Comparison of techniques for measuring pulse-wave velocity in the rat

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Mitchell, Gary F., Marc A. Pfeffer, Peter V. Finn, and Janice M. Pfeffer. Comparison of techniques for measuring pulse-wave velocity in the rat. J. Appl. Physiol. 82(1): 203–210, 1997.—We evaluated methods for measuring average and regional pulse-wave velocity along the aorta in 18-mo-old ether-anesthetized male spontaneously hypertensive rats. Catheter-tip manometers were placed in the ascending and descending thoracic aorta via the right carotid and left femoral arteries, respectively. As the distal catheter was withdrawn at 1-cm intervals, the relationship between distal catheter insertion distance and distance between transducers was determined from the intercept of the insertion distance vs. transmission delay regression line. Methods that assessed the foot-to-foot time delay between pressures accurately predicted the separation between catheters (measured distance of 14.3 cm; intercept of 14.0 ± 0.5 cm; \( P = \text{not significant} \)) were highly reproducible (coefficient of variation of 2.3% for repeated measurements) and showed minimal variability (range 509 ± 30 to 600 ± 29 cm/s) along the full length of the aorta. Methods that made use of the pressure-pressure transfer function were spatially (range of variation of 2.3% for repeated measurements) and showed minimal variability and of the effects of reflected waves.

Aorta: hemodynamics; blood pressure; computer analysis; Fourier analysis

NORMAL AGING results in breakdown of the elastic elements of the conduit vessels, leading to an increase in pulse-wave velocity and premature return of the reflected pressure wave to the proximal aorta in systole (11, 21, 22). The resulting late systolic augmentation of the aortic pressure wave increases the pulsatile load on the left ventricle and contributes to left ventricular hypertrophy even after controlling for mean arterial pressure (6, 24, 31). This process is exacerbated by the presence of hypertension (26) or a diet high in sodium (5) and may be reversible with some forms of antihypertensive treatment (9, 23, 25, 29) or by salt restriction (4). Because hypertension has a cumulative time-dependent effect on the breakdown of the elastic elements of the aorta, an assessment of pulse-wave velocity (2) or of the effects of increased pulse-wave velocity on proximal aortic pressure waveform (7) may provide an index of the duration and severity of hypertension.

To measure pulse-wave velocity, it is necessary to measure two pressures with high-fidelity transducers a known distance apart and to accurately determine the time delay between the recorded pressure waveforms. However, reflected waves modify the pressure waveform as it moves distally through the arterial system, making measurement of the time delay difficult. In the frequency domain, reflected waves create frequency-dependent discrepancies between true phase velocity \( (c_{ph}) \), which is determined by the intrinsic properties of the arterial wall and blood, and measured or apparent phase velocity \( (c_{app}) \) (8, 10, 27, 28). Numerous criteria for averaging \( c_{app} \) across a range of frequencies or harmonics have been proposed (10, 13, 14). The resultant mean \( c_{app} \) should approximate \( c_{ph} \). In the time domain, foot-to-foot pulse-wave velocity \( (c_f) \) has been shown to approximate \( c_{ph} \) (10). However, modification of the advancing pressure wave by reflections complicates the definition of the “foot” of the waveform. Evaluating the precise curvilinear distance between pressure transducers is also difficult, especially in small animals where arterial size limits the use of multisensor catheters with transducers mounted a known distance apart. In the present study, we evaluated a new technique for determining the time delay between simultaneously recorded waveforms. Our goals were to establish a robust method for assessing pulse-wave velocity and to assess the regional variability in pulse-wave velocity along the aorta in the rat.

METHODS

Study animals. Experiments were performed on 18-mo-old ether-anesthetized male spontaneously hypertensive rats obtained from colonies that were bred and maintained in our animal facility. Complete regional analysis of pulse-wave velocity using the pullback procedure described below was performed in seven animals. Reproducibility of pulse-wave velocity measurement techniques at baseline and in response to vasodilation and vasoconstriction was assessed in total of 13 animals.

