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Determination of Aortic Elastic Modulus by Pulse Wave Velocity and Wall Tracking in a Rat Model of Aortic Stiffness

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Key Words
Thoracic aorta · Moens-Korteweg equation · Histomorphometry · Distensibility coefficient · Medial thickness

Abstract
Several methods have been used to evaluate the elastic modulus of the aortic wall in the rat, but these have never been compared when used simultaneously. We measured thoracoabdominal pulse wave velocity (PWV) and changes in thoracic aorta diameter during the cardiac cycle (with wall echo-tracking) in pentobarbital-anesthetized adult male Wistar rats; half of the group had previously received vitamin D_3 plus nicotine (VDN) in order to increase the stiffness of the aortic wall. The Moens-Korteweg elastic modulus (E_{MK}) was calculated from PWV and the ratio of the internal diameter to the medial thickness determined by histomorphometry following in situ pressurized fixation. The incremental elastic modulus (E_{inc}) was calculated from the distensibility coefficient and end-diastolic diameter measured by wall echo-tracking and the medial thickness determined by histomorphometry. Both values were higher in VDN rats than in controls: E_{inc} 8.9 ± 0.5 and 5.7 ± 0.4·10^6 dyne/cm^2, $p < 0.05$; E_{MK} 7.6 ± 0.5 and 4.1 ± 0.5·10^6 dyne/cm^2, $p < 0.05$. E_{inc} was greater than E_{MK} and this was partially due to the fact that the in vivo end-diastolic diameter measured by ultrasound was greater than the mean aortic diameter measured ex vivo by histomorphometry. In conclusion, different methods for the measurement of the elastic properties of the aortic wall gave similar results in controls and in a rat model of aortic stiffness.

Introduction

The wall of the large-diameter elastic arteries (aorta and carotid arteries) becomes progressively stiffer with age, hypertension and other vascular diseases; this leads to a decrease in arterial compliance and an increase in the pulsatile element of pressure [1]. The latter is a strong indicator of cardiovascular risk [2, 3]. The age-related decline in wall distensibility increases aortic input impedance and this adversely affects cardiac function and structure [4].

Direct measurement of the elastic behavior of the aortic wall in vivo is based mainly on two approaches. Measurement of pressure pulse wave velocity (PWV) provides values for elastic modulus via the Moens-Korteweg equation. This was first developed to measure aortic elasticity in large mammal species (humans [5] and dogs [6]), then adapted to smaller species such as the rat [7, 8]. More

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recently, ultrasound technology (using a wall echo-tracking device) has been developed to follow the changes in diameter following a change in pulse pressure. This was applied to the study of the elasticity of the walls of the major peripheral arteries in humans [9, 10], then miniaturized to measure the elastic properties of the wall of the rat carotid artery [11] and aorta [12]. It provides values for the incremental elastic modulus [13].

Although the rat is often used in studies on vascular mechanics, to our knowledge, there has been no direct, simultaneous comparison of the above two methods in this species. This is important, as conclusions based on results using different methods and models may be contradictory.

The goal of the present study was to compare results obtained with two methods (wall echo-tracking Doppler and PWV) simultaneously in the same animal. Experiments were performed using a rat model of high aortic wall stiffness induced by elastocalcinotic arteriosclerosis. Elastocalcinosis is induced by treating young rats with vitamin D_3 and nicotine (VDN) [14], and is accompanied by stiffening of the aortic wall and an increase in characteristic impedance [14–16].

Materials and Methods

Animals

Two-month-old male, outbred Wistar rats (ICO: WI IOPS AF/Han, Iffa-Credo, L'Arbresles, France) were housed under standard conditions (temperature 21 ± 1 °C; humidity 60 ± 10%; lights on from 6.00 a.m. to 6.00 p.m.) and given a standard rodent diet (A04, UAR, Villemoisson-sur-Orge, France; calcium content 150 mmol/kg) and water (Aqua-clear®, Culligan, Northbrook, Ill., USA; calcium 28 μmol/l) ad libitum.

One group of rats (VDN, n = 9) was injected with vitamin D_3 (270,000 IU/kg, i.m; Duphafral® D_3 1000, Duphar B.V., Weesp, the Netherlands) and nicotine (2 × 25 mg/kg, 5 ml/kg, p.o.; Sigma Chemical Company, St. Louis, Mo., USA) to induce elastocalcinosis as previously described [14–16]. Two VDN rats died on the second and third day following treatment. Another group of rats (control, n = 9) received 0.15 *M* NaCl intramuscularly and two gavages of distilled water. Experiments were performed 1 month after treatment in accordance with the guidelines of the French Ministry of Agriculture (permit numbers 03575 and 54-5).

