Published in final edited form as:

Ultrasound Med Biol. 2010 July ; 36(7): 1169–1175. doi:10.1016/j.ultrasmedbio.2010.04.002.

Feasibility of dual Doppler velocity measurements to estimate volume pulsations of an arterial segment

Craig J. Hartley,

Department of Medicine, Section of Cardiovascular Research, Baylor College of Medicine and The Methodist DeBakey Heart and Vascular Center, Houston, TX 77030

Anilkumar K. Reddy,

Department of Medicine, Section of Cardiovascular Research, Baylor College of Medicine and The Methodist DeBakey Heart and Vascular Center, Houston, TX 77030

Sridhar Madala,

Indus Instruments, Houston, TX 77058

Mark L. Entman, and

Department of Medicine, Section of Cardiovascular Research, Baylor College of Medicine and The Methodist DeBakey Heart and Vascular Center, Houston, TX 77030

George E. Taffet

Department of Medicine, Section of Cardiovascular Research, Baylor College of Medicine and The Methodist DeBakey Heart and Vascular Center, Houston, TX 77030

Abstract

If volume flow was measured at each end of an arterial segment with no branches, any instantaneous differences would indicate that volume was increasing or decreasing transiently within the segment. This concept could provide an alternative method to assess the mechanical properties or distensibility of an artery noninvasively using ultrasound. The goal of this study was to determine the feasibility of using Doppler measurements of pulsatile velocity (as opposed to flow) at two sites to estimate the volume pulsations of the intervening arterial segment. To test the concept over a wide range of dimensions, we made simultaneous measurements of velocity in a short 5 mm segment of a mouse common carotid artery and in a longer 20 cm segment of a human brachial-radial artery using a two-channel 20 MHz pulsed Doppler, and calculated the waveforms and magnitudes of the volume pulsations during the cardiac cycle. We also estimated pulse wave velocity from the velocity upstroke arrival times and measured artery wall motion using tissue Doppler methods for comparison of magnitudes and waveforms. Volume pulsations estimated from Doppler velocity measurements were 16% for the mouse carotid artery and 4% for the human brachial artery. These values are consistent with the measured pulse wave velocities of 4.2 m/s and 10 m/s respectively and with the mouse carotid diameter pulsation. In addition, the segmental volume waveforms resemble diameter and pressure waveforms as expected. We conclude that with proper application and further validation, dual Doppler velocity measurements can be used to estimate the magnitude and waveform of volume

© 2010 World Federation for Ultrasound in Medicine and Biology. Published by Elsevier Inc. All rights reserved.

Contact Information: Craig J. Hartley, Ph.D., Professor of Medicine (CVS), Baylor College of Medicine, Methodist Hospital, M/S F-602, Houston, TX 77030, Phone: (713) 798-4195, FAX: (713) 796-0015, cjhartley@ieee.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

pulsations of an arterial segment and to provide an alternative noninvasive index of arterial mechanical properties.

Keywords

blood flow velocity; Doppler ultrasound; vascular compliance; pulse wave velocity

INTRODUCTION

The mechanical properties of arteries, especially their elasticity or stiffness, are important factors in determining vascular impedance and in matching the heart to the vascular system to optimize energy transfer and efficiency (Nichols and O'Rourke, 1998; Nussbacher et al., 1999; Hoeks et al., 1999). Indeed, when arteries become stiffer with age or due to hypertension or atherosclerosis, the heart has to work harder to pump blood (Nichols et al., 1985; Brands et al., 1996). Because the increased load presented by stiff arteries reduces cardiac reserve and may stimulate cardiac hypertrophy, there has been considerable interest in methods to assess vascular stiffness noninvasively (Hickler, 1990; Tardy et al., 1991; Wright et al., 1990). The more common methods include measurement of regional and global pulse wave velocity (*PWV*) using tonometric or ultrasonic Doppler methods to sense the pulse arrival time at two sites separated by a known distance (Avolio et al., 1985; Hartley et al., 1997; Mattace-Raso et al., 2009), and the direct measurement of arterial diameter or cross-sectional area during the pressure pulse using ultrasonic imaging methods (Hokanson et al., 1972; Brands et al., 1996; Hoeks et al., 1999; Kanai et al., 2003; Meinders et al., 2001; Meinders and Hoeks, 2004; Hoeks et al., 1985; Levenson et al., 1981). In general, stiffer vessels pulsate less during the cardiac cycle (Avolio et al., 1985; Cohn, 2006), have a higher *PWV* (Nichols and O'Rourke, 1998; Callaghan et al., 1986; Farrar et al., 1978), and are considered a marker of, and a risk factor for, cardiovascular disease (Saito et al., 2008; Arnett et al., 1994; Weber et al., 2004; Van Merode et al., 1988; Mitchell et al., 2010).