Hemodynamic preparation. The 2-Fr catheter-tip pressure transducers used in this study (model SPR-407, Millar Instruments) were calibrated against a mercury manometer. Before each study, the transducers were balanced and then maintained in a 37°C water bath for ≥1 h to confirm stability of the zero baseline. Atmospheric zeroes were recorded for each study immediately before insertion and after removal of the pressure transducers. The femoral pressure catheter was marked at 1-cm intervals along its distal 20 cm starting from the center of the pressure transducer. After induction of anesthesia with ether, a tracheotomy was performed and the animal was connected to a rodent respirator for maintenance of ventilation and ether anesthesia. The right jugular vein was cannulated to allow for intravenous infusions. The right carotid and left femoral arteries were cannulated with 2-Fr catheter-tip pressure transducers. The tip of the carotid catheter was advanced into the ascending aorta. The femoral catheter was advanced to the 12-cm mark, placing its pressurerecorder into the proximal descending thoracic aorta. Once animals were fully instrumented, an incremental pullback of
the femoral pressure catheter was performed, with proximal and distal pressures recorded at 1-cm intervals starting at 12 cm and pulling back to the 3-cm point, which placed the transducer at approximately the level of the aortic bifurcation. The catheter was then returned to the 5-cm point (2 cm proximal to the aortic bifurcation) for the remainder of the study. This pullback information, obtained over the course of ~2 min, was used to calculate pulse-wave velocity and the distance between the two transducers as detailed below.

After the pullback procedure, a midline thoracotomy was performed and a flow probe was placed around the ascending aorta. The location of the tip of the proximal aortic pressure transducer was visually confirmed and adjusted to be 1–2 mm distal to the downstream edge of the electromagnetic flow probe to avoid interference with the flow measurements. Any necessary adjustments in the location of the proximal transducer were considered in subsequent calculations. To assess repeatability of pulse-wave velocity determinations, four baseline recordings were taken over a period of 10–12 min, during which time the animals were hemodynamically stable. Subsequently, a graded infusion of the vasodilator sodium nitroprusside (0.5–30 µg/min) was administered to reduce the mean arterial pressure into the low-normal range (75–100 mmHg). After return of the mean arterial pressure to baseline, a graded infusion of methoxamine (20–1,600 µg·kg⁻¹·min⁻¹) was titrated to produce a rise in mean arterial pressure first into the moderately (135–175 mmHg) and then into the markedly (175–215 mmHg) hypertensive ranges. Steady-state recordings at each of these three data points were made 1 min after initiation of each new dosage level of nitroprusside and methoxamine. At the completion of each study, the final record to avoid noise in late diastole, a preliminary threshold was scanned to find the maximum value of dP/dt derivative of pressure (dP/dt). Each waveform was digitized at 1,000 samples/s by using a 12-bit simultaneously sampling analog-to-digital converter and analyzed on a microcomputer with custom software. Total arterial compliance was calculated from the diastolic central aortic pressure decay (15). The delays between proximal and distal pressure transducers was determined by measuring the length of a segment of polyethylene tubing that was inserted into the proximal aorta at the level of the proximal transducer and advanced antegrade to the level of the distal pressure transducer.

Data analysis. All hemodynamic data were recorded on an FM data recorder (model XR-310, TEAC) for later analysis. Waveforms were digitized at 1,000 samples/s by using a 12-bit simultaneously sampling analog-to-digital converter and analyzed on a microcomputer with custom software. Total arterial compliance was calculated from the diastolic central aortic pressure decay (15). The delays between proximal and distal pressure waveforms were evaluated by hand and were calculated by four automated techniques, two of which were foot-to-foot techniques. The first automated technique was an adaptive threshold-based approach ("threshold" method) that defined the foot of the pressure waveform by evaluating the first derivative of pressure (dP/dt). Each waveform was scanned to find the maximum value of dP/dt, which was calculated by using a five-point linear least squares fit. To avoid noise in late diastole, a preliminary threshold was initially set at 50% of this maximum value. Starting in late diastole, dP/dt was then searched for the first value that exceeded this threshold. Starting from this point, the first derivative was then searched backward for the earliest point at which dP/dt exceeded 20% of the maximum derivative. This point was taken as the foot of the waveform.

The second approach defined the foot of the waveform as the point of intersection of tangent lines drawn through late diastole and early systole ("intersection" method) (1, 13). The tangent through the upstroke was determined by linear regression of five points, starting with the point defining the foot of the waveform by the threshold approach described above. Next, the waveform was searched backward through its nadir to the diastolic point that was isobaric with the threshold foot, again to avoid any presystolic noise. Linear regression through that and the preceding 16 points (17 ms total) determined the diastolic line. The intersection of the diastolic and systolic tangent lines was taken as the foot of the waveform.

