Opposing changes in thoracic and abdominal aortic biomechanical properties in rodent models of vascular calcification and hypertension

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Ameer OZ, Salman IM, Avolio AP, Phillips JK, Butlin M. Opposing changes in thoracic and abdominal aortic biomechanical properties in rodent models of vascular calcification and hypertension. Am J Physiol Heart Circ Physiol 307: H143–H151, 2014. First published May 16, 2014; doi:10.1152/ajpheart.00139.2014.—This study investigated the effects of hypertension on regional aortic biomechanical and structural properties in three rat models of vascular calcification: the hypertensive Lewis polycystic kidney (LPK; n = 13) model of chronic kidney disease, spontaneously hypertensive rats (SHRs; n = 12), and calcification in normotensive Lewis rats induced by vitamin D3 and nicotine (VDN; n = 8). Lewis and Wistar-Kyoto rats were controls. Thoracic and abdominal aortic stiffness parameters were assessed by tensile testing. In models where aortic stiffness differences compared with controls existed in both thoracic and abdominal segments, an additional cohort was quantified by histology for thoracic and abdominal aortic elastin, collagen, and calcification. LPK and VDN animals had higher thoracic breaking strain than control animals (P < 0.01 and P < 0.05, respectively) and lower energy absorption within the tensile curve of the abdominal aorta (P < 0.05). SHRs had a lower abdominal breaking stress than Wistar-Kyoto rats. LPK and VDN rats had more elastic lamellae fractures than control rats (P < 0.001), which were associated with calcium deposition (thoracic R = 0.37, P = 0.048; abdominal: R = 0.40, P = 0.046). LPK rats had higher nuclear density than control rats (P < 0.01), which was also evident in the thoracic but not abdominal aorta of VDN rats (P < 0.01). In LPK and VDN rats, but not in control rats, media thickness and cross-sectional area were at least 1.5-fold greater in thoracic than abdominal regions. The calcification models chronic kidney disease and induced calcification in normotension caused differences in regional aortic stiffness not seen in a genetic form of hypertension. Detrimental abdominal aortic remodeling but lower stiffness in the thoracic aorta with disease indicates possible compensatory mechanisms in the proximal aorta.

aortic stiffness; hypertension; calcification; regional changes; elastin fragmentation

LARGE ARTERY STIFFNESS has predictive value for cardiovascular events, independent of classical cardiovascular risk factors (37, 48), and is associated with cardiovascular and all-cause mortality (13). It is a driver of increased systolic and widened pulse pressures (46) and impacts left ventricular afterload (14, 15). Increased large artery stiffness may also promote target organ damage through excessive transmission of the pulse to peripheral organs such as the kidney and brain (43, 56). While the outcomes of large artery stiffness are widely studied, the pathophysiology is still poorly understood.

The aorta undergoes significant remodeling in association with cardiovascular disease and aging. This includes vessel dilatation and hypertrophy (12, 28), elastin fragmentation, vascular smooth muscle cell migration, proliferation, and cytoskeletal remodeling (18). Vascular calcification of the aorta is also associated with increased artery stiffness (5, 34) and is a feature of age-linked vascular pathologies such as atheroma and arteriosclerosis as well as an accelerated process seen in diabetes and renal disease (38). There are structural and functional differences in the inherent biomechanical properties of the thoracic and abdominal aorta under normal conditions, as demonstrated by others (29, 60). With cardiovascular disease and aging, there is also strong evidence to suggest that remodeling is also not uniform (47) and that the abdominal aorta is relatively stiffer compared with the more proximal regions of the aorta (20, 22). Regional differences in the diseased aorta maybe due to differential changes in elastin and collagen content (53) and the subsequent impact on the mechanical properties (3). An imbalance in the biomechanical signals within these tissues might provoke vascular remodeling and promote cardiovascular pathologies. Identification of regions of the aorta more susceptible to calcification and wall stiffening may provide valuable insights into understanding the mechanisms underlying cardiovascular disease.