Vascular stiffness increases as a function of distending pressure such that stiffness can change even during the cardiac cycle (Nichols and O'Rourke, 1998; Hermeling et al., 2010). In dogs, *PWV* has been shown to vary by 50% in the central aorta from 4 m/s during diastole to 6 m/s during systole (Anliker et al., 1968). However, *PWV* is usually measured only at end-diastole by timing the upstrokes of velocity, pressure, or diameter measured at two sites before any reflections have returned from the periphery (Nichols and O'Rourke, 1998). When arteries become stiffer with age or disease, systolic pressure often increases and diastolic pressure often decreases to accommodate a normal stroke volume (Lim and Towns, 2009) such that *PWV* measured at end-diastole when wall tension is lower may underestimate the overall increase in vessel stiffness; (Hermeling et al., 2010). The more direct methods of measuring diameter pulsations throughout the cardiac cycle using ultrasound are sensitive to regional variations in stiffness caused by branches and plaques. Thus, an alternative method which can estimate the total volume pulsation averaged along a vessel segment during the entire cardiac cycle would be desirable.

If volume flow Q is measured at two sites separated by a distance L along a branchless elastic tube filled with an incompressible fluid, the temporal averages must be the same, but any transient differences in flow indicate that volume is being added to or removed from the segment between the measurement sites. If the two flows Q_1 and Q_2 are subtracted and integrated, the change in volume ΔV as a function of time can be estimated by:

$$\Delta V = \int (Q_2 - Q_1) dt \quad \text{Eq. 1}$$

The average volume in the segment V is the product of the average area A times the length L , and Q is the luminal average velocity v times A . Thus, the relative change in volume $\Delta V/V$ or the relative change in area $\Delta A/A$ (since L is fixed) is given by:

$$\Delta A/A = \Delta V/V = \int ((v_2 - v_1)/L) dt \quad \text{Eq. 2}$$

where v_1 and v_2 are the luminal average velocities at each site. The general concept is shown schematically in Figure 1. Since A is also a function of time, the relationship between Q and v is not quite constant, but the relationship should hold approximately for small changes in area when the area changes at each end are similar. In real vessels there will also be tapering and branching such that one will not be sampling at the distal end all of the flow that enters the vessel at the proximal end. For Eq. 2 to hold, one has to assume that the waveforms of velocity in the branches which are not sampled are similar to the one being measured, and to correct for any tapering, the distal velocity must be scaled such that the mean velocities are equal at the proximal and distal sites. In addition, since two signals with similar magnitudes and timing are being subtracted, the velocity signals must be taken simultaneously, be very clean, and be of high fidelity. Given all the potential problems, it was not clear whether the concept would be feasible in practice.

Therefore, the purpose of this study was to test the hypothesis that blood velocity can be measured simultaneously and precisely enough at two locations to estimate the volume expansion and contraction of the intervening vessel segment during the cardiac cycle. We chose carotid and brachial arteries because they are typical sites for assessing vascular compliance (Van Merode et al., 1988) and flow mediated dilation (Maltz and Budinger, 2005; Yeboah et al., 2007). For this limited study and to demonstrate feasibility over a wide range of dimensions, we made measurements in a short (5 mm) branchless segment of a mouse common carotid artery and in a longer (20 cm) segment of a human brachial-radial artery and calculated the waveforms and magnitudes of the volume or area changes during the cardiac cycle. We also measured ascending aortic blood velocity in a mouse and calculated the total arterial volume pulsation from an estimate of total arterial length assuming that the outflow is constant.

