In vivo and in vitro mechanical properties of the sheep thoracic aorta in the perinatal period and adulthood

SARAH M. WELLS, B. LOWELL LANGILLE, AND S. LEE ADAMSON

1Departments of Metallurgy and Materials Science, 2Laboratory Medicine and Pathobiology, and 3Obstetrics and Gynecology, and 4Centre for Biomaterials, University of Toronto, 5The Toronto Hospital Research Institute, and 6Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5

Wells, Sarah M., B. Lowell Langille, and S. Lee Adamson. In vivo and in vitro mechanical properties of the sheep thoracic aorta in the perinatal period and adulthood. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1749–H1760, 1998.—The mammalian aorta undergoes rapid remodeling during the perinatal period and more gradual remodeling during subsequent development, but the implications of this remodeling for arterial mechanics are poorly understood. In this study in vivo and in vitro techniques were used to determine the static and viscoelastic properties of the thoracic aortas of 119-day-gestation fetal sheep (full term = 145 days), 21-day-old lambs, and adult sheep at control distending pressures and after 70% increases or 30% decreases in pressure. In the weeks surrounding birth, aortic wall tissue became substantially stiffer (static elastic modulus in vitro increased by 28%, and pressure wave velocity in vivo increased by 61%) but less viscous (pressure wave attenuation in vivo decreased by 46%, and viscoelastic phase angle in vitro decreased by 15%), whereas the wall thickness-to-radius ratio was unchanged. By contrast, modest changes in tissue viscoelasticity from neonatal to adult life were accompanied by a halving of the wall thickness-to-radius ratio from 0.19 ± 0.01 to 0.10 ± 0.01. The relative thinning of the vessel wall, combined with a doubling of blood pressure after birth, resulted in a 265% increase in aortic wall tensile stress over the period of study. We concluded that rapid remodeling in the perinatal period primarily alters the viscoelastic properties of aortic wall tissues, whereas more gradual postnatal remodeling largely affects vessel geometry.

Viscoelasticity; wave propagation; development

The viscoelasticity of arteries greatly influences cardiovascular function, since it determines the interrelationship among blood pressure, blood flow, and vascular dimensions. The viscoelastic properties of the aorta and its major branches are particularly important, because they determine the input impedance that the arterial system presents to pulsatile ventricular outflow, and therefore they are important determinants of cardiac workload.

Arterial viscoelasticity is affected by vessel dimensions and the relative proportions and arrangements of the arterial wall constituents. Thus large changes in arterial viscoelasticity are anticipated in the perinatal period, a time when arterial systems undergo extensive remodeling that is associated with large perinatal changes in cardiovascular function, including a closure of the foramen ovale and ductus arteriosus, loss of the placenta, a marked redistribution of systemic blood flows, and a doubling of central arterial pressure. Perinatal remodeling of most large arteries, including the thoracic aorta, involves rapid connective tissue accumulation and concomitant increases in wall thickness and luminal diameter (5, 6, 22). In sheep the collagen-to-elastin ratio decreases by 45% (calculated from Bendeck and Langille (6)) from the 120-day-gestation fetus to the 21-day-old lamb, reaching nearly adult values at the latter age (2, 17). On the basis of these biochemical and anatomic changes, we hypothesized that large changes in aortic viscoelasticity occur in the perinatal period.

Information on perinatal mechanical properties of the aorta is restricted to measurements of static elastic properties (12, 13, 35, 36, 38); however, dynamic viscoelastic characteristics determine the behavior of the aorta under pulsatile conditions in vivo. In the current study we used in vivo and in vitro techniques to measure aortic viscoelastic properties in the sheep fetus, lamb, and adult ewe. Sheep were chosen because of their suitable size and because their perinatal cardiovascular function has been extensively studied.

We assessed aortic viscoelasticity in vivo by measuring aortic pressure wave transmission characteristics (pressure wave velocity and attenuation). This method assesses aortic viscoelastic behavior under normal in vivo operating conditions: with the aortic wall uninstrumented and tethering undisturbed. In vivo measurements were complemented with standard in vitro arterial mechanical tests to determine the static and dynamic stress-strain relations. Observations of stress-strain behavior in vitro allowed us to separate the effects of changes in material properties of the tissue and changes in vessel dimension on aortic viscoelastic behavior. This was especially important in the current study, since vessel material properties and dimensions change with development. Because in vivo and in vitro measurements of viscoelasticity are seldom performed on the same vessels, we also wanted to compare the agreement between these techniques. We have shown excellent agreement between in vivo and in vitro results for the lamb and adult aorta, whereas the behavior of the fetal aorta was stiffer under in vitro than under in vivo conditions. In vivo and in vitro measurements demonstrate large changes in aortic viscoelasticity with development from fetal to adult life. The thoracic aorta becomes stiffer, less viscous, and more extensible with age, and much of this change occurs during the perinatal period.