The next two techniques used the transfer function between proximal and distal pressures to determine the time delay as previously described (14). Proximal and distal pressure waveforms were transformed to the first 10 harmonics of their respective Fourier series. The real and imaginary components of the transfer function at each harmonic were then calculated by dividing the respective distal pressure component by the proximal aortic pressure component. The regression line of phase vs. frequency for the 10 harmonics of the transfer function phase was then calculated, and the slope of that line was converted to a time delay: \( t = \frac{\text{slope}}{2\pi} \) ("phase-slope" method).

The final technique ("impulse response" method) used the impulse response of the pressure transfer function, which was filtered and inverse transformed by a modification of a previously described technique for calculating the flow/pulse-wave velocity during the pullback procedure. The y-intercept was the distance between the insertion point in the femoral artery and the proximal pressure transducer, i.e., the insertion distance at which the time delay would have become zero if the catheter was placed at the 5-cm point, and the intercept of the regression line was calculated from the linear impulse response of the pressure transfer function, which could then be determined by subtracting the insertion distance from the y-intercept of this regression line. For example, if the catheter was placed at the 5-cm point, and the intercept of the regression line was 13 cm, the transducers would be 8 cm apart. This approach assumed that pulse-wave velocity was relatively constant along the length of the aorta under study. Deviations from this assumption should be evident from a poor fit of the regression line between location and delay and a reduction in the correlation coefficient for the regression line.

Pulse-wave velocity (slope) and distance between transducers (intercept) obtained by using each of the four automatic techniques were compared with a "measured" pulse-wave velocity and the actual distance between transducers (length of polyethylene tubing) at the completion of the study. If an impulsive pressure were introduced at the proximal site at time 0 (14). The timing of the peak of this waveform was therefore taken as the time delay between proximal and distal pressures.

The femoral pullback data were used to calculate pulse-wave velocity and the distance between transducers. Time delays were calculated on five consecutive cardiac cycles at each catheter location, and the results were averaged. If the SD of the delay for the five cardiac cycles at a given location was >1 ms, that location was excluded from the pullback analysis for that method. Linear regression analysis of catheter insertion distance vs. time delay for each catheter position was performed. The (negative) slope of this line was proportional to the spatially and temporally averaged pulse-wave velocity during the pullback procedure. The y-intercept was the distance between the insertion point in the femoral artery and the proximal pressure transducer, i.e., the insertion distance at which the time delay would have become zero if the catheter was placed at the 5-cm point, and the intercept of the regression line was calculated from the linear impulse response of the pressure transfer function, which could then be determined by subtracting the insertion distance from the y-intercept of this regression line. For example, if the catheter was placed at the 5-cm point, and the intercept of the regression line was 13 cm, the transducers would be 8 cm apart. This approach assumed that pulse-wave velocity was relatively constant along the length of the aorta under study. Deviations from this assumption should be evident from a poor fit of the regression line between location and delay and a reduction in the correlation coefficient for the regression line.

Regional variability in pulse-wave velocity along the full length of the descending aorta was assessed by a variety of techniques. First, average pulse-wave velocity for each seg-
and intercept equal to the population means for the given technique. The dashed line, which represents the “gold standard,” is the same in each panel. This line was drawn by setting the slope equal to the negative of the manually measured pulse-wave velocity and the intercept equal to the measured distance between the transducers at the completion of the experiment plus 5 cm (the final location of the femoral catheter). The close fit between the foot-to-foot delays and both the actual and theoretical regression lines suggests that the assumption of a relatively constant pulse-wave velocity along the length of the aorta was reasonable. In contrast, significant deviations from the theoretical regression line were seen for the transfer function-based techniques and the basis for these deviations was explored.

The impulse response technique progressively overestimated the transmission delay as the distal catheter was pulled back into the abdominal aorta and beyond (locations 3–7) (Fig. 1D). This region is expected to be influenced by wave reflections; evidence for this is seen in Fig. 2, which presents a series of impulse response functions for a single representative animal. The initial peak was used to evaluate the time delay between proximal and distal transducers. The smaller secondary peak represents the reflected wave. As the distal catheter was pulled down the aorta, the initial peak occurred later, due to the increased separation between transducers, and the secondary peak occurred earlier, as the distal transducer approached the reflecting site. The progressive overlap between the relatively broad primary and reflected peaks added an additional rightward shift to the primary peak. This becomes apparent when the timing of the peak of the impulse response is compared with the corresponding threshold-based foot-to-foot delays (vertical arrows in Fig. 2) at each position.