METHODS

Experimental procedures

The mouse studies were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. Murine carotid arteries were chosen for the initial test because they are accessible, the Doppler velocity signals are of high fidelity (Hartley et al., 2002; Li et al., 2003), and we have developed methods to measure the vessel wall motion directly using Doppler displacement ultrasound (Hartley et al., 2004) for comparison to the calculated volume waveform. The common carotid artery of a 3 month old anesthetized mouse was exposed for a distance of about 1 cm in the neck and dissected free from surrounding tissue. Two 20 MHz Doppler probes were fastened together 5 mm apart and placed in a holder at a 45° angle to the artery as shown schematically in Figure 1. The sample volumes (SV) and probe positions were adjusted to obtain the maximum Doppler shift frequency with each probe. In-phase (I) and quadrature (Q) Doppler signals were recorded from each probe at a sampling rate of 125 kHz for 2 seconds along with the electrocardiogram *ECG* using an Indus Mouse Vascular Research System (MVRs, Indus Instruments, Houston, TX) (Reddy et al., 2005a; Reddy et al., 2005b).

In a second age-matched anesthetized mouse we recorded ascending aortic velocity, common carotid artery wall motion, and carotid luminal blood velocity simultaneously for comparison of waveforms and magnitudes of actual diameter pulsations and for calculation of total arterial volume expansion. A 20 MHz Doppler probe connected to a three-gate pulsed Doppler (Hartley

et al., 2004) was placed over the skin on the neck at a 60° angle to the common carotid artery in a stable holder, and the Doppler SV and probe positions were adjusted to obtain a blood flow signal. Then the first displacement (wall motion) gate was adjusted to obtain a signal from the near vessel wall and the second displacement gate was adjusted to obtain a signal from the far vessel wall (Hartley et al., 2004). The near and far wall displacement signals were subsequently subtracted to obtain the diameter change signal. A second 10 MHz Doppler probe was held by hand at the cardiac apex to obtain blood velocity signals from the aortic root.

In a 64 year old healthy human male (CJH), blood velocities were measured simultaneously from the brachial artery just below the elbow and from the radial artery 20 cm distal at the wrist using 20 MHz probes hand-held at 45° to the skin surface. After optimizing the waveforms seen on the real-time monitor, *I* and *Q* Doppler signals from each probe were recorded for 10 s as above.

Instrumentation

For simultaneous Doppler measurements and to avoid interference between probes, we used a multichannel 10/20 MHz pulsed Doppler system constructed in our laboratory with simultaneous pulsing at a 125 kHz pulse repetition frequency (Hartley et al., 1978). The *I* and *Q* signals from each velocity or displacement channel were applied to the *I* and *Q* inputs of an Indus MVRS, and all signals were sampled at 125 kHz along with the *ECG* and stored for later analysis. The *I* and *Q* signals from velocity channels were AC coupled at 50 Hz, and the signals from displacement channels were DC coupled. The Indus system displays the spectrum of each Doppler velocity channel simultaneously along with the *ECG* on a monitor in real-time during data acquisition and during analysis of stored signals. From the stored *I* and *Q* velocity signals, the Indus system was set to generate a 256 point (2.05 ms) FFT spectrum every 0.1 ms which was then processed to produce a velocity waveform following the peak or envelope of each spectrum with a frequency resolution of 0.5 kHz. For displacement channels, the Indus system was set to calculate the phase ϕ of each *I* and *Q* signal pair as:

$$\phi = \arctan(Q/I) \quad \text{Eq. 3}$$

since $I = a \cos(\phi)$ and $Q = a \sin(\phi)$ with a being the amplitude of the Doppler signal. The peak velocity and displacement signals were then added to the data file and stored with the raw *I* and *Q* and *ECG* signals. The velocity, displacement, and *ECG* waveforms were subsequently exported as an ASCII file with a temporal resolution of 0.1 ms.

Analysis

The ASCII files containing the *ECG*, displacement, and/or velocity waveforms were then imported into an Excel spreadsheet for further analysis. We then performed calculations in Excel to solve the equations shown above and below. Velocity v was calculated from the Doppler shift Δf using the Doppler equation with a 45° angle θ and a 20 MHz operating frequency f_o :

$$v = c\Delta f / 2f_o \cos\theta \quad \text{Eq. 4}$$

where c is the speed of sound in blood (1560 m/s). We made the assumption that the flow was laminar and that the measured spectral peak velocity was twice the luminal average velocity (Nichols and O'Rourke, 1998). To remove any bias in the signals due to angle or other small errors, we adjusted the offset to assure that the mean velocity over the cardiac cycle was equal in the two velocity channels. Pulse wave velocity at end-diastole was estimated by dividing the separation distance L by the difference in arrival times of the upstrokes in velocity Δt as

shown in Figure 1. The dual velocity signals from the mouse carotid artery appeared clean, but when subtracted there was significant beat-to-beat variability. To improve the signal quality, we marked the peak of the *R-wave* of the *ECG* and performed an ensemble average of the 13 complete cardiac cycles contained in the record by lining up the *R-waves*. We did not average the mouse displacement (wall motion) signals, the aortic velocity signals, or the human velocity signals.