Glossary

- \( a \): Attenuation coefficient (real part of \( \gamma \))
- \( a_1 \): Attenuation coefficient at fundamental (heart rate) frequency
MATERIALS AND METHODS

H1750 DEVELOPMENTAL CHANGES IN AORTIC VISCOELASTICITY

...lines approved by the Canadian Council of Animal Care. Animal surgery and experiments were approved by the Animal Care Committee of Mount Sinai Hospital (Toronto, ON, Canada) and were conducted in accordance with guidelines approved by the Canadian Council of Animal Care. Anesthesia and catheter insertions. Acute experiments were performed on five sheep fetuses at 119 days of gestation (full term = 145 days), five lambs at 21 days of age, and five nonpregnant adult ewes (Dorset-Suffolk crossbreed). Anesthesia was induced in pregnant and nonpregnant ewes with thiopental sodium (Pentothal Sodium, 1 g iv) and in lambs with 5% halothane in oxygen that was delivered through a facemask, then all animals were intubated and artificially ventilated with 1–2.5% halothane in oxygen during surgery and experiments. Ventilator settings were adjusted to maintain stable arterial blood-gas partial pressures. In pregnant ewes the fetal hindlimbs were withdrawn through a small incision in the uterine wall. In the fetuses and lambs, three 3-Fr catheter-tipped pressure transducers (model SPR-407, Millar Instruments, Houston, TX) were inserted into the femoral artery and then were advanced simultaneously by 16.4 ± 0.4 cm in the fetus and 29.2 ± 1.2 cm in the lamb so that the tips lay in the upper thoracic aorta. Identical simultaneous pressure pulsations, viewed on an oscilloscope, confirmed that all three catheter tips were at the same site (i.e., that none had folded back as they were inserted) and that the three sensor and amplifier systems introduced no discernible phase or amplitude differences. The transducer tips were then placed at three equidistant sites by pulling one transducer out by the required sensor separation (Ax) and pulling a second transducer out by twice the desired sensor separation. The mean pressure sensor separation was 3.1 ± 0.01 cm in the fetus and 3.8 ± 0.2 cm in the lamb. These distances were chosen to maximize separation of the sensors while keeping all sensors in the descending thoracic aorta and caudal to where the azygos vein crossed the aorta, which we used as a landmark for the rostral limit of the descending aorta. Sensor position was confirmed at necropsy. For catheterization of the adult aorta, the distance from the femoral artery incision to the lower thoracic aorta (at the diaphragm) was estimated as the distance from the incision in the groin to the lowest rib (38.4 ± 1.7 cm). The transducers were advanced to this position, and identical signals from each were recorded. The catheters were withdrawn and tied together with equidistant spacing of 6.07 ± 0.07 cm and advanced back into the femoral artery until the most distal catheter was at the original position. Correct placement of sensors was confirmed at necropsy. The mean distance from the azygos vein to the most proximal pressure sensor (P1) was 0.7 ± 0.7 cm in the fetus, 0.5 ± 1.3 cm in the lamb, and 6.7 ± 1.7 cm in the adult. The mean distance from the diaphragm to the most distal pressure sensor (P3) was 0.9 ± 0.6 cm in the fetus, 2.6 ± 0.7 cm in the lamb, and 2.5 ± 1.2 cm in the adult. Catheter-tipped transducers provide high-fidelity pulsatile pressures, but they are prone to baseline drift; therefore, mean arterial pressure was monitored using a fluid-filled manometer (model P23XL, Spectramed, Oxnard, CA) via a polyvinyl catheter advanced into the abdominal aorta via the right femoral artery. This catheter was also used to obtain arterial blood samples to determine blood-gas partial pressures and pH. A second polyvinyl catheter was advanced through the femoral vein into the inferior vena cava to infuse vasoactive agents. The animals were allowed to stabilize for 30 min before the experiments began. The fetuses were studied with their hindlimbs exteriorized.

Data collection. Pulsatile aortic blood pressures, mean aortic pressure, and heart rate were monitored (determined from a pulsatile pressure with a tachometer (model 7P4, Grass Instruments, Quincy, MA)) and continuously recorded on a strip chart recorder (model 78D, Grass) and stored on magnetic tape (model 4000, Vetter, Rebersburg, PA) for subsequent computer analysis. Arterial blood samples (1 ml) were collected immediately before each drug infusion and analyzed immediately at 37°C for blood-gas partial pressures and pH with a blood-gas analyzer (model 178B, Corning Medical, Medfield, MA).

Changes in mean arterial pressure. Mean arterial blood pressure (MABP) was monitored by recording pulsatile blood pressure through the femoral artery before a bolus injection of norepinephrine. MABP was increased 70% by norepinephrine bitartrate (Levophed, Winthrop, Aurora, ON, Canada) and decreased 30% by sodium nitroprusside (Nipride, Hoffmann-La Roche, Etobicoke, ON, Canada). Initial infusion
rates of norepinephrine bitartrate were 1.67 µg·min⁻¹·kg⁻¹ for the fetus, 1.2 µg·min⁻¹·kg⁻¹ for the lamb, and 0.8 µg·min⁻¹·kg⁻¹ for the adult. Initial infusion rates of sodium nitroprusside were 8 µg·min⁻¹·kg⁻¹ for the fetus and the lamb and 1.2 µg·min⁻¹·kg⁻¹ for the adult. Drug infusion rates were adjusted to achieve the desired blood pressure changes. In each animal a 5% dextrose solution (the vehicle for sodium nitroprusside) was infused as a control. The order of drug and dextrose administration followed sequentially from a 3 × 3 Latin square. Each infusion was followed by a 30-min recovery period before the next drug was administered.

Analysis of in vivo data. Taped data were digitized at 500 Hz with a data acquisition program (Viewdac, version 2.1, Keithley Instruments, Tauton, MA). Five consecutive cardiac cycles were chosen at 4 min into the 5-min infusion for control experiments and at the most stable region at the target mean blood pressure during the drug infusions. A Fourier transformation was performed on each pulsatile pressure waveform, and the propagation constants from each of the five waves were averaged at each harmonic frequency.

