Measurement of aortic input impedance in mice: effects of age on aortic stiffness

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Reddy, Anilkumar K., Yi-Heng Li, Thuy T. Pham, Lyssa N. Ochoa, Maria T. Treviño, Craig J. Hartley, Lloyd H. Michael, Mark L. Entman, and George E. Taffet. Measurement of aortic input impedance in mice: effects of age on aortic stiffness. Am J Physiol Heart Circ Physiol 285: H1464–H1470, 2003. First published May 29, 2003; 10.1152/ajpheart.00004.2003.—Mice are used with increasing frequency as models of human cardiovascular diseases, but significant gaps exist in our knowledge of vascular function in the aging mouse. We determined aortic input impedance spectra, pulse wave velocity, and augmentation index in adult (8-mo-old) and old (29-mo-old) mice to determine whether arterial stiffening occurred with age in mice as it does in humans. Pressure and blood velocity signals measured simultaneously from the same location in the ascending aorta were used to determine input impedance spectra (0–10 harmonics). The first minimum of the impedance modulus occurred at the second harmonic in adult mice but shifted to the fourth harmonic in old mice. Characteristic impedance (average of 2nd–10th harmonic) was 57% higher in old mice: 471 ± 62 vs. 299 ± 10 (SE) dyn·s·cm⁻³ (P < 0.05). Pulse pressure and augmentation index, determined from the aortic pressure signals, were also higher in old mice: 42 ± 2.2 vs. 29 ± 4.9 mmHg (P < 0.05) and 37 ± 5 vs. 14 ± 2% (P < 0.005). Aortic pulse wave velocity measured from the timing of upstrokes of the Doppler velocity signals was 45% higher in old mice: 416 ± 22 vs. 286 ± 14 cm/s (n = 3, P < 0.01). These results reproduce age-related findings reported in humans and confirm that mice may be used as models of age-related vascular stiffening.

The proximal aorta serves an important buffering function, becoming distended during cardiac ejection and propelling blood during diastole. Alterations in aortic structure and function occur with advancing age in healthy humans, which may affect this buffering function. Degeneration and fragmentation of elastin fibers and an increase in less compliant forms of collagen occur with advancing age, leading to progressive elongation and dilatation of the aorta with stiffening of the aortic wall (16, 22, 34). Increases in aortic input impedance, characteristic impedance (Zc), pressure augmentation index, and aortic pulse wave velocity (PWV) and early return of wave reflections have been observed in the aging human (2, 15, 22, 23, 32). With increasing arterial stiffness, pressure waves travel more rapidly and return early, while the aortic valve is still open (22, 26). This increase in afterload is sensed by the heart and may be a stimulus for age-related cardiac hypertrophy (6, 28, 29).

Age-related arterial stiffening is an important epidemiological phenomenon. Recent studies have demonstrated that arterial stiffness estimated from aortic PWV is an independent predictor of cardiovascular mortality in patients with hypertension or end-stage renal disease independent of gender, lipoprotein abnormalities, and diabetes (4, 5, 17, 18). Increased arterial stiffness is also an early marker of asymptomatic atherosclerosis and structural arterial changes resulting from hypertension. Therefore, in addition to reduction of blood pressure, recent antihypertensive pharmacotherapy has focused on decreasing arterial stiffness as a specific treatment target (33). Obviously, if therapeutic interventions are to be directed specifically at the pathogenic basis for age-related arterial stiffening, models to understand this process are necessary. Although the effect of aging on arterial stiffness is inconclusive in dogs (37), arterial stiffening is seen with age in the absence of atherosclerosis in rats (8), pigs (11), and monkeys (27). The advancements in genetics and relatively short generation time have made mice the animals of choice in which to model human cardiovascular diseases. However, challenges in making cardiovascular measurements in mice because of their small size have left significant gaps in our knowledge of vascular aging and vascular function in the mouse. This study was undertaken to determine whether mice are similar to humans with respect to the manifestation of the vascular aging phenotype.