The wall motion or phase signals ϕ were converted to displacement Δd by solving the equation:

$$\Delta d = c\Delta\phi / 4\pi f_o \cos\theta \quad \text{Eq. 5}$$

where θ is the angle between the direction of motion and the sound beam. Eq. 5 is similar in form to Eq. 4 given that $\Delta\phi = 2\pi\Delta f t$. Thus, when the motion is parallel to the sound beam ($\cos\theta = 1$), each complete revolution of the Doppler vector ($\Delta\phi = 2\pi$) corresponds to a tissue displacement of 1/2 wavelength or $39\text{ }\mu\text{m}$ at $f_o = 20\text{ MHz}$. We have shown previously that the resolution of this method is on the order of $0.1\text{ }\mu\text{m}$ for carotid artery wall motion in mice (Hartley et al., 2004).

RESULTS

Figure 2 shows ensemble averaged proximal and distal velocity signals from a mouse carotid artery taken 5 mm apart simultaneously, and Figure 1B shows an expanded view of the upstrokes. In Figure 2, the distal signal is shown inverted for clarity. The percent area change waveform with a 16% magnitude was calculated in Excel using Eq. 2 and plotted on the scale shown to the right. This reduces to an absolute diameter pulsation of $31\text{ }\mu\text{m}$ assuming a carotid artery diameter of $400\text{ }\mu\text{m}$ (Hartley et al., 2004). Although the proximal and distal velocity signals look nearly identical at the scale shown in Figure 2, there is a 1.2 ms delay between the upstrokes as seen in Figure 1B corresponding to a *PWV* at end-diastole of 4.2 m/s given the 5 mm distance between the sample volumes.

Figure 3 shows brachial and radial velocity signals taken 20 cm apart on the arm of one of the authors (CJH). The percent area waveform of 4% magnitude was calculated and plotted as in Fig. 2. There is a 20 ms delay in the upstrokes of velocity corresponding to a *PWV* at end-diastole of 10 m/s.

Figure 4 shows carotid artery velocity, carotid artery wall motion, and ascending aortic velocity taken simultaneously and noninvasively from an anesthetized mouse. There is a 5 ms delay in the upstrokes of the aortic and carotid velocity signals taken about 20 mm apart corresponding to a *PWV* of $\sim 4\text{ m/s}$. The total excursion of the near vessel wall was about $33\text{ }\mu\text{m}$, and the far wall motion (not shown) was nearly zero such that it was not necessary to subtract the far wall signal to obtain the diameter change. It has been our experience in mice that because of anatomy and the lack of probe pressure against the skin, the near carotid wall moves much more than the far wall which is partially supported by the trachea (Hartley et al., 2004).

Figure 5 shows aortic velocity, carotid diameter change, and the calculated total arterial volume change in the same mouse as in Figure 4. The aortic signal was taken just past the aortic valve to avoid the valve click seen in Figure 4. The total arterial volume change waveform of 15% was calculated from the aortic velocity signal and its mean using an estimated arterial length of 7 cm.

DISCUSSION

We have demonstrated the feasibility of using the continuity principle with differential velocity measurements to calculate the magnitude and waveform of the volume pulsations of an arterial segment during the cardiac cycle. The total arterial volume pulsation during a cardiac cycle is equal to left ventricular stroke volume, and the percentage pulsation is equal to stroke volume divided by total arterial blood volume. Since both stroke volume and blood volume are proportional to body weight in mammals (Dawson, 1991), volume pulsations expressed as a percentage are expected to be similar in mammals of different sizes, and are on the order of 10 to 20% in man and dogs (Bergel, 1972). Pulse wave velocity is also expected to be similar in mammals independent of body weight (Dawson, 1991), and we have measured values between 3 and 5 m/s in the descending aortas of normal mice (Hartley et al., 1997). It is also accepted that the distensibility of vessels decreases and *PWV* increases with distance from the heart (Latham et al., 1985; Nichols and O'Rourke, 1998; Bergel, 1972). Reported values for end-diastolic *PWV* in dogs and humans range from 4 m/s at the aortic root to 15 m/s in the smaller peripheral arteries (Nichols and O'Rourke, 1998).