The aim of the present study was to investigate the impact of vascular calcification and hypertension on regional (thoracic and abdominal) aortic biomechanical function, the net stiffness gradient, and the vascular wall structure. Regional aortic parameters were measured in three rodent pathologies known to lead to large artery remodeling: a model of chronic kidney disease, the Lewis polycystic kidney (LPK) rat (45), in which hypertension, aortic stiffness, and calcification have previously been demonstrated (40); the vitamin D3/nicotine (VDN)-administered normotensive rodent model of vascular calcification and arterial stiffness (4); and the spontaneously hypertensive rat (SHR), a genetically hypertensive model of vascular remodeling, in which regional differences in aortic stiffness using pulse wave velocity have been shown alongside vascular calcification (25, 39). Control Lewis and Wistar-Kyoto (WKY) rats were also studied. It was hypothesized that the models would show a varying degree of accelerated vascular stiffening between the thoracic and abdominal regions of the aorta.

MATERIALS AND METHODS

Animals. All animals were obtained from the Animal Resource Centre (Murdoch, WA, Australia). Rats were fed with normal rat chow and water ad libitum and housed on 12:12-h light-dark cycle. All experimental protocols and procedure were approved by the institution’s Animal Ethics Committee.

For the hemodynamic and tensile testing analysis, the following six groups of animals were used: mixed sex 12- to 14-wk-old LPK rats (n = 13) and age-matched control strain Lewis rats (n = 14), male Lewis rats administered VDN and studied at 14 wk of age (n = 8) and age-matched control Lewis rats administered with vehicle (n = 9); and mixed sex 17- to 18-wk-old SHRs (n = 12) and age-matched...
control WKY rats (*n* = 12). Not all parameters were tested for each animal.

The LPK model is as a result of a mutation in the never in mitosis A-related kinase 8 (Nek8) gene (33), which is a form of nephronophthisis (nephrocystin protein 9) and has a phenotype presentation that resembles human autosomal recessive polycystic kidney disease (45). VDN calcification was induced as previously described (41). Briefly, Lewis rats received an intramuscular dose of vitamin D3 (300,000 IU/kg cholecalciferol, equivalent to 7.5 mg/kg, by intramuscular injection, Sigma-Aldrich) and nicotine (25 mg/kg by oral gavage, twice, 9 h apart, Sigma-Aldrich) at 8 wk of age. The VDN control group of Lewis rats received saline and drinking water equivalent at twice, 9 h apart, Sigma-Aldrich) and nicotine (25 mg/kg by oral gavage, twice, 9 h apart, Sigma-Aldrich) at 8 wk of age. The VDN control group of Lewis rats received saline and drinking water equivalent at the same age. Animals were anesthetized, and hemodynamic measurements taken 6 wk after VDN administration.

**Hemodynamic measurements.** Basal hemodynamic measurements were determined in urethane-anesthetized animals (1.3 g/kg ip, Sigma-Aldrich, maintained with intravenous urethane as required). Body temperature was kept at 37 ± 0.5°C. A tracheotomy was performed, and the rodent remained spontaneously breathing. The right femoral artery and right jugular vein were cannulated for arterial blood pressure recording and fluid administration, respectively. After a 30-min stabilization period, baseline blood pressure and heart rate were obtained. All data were acquired using a CED1401 system (Spike2, version 7, CED, Cambridge, UK). Average systolic, diastolic, pulse, and mean arterial pressures were measured over a 20-min interval.

**Biomechanical testing.** After baseline hemodynamic parameters had been recorded, animals were euthanized [pentobarbitone sodium (1.3 g/kg ip), perfused via the left ventricle with heparinized 0.9% saline followed by 4% formalin for 24 h, and then placed into 70% ethanol until further processing. The aorta was dissected as above and postfixed for paraffin embedding using an automated tissue processor (Leica ASP2005). Paraffin-embedded sections (5 μm) were obtained using a microtome. Sections of thoracic and abdominal segments were cut at the same relative locations as those used for the tensile testing.

Aortic sections were then stained for elastin (Shikata’s orcein), collagen deposition and smooth muscle cell nuclei (Martius scarlet blue), and calcium deposition (von Kossa) as previously described (40, 49). Images were acquired at ×20 magnification (Zeiss Axiovision, version 4.8.2.0, Carl Zeiss Microimaging, Gottingen, Germany), and histomorphometric analysis was performed using customized automated image-processing software (ImageJ, version 1.47d, National Institutes of Health).