The analysis of regional pulse-wave velocity provided an explanation for the decreased slope and intercept of the pullback regression line obtained from the phase-slope delays. Regional pulse-wave velocities obtained by this and the threshold-based foot-to-foot technique were evaluated (Fig. 3A). As suggested in Fig. 1, regional $c_f$ was relatively constant along the aorta. In contrast, there was a region of low $c_f$ in the mid-aorta.

Table 1. Derived slopes and intercepts from pullback data

<table>
<thead>
<tr>
<th>Slope, cm/s</th>
<th>Measured</th>
<th>Threshold</th>
<th>Lines</th>
<th>Phase</th>
<th>Impulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept, cm</td>
<td>552 ± 42</td>
<td>536 ± 54</td>
<td>537 ± 52</td>
<td>502 ± 40*</td>
<td>474 ± 35*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 pullback analyses. Measured slope was obtained by dividing measured distance between proximal and distal transducers by manually determined foot-to-foot delay between proximal and distal pressures when distal transducer was placed at 5-cm location. Measured intercept was obtained by adding 5 cm to distance between transducers at completion of study. *Significantly different compared with measured values by repeated measures analysis of variance with Scheffe testing of individual means, P < 0.05.
(locations 7–8; Fig. 3A) detected by the phase-slope method. This region of low $c_{app}$ (longer delays) produced a small offset along the time axis in the mid-aorta and resulted in a counterclockwise rotation of the pullback regression line, accounting for the diminished slope and $y$-intercept obtained in the pullback analysis (Fig. 1, Table 1).

The basis for the region of low $c_{app}$ in the mid-aorta was established by evaluating the $c_{app}$ contour map (Fig. 4). Considerable regional and frequency-dependent variation in $c_{app}$ was found. There were markedly elevated values at low frequencies due to the effects of reflected waves, which rapidly reached a minimum then oscillated about a value that was approximately the same as $c_f$. Additionally, for each harmonic, acceleration of $c_{app}$ was seen in regions 5–6 in the distal aorta, suggesting a region of wave reflection. A second region of accelerated $c_{app}$ was seen in the proximal aorta (regions 10–11) in the higher frequency range (6th–7th harmonics), suggesting a proximal reflecting site, since this peak was higher than the distal peak (regions 5–6) in the same harmonics. Between these peaks was a deep trough of low pulse-wave velocity that started at the 3rd harmonic at position 11 and hooked around between the proximal and distal peaks ending at the 10th harmonic at position 7–8. As a result of this trough, $c_{TT}$ and $c_{SD}$ were reduced in regions 8–9 and

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Fig. 1. Aortic pullback delays for threshold (A), intersecting lines (B), phase-slope (C), and impulse-response (D) methods ($n = 7$). See text for details.

Fig. 2. Aortic pullback delays for threshold (A), intersecting lines (B), phase-slope (C), and impulse-response (D) methods ($n = 7$). See text for details.

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The effect of averaging criteria on regional variation in apparent phase trend was observed for qualitatively similar to that for pulse-slope technique (Fig. 3). The regional heterogeneity was less. The regional variability was significantly different for 2 methods. There were no significant differences among locations for threshold method.* Significantly different at $P < 0.05$ compared with: $\star$ significanly different at $P < 0.05$ for each technique). The phase-slope method did, however, have a higher variability ($3.6 \pm 1.3\%$) than either of the foot-detection techniques ($P < 0.05$).

The performance of each of the techniques was evaluated over the wide range of vasodilation and vasoconstriction produced by nitroprusside and methoxamine, respectively (Fig. 5). As expected, vasodilation, with its associated reduction in the magnitude of reflected waves, produced a striking concordance between the four techniques. Conversely, vasoconstriction augmented the differences between the techniques, with the transfer function-based techniques progressively underestimating pulse-wave velocity compared with the foot-detection algorithms. In addition, during the two levels of methoxamine infusion, the impulse-response technique returned markedly erroneous results in 5 of 26 analyses involving 4 of the 13 animals, accounting for the relatively wide SE values for that technique (Fig. 5). These sporadic results were produced by the marked difference in harmonic content between proximal and distal waveforms. With vasoconstriction, the harmonic content of the distal waveform was augmented in the higher frequencies (4th-8th harmonics) due to the brisk monophasic upstroke produced by superimposition of the forward and reflected waves. As a result, there were sporadic harmonics with considerable power in the distal waveform, which fell below the resolution of the measuring system in the proximal waveform. The resulting spike in the transfer function produced an augmented harmonic in the impulse response that obscured all useful data.