True wave propagation coefficient. The true wave propagation coefficient (γ) describes the transmission characteristics of a pressure wave harmonic as it travels through an artery

\[ P_x = P_0 e^{-\gamma x} = P_0 e^{-a x + i \omega x} \]

where \( P_0 \) is a pressure downstream of \( P_x \) by a distance \( x \), \( \omega \) is the angular frequency (s⁻¹), \( a \) is the wave attenuation coefficient (cm⁻¹), and \( c \) is the true phase velocity (cm/s) of wave propagation. The propagation coefficient for each harmonic of the pressure wave was determined using the three-pressure method (18), which mathematically removes the effects of wave reflections. Accordingly (18)

\[ \gamma = \frac{1}{\Delta x} \cosh^{-1} \left( \frac{P_1 + P_3}{2P_2} \right) \]

where \( P_1 \), \( P_2 \), and \( P_3 \) are the corresponding complex pressure harmonics measured at proximal, middle, and distal sites, respectively, and \( \Delta x \) is the pressure sensor separation (18).

From the real and imaginary parts of the complex propagation coefficient, we obtained the attenuation coefficient (\( a \), in cm⁻¹), a measure of viscous damping of the pressure wave, and \( c \) (in cm/s), an indicator of vessel wall stiffness (7, 31).

For comparison among age groups or different blood pressures, we compared the attenuation coefficient at the first harmonic (heart rate frequency; \( a_1 \)) and we compared phase velocity (\( c \)) averaged over frequencies >5 Hz in the fetus and adult and >15 Hz in the lamb. The 5-Hz limit was chosen for fetuses and adults, because accurate phase differences between recording sites could not be determined at lower frequencies because of the proximity of the recording sites. A 15-Hz cutoff was used for lambs because \( c \) values declined substantially below this frequency (see Fig. 3).

We assessed the ability of the three-pressure method to remove wave reflection effects and provide the true viscoelasticity estimates (attenuation and phase velocity). The \( c \) was compared with the apparent phase velocity (\( c_{app} \), i.e., the velocity of pressure harmonics in the presence of reflections (\( c_{app} = \omega 2\Delta x / \phi \), where \( 2\Delta x \) is the spatial separation of \( P_1 \) and \( P_3 \) and \( \phi \) is the phase difference between corresponding harmonics at the sites). This comparison tested whether reflection effects present in \( c_{app} \), i.e., large oscillations in the frequency domain, were eliminated in \( c \). The \( c_{app} \) and \( c \) were also compared with the foot-foot velocity (\( V_f \)), the velocity determined by dividing the separation of the two most distant sensors (\( P_1 \) and \( P_3 \)) by the time delay between the initial rise in arterial pressures (i.e., the “foot” of the pressure wave) recorded at these sites (31). \( V_f \) is largely unaffected by reflections, because the pressure wave foot is produced by the high-frequency components of the wave, and reflections of high-frequency waves are highly attenuated before returning to the thoracic aorta (31). Therefore, \( V_f \) theoretically approximates \( c \) at high frequencies.

In Vitro Experiments

Aortic samples were collected from animals at the end of the in vivo experiments, as well as from additional animals. All animals were heparinized (1 ml, 10,000 USP U/ml) and killed with an overdose of anesthetic (pentobarbital sodium, Euthanyl, MTC Pharmaceuticals, Cambridge, ON, Canada). The in situ length of the thoracic aorta was measured from where the azygos vein crossed the aorta (just distal to the ductus arteriosus or ductal ligament) to the diaphragm. Two reference sutures were sewn to the adventitia, and the distance between them was recorded. The intercostal side branches were tied off, the vessel was excised, and its retracted length was recorded (i.e., the length between the reference sutures). The vessel was placed into Tyrode solution (in g/l: 8.0 NaCl, 0.20 KCl, 0.20 CaCl₂, 0.077 MgCl₂, 1.00 NaHCO₃, 0.045 NaH₂PO₄, 1.0 glucose) containing 30 ml/g of sodium nitroprusside at 4°C.

Viscoelastic modulus. The central 5–10 cm of the thoracic aorta was mounted at in situ longitudinal strain in a mechanical testing apparatus, as described by Weygang et al. (41) for in vitro mechanical (pressure-diameter) testing. A catheter-tipped pressure transducer (3-Fr, Millar) was inserted into the vessel lumen through an access port until it lay near the middle of the segment. Pulsatile changes in vessel diameter were measured using a sonomicrometer (model VF-1, Pulsed Doppler Flow/Dimension System, Valpey Fisher, Hopkinton, MA) and a pair of 1-mm-diameter sonomicrometer crystals (model LMT-505, Crystal Biotech, Hopkinton, MA) that were sutured to the adventitia where the pressure sensor was located. The vessel was pressurized from a reservoir and immersed in a bath of 37°C Tyrode solution with 30 mg/l of sodium nitroprusside to maintain relaxed vascular smooth muscle tone.

Single pressure pulses of <20 mmHg and lasting 1–1.5 s were produced by injecting Tyrode solution into the vessel with a syringe connected to a side port of the vessel mount. Pressure and diameter signals were acquired at 500 Hz for a 2-s interval that included the pressure and diameter transients produced by the injection. Signals were acquired by a Macintosh IIfx computer equipped with an analog-to-digital converter card (NB-MIO-16L, National Instruments, Austin, TX) using LabVIEW 2.2.1 data acquisition software (National Instruments). Injections were repeated five times. The complex viscoelastic modulus (\( E^{*} \)) was determined (see Analysis of in vitro data) for each vessel at control MABP, MABP reduced by 30%, and MABP increased by 70%.

The accuracy of dynamic diameter measurements obtained using the sonomicrometer transducers and amplifier system was determined by comparing them with measurements obtained using a linearly variable differential transducer of a servo-hydraulic test system (model 1331 load frame and series 8501 controller, Instron). The sonomicrometer system
had a positive phase shift, which varied only slightly (from ~3° to 4°) over the frequency range of 2.5–5 Hz used in the in vitro portion of our study. We did not correct for this small phase shift.