METHODS

Instrumentation and signal processing. A 10-MHz (1-mm-diameter) pulsed Doppler probe was used to measure ascending aortic flow velocity, and a 20-MHz (1-mm-diameter) pulsed Doppler probe was used to measure aortic arch and abdominal aortic flow velocity. Both probes were driven by a

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modular ultrasonic pulsed Doppler instrument at a pulse repetition frequency of 62.5 kHz. Ascending aortic blood pressure was measured by a pressure catheter (0.36 mm diameter; PressureWire3, RADI Medical System, Uppsala, Sweden). The RADI catheter was designed as an angioplasty guide wire and has a 3-cm-long floppy wire tip distal to the pressure sensor. The distal tip was removed before the catheter was used in the mouse. Because the RADI signal processor contains filters to smooth the recorded signal and adds a 10-ms time delay, a custom signal conditioner was built to interface the catheter to our pressure amplifier module (frequency response 0–2 kHz). The RADI catheter contains a sensor with a two-element resistive bridge. Our simple interface added two more resistors and a differential amplifier to form a standard four-element Wheatstone bridge matching the industry-standard sensitivity of 5 µV·mmHg⁻¹·Vexc⁻¹ (where Vexc is excitation voltage). The catheter and pressure amplifier were calibrated for each experiment from 0 to 250 mmHg using a mercury manometer.

A computer-based real-time Doppler spectrum analyzer (DSPW, Indus Instruments, Houston, TX) was used to acquire the Doppler audio signals at a sampling rate of 125 kHz along with the pressure and ECG signals, which were sampled at 4 kHz. Typically, 2 s of raw data were acquired and stored for analysis off-line. A 256-point fast Fourier transform (~2-ms data segment resulting in 500-Hz frequency resolution) was performed every 0.1 ms (temporal resolution) on the raw Doppler audio signals and displayed on the screen as a spectrogram, along with the pressure and ECG signals.

Velocity (v) was calculated from the Doppler shift frequency (∆f) using the Doppler equation: \( v = c \times \Delta f / 2f_0 \times \cos(\theta) \), where c is the speed of sound in blood (1,540 m/s), f0 is the ultrasonic frequency (10 or 20 MHz), and θ is the angle between the sound beam and the velocity vector. No angle correction was needed in this study (θ = 0).

Animals. Old (29-mo-old, n = 9) and adult (8-mo-old, n = 8) male B6D2F1 mice were purchased from the National Institute of Aging colonies maintained by Harlan Sprague Dawley. They were caged individually in a dedicated specific-pathogen-free room and allowed to recover for 2 wk after transport. They were examined for obvious signs of disease or weight loss. All mice were housed in the animal facility at the Fondren-Brown vivarium of Baylor College of Medicine, approved by American Association for Accreditation of Laboratory Animal Care and cared for in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revised 1996). Animals were kept in a room at controlled temperature (24°C) and lighting (14:10-h light-dark cycle) with free access to food and water.

Measurement of blood pressure and flow velocity in the ascending aorta. Blood pressure and flow velocity signals from 11 mice (group I: 5 adult and 6 old) were used to determine aortic input impedance and augmentation index. The mice were anesthetized with a continuous flow (20 ml/min) of a gas mixture containing 1% isoflurane combined with 99% oxygen (excess gas evacuated). The neck and xiphoid areas were shaved, and the anesthetized mouse was placed in a supine position with its paws taped to electrodes on a temperature-controlled ECG board. The right carotid artery of the mouse was isolated and tied off distally, and the proximal end was temporarily occluded. A small cut was made in the artery, and the RADI pressure catheter was inserted and held in place with a suture loosely tied over the artery-catheter overlap region. The proximal end of the artery was then opened, and the catheter was advanced into the ascending aorta as close as possible to the aortic root. A second suture was tied over the artery-catheter overlap to prevent any blood leakage as the catheter was advanced. To obtain the ascending aortic flow velocity signal, a 10-MHz Doppler probe was placed below the xiphoid process and oriented toward the left ventricular outflow tract. The probe was positioned such that the angle (θ) was close to zero, and the range gate depth was adjusted between 6 and 9 mm to obtain maximal velocity (14). Care was taken to ensure that the sample volume was placed very close to the location of the pressure sensor such that the foot of the velocity waveform was aligned with the foot of the pressure waveform to avoid potential errors in phase relation between pressure and velocity signals (36).