The values reported here for pulse wave velocity and for total arterial, carotid artery, and brachial artery volume pulsations in mice and humans are in good agreement with the accepted values for mammals. The estimated values for *PWV* were 4 m/s in the mouse aorta, 4.2 m/s in the mouse carotid artery, and 10 m/s in the human brachial artery. Values reported for aortic *PWV* range from 5 m/s in young to 12 m/s in old humans with brachial *PWV* being 10 to 15 m/s and largely independent of age (Nichols and O'Rourke, 1998). The calculated area pulsation of 15% for the total arterial system of a mouse is within the accepted values for mammals, but our 7 cm estimate for total arterial length could be higher or lower than the true effective value. The absolute carotid diameter pulsation of 31 μm calculated from velocities is close to the value of 33 μm measured directly in the second mouse.

Vessel distensibility $\Delta V/V\Delta P$ is related to *PWV* by:

$$\rho\Delta V/V\Delta P=1/(PWV)^2 \quad \text{Eq. 6}$$

where ρ is the density (Nichols and O'Rourke, 1998). Given the observed range of *PWV*, arterial distensibility can change by an order of magnitude from the aorta to the periphery such that central vessels are expected to pulsate much more than peripheral vessels and to provide the majority of total arterial compliance. The calculated area pulsation for the human brachial artery of 4% is much smaller than the 16% pulsation of the mouse carotid artery. Although we did not measure the actual diameter pulsations, the estimated *PWVs* of 10 m/s in the human brachial artery and 4.2 m/s in the mouse carotid artery are consistent with the differences in pulsations given that distensibility is proportional to the inverse square of *PWV*. This suggests that volume pulsation as measured by $\Delta V/V$ for a given pulse pressure ΔP could be a more sensitive index of vessel stiffness than end-diastolic *PWV*.

The shape of the volume or area wave in an arterial segment is expected to resemble the pressure wave averaged over the length of the segment (Hartley et al., 2004; Meinders and Hoeks, 2004; Nichols and O'Rourke, 1998) with some distortion due to the non-linear compliance as a function of distending pressure (Nichols and O'Rourke, 1998; Anliker et al., 1968; Hermeling et al., 2010). The longer the segment the more smoothing is expected, and this is apparent in the area signals shown in Figures 2, 3 and 5. Thus, the shapes and magnitudes of the area waves calculated from dual velocity signals along arterial segments of different lengths, sizes, and distances from the heart are in general agreement with published results (Hoeks et al., 1985; Hoeks et al., 1999; Meinders et al., 2001; Hartley et al., 2004).

Potential sources of error include the Doppler sample rate, the number of points in the FFT, the overlap of the FFTs, and the ability to identify the upstroke of velocity. The FFT display from which the velocity envelope signal is generated is broadened in time by the width of the FFT sample resulting in a distortion of the actual peak velocity signal. We used a 256 point FFT with a 125 kHz sampling frequency resulting in a width of $256/125 = 2.05$ ms with a new FFT being calculated every 0.1 ms. Even with the high heart rates of a mouse (300 to 600 BPM), this would result in a relatively minor distortion in the shape of the area or volume waveform. When comparing arrival times of the leading edges or upstrokes of velocity, the 0.1 ms FFT rate defines the temporal resolution. A more real limitation especially when measuring *PWV* in short segments is that the actual upstroke of velocity at a peripheral arterial site is not always sharp enough to identify its timing to within 0.1 ms. This is apparent in the expanded view of the velocity upstrokes shown in Figure 1B, where the rounded starts and slight differences in the shapes of the waves makes the measurement of Δt ambiguous. However, when calculating volume waves, precise identification of the upstrokes is not necessary.