Static elastic modulus. For measurements of the static elastic modulus \(E_{\text{stat}}\), the bath was drained, and the external diameter \(D_e\) of the vessel was measured with a video-camera (model TMC-7i, PULNiX America, Sunnyvale, CA) mounted above the vessel and interfaced with a video-dimension analyzer (model TMC-7i, Instrumentation for Physiology and Medicine, San Diego, CA). Intraluminal pressure was cycled from 0 to the peak test pressure 20 times (1 cycle/min) to precondition the vessel (8). Pressure provided by the reservoir was then elevated in 10-mmHg increments from 10 mmHg to 100 mmHg for fetal vessels, to 150 mmHg for lamb vessels, or to 170 mmHg for adult vessels. \(D_e\) was measured 2 min after each step in pressure, when a stable maximum diameter was reached. The vessel was superfused with Tyrode solution to keep the vessel moist throughout the test. Analysis of in vitro data. A Fourier transformation was performed on the pressure and diameter transients produced by the fluid injections, and the complex viscoelastic modulus \(E^*\) for a thick-walled vessel was calculated for each harmonic (7, 31)

\[
E^* = \left[\frac{3R_e^2R_i}{2(R_e^2 - R_i^2)} \cdot \Delta P \cdot e^{i\phi} \right] = E_{\text{dyn}} \cdot e^{i\psi}\]  

(3)

where \(R_e\) and \(R_i\) are the external and internal radii of the vessel, respectively, \(\Delta P\) is the amplitude of the pressure harmonic, \(\Delta R_e\) is the amplitude of the radius harmonic (one-half the amplitude of the diameter harmonic), and \(\phi\) is the phase angle between the corresponding pressure and radius harmonics. The viscoelastic moduli and phase angles were averaged for all values between 2.5 and 5 Hz.

For comparisons with in vivo data, the in vivo dynamic elastic modulus \(E_{\text{dyn}} = E_{\text{cosi}}\) (the real part of \(E^*\)), was compared with \(E_{\text{dyn}}\) calculated from the in vivo \(\text{c}(t)\) for a thick-walled vessel (substituting for \(D_e\)) (31)

\[
\sigma_{\text{circ, stat}} = \frac{P}{R_e^2 - R_i^2} \left(\frac{2}{R_i} - \frac{R_i}{R_e}\right) \]  

(5)

where \(P\) is the intraluminal pressure and \(R_i = R_e - h\), where \(h\) is the wall thickness computed at each pressure level, assuming incompressibility.

Circumferential strain \(\epsilon_{\text{circ}}\) was calculated from the \(D_e\) at 10 mmHg (\(D_0\), the lowest pressure at which the vessel was not collapsed) and from the measured change in \(D_e\) (\(D_e - D_0\))

\[
\epsilon_{\text{circ}} = \frac{D_e - D_0}{D_0} \times 100\% \]  

(6)

E_{\text{stat}} values were calculated as the slope of the stress-strain curve at the stresses corresponding to the blood pressures measured in vivo for that animal. Also, the initial and final slopes of the stress-strain curve (representing the moduli dominated by elastin and collagen, respectively) were computed as the slope of a linear regression fitting the first and last three data points. The "elbow" of the aortic stress-strain curve was taken as the strain at which the tangents fitting the first and last three points intersected.

Statistical Analysis

Values are means ± SE; \(n\) is the number of animals (except in Fig. 2, where \(n\) is the number of waves). Statistical comparisons between age groups were made at control distending pressures only by using a one-way ANOVA followed by Student-Newman-Keuls test. Statistical comparisons within age groups tested the significance of changes from control caused by changing distending pressure. Paired \(t\)-tests with a Bonferroni correction for two comparisons were employed. A significant difference was concluded when \(P < 0.05\).

There were two circumstances under which data were disregarded. The in vitro \(E_{\text{stat}}\) (under all 3 distending pressures) from one fetus was omitted, since it was >2 SD greater than the mean value for that age group. This animal was not in the in vivo study. Second, in vivo wave transmission calculations for one adult during noradrenaline infusion were not performed. In this case, MABP was very high and the pressure signals were clipped by the tape recorder.

RESULTS

Between 119 days gestation and adulthood, body weight increased 24-fold, thoracic aortic length increased 3-fold, and aortic wall thickness-to-radius ratio was approximately halved under physiological distending pressures (Table 1). MABP was lower in the fetus than in the lamb and the adult (by 27 and 41%, respectively), which were not significantly different from each other (Table 2). The in-

<table>
<thead>
<tr>
<th>Table 1. Anatomic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Fetus</td>
</tr>
<tr>
<td>Lamb</td>
</tr>
<tr>
<td>Adult</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 5–12\). Vessel length was measured in situ from azygous vein to diaphragm. \(h_v\), Vessel wall thickness measured via calipers from 5 locations on each of 3 cross-sections of vessel cut into quarters; \(h_e\), wall thickness of vessel calculated from \(h_v\) at its in vivo length and under control distending pressure by assuming incompressibility; \(D_e\), vessel external diameter measured via video-dimension analyzer at middle of segment at 5–10 mmHg distending pressure; \(R_e\), vessel external radius \((D_e/2)\). For each variable, values with same symbols (a, b, c) are not significantly different.
crease in MABP, along with changes in aortic dimensions from the fetus to the adult, caused a 265% increase in aortic circumferential wall stress with age from the fetus to the adult (see Table 5).