Conclusion. Aortic input impedance is defined as the ratio of pressure to luminal average blood velocity (vavg). The maximum frequency [spatial peak velocity (vpeak)] envelope of the spectrogram was automatically calculated off-line by the DSPW software. A 1,000-Hz (~8 cm/s) high-pass filter was applied temporarily to remove the clutter (wall motion artifacts) around zero before calculation of the envelope. Because we know that no outflow occurs after aortic valve closure, the envelope in the diastolic phase was manually set to zero (Fig. 1). The vpeak envelope was calculated every 0.1 ms and smoothed using a 41-point filter, resulting in a low-pass cutoff frequency of ~244 Hz. The luminal vavg depends on the velocity profile at the measurement site in the vessel. If the velocity profile in the lumen of the ascending aorta is flat (blunt or plug), then vavg = vpeak, and vpeak can be used to calculate input impedance. However, Fig. 1. Ascending aortic blood pressure and flow velocity signals in an adult and an old mouse. Aortic pressure was measured with a RADI PressureWire3 catheter inserted into the aorta via the right carotid artery. Aortic flow velocity was measured with a 10-MHz pulsed Doppler probe, with the sample volume placed in the vicinity of the pressure sensor. Pressure and velocity signals were acquired simultaneously. Stippled region under the first 2 cycles of the peak velocity waveform (vpeak) shows uniform distribution of velocity ranging from zero to peak, indicating a parabolic velocity profile. Under this condition, luminal average blood velocity (vavg) is calculated as one-half of the vpeak waveform.
if the velocity profile is parabolic, as we estimate it to be in the mouse aorta, then $v_{avg} = \frac{1}{2}v_{peak}$ (9, 22). Thus we calculated $v_{avg}$ as $\frac{1}{2}v_{peak}$ to determine input impedance. Typical aortic pressure and spectral Doppler $v_{peak}$ signals from an adult and an old mouse are shown in Fig. 1. Four contiguous cycles of pressure and $v_{avg}$ waves were converted to the frequency domain using the discrete Fourier transform (24). The modulus of impedance was calculated as the ratio of the pressure modulus to the velocity modulus, and the phase of impedance was calculated as the phase of velocity subtracted from the phase of pressure at zero frequency and the first 10 harmonics. $Z_c$ was estimated as the average of the 2nd to 10th harmonic of the impedance moduli (19).

Aortic blood pressure indexes. From the recorded aortic pressure waveforms, we calculated systolic pressure, diastolic pressure, pulse pressure, and augmentation index. The aortic augmentation index was used to quantify the age-associated augmentation of the late systolic portion of the arterial pressure wave (15, 22, 32). The first concavity on the upstroke of the pressure wave, which separates the initial pressure rise from that in late systole, is defined as the augmentation point of the pressure wave (32). The temporal position of the augmentation point was determined by a local minimum (past the foot of the pressure wave) in the first derivative of the pressure wave (32). The augmentation index was defined as the height from the augmentation point to the systolic peak of the pressure waveform divided by the pulse pressure (Fig. 2) and expressed as a percentage (15, 32).

Measurement of velocity signals to determine aortic PWV.
The 20-MHz Doppler probe was placed in the second intercostal space to the right of the sternum and angled to record velocity in the aortic arch moving away from the probe at a depth of 2–4 mm. A mark was made 40 mm distally on the abdomen. A measurement was then taken at the mark from the abdominal aorta, with the probe angled toward the heart at a depth of 2–3 mm. Aortic PWV was calculated by dividing the separation distance (40 mm) by the difference in arrival times of the velocity pulse timed with respect to the ECG.