Because the measured velocities at the two sites are often nearly equal, and the differences can be small as illustrated in Figure 2, the signals must be acquired simultaneously and be of high fidelity with minimal noise. The ultrasonic flow/dimension system we used was custom built in our laboratory for use with mice where high spatial and temporal resolutions are essential (Hartley et al., 1978; Hartley et al., 2002; Hartley et al., 2007). Even so, we ensemble averaged the signals from the mouse carotid artery because the small beat-to-beat differences in velocity at the two sites or variations in actual stroke volume caused the baseline and magnitude of the calculated area wave to change with each cardiac cycle. The dual velocity method worked much better in the human arm where the signals could be obtained farther apart and the differences in velocity were greater. However, few clinical ultrasound systems are able to record high quality spectral Doppler signals from more than one probe or sample volume at a time, and this may limit the adoption of this methodology for estimating vascular pulsatility and stiffness in the clinic.

An advantage of the dual-velocity method is that it directly estimates pulsations in volume or area and not diameter, and since the units cancel, absolute vessel areas or volumes are not needed in the calculation of the percent volume change during the cardiac cycle. Because the method averages the volume or area change along a defined vascular segment, it is less sensitive to irregularities in wall geometry including focal or asymmetric plaques. However, this limited study was designed only to show feasibility, and more detailed experiments and careful validation against a "gold standard" will be needed to establish the proper protocols and the accuracy, reproducibility, and limitations of the method under clinically relevant conditions.

CONCLUSIONS

We have shown the feasibility of calculating the percent area or volume pulsations of an arterial segment from the segment length and the instantaneous difference in the velocity of blood entering and exiting the segment. The method was tested experimentally using Doppler ultrasound in segments ranging in diameter from 0.4 to 6 mm and in length from 0.5 to 20 cm and generated values of volume pulsation in close agreement with measured and expected values. The area or volume waveforms resemble pressure and diameter waveforms, the magnitudes are quantitative as a percentage of volume, and the calculations do not require knowledge of the absolute volume of the segment. In addition, distensibility is expected to be more sensitive to changes in vessel stiffness than end-diastolic pulse wave velocity. If proven accurate and reproducible in validation studies, this non-imaging and non-invasive method could provide an alternative means to assess regional vascular distensibility and compliance in man and in animal models of vascular disease.

Acknowledgments

This work was supported in part by National Institutes of Health Grants R01-HL22512, P01-HL42550, R01-AG17899, R41-HL76928, and K25-HL73041.