Effects of Drug Infusions on Blood and Cardiovascular Variables

Norepinephrine increased mean arterial pressure by ~65–70% and nitroprusside decreased arterial pressure by ~33–35%, changes that were similar to the desired +70% and −30% changes in MABP. The shapes of pressure waveforms in the midthoracic aorta did not vary substantially with age, and they demonstrated similar changes in shape during drug infusions (Fig. 1, Table 2), except that the lamb pressure waveform exhibited a prominent dicrotic notch that was larger with nitroprusside infusion and smaller with norepinephrine infusion (Fig. 1). At all ages, arterial blood gas partial pressures and pH were stable for the duration of the study (Table 3).

Validation of Three-Pressure Method

To make valid inferences about aortic wall properties from pressure wave propagation characteristics, it was necessary to ensure that the three-pressure method eliminated the effects of wave reflections from peripheral vascular beds. A prominent effect of wave reflections is large oscillations in $c_{app}$ with frequency. For all ages and blood pressure conditions, $c_{app}$ oscillated with frequency about $V_f$ (Fig. 2). These oscillations were enhanced during norepinephrine infusion and diminished during nitroprusside infusion, findings that are consistent with wave reflection theory (31). The three-pressure method markedly reduced these effects of wave reflections to yield the $c$ spectra (Fig. 3A), although oscillations with frequency of $c$ were not completely removed. These oscillations were greatest in the adult when mean arterial pressure was elevated with norepinephrine, which likely increased wave reflections through peripheral vasoconstriction (23, 34). When $c$ values were averaged over high frequencies, values were not significantly different from $V_f$ values for all ages and all blood pressure conditions (Fig. 3B). These results indicate that the three-pressure method largely

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>Control</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>27.0 ± 1.3*</td>
<td>40.4 ± 2.9a</td>
<td>66.2 ± 2.7*</td>
</tr>
<tr>
<td>Lamb</td>
<td>37.2 ± 2.1*</td>
<td>55.6 ± 3.9a</td>
<td>93.6 ± 8.4*</td>
</tr>
<tr>
<td>Adult</td>
<td>44.4 ± 3.8*</td>
<td>68.4 ± 6.0b</td>
<td>114.0 ± 7.7*</td>
</tr>
<tr>
<td>HR, s⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>2.22 ± 0.05</td>
<td>2.33 ± 0.06a</td>
<td>3.12 ± 0.20</td>
</tr>
<tr>
<td>Lamb</td>
<td>2.46 ± 0.16a</td>
<td>2.74 ± 0.18a</td>
<td>2.47 ± 0.11</td>
</tr>
<tr>
<td>Adult</td>
<td>1.85 ± 0.30</td>
<td>1.64 ± 0.22b</td>
<td>1.65 ± 0.15</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>11.7 ± 1.4*</td>
<td>16.2 ± 1.6a</td>
<td>27.7 ± 2.2*</td>
</tr>
<tr>
<td>Lamb</td>
<td>20.8 ± 1.7*</td>
<td>30.8 ± 1.2b</td>
<td>43.8 ± 1.2*</td>
</tr>
<tr>
<td>Adult</td>
<td>16.6 ± 2.3</td>
<td>20.8 ± 1.3c</td>
<td>36.0 ± 3.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 in each age group. MABP, mean arterial pressure; HR, heart rate; PP, pulse pressure; NP, nitroprusside; NE, norepinephrine. Under control conditions, different symbols (a, b, c) indicate significant differences between age groups (P < 0.05). *Significantly different from control (P < 0.05) within each age group.

Fig. 1. Typical pressure signals recorded in midthoracic aorta in fetus, lamb, and adult during infusion of vehicle (Control), nitroprusside (NP), and norepinephrine (NE). Waveforms are aligned by their mean values (mean arterial blood pressures are shown in Table 2). Traces are examples of digitized (500-Hz) waveforms used in computer analysis.
removed wave reflection effects and thus determined \( c \) and true attenuation coefficient.

**Comparison of In Vivo and In Vitro Data**

To test the agreement between in vivo and in vitro data, we compared \( E_{\text{dyn}} \) obtained in vitro with \( E_{\text{dyn}} \) calculated from the vessel dimensions and the phase velocity measured in vivo (Eq. 4). These values were consistent for lamb and adult vessels; values agreed within 2% for adults and within 14% for lambs (Table 4). In contrast, the fetal aorta was much stiffer in vitro under static and dynamic conditions. \( E_{\text{dyn}} \) of the fetal aorta was 200% higher in vitro than in vivo (Table 4), and \( E_{\text{stat}} \) in vitro (Table 5) was more than twice \( E_{\text{dyn}} \) in vivo (Table 4), even though in theory \( E_{\text{stat}} \) is less than \( E_{\text{dyn}} \) for viscoelastic materials.

**Viscoelasticity of the Thoracic Aorta Under Control Blood Pressure Conditions**

The thoracic aorta became progressively less viscous with increasing age. Pressure wave attenuation in the thoracic aorta significantly decreased by \( \sim 70\% \) from fetal life to adulthood in vivo (Fig. 4, control MABP; Table 5); furthermore, the phase angle of the viscoelastic modulus \( \phi \) decreased by 36% from fetal life to adulthood at control distending pressures in vitro (Table 5).