Internal and external diameters, media thickness, and cross-sectional area (CSA) were measured. Wall thickness measurements were averaged across at least eight locations evenly distributed around the ring. Total elastin, collagen, and calcium were quantified by applying a threshold to construct a binary image from the appropriate color (red, blue, and black, respectively) and measuring the dark-to-bright ratio. Nuclei were automatically counted by a built-in software algorithm. Densities of elastin, collagen, calcium, and nuclei were expressed as percentages of the total CSA. The elastin-to-collagen ratio was also calculated. The medial elastin network was characterized by the relative area occupied by elastin lamellae and interlamellae elastin, cleaned of perivascular material and kept in saline at 37°C for tensile testing. Nonbranching segments of ~4 mm length were cut from the thoracic and abdominal aorta. Aortic rings were mounted between two custom-made jigs comprising 26-gauge pins fed through the vessel lumen and tested in uniaxial strain (Instron 5542, Instron Bluehill software, version 2.15). Rings were initially cycled between an equivalent force of 80 and 120 mmHg at a speed of 6 mm/min until steady-state hysteresis was achieved (8–12 cycles) to remove inherent smooth muscle tension (52). The aortic segment was then stretched at a rate of 2 mm/min to failure, and the tensile load was recorded.

The stress-strain curve was quantified as shown in Fig. 1. The incremental elastic modulus (*E*) was calculated for the first (E*low*) and last (E*high*) 10% strain of the stress-strain relationship. These two regions of incremental elastic modulus can be taken as largely dominated by elastin (E*low*) and collagen fibers (E*high*). The difference between the area under the curve (AUC) of the thoracic and abdominal stress-strain relationship of the same animal was calculated (ΔAUC) as a reflection of the stiffness gradient along the length of the aorta.

**Histomorphometry.** Based on the biomechanical tensile testing experimentation results, a separate cohort of LPK (*n* = 9), Lewis control (*n* = 6), and VDN (*n* = 7) rats was tested for histomorphometric evaluation of the thoracic and abdominal segments. Animals were anesthetized [urethane (1.3 g/kg ip)], perfused via the left ventricle with heparinized 0.9% saline followed by 4% formalin for 24 h, and then placed into 70% ethanol until further processing. The aorta was dissected as above and postfixed for paraffin embedding using an automated tissue processor (Leica ASP2005). Paraffin-embedded sections (5 μm) were obtained using a microtome. Sections of thoracic and abdominal segments were cut at the same relative locations as those used for the tensile testing.

Aortic sections were then stained for elastin (Shikata’s orcein), collagen deposition and smooth muscle cell nuclei (Martius scarlet blue), and calcium deposition (von Kossa) as previously described (40, 49). Images were acquired at ×20 magnification (Zeiss Axiovision, version 4.8.2.0, Carl Zeiss Microimaging, Gottingen, Germany), and histomorphometric analysis was performed using customized automated image-processing software (ImageJ, version 1.47d, National Institutes of Health).

**Table 1. Characteristics and baseline hemodynamic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lewis Rats</th>
<th>LPK Rats</th>
<th>Vehicle-Treated Lewis Rats</th>
<th>VDN Rats</th>
<th>WKY Rats</th>
<th>SHRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats/group</td>
<td>8</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>3/5</td>
<td>4/8</td>
<td>9/0</td>
<td>8/0</td>
<td>8/4</td>
<td>8/4</td>
</tr>
<tr>
<td>Age, wk</td>
<td>12 to 14</td>
<td>12 to 14</td>
<td>14</td>
<td>14</td>
<td>17 to 18</td>
<td>17 to 18</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>301 ± 77</td>
<td>209 ± 49</td>
<td>362 ± 14</td>
<td>352 ± 37</td>
<td>296 ± 59</td>
<td>277 ± 71</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>123 ± 8</td>
<td>197 ± 36</td>
<td>115 ± 8</td>
<td>122 ± 10</td>
<td>149 ± 19</td>
<td>171 ± 23*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>49 ± 14</td>
<td>65 ± 20</td>
<td>81 ± 9</td>
<td>89 ± 11</td>
<td>76 ± 20</td>
<td>89 ± 19</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>86 ± 6</td>
<td>111 ± 19</td>
<td>98 ± 8</td>
<td>105 ± 10</td>
<td>100 ± 17</td>
<td>116 ± 14</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>74 ± 13</td>
<td>132 ± 34</td>
<td>34 ± 2</td>
<td>33 ± 2</td>
<td>73 ± 23</td>
<td>82 ± 31</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>353 ± 55</td>
<td>360 ± 51</td>
<td>370 ± 35</td>
<td>370 ± 33</td>
<td>332 ± 32</td>
<td>340 ± 35</td>
</tr>
</tbody>
</table>

Values are means ± SD. For the hemodynamic and tensile testing analysis, the following six groups of animals were used: Lewis and control Lewis polycystic kidney (LPK) rats, Lewis rats treated with vehicle and vitamin D3 and nicotine (VDN), and Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHRs).