REFERENCES

- Anliker M, Histan MB, Ogden E. Dispersion and attenuation of small artificial pressure waves in the canine aorta. *Circ Res* 1968;23:539–551. [PubMed: 5677945]
- Arnett DK, Evans GW, Riley WA. Arterial stiffness: A new cardiovascular risk factor? *Am J Epidemiol* 1994;140(8):669–682.
- Avolio AP, Fa-Quan D, Wei-Qiang L, Yao-Fei L, Zhen-Dong H, Lian-Fen X, et al. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 1985;71(2):202–210. [PubMed: 3965165]
- Bergel, DH. *Cardiovascular Fluid Dynamics*. New York: Academic Press; 1972.
- Brands PJ, Hoeks APG, Rutten MCM, Reneman RS. A noninvasive method to estimate arterial impedance by means of assessment of local diameter change and the local center-line blood flow velocity using ultrasound. *Ultrasound in Med & Biol* 1996;22(7):895–905. [PubMed: 8923708]
- Callaghan FJ, Geddes LA, Babbs CF, Bourland JD. Relationship between pulse-wave velocity and arterial elasticity. *Med Biol Eng Comput* 1986;24:248–254. [PubMed: 3747623]
- Cohn JN. Arterial stiffness, vascular disease, and risk of cardiovascular events. *Circulation* 2006;113:601–603. [PubMed: 16461830]
- Dawson, TH. *Engineering design of the cardiovascular system of mammals*. Englewood Cliffs: Prentice Hall; 1991.
- Farrar DJ, Green HD, Bond MG, Wagner WD, Gobbee RA. Aortic pulse wave velocity, elasticity, and composition in a nonhuman primate model of atherosclerosis. *Circ Res* 1978;43:52–62. [PubMed: 95906]
- Hartley CJ, Hanley HG, Lewis RM, Cole JS. Synchronized pulsed Doppler blood flow and ultrasonic dimension measurement in conscious dogs. *Ultrasound in Med & Biol* 1978;4:99–110. [PubMed: 734798]
- Hartley CJ, Reddy AK, Madala S, Entman ML, Michael LH, Taffet GE. Noninvasive ultrasonic measurement of arterial wall motion in mice. *Am J Physiol Heart Circ Physiol* 2004;287:H1426–H1432. [PubMed: 15317682]
- Hartley CJ, Reddy AK, Madala S, Michael LH, Entman ML, Taffet GE. Effects of isoflurane on coronary blood flow velocity in young, old, and ApoE^{-/-} mice measured by Doppler ultrasound. *Ultrasound in Med & Biol* 2007;33(4):512–521. [PubMed: 17346873]
- Hartley CJ, Taffet GE, Michael LH, Pham TT, Entman ML. Noninvasive determination of pulse-wave velocity in mice. *Am J Physiol Heart Circ Physiol* 1997;273:H494–H500.
- Hartley CJ, Taffet GE, Reddy AK, Entman ML, Michael LH. Noninvasive cardiovascular phenotyping in mice. *ILAR Journal* 2002;43(3):147–158. [PubMed: 12105382]
- Hermeling E, Hoeks APG, Winkens MHM, Waltenberger JL, Reneman RS, Kroon AA, Reesink KD. Noninvasive assessment of arterial stiffness should discriminate between systolic and diastolic pressure ranges. *Hypertension* 2010;55:124–130. [PubMed: 19933922]
- Hickler RB. Aortic and large artery stiffness: current methodology and clinical correlations. *Clin Cardiol* 1990;13:317–322. [PubMed: 2189612]
- Hoeks APG, Brands PJ, Willigers JM, Reneman RS. Non-invasive measurement of mechanical properties of arteries in health and disease. *Proc Instn Mech Engrs* 1999;213(H):195–202.
- Hoeks APG, Ruijsen CJ, Hick P, Reneman RS. Transcutaneous detection of relative changes in artery diameter. *Ultrasound in Med & Biol* 1985;11:51–59. [PubMed: 3892817]
- Hokanson DE, Mozersky DJ, Sumner DS, Strandness DE Jr. A phase-locked echo tracking system for recording arterial diameter changes in vivo. *J Appl Physiol* 1972;32:728–733. [PubMed: 5038866]
- Kanai H, Hasegawa H, Ichiki M, Tezuka F, Koiwa Y. Elasticity imaging of atheroma with transcutaneous ultrasound: preliminary study. *Circulation* 2003;107(24):3018–3021.