Statistical analysis. Values are means ± SE for the number of animals in adult and old groups. Differences were determined by unpaired Student’s t-test, and statistical significance was defined by $P < 0.05$.

RESULTS

The data are summarized in Table 1. There were no significant differences in the heart rate, body weight, mean aortic velocity, mean aortic pressure, and diastolic pressure between adult and old group I mice. However, we found significant differences between adult and old mice in systolic blood pressure (116 ± 4.5 and 88 ± 8.8 mmHg for old and adult mice, respectively, $P < 0.05$) and pulse pressure (42 ± 2.2 and 29 ± 4.9 mmHg for old and adult mice, respectively, $P < 0.05$). Analysis of the pressure waveforms showed a significant increase in augmentation index in old mice compared with adult mice (37 ± 5 vs. 14 ± 2%, $P < 0.005$). The impedance moduli and phase of adult and old mice plotted vs. harmonic number are shown in Fig. 3. There was an increase in the impedance modulus of old mice at all harmonics. $Z_c$ was significantly higher in old than in adult mice ($471 ± 62$ vs. $299 ± 10$ dyn·s·cm⁻³, $P < 0.05$), a 57% increase. The first minimum of the impedance modulus occurred at the second harmonic in adult mice and at the fourth harmonic in old mice (Fig. 3). Impedance at the first harmonic (heart rate) was significantly higher in older mice (972 ± 73 vs. 607 ± 53, $P < 0.005$), indicating early peripheral wave reflections. Although not significant, the phase of impedance was modified by age, with the first minimum occurring at the first harmonic in the old mice and at the second harmonic in the adult mice. Total peripheral resistance (impedance modulus at zero frequency) tended to be higher in old mice but had significant variation and was not statistically different (9,620 ± 1,051 vs. 7,298 ± 1,503 dyn·s·cm⁻³, not
significant). Aortic PWV, measured noninvasively in group II mice, was significantly higher in old than in adult mice (416 ± 22 vs. 286 ± 14 cm/s, P < 0.005).

DISCUSSION

Human aging leads to progressive arterial stiffening (16, 22). There are a number of changes that are well described and have been implicated in the increase in arterial stiffness with age, including degeneration and fragmentation of elastin fibers in the media, a relative increase in collagen and extracellular matrix substance in the media, cross-linking of these proteins after nonenzymatic glycation, and increased vasoconstriction (16, 22, 34). However, atherosclerosis also increases vessel stiffness, and in humans it is difficult to resolve the relative importance of atherosclerosis from aging per se. We used 8- and 29-mo-old mice to represent adult and old populations, respectively, to study the effects of age on arterial stiffness. Several indexes, including aortic input impedance, Zc, PWV, and augmentation index, that have been previously described in human studies were used to characterize the aortic elastic wall properties in the adult and old mice.

The invasive studies performed were focused on the aorta and central arteries because, in other species, that is where the aging changes are observed to be largest. Medial hypertrophy, with an increase in matrix and cellular elements, contributes to increased vessel wall thickness and stiffness. Goyal (10) showed that the same constellation of changes occurs in the mouse aorta. Aging increases aortic wall thickness and collagen concentration in mice (10). Also, in the apolipoprotein E-null mice (used as a model of human atherosclerosis), there is clear evidence of aortic stiffening (14, 35), underscoring the additive and confounding interaction between age and atherosclerosis.
Aortic input impedance. Aortic input impedance is a means of characterizing the hydraulic external load on the left ventricle. Because it incorporates the pulsatile and the steady components of the hydraulic load, it provides a more complete description of the load by including factors related to aortic stiffness. Aortic input impedance has been measured in humans and small and large animals (3, 7, 19, 20, 22, 25) but, to our knowledge, has not been measured in mice.

We used velocity, instead of flow, to calculate the aortic input impedance in mice, because we can measure velocity noninvasively. Flow measurement, on the other hand, is an invasive procedure requiring the placement of a flowmeter around the aorta of the mouse, which can also change the system. Solomon et al. (30) and Spencer et al. (31) showed that the mean impedance modulus, peripheral resistance, and $Z_c$, determined from flow measured by electromagnetic flowmeter and flow velocity measured by Doppler ultrasound, were very similar. Because velocity (like pressure) is independent of body weight or size, impedance calculated as pressure / velocity (instead of pressure / flow) is also independent of body weight or size, thereby allowing direct comparison between species (22, 25).