*P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001 compared with the respective control group, as evaluated by a two-tailed unpaired *t*-test.

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parameters were determined using two-tailed paired differences (thoracic compared with abdominal aortic samples) in t-tests (Bonferroni correction) to identify strain differences. Regional ANOVA considering both strain and sex with post hoc unpaired otherwise stated. Between strain differences were identified using Automated software.

Values are means ± SD. The incremental elastic modulus (E) was calculated for the first (E_low) and last (E_high) 10% strain of the stress-strain relationship. AUC, area under the stress-strain curve. *P < 0.05 and **P < 0.01 compared with Lewis rats for the corresponding aortic segment. †P < 0.05, ‡P < 0.01, and ††P < 0.001 compared with thoracic sections within groups.

The mean thickness of each elastin lamella, and the mean interlamellae distance. Elastin lamellae fracture points were quantified by manual counting of lamellae elastin discontinuation around the aortic section, which was normalized to the number of elastin lamellae and the averaged inner and outer circumferences to control for the increase in lamella population. Quality control in the application of a threshold to obtain a binary image was made by visual inspection and comparison with the original image to evaluate the integrity of the customized automated software.

Statistical analysis. All data are expressed as means ± SE unless otherwise stated. Between strain differences were identified using ANOVA considering both strain and sex with post hoc unpaired t-tests (Bonferroni correction) to identify strain differences. Regional differences (thoracic compared with abdominal aortic samples) in parameters were determined using two-tailed paired t-tests.

Tensile testing stress/strain data points were fitted to a third-order polynomial (cubic) function, and a 25-point construct of that curve was normalized to the number of elastic lamellae breaks using Pearson correlation analysis followed by linear regression. All statistical analysis was performed using GraphPad Prism (version 6, GraphPad Software, La Jolla, CA).

RESULTS

Hemodynamic measurements. LPK and SHR animals had significantly higher systolic blood pressure compared with their respective control animals, with LPK animals also having

![Fig. 2. Aortic stress-strain curves obtained through tensile testing. A–C: thoracic aortic rings. D–F: abdominal aortic rings. A and D: Lewis and Lewis polycystic kidney (LPK) rats. B and E: Lewis rats administered vehicle and vitamin D3 and nicotine (VDN). C and F: Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). A significant rightward shift of the stress-strain curves of LPK and VDN thoracic aortas were observed relative to their control rats. A significant leftward shift was observed in the LPK abdominal aorta relative to control Lewis rats. No significant differences were observed between the thoracic and abdominal aorta of SHRs compared with control WKY rats. ***P < 0.001.](https://ajpheart.physiology.org/)
a higher mean arterial pressure (Table 1). Diastolic blood pressure and heart rate, however, were not significantly different from control animals. There were no differences in measured baseline hemodynamic measures in VDN rats compared with control rats (Table 1).

**Biomechanical testing.** The third-order polynomial provided a good fit to the stress-strain data, with all curves in individual animals demonstrating $R^2 \geq 0.995$.

LPK rats had a higher breaking strain and lower $E_{\text{low}}$ than their control rats in thoracic segments (Table 2 and Fig. 2A). Conversely, the abdominal segments showed lower breaking strain and higher $E_{\text{low}}$ than control rats (Table 2 and Fig. 2D). VDN rats also showed a higher breaking strain than control rats in the thoracic aorta but did not differ in $E_{\text{low}}$ (Table 2 and Fig. 2B). The abdominal AUC, a measure of absorbed energy, was lower for LPK and VDN rats compared with their respective control rats (Table 2 and Fig. 2). There were no significant differences in any tensile testing parameter for SHRs and WKY rats (Table 2 and Fig. 2, C and F) other than the abdominal aortic breaking stress being greater in SHRs ($P < 0.05$).