In vitro stress-strain relations and in vivo phase velocities indicate that the thoracic aorta became progressively stiffer with increasing age. The in vitro \( E_{\text{stat}} \) of the fetal aorta was significantly less than that of the adult aorta under control distending pressures (Table 5, Fig. 5, control distending pressure), and calculations based on age-related changes in phase velocity and vessel dimensions were consistent with an increase in dynamic stiffness of the aortic wall with age. In vivo \( c \) was 22% lower in the fetal aorta than in the adult aorta (Table 5). A 60% increase in phase velocity from the fetus to the lamb was caused by a large increase in dynamic wall stiffness \( (E_{\text{dyn}} \text{ in vivo}) \), whereas from the lamb to the adult, phase velocity was decreased due to a 50% decrease in relative wall thickness with relatively unchanged dynamic wall stiffness (Tables 1 and 4; see Eq. 4). Thus dynamic wall stiffness is increased during development from fetal to adult life, with most of these changes occurring during the perinatal period.

**Pressure Dependence of Aortic Viscoelasticity**

Measures of aortic wall stiffness, i.e., \( c \) and \( V_f \) in vivo, were significantly increased when blood pressure was elevated in the fetus and adult, but not in the lamb (Fig. 3B). Elevated distending pressures in vitro significantly increased \( E_{\text{dyn}} \) at all ages (Fig. 6) and increased \( E_{\text{stat}} \) at all ages, although the increase was significant only for the fetus (Fig. 5). Thus aortic wall stiffness tended to increase with elevated distending pressures.

---

**Table 3. Blood gases and pH**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Pco₂, mmHg</th>
<th>P0₂, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>7.36±0.03</td>
<td>51.5±5.1</td>
<td>21.5±1.5</td>
</tr>
<tr>
<td>Lamb</td>
<td>7.41±0.06</td>
<td>48.4±12.0</td>
<td>510.4±27.1</td>
</tr>
<tr>
<td>Adult</td>
<td>7.58±0.02</td>
<td>29.6±1.3</td>
<td>419.5±58.5</td>
</tr>
</tbody>
</table>

Values are means ± SE taken immediately before first drug infusion; \( n = 5 \) in each age group. There were no significant differences between initial values and final values (measured before final drug infusion, not shown) in any age group, \( P < 0.05 \).
With increased MABP in vivo, pressure wave attenuation, a measure of aortic wall viscosity, significantly decreased by 41% in the lamb aorta and also tended to decrease in the fetus (P < 0.05; Fig. 4). However, attenuation in the adult aorta was unchanged with increased MABP (Fig. 4).

Static Stress-Strain Relations In Vitro

There were marked age-related changes in the aortic static stress-strain curves (Fig. 7). Aortic wall stiffness at low strain (initial slope of curve) was almost doubled between the fetus and the lamb, then showed no further changes with postnatal age (Fig. 8A). In contrast, static wall stiffness at high strain (final slope of curve) was unchanged during the perinatal period and almost doubled during postnatal development between the lamb and the adult (Fig. 8B).

The operating strain (strain at control distending pressure) was doubled in the adult aorta compared with the fetus and lamb, in which operating strains were not significantly different (Fig. 7). This observation may be due in part to a rightward shift of the stress-strain curve elbow, defined as the strain at which the tangents fitting the first and last three points intersect. The elbow of the curve was increased significantly with age from 20.2 ± 2.4% in the fetus and 25.8 ± 0.8% in the lamb to 55.7 ± 6.5% in the adult, indicating that the thoracic aorta becomes more extensible in the circumferential direction during postnatal development.

**DISCUSSION**

This study is the first to describe developmental changes in aortic wall viscoelasticity in the perinatal and postnatal period. In vitro experiments directly determined the E* of the aorta, which explicitly characterizes the dynamic stiffness and the intrinsic viscosity.
of the aortic wall tissue. In vivo measurements of pressure wave attenuation and velocity provided assessments of viscoelastic wall properties that were indirect but had the advantage that the uninstrumented vessel could be examined in its natural setting. In general, compatible results were obtained using these two approaches. For example, a rise in vitro elastic modulus and in vivo phase velocity with age indicated age-dependent stiffening of the aorta, whereas decreases in the in vitro phase angle of the viscoelastic modulus and the in vivo attenuation coefficient indicated that the aorta becomes less viscous with age. In addition, well-established relations (7, 31) permitted computation of $E_{\text{dyn}}$ (real part of $G$) from $G$ determined in vivo. We found that the in vivo $E_{\text{dyn}}$ determined in this way was very similar to direct in vitro measurements for lambs and adult sheep. Agreement between these two methods was also reported by Li et al. (25) for the adult canine femoral artery. However, large discrepancies were seen between in vivo and in vitro estimates of $E_{\text{dyn}}$ for the fetus. We infer that these discrepancies were due to effects of vessel isolation on wall mechanics at this age. We did not determine why the fetal aorta was stiffer in vitro; however, fetal aortic tissue may have behaved differently in vitro due to age-dependent differences in the degree of tissue hydration in vitro (11) and/or age-related differences in the degree of smooth muscle cell relaxation induced by sodium nitroprusside (4). Furthermore, mechanical coupling to surrounding tissues (tethering) may have a greater impact on the fetal aorta, because this age precedes the rapid perinatal growth of the aorta (6, 22), the abrupt postnatal rise in arterial pressure, and the aeration of the fluid-filled lung at birth.

Although changes in the viscoelasticity of the aorta during development were large, equally striking changes in vessel geometry also affected wall mechanics. Thus a halving of the wall thickness-to-radius ratio, coupled with a 70% increase in blood pressure, resulted in a 265% increase in aortic wall stress at control arterial pressure from fetal to adult life. Previous studies demonstrated a striking consistency in the tensile wall stress imposed on adult thoracic aorta of a very wide range of mammalian species (43). This finding, coupled with observations that changes in blood pressure modulate medial growth (16, 24), has led to the inference that tensile arterial wall stress is controlled around a well-defined set point. If this is true, then our data indicate that the set point is age dependent. An alternative explanation is that wall

![Fig. 4](image_url)  
**Fig. 4.** Mean in vivo wave attenuation coefficients ($a_1$) in fetal, lamb, and adult thoracic aorta with altered mean arterial blood pressures ($\Delta MABP$). Values are means ± SE at heart rate frequency (1st harmonic); $n = 5$ in each age group, except adults during norepinephrine infusion, where $n = 4$. Points joined by solid lines are significantly different from control; points joined by dashed lines do not significantly differ. Statistical comparisons of attenuation were made between age groups at their control blood pressures. Values labeled with same letter (a, b) are not significantly different.

