difference between measured \( (P_{ao}) \) and fitted \( (P_{fit}) \) pressure was quantified by the root mean square error (RMSE) calculated as

\[
\text{RMSE} = \sqrt{\frac{\sum (P_{ao} - P_{fit})^2}{N}}
\]

with \( N \) as the number of sample points in the cycle and by the relative area difference (%AD)

\[
\%\text{AD} = 100 \frac{\sum |P_{ao} - P_{fit}|}{\sum P_{ao}}
\]

which represents the absolute area between the measured and fitted pressure curve, respectively, normalized for the area under \( P_{ao} \).

Total arterial compliance was further assessed using earlier described methods, including the PP method (\( C_{PPM} \)) (26), the area method (\( C_{area} \)) (12), and the ratio of SV and PP (\( C_{SV/PP} \)) (3).

**Statistical analysis.** The statistical analysis related to the four-element windkessel model fitting was described earlier in MATERIALS AND METHODS. Paired \( t \) tests were applied to assess the effect of VCO or AOO on arterial mechanical parameters. Linear regression and Pearson correlation analysis were used to quantify linear relations between parameters. To assess the effect of VCO or AOO on \( Z_{in} \) modulus or phase, one-way repeated measures ANOVA was performed. When the ANOVA reached statistical significance (\( P < 0.05 \)), post hoc analysis was done at each harmonic frequency using Bonferroni correction to assess the statistical significance level of the measured difference. All statistics were performed using SPSS (version 11.5, SPSS, Chicago, IL).

**RESULTS**

**Hemodynamic analysis.** Hemodynamic data measured in the mouse at baseline, after preload reduction (VCO), and during afterload increase (AOO) are summarized in Table 1. VCO lowers systolic, diastolic, and mean arterial blood pressure, as...
well as reduces PP, SV, and CO. AOO, on the other hand, increases systolic, diastolic, and mean arterial blood pressure and PP, while maintaining SV and CO. There is no change in heart rate with any of the interventions. \( E_{\text{max}} \) measured at VCO is \( 3.23 \pm 1.02 \text{ mmHg} / \mu \text{L} \), with \( V_0 = -19.9 \pm 8.6 \mu \text{L} \). AOO increases the slope of the ESPVR to \( 5.53 \pm 1.53 \mu \text{L} / \mu \text{L} \) and induces a rightward shift in \( V_0 (1.62 \pm 13.51 \mu \text{L}) \) (see Fig. 2 for representative sample data).

Figure 3 displays the measured \( Z_0 \) spectra in terms of modulus and phase angle, as well as the group average. For this average curve, total systemic vascular \( R \), which represents the steady-state (0 Hz) component of the \( Z_0 \) spectrum, is \( 0.41 \pm 0.13 \text{ mmHg} / \mu \text{L}^{-1} \cdot \text{s} \). The phase angle crosses the frequency axis at about 29 Hz. The average modulus of the fifth to tenth harmonic (\( Z_{0-10} \)) is \( 0.026 \pm 0.010 \text{ mmHg} / \mu \text{L}^{-1} \cdot \text{s} \). VCO occlusion has no effect on systemic vascular \( R \) or on \( Z_{0-10} \), and no clear effect on \( Z_0 \) modulus or phase angle. AOO, on the other hand, has a significant effect on the modulus of \( Z_0 (P < 0.001) \). Post hoc analysis demonstrates an increase in the value of systemic vascular \( R \), and the modulus of \( Z_0 \) is higher in the intermediate frequency range (20–40 Hz). \( Z_{0-10} \) is higher than at baseline (Table 2), and the minimum in \( Z_0 \) is shifted toward higher frequencies (~60 Hz).

In addition to the compliance obtained from the four-element windkessel fit, three other methods (the area method, the PP method, and the ratio of SV to PP) to estimate total arterial compliance were applied, with results listed in Table 2. There is a good correlation among the estimates obtained by all three methods (analysis on pooled BL, VCO, and AOO data), varying from 0.86 (\( C_{\text{area}} \) vs. \( C_{\text{SV/PP}} \), \( P < 0.001 \)) to 0.96 (\( C_{\text{PPM}} \) vs. \( C_{\text{SV/PP}} \), \( P < 0.001 \)). Despite high overall correlation, the values for estimated compliance derived by \( C_{\text{PPM}} \) method were somewhat lower than those derived by the other two methods.