Finally, the correlation between pulse-wave velocity and total arterial compliance was assessed during vasodilation and vasoconstriction as an independent indicator of the accuracy of the four techniques. Although the correlation between single measurements of pulse-wave velocity and total arterial compliance is poor, because of the stronger effects of volume of the arterial system on compliance than on pulse-wave velocity, the directional changes in the two parameters should be opposite and highly correlated when mean arterial pressure is varied. We found a strong correlation between pulse-wave velocity and compliance when either of the foot-detection algorithms was used ($R = -0.79$; $P < 0.05$ for each technique). The phase slope and impulse-response techniques produced lower correlation coefficients that were, however, still statistically significant ($R = -0.50$ and $-0.39$, respectively).

The repeatability of each of the techniques during a series of four consecutive steady-state measurements was assessed in 13 animals with the catheter at the final 5-cm location. To focus on variability in the delay measurement, the actual measured distance between transducers was used for all techniques. Under basal conditions, mean pulse-wave velocity was comparable for all but the impulse response method, which overestimated the transmission delay and thus underestimated pulse-wave velocity, for the reasons described above (threshold of $598 \pm 65$; intersecting lines of $604 \pm 61$; phase slope of $600 \pm 50$; impulse response of $565 \pm 54$ cm/s; $P < 0.001$). Repeatability, as assessed by the coefficient of variability, was not different for threshold (2.3 \pm 1.3\%), intersecting lines (2.3 \pm 1.1\%), and impulse response (2.5 \pm 1.2\%) methods. The phase-slope method did, however, have a higher variability (3.6 \pm 1.3\%) than either of the foot-detection techniques ($P < 0.05$).

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DISCUSSION

This study is the first to compare various methods for measuring pulse-wave velocity in the rat. We have described a new technique for determining the curvilinear distance between the proximal and distal pressure transducers and have presented a simple yet robust technique for determining the time delay between proximal and distal pressure waveforms. We compared this new foot-detection algorithm to three previously described methods and found that its accuracy and reproducibility met or exceeded that of the more complex methods. We have further described the regional variability in pulse-wave velocity ($c_{ff}$ and $c_{app}$) along the length of the aorta and evaluated the effects of averaging criteria on regional $c_{app}$.

Although the concept of pulse-wave velocity measurement is quite simple, implementation of a robust method for making the measurement can be complex due to limitations in determining both the transmission delay and the distance between pressure sensors. We have presented a simple pullback method for accurately determining the curvilinear distance between the transducers. The method assumes that pulse-wave velocity is reasonably constant along the segment of the aorta under study. The accuracy of the method was likely dependent on having at least one pair of recordings taken with the transducers as closely spaced as possible to minimize errors that could result from extrapolating the regression line to the location intercept. By keeping the distal catheter just beyond the highly curved proximal aorta when the transducers were at their closest point, however, we eliminated the ambiguity of establishing the actual curvilinear distance between transducers in that region, which presents a problem even when the transducers are mounted a fixed distance apart on a single catheter (13, 29).

Measurement of the transmission delay is complicated by the small magnitude of the delay and by the dissimilar nature of the proximal and distal waveforms due to the presence of reflected waves in the arterial system. Attempts to lengthen the transmission delay by increasing the separation between transducers increases the effects of reflected waves on the waveforms. The foot of the pressure waveform, which is too early to be significantly affected by reflections, is often used as a landmark for determining the delay. The resulting $c_{ff}$ has been shown to correlate well with $c_{ph}$ calculated from the Moens-Korteweg equation (10) and eliminates the need to average apparent phase velocities over an empirically determined range of harmonics. However, there is no precise definition for what constitutes the “foot” of the waveform. Many investigators have drawn (or computed) tangent lines through the last part of
diastole and the first part of systole and used the intersection between these lines as the foot. To avoid contamination by the reflected wave, the line through the upstroke of pressure must be limited to the earliest portion of systole. If not, the reflected wave may influence the slope of the line enough to modify the intersection point. The initial point to be used in defining the upstroke must also be qualified. The points immediately after the absolute pressure minimum cannot be used because of the possibility of false triggering due to noise in late diastole, where the pressure tracing can be relatively flat. If such an absolute minimum pressure occurred even 2–3 ms before the actual upstroke, the slope of the line would be considerably blunted, since the entire line is based on only 5 ms.