Measurement of Thoracoabdominal PWV

The procedures for PWV measurement have been described in detail elsewhere [8, 15, 16]. Briefly, polyethylene cannulas (0.96 mm outer diameter/0.58 mm inner diameter) were implanted under sodium pentobarbital anesthesia (60 mg/kg, i.p.) into the descending thoracic aorta and the abdominal aorta. The aortic cannulas were connected to a pressure recording system, the dynamic frequency response of which is flat with a phase lag of $<-6°$ up to 30 Hz, and then slightly underdamped [17]. Pressure signals were converted into digital form and recorded on-line at a sampling rate of 256 Hz. Parameters were determined beat-to-beat and averaged over periods of 4 s every 30 s for 30 min. An algorithm detected the maximal and minimal values of each pressure signal, and calculated the mean aortic blood pressure (mm Hg) from the waveform area, pulse pressure as the diastolo-systolic difference and heart rate (beats/min) by counting the number of cycles over the 4-second period.

PWV (cm/s) was calculated as the distance between the two cannula tips – measured in situ following postmortem fixation by sticking a damp cotton thread onto the aorta – divided by the transit time. Transit times (ms) were measured on-line by an algorithm which systematically shifted the peripheral pressure waveform in time with respect to the central pressure waveform and determined the value of the time shift giving the highest correlation [8, 15–17]. The accuracy of the determination of transit time was improved by performing the calculation for the entire waveform, using least squares analysis of the differences in amplitude between the central and peripheral signals and by creating intermediate points for the peripheral signal by linear interpolation. As the sampling rate was 1/3.9 ms and 10 intermediate points were created, the theoretical resolution of the calculated transit time was 0.39 ms, which gives a $<5\%$ error for the lowest value of transit time measured.

Measurement of Thoracic Aorta Distension

The measurement of the thoracic aorta diameter and changes in diameter during the cardiac cycle (distension) has been described in detail elsewhere [12, 13]. A vessel wall echo-tracking system attached to a conventional B-mode ultrasound system (B = brightness, Pie 480, 7.5-MHz linear array; Pie Medical, Maastricht, the Netherlands) was used. The thoracic aorta was visualized in B-mode approximately 10 mm above the diaphragm and an M-line was positioned perpendicular to the vessel wall. With a sample frequency of 30 MHz and an emission frequency of 1,653 Hz, 1 s of data correspond to 5–9 cardiac cycles (1–2 respiratory cycles). The data were analyzed with an algorithm which allowed visual identification of the anterior and posterior wall boundaries by two markers, thus delimiting the sample window for data processing. End-diastolic diameter and distension were assessed with a precision of 10 μm for each cardiac cycle and then averaged [12, 13]. Recordings showing more than 10% variation for distension measurements were discarded. The coefficients of variation of 5 consecutive measurements performed on a given day by a given investigator were less than 3% for end-diastolic diameter and less than 5% for distension; the coefficients of variation of measurements performed on successive days (same investigator) were 2 and 3% for end-diastolic diameter and distension, respectively.

Aortic distensibility [distensibility coefficient (DC), relative increase in volume (ΔV) for a given increase in aortic pulse blood pressure (ΔP)] and compliance [compliance coefficient (CC), absolute increase in ΔV for a given ΔP] were calculated by the equations given below, assuming that a volumetric increase is due to vascular distension (radial, ΔD) rather than (axial) elongation [10] and that the vascular cross section is perfectly circular:

$$DC = \Delta V/(V\Delta P) \approx \Delta A/(A_{dia}\Delta P) \approx (2\,\Delta D/D_{dia})/\Delta P$$

$$CC = \Delta V/\Delta P \approx \Delta A_{dia}/\Delta P \approx (\pi\, D_{dia}\Delta D)/(2\,\Delta P),$$

where D_{dia} and A_{dia} are the end-diastolic diameter and area, respectively, and ΔD and ΔA represent the change in diameter (distension) and lumen area during the cardiac cycle, respectively. The incremen-

tal elastic modulus of the thoracic aorta (E_{inc}; 10^6 dyne/cm^2) was calculated as follows [13]: $E_{inc} = D_{dia}/(h \cdot DC)$, where h is the medial thickness (mm) (see below).