At baseline, effective \( E_a \) is \( 4.46 \pm 1.62 \text{ mmHg} / \mu \text{L} \), so that \( E_a / E_{\text{max}} = 1.44 \pm 0.43 \). This ratio remains unchanged with VCO (\( E_a / E_{\text{max}} = 1.36 \pm 0.58 \), \( P = 0.47 \)) and AOO (\( E_a / E_{\text{max}} = 1.21 \pm 0.37 \), \( P = 0.50 \)).

**DISCUSSION**

In this study, we used a custom-made, dual-sensor conductance catheter to simultaneously acquire all components of cardiovascular function, i.e., ventricular function and aortic \( Z_0 \), in the mouse. The use of a conductance catheter for the assessment of murine cardiac function has been previously described (7, 21). The novelty of our study involves the use of the catheter that is equipped with an extra pressure sensor to simultaneously acquire aortic \( Z_0 \). This implies that aortic flow can be obtained as the derivative of ventricular volume, as shown in Fig. 1. This was possible in all data sets, provided that volumes were filtered before numerical differentiation. The resulting \( Z_0 \) patterns show all characteristics of the im-

---

**Table 1. Hemodynamic data measured in mice at baseline, after preload reduction, and during afterload increase**

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>VCO</th>
<th>AOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>17</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>607 ± 42</td>
<td>608 ± 43</td>
<td>609 ± 43.3</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>117.9 ± 13.6</td>
<td>73.6 ± 6.1</td>
<td>176.9 ± 11.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>71.7 ± 12.1</td>
<td>45.6 ± 9.3</td>
<td>97.2 ± 12.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. BL, baseline; VCO, vena cava occlusion; AOO, aortic occlusion; pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; \( P_{ao} \), LV end-systolic pressure; CO, cardiac output; SV, stroke volume; \( E_{\text{max}} \), \( V_0 \), slope and intercept of the end-systolic pressure-volume (PV) relation; \( V_{ao} \), LV end-diastolic volume; \( P_{ao} \), LV end-diastolic blood pressure. *\( P < 0.05 \); †\( P < 0.01 \); ‡\( P < 0.001 \) vs. BL.
In humans, the progressive stiffening of the arterial tree with age or in cardiovascular disease (hypertension, diabetes) has an impact on arterial hemodynamics. In terms of pressure and flow-wave phenomena, the increase in PWV (which may itself as an increase in SBP, often reaching values designated as cut-off values for hypertension (>140 mmHg), and a decrease in DBP (<80 mmHg). This phenomenon is called “isolated systolic hypertension.”

Whereas Zin was measured earlier in larger rodents such as rats (13, 15), the assessment of arterial properties in mouse models is fairly new and made possible through the further miniaturization of (pressure) sensor technology and new developments in ultrasound technology. Recently, Reddy et al. (20) studied the effects of aging on arterial stiffness and reported data on aortic Zin, PWV, and AIX in adult and old mice. Velocities were measured with ultrasound and pressures with the RADI-wire system commonly used in interventional

### Table 2. Arterial function parameters assessed in the mouse at baseline reduced filling, and increased afterload

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BL</th>
<th>VCO</th>
<th>AOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pp/Pa (−)</td>
<td>0.60±0.10</td>
<td>0.48±0.09†</td>
<td>0.67±0.06</td>
</tr>
<tr>
<td>AIX, %</td>
<td>116.7±7.8</td>
<td>103.1±8.3‡</td>
<td>145.9±19.5†</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>3.50±0.94</td>
<td>3.19±1.10</td>
<td>5.95±1.62*</td>
</tr>
<tr>
<td>Ees, mmHg/μl</td>
<td>4.46±1.62</td>
<td>4.24±1.92</td>
<td>6.02±1.43†</td>
</tr>
<tr>
<td>Z0,F,V, mmHg/μl−1⋅s</td>
<td>0.025±0.007</td>
<td>0.022±0.008</td>
<td>0.040±0.011*</td>
</tr>
<tr>
<td>Z0,T,D, mmHg/μl−1⋅s</td>
<td>0.024±0.007</td>
<td>0.023±0.007</td>
<td>0.036±0.008*</td>
</tr>
<tr>
<td>CSVPP, μl/mmHg</td>
<td>0.64±0.15</td>
<td>0.63±0.29</td>
<td>0.29±0.07‡</td>
</tr>
<tr>
<td>CVPP, μl/mmHg</td>
<td>0.38±0.09</td>
<td>0.46±0.20*</td>
<td>0.15±0.04‡</td>
</tr>
<tr>
<td>Cm, μl/mmHg</td>
<td>0.47±0.12</td>
<td>0.70±0.42*</td>
<td>0.29±0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Pp/Pa, ratio of backward to forward wave; AIX, augmentation index; PWV, pulse-wave velocity; Ees, effective arterial elastance; Z0,F,V, frequency and time domain estimate of Zin; CSVPP, CVPP, Cm, total arterial compliance estimated by the ratio of stroke volume (SV) and pulse pressure (PP), the pulse pressure method and the area method, respectively. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. BL.