![Fig. 5](image_url)  
**Fig. 5.** In vitro circumferential static elastic moduli ($E_{\text{stat}}$) at distending pressures corresponding to in vivo blood pressures for fetus ($n = 9$), lamb ($n = 6$), and adult ($n = 8$). Values are means ± SE. Points joined by solid lines are significantly different from control; points joined by dashed lines do not significantly differ. Statistical comparisons of elastic moduli were made between age groups at their control distending pressures. Values labeled with same letter (a, b) are not significantly different.

Table 5. Summary of in vivo and in vitro measurements at control distending pressures

<table>
<thead>
<tr>
<th>In Vitro</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_{\text{circ}}$, $\times 10^6$ dyn/cm²</td>
<td>$E_{\text{stat}}$, $\times 10^6$ dyn/cm²</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>Fetus 0.20 ± 0.02* (10)</td>
<td>2.16 ± 0.27* (9)</td>
</tr>
<tr>
<td>Lamb 0.30 ± 0.04* (6)</td>
<td>2.76 ± 0.26* (6)</td>
</tr>
<tr>
<td>Adult 0.73 ± 0.11* (8)</td>
<td>3.21 ± 0.30* (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of animals in parentheses. $\sigma_{\text{circ}}$, Circumferential stress; $E_{\text{stat}}$, static elastic modulus; $E_{\text{G}}$, magnitude of complex viscoelastic modulus; $\theta$, phase angle of complex viscoelastic modulus; $c$, mean true phase velocity; $a_1$, attenuation coefficient at fundamental (heart rate) frequency. Within each variable, values with same symbols (a, b, c) are not statistically different ($P < 0.05$).
stress is one of several factors that modulate growth of wall thickness, without reference to a predetermined set point.

Although geometry and tissue properties of the aorta changed during development, the relative importance of these changes in determining viscoelasticity at control pressures was age dependent. Changes in tissue mechanical properties at control distending pressures were concentrated in the perinatal period: 50% of the change in wall viscosity and 60% of the change in static wall stiffness occurred in the 6 wk surrounding birth.

In contrast, aortic dimensions increased symmetrically over this time, and the wall thickness-to-radius ratio did not change. However, the wall thickness-to-radius ratio fell by 50% during later postnatal development. Thus wall viscoelasticity at control distending pressures is greatly influenced by changes in material properties in the perinatal period, whereas remodeling of vessel geometry dramatically affects the vessel during later development.

Changes in the material properties of the aorta in the weeks surrounding birth are not surprising, since this is a period of rapid growth and remodeling of the vessel. We chose the ages at which to examine fetal sheep and lambs to coincide with our previous studies of perinatal accumulation of arterial wall constituents (6). In those experiments, we observed a fourfold increase in elastin content and a twofold increase in collagen content between the 120-day-gestation fetus and the 21-day-old lamb. Elastin is thought to contribute most to the elastic modulus of arteries at low and moderate blood pressures, with collagen making increasing contributions at higher pressures (10, 37, 42). Consequently, the accumulation of elastin in the weeks surrounding birth may explain why the initial slope of the stress-strain curve is almost doubled during perinatal development.

In addition, there may be developmental changes in the biochemical structure of elastin that could affect its mechanical properties (32). Despite the increase in connective tissue content during the perinatal period, there was no change in smooth muscle cell content (6); hence, the overall cellularity of the wall was reduced. Internal viscosity of the arterial wall tissue is normally attributed to cellular constituents, mainly smooth muscle cells (31); therefore, the decreased cellularity of the aortic wall likely accounts for the trend toward a...
Developmental changes in geometry and composition of the aorta affect its mechanical properties, but these properties are also affected by developmental increases in arterial pressure, which alter the point on each stress-strain curve at which the vessel operates. Our in vitro data suggest that fetal and lamb vessels exhibit the same strain at baseline blood pressures; however, this inference must be made cautiously given differences between in vitro and in vivo stress-strain relations for the fetal aorta. On the other hand, the more than doubling of strain at baseline blood pressure between neonatal and adult life is undoubtedly genuine. This increase is due in part to the postnatal rise in arterial pressure, but it is also caused by a developmental increase in aortic circumferential extensibility as shown by a rightward shift of the stress-strain curve.

Changes in wall tissue organization, as well as composition, may be responsible for age-related changes in the aortic stress-strain curve. For example, we recently showed that the fenestrae that perforate elastic lamellae increase dramatically in number and size during postnatal development (44). This relative reorganization of a wall constituent that bears much of wall tension at baseline blood pressure is expected to affect wall mechanics, because stresses are concentrated adjacent to perforations through materials under stretch (20). Consequently, increased fenestration of elastic lamellae is expected to increase the tensile load on elastic tissues and thereby influence the material properties of this medium. Collagen fibers may also demonstrate structural alterations with maturation through changes in cross-linking. Collagen cross-link formation, through the conversion of reactive aldmine cross-links into stable, nonreducible cross-links, increases during development and maturation of other tissues, including skin and pericardium (3, 14, 33, 39), and has been associated with alterations in tissue mechanical properties (15, 33, 40). Unfortunately, the effects of maturation of collagen on arterial wall properties have received little study.