Aortic Wall Geometry and Calcium Content

At the end of the experiment, rats were perfused in situ at their individual peripheral mean aortic blood pressure with 10% formol containing phosphate-buffered saline. A 1-cm sample of the thoracic descending aorta was excised (at the same locus where distension was measured) and immersed in 10% formol, dehydrated then embedded in paraffin. Three sections (thickness 20 μm) were cut and stained with hematoxylin-eosin for the measurement (Saisam®, Microvision Instruments, Evry, France) in a double-blind fashion of mean internal diameter (D_i; mm) and medial thickness (distance between the external and internal elastic laminae).

The elastic modulus of the thoracoabdominal aortic wall was calculated from the Moens-Korteweg equation (E_{MK}; 10^6 dyne/cm^2): $E_{MK} = (PWV^2 \cdot D_i \cdot \rho)/h$, where ρ is the blood density and is equal to 1.05 g/cm^3.

Another 1-cm sample of the thoracic aorta was excised and the wall calcium content was determined by atomic absorption spectrophotometry (AA10, Varian Ltd., Melbourne, Australia) following mineralization and nitric acid digestion [18].

Statistics

Results are expressed as means ± SEM. The statistical significance of the differences between group means was evaluated by ANOVA plus the Bonferroni test. The null hypothesis was rejected at a probability level of $p < 0.05$. Linear regression was performed using standard parametric models.

Results

Body weight was similar in control and VDN rats, as was heart rate. VDN rats remained normotensive, as central and peripheral aortic mean blood pressures were similar to those of controls. Central and peripheral pulse pressures were 30 and 20% higher in VDN rats than in controls. In VDN rats, PWV increased by 35%, whereas distension was reduced by 20% and DC and CC by 35%. These results are shown in table 1.

VDN induced a 14-fold increase in the calcium content of the wall of the thoracic aorta (VDN rats 327 ± 54 μmol/g dry weight, controls 24 ± 5 μmol/g dry weight, $p < 0.0001$). The results of thoracic aortic wall geometry and elastic moduli in control and VDN rats are summarized in table 2. There were no differences in aortic internal diameter between control and VDN rats, whether it was measured in situ by the wall echo-tracking device (D_{dia}) or ex vivo by histomorphometry (D_i). However, D_{dia} was 20% higher than D_i in both groups of rats. Medial thickness (histomorphometry) was similar in both groups.

E_{MK} increased by 1.85-fold and E_{inc} by 1.56-fold in VDN rats. E_{inc} was 39 and 17% higher than E_{MK} in con-

Table 1. Body weight, heart rate, aortic blood pressures, PWV, distension, DC and CC in anesthetized control and VDN rats

	Control (n = 9)	VDN (n = 7)	p
Body weight, g	469 ± 30	449 ± 32	0.6626
Heart rate, beats/min	310 ± 13	323 ± 7	0.4461
Central aortic blood pressure, mm Hg			
Systolic	117 ± 3	123 ± 4	0.1168
Mean	103 ± 2	104 ± 3	0.7232
Diastolic	88 ± 2	85 ± 3	0.4643
Pulse	29 ± 1	38 ± 3	0.0058
Peripheral aortic blood pressure, mm Hg			
Systolic	126 ± 3	132 ± 6	0.3078
Mean	101 ± 2	103 ± 4	0.7339
Diastolic	86 ± 2	84 ± 4	0.5788
Pulse	40 ± 2	49 ± 5	0.0876
PWV, cm/s	399 ± 17	545 ± 26	0.0002
Distension, μm	213 ± 7	170 ± 11	0.0040
DC, 10^{-3} /kPa	51 ± 3	32 ± 2	0.0002
CC, 10^{-3} mm^2/kPa	129 ± 10	85 ± 12	0.0108

Table 2. Thoracic aortic wall geometry and elastic moduli in control and VDN rats

	Control	VDN	p
Internal diameter, mm			
Histomorphometry mean diameter (D_i)	1.51 ± 0.04	1.51 ± 0.06	0.8981
Ultrasound diastolic diameter (D_{dia})	1.79 ± 0.05	1.78 ± 0.05	0.8979
Medial thickness, μm	63 ± 1	62 ± 2	0.8009
Elastic modulus, 10^6 dyne/cm^2			
E_{MK}	4.1 ± 0.5	7.6 ± 0.5	<0.0001
E_{inc}	5.7 ± 0.4	8.9 ± 0.5	0.0013

trols and VDN rats, respectively. When E_{inc} was calculated using D_i instead of D_{dia}, it was not significantly different from E_{MK} ($4.8 \pm 0.3 \cdot 10^6$ dyne/cm^2 in controls and $7.6 \pm 0.7 \cdot 10^6$ dyne/cm^2 in VDN rats). Using pooled values, E_{MK} and E_{inc} were significantly correlated (intercept = $0.37 \pm 1.53 \cdot 10^6$ dyne/cm^2, slope = $0.743 \pm 0.209 \times E_{inc}$, $p = 0.0031$, $R^2 = 0.475$, $n = 16$). Within control and VDN groups, however, no significant correlation was found.