### Table 3. Four-element WK parameters assessed in the mouse at baseline, reduced filling, and increased afterload

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BL</th>
<th>VCO</th>
<th>AOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>R, mmHg/μl−1⋅s</td>
<td>0.41±0.13</td>
<td>0.39±0.17</td>
<td>0.62±0.09†</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.16±0.06%)</td>
<td>(0.26±0.13%)</td>
<td>(0.54±0.24%)</td>
</tr>
<tr>
<td>C, μl/mmHg</td>
<td>0.50±0.15</td>
<td>0.75±0.43*</td>
<td>0.20±0.06†</td>
</tr>
<tr>
<td>95% CI</td>
<td>(1.59±0.52%)</td>
<td>(2.76±1.23%)</td>
<td>(4.20±3.44%)</td>
</tr>
<tr>
<td>Zs, mmHg/μl−1⋅s</td>
<td>0.021±0.005</td>
<td>0.020±0.007</td>
<td>0.063±0.019*</td>
</tr>
<tr>
<td>95% CI</td>
<td>(2.32±0.97%)</td>
<td>(2.53±0.65%)</td>
<td>(6.26±4.39%)</td>
</tr>
<tr>
<td>L, mmHg/μl−1⋅s²</td>
<td>0.15±0.006</td>
<td>0.011±0.005</td>
<td>0.008±0.004</td>
</tr>
<tr>
<td>95% CI</td>
<td>(8.00±44.4%)</td>
<td>(128.4±124.9%)</td>
<td>(51.5±24.8%)</td>
</tr>
</tbody>
</table>

Values are means ± SD. R, total systemic vascular resistance; C, total arterial compliance; Zs, aortic characteristic impedance; L, total arterial inductance; WK, windkessel. For each parameter, the 95% confidence interval (95% CI) on the estimated parameter is given as percentage of the parameter value. *P < 0.05; †P < 0.001 vs. BL.
cardiology. Reddy et al. (20) reported significant anticipated age-related changes in SBP (from 88 to 116 mmHg), PP (from 29 to 42 mmHg), PWV (from 2.86 to 4.16 m/s), and AIx (from 14 to 37%) in adult and old mice (20). The minimum in Z_o increased from the second to the fourth harmonic in the older mice, further demonstrating the feasibility of measuring age-related changes in arterial function with the applied methodology. Note, however, that the absolute values of blood pressure, PP, and PWV (in both adult and old mice) are somewhat lower than one would expect in conscious animals, possibly due to specifics of the experimental preparation and the anesthetics used, which may also be responsible for the relatively low heart rate (400 beats/min) in their setting. Given the high (nonlinear) dependency of arterial mechanical parameters on blood pressure (11), the reported numbers on PWV and Z_o may therefore be an underestimation of their values in the old, conscious animal.