In all groups, including the control groups, there was a significant rightward shift of the abdominal stress-strain relationship compared with the thoracic segment (Fig. 3, A–F), which, irrespective of the magnitude, represents the biomechanical tensile pattern of the stiffer abdominal aorta relative to the more compliant thoracic segment. The net energy gradient ($\Delta\text{AUC}$), a representative of the overall energy absorbed through the thoracic to abdominal aorta, was significantly different from control rats for both LPK and VDN rats (Fig. 3, G and H, respectively) but not SHRs (Fig. 3I).

**Histomorphometry.** Vascular remodeling and hypertrophy were evident in thoracic and abdominal aortic sections of LPK and VDN rats (Fig. 4 and Table 3). The LPK thoracic aorta exhibited a marked increase in wall thickness, CSA, wall-to-lumen ratio, numbers of elastic lamellae, collagen density, nuclear density, nucleus CSA, and calcium density relative to Lewis rats. Total elastin density and nuclei per micrometer squared were significantly lower in LPK compared with Lewis rats. The thoracic elastin-to-collagen ratio was greater in Lewis compared with LPK rats; however, this was due to a sex effect, with female Lewis rats having a higher thoracic elastin-to-collagen ratio of $5.1 \pm 0.8$. Qualitatively, the structure of the elastin lamellae of LPK rats was considerably disorganized and had detectable elastin lamellae fractures (Fig. 4). Calcium deposits appeared as relatively small patches of medial calci-
Fig. 4. Representative histological thoracic and abdominal aortic sections in Lewis (A,i–F,i), LPK (A,ii–F,ii), and VDN (A,iii–F,iii) rats stained for elastin (red, Shikata’s orcein), collagen (blue, Martius scarlet blue), and nuclei (dark magenta, Von Kossa) and calcium (black, Von Kossa). A thickened media, marked reduction in lamellae and interlamellae elastin densities, and increased collagen density were observed in LPK rats. The black arrows in A,ii, D,ii, A,iii, and D,iii show examples of elastic lamellae fracture points in LPK and VDN rats. Significant calcification areas were identified in LPK and VDN rats (C,ii, F,ii, C,iii, and F,iii). Quantitative analysis of the images is shown in Table 3.
Table 3. Histomorphometric evaluation of the thoracic and abdominal aortic sections in Lewis, LPK, and VDN rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lewis Rats</th>
<th>LPK Rats</th>
<th>VDN Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thoracic sections</td>
<td>Abdominal sections</td>
<td>Thoracic sections</td>
</tr>
<tr>
<td>Number of sections/group</td>
<td>6</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Media thickness, μm</td>
<td>76 ± 7</td>
<td>61 ± 4</td>
<td>156 ± 13e</td>
</tr>
<tr>
<td>Medial CSA, mm²</td>
<td>0.38 ± 0.07</td>
<td>0.25 ± 0.04</td>
<td>0.66 ± 0.04b</td>
</tr>
<tr>
<td>External diameter, μm</td>
<td>1606 ± 93</td>
<td>1080 ± 91d</td>
<td>1914 ± 157</td>
</tr>
<tr>
<td>Internal diameter, μm</td>
<td>1509 ± 66</td>
<td>986 ± 176e</td>
<td>1485 ± 56</td>
</tr>
<tr>
<td>Wall-to-lumen width ratio</td>
<td>0.104 ± 0.005</td>
<td>0.114 ± 0.008</td>
<td>0.196 ± 0.007c</td>
</tr>
<tr>
<td>Number of lamellae</td>
<td>7.8 ± 0.3</td>
<td>6.5 ± 0.2e</td>
<td>9.1 ± 0.2c</td>
</tr>
<tr>
<td>Total elastin density, %</td>
<td>72 ± 2</td>
<td>64 ± 2</td>
<td>47 ± 2c</td>
</tr>
<tr>
<td>Lamellae elastin density, %</td>
<td>35 ± 3</td>
<td>32 ± 1</td>
<td>23 ± 1c</td>
</tr>
<tr>
<td>Interlamellae elastin density, %</td>
<td>38 ± 4</td>
<td>34 ± 4</td>
<td>23 ± 4d</td>
</tr>
<tr>
<td>Lamellae-to-interlamellae ratio</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Elastin lamellae, μm</td>
<td>4.1 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Elastin lamellae spacing, μm</td>
<td>7.3 ± 0.3</td>
<td>6.8 ± 0.2</td>
<td>12.3 ± 5f</td>
</tr>
<tr>
<td>Lamellae fractures</td>
<td>0.03 ± 0.01</td>
<td>0.08 ± 0.01e</td>
<td>0.16 ± 0.01c</td>
</tr>
<tr>
<td>Collagen density, %</td>
<td>21 ± 3</td>
<td>27 ± 5</td>
<td>42 ± 6b</td>
</tr>
<tr>
<td>Elastin-to-collagen ratio</td>
<td>3.6 ± 0.6</td>
<td>2.9 ± 0.6</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Nuclear density, %</td>
<td>2.8 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>3.6 ± 0.2b</td>
</tr>
<tr>
<td>Nucleus CSA, μm²</td>
<td>11.9 ± 0.4</td>
<td>11.6 ± 0.3</td>
<td>15.7 ± 0.4c</td>
</tr>
<tr>
<td>Number of nuclei/μm²</td>
<td>0.084 ± 0.003</td>
<td>0.086 ± 0.002</td>
<td>0.064 ± 0.002c</td>
</tr>
<tr>
<td>Calcium density, %</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.6 ± 0.4c</td>
</tr>
<tr>
<td>Calcium deposit average, μm²</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>2.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. CSA, cross-sectional area. The difference in the thoracic elastin-to-collagen ratio between LPK and Lewis rats was due to a sex effect (female Lewis rats: 5.1 ± 0.8). *P < 0.05, **P < 0.01, and ***P < 0.001 compared with Lewis rat for the corresponding aortic segment. aP < 0.05, bP < 0.01, and cP < 0.001 compared with thoracic sections within groups.