Avolio (1) found that ascending aortic impedance curves determined from aortic pressure and velocity in humans, sheep, dogs, rabbits, and guinea pigs were quite similar to each other, with minor differences that were attributed to the differences in the shape and size of their bodies. Impedance determined from aortic pressure and flow can be expressed in terms of velocity by normalizing flow to aortic cross-sectional area. Thus, for example, when expressed in terms of velocity, the estimated value of $Z_c$ in humans (~426 dyn·s·cm⁻³) (21), dogs (~505 dyn·s·cm⁻³) (37), rabbits (~320 dyn·s·cm⁻³), and guinea pigs (~315 dyn·s·cm⁻³) (3) is of the same order of magnitude as that in mice (~299 dyn·s·cm⁻³).

Impedance is properly calculated using the luminal $v_{avg}$. If the velocity profile is flat (narrow Doppler frequency spectrum or range of velocities), then $v_{avg}$ is close to $v_{peak}$. In humans and large animals, the velocity profile was found to be flat in the ascending aorta both experimentally and by the calculated inlet length (22). Inlet length ($L$), defined as the distance from the aortic valve at which the velocity profile becomes parabolic, is estimated as $L = 4.2a/r^2$ (where $a$ is the mean velocity in cm/s and $r$ is the radius of the vessel in cm) (22). With an aortic radius of 0.05 cm and a mean velocity of 20 cm/s, the inlet length in mice is ~0.2 cm. This indicates that the velocity profile becomes nearly parabolic in the ascending aorta of mice, in contrast to humans and large animals. We found that the aortic velocity signals measured in mice contained a broad frequency spectrum or range of velocities. Because the Doppler sample volume covers the entire lumen of the 1-mm-diameter aorta at the measurement site, the broad range of velocities (Fig. 1) suggests a nearly parabolic velocity profile (where $v_{avg} \approx \frac{1}{2}v_{peak}$) (9, 22).

We therefore calculated impedance using $\frac{1}{2}v_{peak}$ as an estimate of average aortic velocity.

Nichols et al. (23) found differences in impedance spectra between groups of young and old patients without discernible vascular disease. In our study, we found that the $Z_c$ and impedance at the first harmonic increased significantly with age, and the first minimum of the impedance modulus shifted from the second harmonic in adult mice to the fourth harmonic in old mice. Because pressure and velocity waves travel faster in a stiffer vessel, the reflections of these waves arrive earlier from the periphery and augment the respective waves during systole, rather than during diastole, thus resulting in increases in the impedance modulus at lower frequencies. Although no significant differences were found in phase, the first minimum of phase in old mice occurred at the first harmonic, indicating that pressure and velocity signals are out of phase earlier (19) than in adult mice. Thus our data from mice support the finding that $Z_c$ and the location of the first minimum increase with age, as observed in humans (23). The findings of our study show that the aging mouse model may be representative of the aging human.

The focus of this study was to evaluate the large artery stiffness and not peripheral vascular resistance. However, the lack of differences in peripheral vascular resistance between adult and old mice warrants comment. We found no differences in mean pressure or mean velocity between adult and old mice. This may suggest that peripheral vascular resistance and cardiac output of adult and old mice are similar. On the other hand, if aortic diameter is larger in old mice, then cardiac output under anesthesia could be higher. Previously, we showed that if flow in a vessel is reduced, the diameter constricts to maintain velocity (12). In this study, we did not measure cardiac output and cannot rule out the possibility that aortic diameter of old mice may be different from that of adult mice, as it is in older humans. We are not confident in the accuracy of any noninvasive method to measure vessel diameter in vivo in mice, and postmortem diameter, which we measured in previous studies (12), is unloaded and therefore less valuable. Thus we cannot

![Fig. 4. $Z_c$ and the product of blood density (1.06 g/cm³) and pulse wave velocity ($\rho$-PWV). Values are means ± SE. $Z_c$ and $\rho$-PWV were not significantly different from each other in adult and old mice.](http://ajpheart.physiology.org/)

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make any conclusions regarding peripheral vascular resistance.