Developmental remodeling of the aorta is expected to affect central hemodynamic function. The property of the descending thoracic aorta that most directly influences central hemodynamics is its characteristic impedance. Characteristic impedance of the aorta is the impedance to pulsatile flow observed in the vessel in the absence of reflections from downstream sites, and it is determined by the aortic dimensions and viscoelasticity. Previously, we reported that the characteristic impedance of the descending aorta was unchanged from late fetal to early neonatal life (1, 21). Its invariance during perinatal development is very surprising given the extensive changes in these properties that we now report. We infer that the tendency for increased aortic diameter to decrease characteristic impedance is offset by the tendency for increased aortic wall stiffness to increase characteristic impedance. In contrast, the large increase in aortic diameter during postnatal development is likely responsible for the lower aortic characteristic impedance that is typical of adults (31) [−80% lower than in fetuses or lambs (1, 21)]. A by-product of the decrease in impedance is that cardiac workload associated with accelerating and decelerating blood flow during the cardiac cycle is reduced. High pulsatile energy losses during perinatal development may be an inescapable consequence of a small-caliber vessel with a highly cellular wall (high viscous losses) that is a requirement for rapid tissue synthesis in the growing aorta.

Our findings are consistent with the limited previous data on mechanical properties of the developing aorta. Although there are no previous data on viscoelasticity of fetal or lamb vessels, information on $E_{\text{stat}}$ and $V_f$ measured in the aorta is available at these ages. Our aortic static elastic moduli measured in vitro for the fetus ($2.16 \times 10^6$ dyn/cm$^2$ at 119 days gestation) and lamb ($2.76 \times 10^6$ dyn/cm$^2$ at 21 days of age) were similar to in vivo measurements obtained by Pagani et al. (35) in unanesthetized fetal sheep ($2.58 \times 10^6$ dyn/cm$^2$ at 130 days gestation) and newborn lambs ($2.42 \times 10^6$ dyn/cm$^2$ at 1–3 days of age) (35). Similarly,
our \( V_i \) values in vivo in the fetus (3.6 m/s at 119 days gestation) and lamb (5.2 m/s at 21 days of age) were similar to previous in vivo measurements in unanesthetized fetuses (3.9 m/s at 123–127 days gestation) and lambs (5.8 m/s at 7 days of age) (1, 21). It is likely that the small differences in the values are largely attributable to age differences. In adult vessels, static elastic and viscoelastic moduli and pressure wave transmission coefficients have been measured previously in various species, and our results are similar to previous reports. In particular, our \( E_{\text{static}} \) in vitro (3.2 \( \times 10^6 \) dyn/cm\(^2\)) was similar to that obtained in unanesthetized adult sheep in vivo (3.7 \( \times 10^6 \) dyn/cm\(^2\)) (35), and our viscoelastic modulus in vitro (3.5 \( \times 10^6 \) dyn/cm\(^2\)) was similar to values obtained in the canine thoracic aorta (3 \( \times 10^6 \) to 4.7 \( \times 10^6 \) dyn/cm\(^2\)) (7, 19). Our wave attenuation coefficient (0.021 cm\(^{-1}\)) and \( V_i \) (4.7 m/s) in vivo were also similar to measurements obtained in the canine thoracic aorta (0.01 cm\(^{-1}\) at heart rate frequency and 4.1–4.8 m/s, respectively) (29, 30).

In summary, we have demonstrated developmental changes in aortic wall viscoelasticity in the perinatal period through in vivo pressure wave transmission characteristics and in vitro mechanical testing. The use of in vivo measurements allowed us to observe the developmental changes in viscoelastic behavior of vessels under normal operating conditions, with the aortic wall uninstrumented and tethering undisturbed. By complementing these measurements with in vitro dynamic and static stress-strain relations, we were able to assess the developmental changes in the material properties of the vessel wall that underlie our observations in vivo. Developmental changes in aortic viscoelasticity were caused by remodeling of the wall structure and not simply by a developmental rise in arterial wall stress along a stress-strain curve that remained unchanged with age. We also tested the agreement between measurements obtained using in vivo and in vitro techniques. Our results show excellent agreement between in vivo and in vitro measurements in postnatal ages, whereas fetal vessels were stiffer in vitro. Nevertheless, both methods suggest large developmental changes in aortic viscoelasticity from fetal to adult life: the vessel became progressively stiffer, less viscous, and more extensible with development at normal in vivo blood pressures. These changes in aortic viscoelasticity are largely effected by changes in the material properties of the tissue in the perinatal period and thus coincide with major developmental adjustments in hemodynamic function at birth. In contrast, postnatal remodeling largely influences vessel geometry and results in an increase in the tensile stress borne by aortic tissue.

We are grateful to Kathie Whiteley for excellent technical assistance, Christopher Pereira for writing the LabVIEW data acquisition program, and Dr. Greg Wilson for generously allowing us the use of laboratory space and equipment. We are also grateful to Drs. J. Michael Lee and David Courtman for helpful discussions and advice. This work was supported by a grant from the Heart and Stroke Foundation of Ontario and the Medical Research Council of Canada. S. L. Adamson and B. L. Langille are Career Investigators of the Heart and Stroke Foundation of Ontario, and S. M. Wells is an awardee of a Medical Research Council of Canada Studentship. Address for reprint requests: S. L. Adamson, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Rm. 138-P, 600 University Ave., Toronto, ON, Canada M5G 1X5.

Received 13 November 1997; accepted in final form 30 January 1998.

REFERENCES