We tested a related, though less complex (threshold) algorithm that used \( \frac{dP}{dt} \) alone to define the foot of the waveform. To avoid the possibility of false triggering due to late diastolic noise, we found this point by searching backward from the point at which \( \frac{dP}{dt} \) reached 50% of its maximal value. We searched forward to find the initial 50% threshold to avoid the possibility of triggering on the inflection point between primary and secondary pressure peaks in pressure waveforms with late systolic augmentation due to a reflected wave. This approach was computationally related to the intersecting lines technique. However, it avoided the need to fit a line to the intrinsically curvilinear diastolic portion of the pressure waveform, which can also be affected by reflected pressure waves. We based the threshold for each waveform (proximal or distal) on a percentage of peak \( \frac{dP}{dt} \) for that waveform, avoiding the possibility that a distal pressure waveform, adjacent to a reflecting site, would reach the threshold prematurely due to its steeper upstroke. The threshold and intersecting lines techniques gave results that were no different from manually determined values during the pullback procedure. Their repeatability was comparable during multiple steady-state recordings, and they both gave reliable results over a wide range of vasoconstriction and vasodilation.

We compared these foot-to-foot techniques to two additional techniques that were based on the pressure-pressure transfer function. Because the latter techniques use the entire waveform to assess the delay between proximal and distal pressure recordings, they are more susceptible to the effects of wave reflection (20). The phase-slope technique used linear regression to effectively average out the oscillations of phase between the 1st and 10th harmonics (14). This proved comparable to averaging \( c_{app} \) over approximately the 5th through 10th harmonics, as has been done by others (29). The difference in frequency band required to produce a comparable \( c_{app} \) resulted from the differential weight that low- and high-frequency bands had when phase shift rather than phase velocity was averaged. The phase-slope technique was relatively insensitive to the marked increases in \( c_{app} \) in the low-frequency range because these large velocities corresponded to relatively small deviations in phase. Thus marked differences in \( c_{app} \) in the low-frequency range minimally affected the slope of the phase-frequency regression line. In contrast, when averaging was done across harmonics of \( c_{app} \), the low-frequency values, which may be severalfold greater than \( c_{ph} \) or \( c_{ts} \), had a greater effect on the mean.

Several studies have documented regional variation in and discordance between \( c_{ph} \) and \( c_{app} \) in the aortas of humans (14, 29), baboons (12), and dogs (17). Although there are differences in the patterns of variation between these studies, there is a consensus that reflected waves give rise to the variations in \( c_{app} \) (8, 10, 27, 28). Figures 3 and 4 illustrate the basis for discrepancies between \( c_{ph} \) and \( c_{app} \), both in our study and when different studies are compared. Clearly, the frequency band over which \( c_{app} \) is averaged can significantly influence the value of \( c_{app} \). Unfortunately, the appropriate criteria for averaging are not clear. The data presented in Fig. 4 suggest that, up to the 10th harmonic, \( c_{app} \) clearly has not begun to converge to \( c_{app} \), making the choice of averaging criteria difficult. In the study of Newman et al. (19), the high-frequency content of the pressure waves was enhanced by introduction of a pressure impulse. As a result, meaningful values for \( c_{app} \) could be calculated to very high frequencies. In that study, \( c_{app} \) clearly converged to \( c_{ph} \), which was independently assessed through analysis of characteristic impedance and pressure-diameter relations. However, examination of their plot of \( c_{app} \) vs. frequency indicates that this convergence occurred beyond the 10th harmonic. Averaging of values below that range (<20 Hz in their study) would have likely underestimated \( c_{ph} \) substantially in the abdominal aorta where their measurements were taken (see Fig. 5 of Ref. 19). As a result of this regional variation in \( c_{app} \), the phase-slope method gave erroneous results during the pullback procedure. Additionally, this method proved more variable than the foot-to-foot techniques during repeated steady-state recordings and progressively underestimated pulse-wave velocity during vasoconstriction.

The impulse response method was also less reliable than the foot-based methods. If a greater number of harmonics could be calculated and the filtering effect reduced, resulting in primary and secondary peaks that more closely resemble true impulses, then this technique might prove more reliable. Unfortunately, as noted above, the modulus of pressure rapidly approaches the resolution of the measurement system by the 10th harmonic. Therefore, it would be necessary to introduce a waveform with a higher content of power in the high-frequency band to further refine this method (19).

In conclusion, pulse-wave velocity can be reliably measured in small-animal models such as the rat. Unless the goal of the analysis is to study reflected waves, foot-to-foot techniques are more reliable than methods that assess the entire pressure waveform. Careful analyses of pulse-wave velocity should further our understanding of the complex interaction between heart and conduit vessels.