Both E_{MK} and E_{inc} were significantly correlated with the calcium content of the thoracic aortic wall when values from VDN and control rats were pooled (E_{MK}: intercept = $4.1 \pm 0.5 \cdot 10^6$ dyne/cm^2, slope = $0.9 \pm 0.2 \cdot 10^4$ dyne$\cdot$g$\cdot\mu$mol$^{-1}\cdot$cm^{-2}, $p = 0.0021$, $R^2 = 0.503$; E_{inc}: intercept = $5.3 \pm 0.4 \cdot 10^6$ dyne/cm^2, slope = $0.9 \pm 0.2 \cdot 10^4$ dyne$\cdot$g$\cdot\mu$mol$^{-1}\cdot$cm^{-2}, $p = 0.0002$, $R^2 = 0.652$; $n = 16$). There was a significant correlation for E_{inc}, but not for E_{MK}, within the VDN group (E_{inc}: intercept = $5.6 \pm 0.9 \cdot 10^6$ dyne/cm^2, slope = $0.9 \pm 0.3 \cdot 10^4$ dyne$\cdot$g$\cdot\mu$mol$^{-1}\cdot$cm^{-2}, $p = 0.0280$, $R^2 = 0.581$, $n = 7$).

Discussion

The two methods used to evaluate the elastic properties of the aortic wall revealed similar increases in the elastic modulus of the aortic wall in VDN rats, a model of increased aortic stiffness [14–16]. Treatment of young rats with VDN induces calcium deposition on the medial elastic fibers, leading to fragmentation of the elastic network [19]. Elastocalcinosis is the major factor responsible for stiffening of the aortic wall (as revealed by increases in aortic characteristic impedance, PWV and elastic modulus), as no changes in aortic mean pressure, geometry or wall stress were observed [14–16]. The degree of wall stiffness is significantly correlated with the degree of medial elastocalcinosis [15]. In the present study, the increase in wall stiffness (+37% increase in PWV) was associated with a decrease in distensibility, of the same order of magnitude (–37% for DC); moreover, increases in Moens-Korteweg and incremental elastic moduli were correlated with the increase in calcium content of the thoracic aortic wall.

E_{inc} was greater than E_{MK} in both VDN (+17%) and control (+39%) rats. This may arise from the fact that E_{inc} is measured over a short section of the thoracic aorta and E_{MK} over the entire descending thoracic and abdominal aorta. However, this is not a satisfactory explanation, as the aortic wall stiffens progressively with increasing distance from the heart and so it would be expected that E_{MK} is greater than E_{inc}.

Differences between E_{inc} and E_{MK} may be partially explained by the fact that D_{dia} was 20% greater than D_i. This is apparently paradoxical, as D_{dia} is measured at diastolic pressure and D_i at mean pressure. However, others have reported a similar difference, for example Girerd et al. [20], who used pressurized fixation conditions similar to those used in the present report. This paradox may be explained by (1) the existence of flow-dependent mechanisms that may have contributed in vivo to thoracic aorta dilation and (2) tissue shrinkage due to formol fixation, which may have induced a significant decrease in the ex vivo arterial wall diameter [21, 22]. Concerning possible tissue shrinkage, it is surprising that differences between D_{dia} and D_i were similar in controls and VDN rats, as the aortic wall of the latter group is substantially stiffer and therefore may be expected to shrink less on fixation.

In conclusion, two different techniques for the measurement of the elastic properties of the aortic wall gave similar results in controls and in a rat model of aortic stiffness. In vivo end-diastolic diameters measured by ultrasound are greater than the mean aortic diameter measured ex vivo by histomorphometry.