In our model, aortic banding induces a pressure-modulated increase in arterial stiffness (Z_o; decrease in total arterial compliance), increasing SBP, PP, and AIx up to 177 mmHg, 80 mmHg, and 146%, respectively. Although our model does not account for changes in aortic structure and function related to aging or cardiovascular disease, these values do resemble what is observed in older subjects and in isolated systolic hypertension. When we make some assumptions on the dimensions of the mouse aorta [i.e., assuming a constant diameter of 1.2 mm (8)], it is possible to further quantitatively compare some of our data to what was found by Reddy et al. In particular, the Z_o values reported by Reddy et al. (20) would vary from 0.020 (adult) to 0.031 (old) mmHg·μl^{−1}·s, which is somewhat lower than what we found, but which may again be attributed to the lower pressures in their setting. Converting Z_0 data into the more intuitively interpretable PWV, the increase in Z_0 with AOO is equivalent with an increase in PWV from 3.50 to 5.95 m/s. Given the fact that the proximal aortic cross-sectional area is expected to increase with AOO, the estimated value of 5.95 m/s is even expected to underestimate PWV during AOO. Again, these values are close to what is found in human pathophysiological conditions associated with increased vessel stiffening. These data at least suggest that aortic banding may be an interesting mechanical model to study (chronic) effects of increased afterload in the mouse, and that the conductance catheter-based methodology for assessing arterial function parameter is sensitive enough to detect pressure-modulated increase of arterial stiffness.

There are also some important methodological differences between our study and that of Reddy et al. (20). We approximated aortic flow by differentiating an averaged and filtered ventricular volume tracing, operations that have an effect on the (high) frequency contents of the signal, and hence potentially on some of our results. Analysis of the power spectrum of the volume and flow data demonstrated that averaging of the time series (construction of ensemble average), an operation that is common in the analysis of hemodynamic data, smoothed the data by reducing the high-frequency contents above 100 Hz (greater than the tenth harmonic). As explained before, the Savitsky-Golay filter smoothing reduced the power only for frequencies higher than 100 Hz. We are thus confident that the followed procedure preserved the information contained in the first 10 harmonics (the harmonic range used to derive Z_0). Also, the methodology did not seem to have an impact on the assessment of Z_0, which depends on the high-frequency components of pressure and flow and is therefore susceptible to filter settings. We used both a time and frequency domain method to assess Z_0, with acceptable agreement between both methods as expressed by the linear regression and Bland-Altman analysis (Fig. 4). The mean difference and limits of the agreement between the two methods are 0.00087 and −0.00911 per 0.0108 mmHg·μl^{−1}·s, respectively, and paired t-test indicates no difference (P = 0.26) between the two approaches. Although this is not a fool-proof validation of the methodology (which would require simultaneous measurement of velocity or flow), it is an indication that the frequency contents of the flow signal is sufficiently high for the calculation of impedance spectra. Reddy et al. (20) calculated impedance from flow velocities in the aorta measured with ultrasound equipment, which certainly has the advantage of being noninvasive. On the other hand, the sampling volume covers most of the aortic cross-section, which has the advantage that all velocities within the volume are being sampled but which also has the drawback that there is no information on the precise spatial distribution of these captured velocities. As such, one needs to make assumptions about the shape of the velocity profile (flat vs. parabolic), which may not be easily predictable in complex bending, three-dimensional geometries such as the aorta and which may be modified by physiological or pathological alterations in SV or heart rate. The similarities in the Z_o spectrum in large (including humans) and small mammals has been documented before, and it has been demonstrated that, when scaled for heart rate and

Fig. 4. Relation between characteristic impedance estimated in the frequency (Z_{o-FD}) and time domain (Z_{o-TD}). A: linear regression analysis; B: Bland-Altman plot. Open circles, baseline; filled squares, VCO; filled triangles, aorta occlusion.
Zo, aortic Zin patterns are similar throughout a wide range of mammals (13, 14, 36). Although this work does not include a direct comparison with data from other mammals, it is anticipated that normalized Zin in the mouse is in close agreement with normalized data from other mammals. It has also been previously shown that the Zin spectrum, mainly within the low to midfrequency region, of large mammals, including humans, is well described by windkessel models, particularly by the four-element windkessel model (27). It is therefore anticipated that the same lumped parameter models, with appropriately scaled parameter values, would adequately describe the arterial system. Our data confirm these predictions, although the agreement between measured pressure and the pressure predicted by the four-element windkessel model decreased somewhat with aortic banding. We speculate that this is due to the application of the occluder around the aorta, which creates a strong reflection site relatively close to the heart, resulting in an in vivo Zin spectrum (see also Fig. 3) that is less well described by a lumped parameter model. In this study, data were fitted to the four-element windkessel model, but it is expected that similar results will be obtained using lumped parameter models with other configurations. Also, all known methods for the assessment of total arterial compliance seem to apply to the mouse. As we previously reported in dogs (24), the ratio of SV and PP yields an estimate of total arterial compliance, which is higher than compliance given by any other method. In agreement with earlier findings (24), the area method systematically yields higher compliance estimates than the PP method, and the overestimation is inversely related to the value of the reflection coefficient (r = −0.31, P < 0.05), i.e., the higher the reflection coefficient, the closer CPPM and Carea.