**Aortic PWV.** Our second independent measure of aortic stiffness is aortic PWV. Several human studies have demonstrated that aging increases aortic PWV, even in healthy humans free of atherosclerosis (6, 25, 32). Avolio et al. (2) found that PWV increased with age in urban and rural Chinese populations, despite the dietary differences. We observed a 1.5-fold increase in PWV of old vs. adult (group II) mice. This is similar to the 1.7-fold increase in PWV observed in 80-yr-old vs. 30-yr-old male human subjects (PWV = 8.2 × age + 314) (32). In the apolipoprotein E-null mouse, atherosclerotic lesions cover >20% of the proximal aortic wall at 4 mo and 50% at 13 mo (35). The aortic PWV in the 13-mo-old apolipoprotein E-null mice was 428 cm/s (14). This is similar to that in our 29-mo-old mice, which are free of spontaneous atherosclerosis (13). Therefore, the increase in aortic stiffness observed in our mice is related to the aging process. In humans, the two processes (aging and atherosclerosis) are likely to be additive.

\[ Z_c = \rho \cdot \text{PWV} \] (where \( \rho \) is blood density, \(-1.06 \text{ g/cm}^3\)) (22, 25). With the use of this equation, PWV (282 ± 9 and 444 ± 58 cm/s in adult and old mice, respectively) calculated from \( Z_c \) values of group I mice was very similar to PWV (286 ± 14 and 416 ± 22 cm/s in adult and old mice, respectively) measured in group II mice. Despite use of different groups of mice, the changes observed in these parameters (\( Z_c \) and PWV) with age were consistent with the above relation (Fig. 4), implying that \( Z_c \) can be calculated from the PWV and vice versa, and providing strong confirmation that using \( \frac{1}{2}v_{\text{peak}} \) for impedance calculations is correct.

**Augmentation index.** The augmentation index is a way to assess the early pressure wave reflection, which increases the pressure between the augmentation point and the systolic peak. Kelly et al. (15) showed that the augmentation index of carotid pressure increased ∼3.2-fold in 71-yr-old compared with 31-yr-old subjects, and Vaitkevicius et al. (32) observed a 4.7-fold increase in carotid augmentation index in 80-yr-old vs. 30-yr-old male subjects. By comparison, we observed a 2.6-fold increase in the augmentation index in old mice compared with adult mice. Therefore, the age-related increase of aortic augmentation index is a common phenomenon in mice and humans.

**Pulse pressure.** Another independent measure of aortic stiffness is aortic pulse pressure. The pulse pressure was increased by 47% in the old mice compared with the adult mice. Pulse pressure was likely augmented with age by the noncompliance of the aorta to receive cardiac output and the addition of pressure waves reflected from the periphery. Pulse pressure is also increased with age in humans and other species (21). This study also reveals that investigation and manipulation of the cardiovascular system in mice need not be limited to the heart. Although measurement of systolic blood pressure has been performed by many, impedance has not been previously reported in the mouse. The frequency of technical failures in this study was low, inasmuch as the modified RADI pressure wire fit comfortably in the carotid artery, and the Doppler assessment of flow velocity is routine in our laboratory. The signals obtained were of high fidelity and needed minimal smoothing or filtering before analysis. Thus we conclude that aortic input impedance may be used to determine aortic function in cardiovascular models of mice.

In summary, an age-related increase in aortic stiffness occurs in mice, resulting in increased aortic input impedance, \( Z_c \), PWV, pulse pressure, and augmentation index. The measurement techniques that have been developed to evaluate aortic stiffness in humans can be directly applied to the mouse and the findings interpreted appropriately.

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**DISCUSSIONS**

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