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References

1 Nichols WW, O'Rourke MF: in McDonald's Blood Flow in Arteries. London, Edward Arnold, 1990, pp 399–402.
2 Safar ME: Pulse pressure, arterial stiffness, and cardiovascular risk. Curr Opin Cardiol 2000; 15:258–263.
3 Vaccarino V, Holford TR, Krumholz HM: Pulse pressure and risk for myocardial infarction and heart failure in the elderly. J Am Coll Cardiol 2000;36:130–138.
4 Nichols WW, O'Rourke MF, Avolio AP, Yaginuma T, Murgo JP, Pepine CJ, Conti CR: Effects of age on ventricular-vascular coupling. Am J Cardiol 1985;55:1179–1184.
5 Bazett HC, Dreyer NB: Measurements of pulse wave velocity. Am J Physiol 1922–1923;63: 94–116.
6 Dow P, Hamilton WF: An experimental study of the velocity of the pulse wave propagated through the aorta. Am J Physiol 1939;125:60–62.
7 Chillon JM, Capdeville-Atkinson C, Lartaud I, Guillou J, Mertes PM, Atkinson J: Chronic antihypertensive treatment with captopril plus hydrochlorothiazide improves aortic distensibility in the spontaneously hypertensive rat. Br J Pharmacol 1992;107:710–714.
8 Marque V, Kieffer P, Atkinson J, Lartaud-Idjouadiene I: Elastic properties and composition of the aortic wall in old spontaneously hypertensive rats. Hypertension 1999;34:415–422.
9 Hoeks APG, Ruissen CJ, Hick P, Reneman RS: Transcutaneous detection of relative changes in artery diameter. Ultrasound Med Biol 1985;11:51–59.
10 Reneman RS, van Merode T, Hick P, Muytjens AMM, Hoeks APG: Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol 1986;12:465–471.
11 Hayoz D, Rutschmann B, Perret F, Niederberger M, Tardy Y, Mooser V, Nussberger J, Waeber B, Brunner H: Conduit artery compliance and distensibility are not necessarily reduced in hypertension. Hypertension 1992; 20:1–6.
12 Van Gorp AD, van Ingen Schenau DS, Willigers J, Hoeks APG, de Mey JGR, Struyker-Boudier HAJ, Reneman RS: A technique to assess aortic distensibility and compliance in anesthetized and awake rats. Am J Physiol 1996;270: H780–H786.
13 Brouwers-Ceiler DL, Nelissen-Vrancken HJ, Smits JF, De Mey JG: The influence of angiotensin II-induced increase in aortic wall mass on compliance in rats in vivo. Cardiovasc Res 1997;33:478–484.
14 Atkinson J, Poitevin P, Chillon JM, Lartaud I, Levy B: Vascular calcium overload produced by vitamin D_3 plus nicotine treatment diminishes arterial distensibility in rats. Am J Physiol 1994;266:H540–H547.
15 Niederhoffer N, Lartaud-Idjouadiene I, Giummelly P, Duvivier C, Peslin R, Atkinson J: Calcification of medial elastic fibers and aortic elasticity. Hypertension 1997;29:999–1006.
16 Lartaud-Idjouadiene I, Lompre AM, Kieffer P, Colas T, Atkinson J: Cardiac consequences of prolonged exposure to an isolated increase in aortic stiffness. Hypertension 1999;33:63–69.
17 Niederhoffer N, Marque V, Lartaud-Idjouadiene I, Duvivier C, Peslin R, Atkinson J: Vasodilators, aortic elasticity, and ventricular end-systolic stress in nonanesthetized, unrestrained rats. Hypertension 1997;30:1169–1174.
18 Henrion D, Chillon JM, Godeau G, Muller F, Capdeville-Atkinson C, Hoffman M, Atkinson J: The consequences of aortic calcium overload following vitamin D_3 plus nicotine treatment in young rats. J Hypertens 1991;9:919–926.
19 Niederhoffer N, Bobryshev YV, Lartaud-Idjouadiene I, Giummelly P, Atkinson J: Aortic calcification produced by vitamin D_3 plus nicotine. J Vasc Res 1997;34:386–398.
20 Girerd X, Mourad JJ, Acar C, Heudes D, Chiche S, Bruneval P, Mignot JP, Billaud E, Safar M, Laurent S: Noninvasive measurement of medium-sized artery intima-media thickness in humans: In vitro validation. J Vasc Res 1994;31:114–120.
21 Gussenhoven EJ, Essed CE, Lancee CT, Mastik F, Frietman P, van Egmond FC, Reiber J, Bosch H, van Urk H, Roelandt J, Bom N: Arterial wall characteristics determined by intravascular ultrasound imaging: An in vitro study. J Am Coll Cardiol 1989;14:947–952.
22 Tobis JM, Mallery J, Mahon D, Lehmann K, Zalesky P, Griffith J, Gessert J, Moriuchi M, McRae M, Dwyer ML: Intravascular ultrasound imaging of human coronary arteries in vivo. Analysis of tissue characterization with comparison to in vitro histological specimens. Circulation 1991;83:913–926.