It is common, especially in studies on heart-arterial interaction, to characterize the arterial system in terms of the effective Ea (4, 10), whereas the ventricle is characterized by the slope of the end-systolic PV relation Emax. The link between the Eα/Emax framework and ventricular mechanoenergetics further favored the widespread use of Eα/Emax as the heart-arterial coupling parameter (29, 30). Theoretical work (2), which was subsequently confirmed in large mammals (33, 34), has shown that Eα/Emax is 0.5 to 1 in the normal heart, with the left ventricle operating close to optimal efficiency or stroke work, respectively. In heart failure, coupling deteriorates, resulting in progressive increase of Eα/Emax. In our data set, average Eα/Emax was 1.44 ± 0.43 at baseline, which is higher than the value of 0.49 reported earlier by Georgakopoulos et al. (7). On the other hand, the values for Eα and Emax are similar to recent findings of Reyes et al. (21) who reported Eα and Emax values of 5.9 and 3.1 mmHg/μl, respectively (Eα/Emax about 1.9) (21).

We attribute the difference between these and our previous data at least in part to the animal preparation, which we consider to be much more physiological in this setting. Since these initial experiments, the preparation has undergone substantial modifications, including the use of different anesthesia, fluid supplementation, optimized ventilation parameters, and minimal surgery. This has subsequently resulted in increased systolic pressure, end-diastolic volume, ejection fraction, and maximal first derivative of pressure with a simultaneous decrease in heart rate compared with our first study.

There are some limitations inherent to our study. First, the outcome of the study, in terms of absolute values for the different cardiovascular parameters, highly depends on appropriate calibration of the conductance catheter, which was based on an aortic flow probe around the thoracic aorta. It is, however, technically very difficult to place a flow probe on the ascending aorta of the mouse with a conductance catheter in the heart so as not to distort the signals. The values that we found for Emax and Ea are significantly lower than those previously reported by our group (7) (using earlier prototype conductance catheters in other animal preparation settings) but they are in good agreement with recent data of Reyes et al. (21) obtained with a dual-frequency conductance catheter system. Second, it has been demonstrated that, especially in conditions of enhanced contractility (35), the ESPVR may be better approximated by a nonlinear (e.g., quadratic) curve, particularly in small mammals such as the rat (22). The data presented in Fig. 2 demonstrate that the ESPVR is also curvilinear in the mouse. However, given the fact that the focus of the paper is in quantification of arterial function with eventual extension to ventriculoarterial coupling using the Eα/Emax framework (where linear ESPVR relation is assumed), we chose to use the simple linear approximation of the ESPVR, using all transient beats during AOO and VCO to avoid bias by solely selecting beats at high or low pressures. Third, experiments were conducted in an open thorax setting, which affects intrapleural pressure and thus transmural pressure across the wall of the thoracic aorta. This may have an effect on pressure-dependent mechanical properties, and thus on Zin, PWV, and AIx. On the other hand, we did experiments with the conductance catheter placed into the heart through the right carotid and recorded PV data before and after the diaphragmatic incision and found no changes in the values. Fourth, although data were measured in mice of different strains, the groups are too small for subgroup analysis of arterial system properties in a statistically reliable way. Finally, in terms of practical applicability of our technique, its invasive character remains a drawback, making the technique less suitable for follow-up measurements in chronic experiments. In these settings, ultrasound technology may be more suitable, especially now that it has been shown that diameter distension of superficial arteries can be measured in the mouse (9). The morphology of these diameter distension tracings closely resembles intra-arterial pressure morphology and may offer possibilities to obtain central pressure in a noninvasive way.

In conclusion, we have shown that a dual-pressure conductance catheter can be used to assess both cardiac and arterial parameters. Because no additional methodology is required when PV studies are performed in mice, this technique provides for complete characterization of the cardiovascular system. This should be useful in the assessment of genetic models of heart failure and potential therapies.

REFERENCES