- Latham RD, Westerhof N, Sipkema P, Rubal BJ, Reuderink P, Murgo JP. Regional wave travel and reflections along the human aorta: a study with six simultaneous micromanometric pressures. *Circulation* 1985;72(6):1257–1269. [PubMed: 4064270]
- Levenson JA, Peronneau PA, Simon A, Sarar ME. Pulsed doppler: determination of diameter, blood flow velocity, and volumetric flow of brachial artery in man. *Cardiovasc Res* 1981;15(3):164–170. [PubMed: 6455197]
- Li Y-H, Reddy AK, Taffet GE, Michael LH, Entman ML, Hartley CJ. Doppler evaluation of peripheral vascular adaptations to transverse aortic banding in mice. *Ultrasound in Med & Biol* 2003;29(8):1281–1289. [PubMed: 14553805]
- Lim MA, Townsden RR. Arterial compliance in the elderly: Its effect on blood pressure measurement and cardiovascular outcomes. *Clin Geriatr Med* 2009;25:191–205. [PubMed: 19555866]
- Maltz JS, Budinger TF. Evaluation of arterial endothelial function using transit times of artificially induced pulses. *Physiol Meas* 2005;26(3):293–307. [PubMed: 15798303]
- Mattace-Raso FUS, van der Cammen TJM, Hoffman A, van Popele NM, Bos ML, Schalekamp MADH, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam study. *Circulation* 2009;113:657–663. [PubMed: 16461838]
- Meinders JM, Hoeks APG. Simultaneous assessment of diameter and pressure waveforms in the carotid artery. *Ultrasound in Med & Biol* 2004;30(2):147–154. [PubMed: 14998666]
- Meinders JM, Kornet L, Brands PJ, Hoeks APG. Assessment of local pulse wave velocity in arteries using 2D distension waveforms. *Ultrasonic Imaging* 2001;23(4):199–215. [PubMed: 12051275]
- Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial stiffness and cardiovascular events - The Framingham Heart Study. *Circulation* 2010;121:505–511. [PubMed: 20083680]
- Nichols, WW.; O'Rourke, MF. McDonald's Blood Flow in Arteries: Theoretical, Experimental, and Clinical Principles. 4 ed.. Edward Arnold: London; 1998.
- Nichols WW, O'Rourke MF, Avolio AP, Yaginuma T, Murgo JP, Pepine CJ, et al. Effects of age on ventricular-vascular coupling. *Am J Cardiol* 1985;55:1179–1184. [PubMed: 3984897]
- Nussbacher A, Gerstenblith G, O'Connor FC, Becker LC, Kass DA, Schulman SP, et al. Hemodynamic effects of unloading the old heart. *Am J Physiol Heart Circ Physiol* 1999;277:H1863–H1871.
- Reddy AK, Jones AD, Martino C, Caro WA, Madala S, Hartley CJ. Pulsed Doppler signal processing for use in mice: design and evaluation. *IEEE Trans Biomed Eng* 2005;52(10):1764–1770. [PubMed: 16235662]
- Reddy AK, Taffet GE, Li Y-H, Lim S-W, Pham TT, Pocius J, et al. Pulsed Doppler signal processing for use in mice: Applications. *IEEE Trans Biomed Eng* 2005;52(10):1771–1783. [PubMed: 16235663]
- Saito M, Okayama H, Nishimura K, Ogimoto A, Ohtsuka T, Inoue K, et al. Possible link between large artery stiffness and coronary flow velocity reserve. *Heart* 2008;94(6):690–691. [PubMed: 18480345]
- Tardy Y, Meister JJ, Perret F, Brunner HR, Arditi M. Non-invasive estimate of the mechanical properties of peripheral arteries from ultrasonic and photoplethysmographic measurements. *Clin Phys Physiol Meas* 1991;12(1):39–54. [PubMed: 2036772]
- Van Merode T, Hick PJJ, Hoeks APG, Rahn KH, Reneman RS. Carotid artery wall properties in normotensive and borderline hypertensive subjects of various ages. *Ultrasound in Med & Biol* 1988;14:563–569. [PubMed: 3059645]
- Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, et al. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* 2004;109:184–189.
- Wright JS, Cruickshank JK, Kontis S, Dore C, Gosling RG. Aortic compliance measured by non-invasive Doppler ultrasound: description of a method and its reproducibility. *Clin Sci* 1990;78:463–468. [PubMed: 2162271]
- Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: The cardiovascular health study. *Circulation* 2007;115:2390–2397. [PubMed: 17452608]

Fig. 1.

A. Drawing showing two pulsed Doppler probes measuring velocities v_1 and v_2 at sample volumes SV_1 and SV_2 separated by distance L along an artery. The difference in arrival times between the upstrokes of velocity (Δt) can be used to estimate pulse wave velocity (PWV). B. The upstrokes of actual velocity signals taken 5 mm apart on a mouse carotid artery.

Fig. 2.

Simultaneous measurements of proximal and distal velocities taken 5 mm apart along an exposed mouse carotid artery. Also shown are the ECG used for timing and the calculated area (or volume) wave expressed as a percent using the scale to the right. The velocity waves are taken from the envelopes of the Doppler spectra and ensemble averaged over 13 cardiac cycles, and the distal velocity wave is shown inverted for clarity. An expanded view of the velocity upstrokes is shown in Fig. 1B.

Fig. 3. Brachial and radial artery velocities recorded simultaneously 20 cm apart on the arm of a human volunteer and the calculated area or volume wave expressed as a percent using the scale to the right.

Fig. 4.

Simultaneous measurements of carotid artery blood velocity, carotid artery near wall displacement, aortic blood velocity, and *ECG* taken noninvasively from an anesthetized mouse. The carotid signals were taken from two Doppler range gates using a 20 MHz probe held at 60° to the vessel axis, and the aortic spectral Doppler signal was taken using a 10 MHz probe held at the cardiac apex with the sample volume placed at the aortic valve. The vertical line indicates the start of cardiac ejection.

Fig. 5. ECG, peak aortic velocity, carotid diameter change, and total arterial volume change calculated from aortic velocity and an estimate of arterial length (7 cm) in a mouse. The baselines for ECG and for volume and diameter change are arbitrary and are offset for clarity.