Calcification heterogeneously distributed in the tunica media (Fig. 4). The abdominal aorta of LPK rats showed the same differences to Lewis rats, with the exception that the number of elastin lamellae and collagen density were not significantly different (Fig. 4 and Table 3). LPK abdominal aorta media thickness, CSA, and elastin lamellae thickness were markedly smaller than that of the thoracic aorta; however, this was not seen in Lewis rats. Both Lewis and LPK rats had an increasing number of elastin breaks in the abdominal aorta and a reduced number of elastin lamellae (Table 3).

The VDN thoracic aorta demonstrated increased CSA, numbers of lamellae, and nuclear density compared with control Lewis rats (Table 2). A trend toward thinner lamellae (P = 0.06) and markedly more lamellae fractures were observed in VDN rats (Fig. 4 and Table 2). Thoracic elastin and collagen did not differ from control rats; however, the elastin-to-collagen ratio was lower in the thoracic VDN aorta (Table 2). A trend toward increased calcium density in the VDN thoracic aorta compared with control Lewis rats was observed (P = 0.22; Table 3). The VDN abdominal aorta showed increased elastin lamellae fractures and higher tissue calcium density (Table 3). In VDN rats, this appeared as heterogeneous patches of medial calcification of the abdominal aorta that were significantly larger than deposition in control Lewis rats (Fig. 4). A positive correlation between number of elastin lamellae breaks and calcium density (in %) was observed in both thoracic and abdominal aortic sections of all groups combined (Fig. 5).

In all three groups (Lewis, LPK, and VDN), the abdominal aorta had a smaller internal and external diameter compared with the thoracic aorta. In LPK and VDN rats, medial CSA was less in the abdominal aorta compared with the thoracic aorta, and, in LPK rats, abdominal media thickness was also less. In all three groups, the abdominal aorta had a higher number of lamellae fractures compared with the thoracic aorta, and the LPK abdominal aorta had smaller elastic lamellae compared with thoracic segments. In VDN rats, nuclear density was less in the abdominal aorta compared with the thoracic aorta.

DISCUSSION

This tensile and histomorphological analysis has shown regional differences between the thoracic and abdominal segments of the aorta. However, these differences were not the same across all rodent models of vascular remodeling.

The hemodynamic measurements confirmed findings of previous studies that both LPK rats (45) and SHRs (32) were hypertensive, whereas VDN treatment induced vascular calcification in the absence of blood pressure changes (42).

The tensile tests that were undertaken in this study reflect the passive biomechanical properties of the aorta, primarily collagen and elastin (6, 7, 55, 59). The values of $E_{low}$ and $E_{high}$ are predominantly representative of the elastin and collagen tensile strength, respectively. There were increases in abdominal aortic tensile testing parameters relative to control rats for LPK rats, VDN rats, and SHRs. This corresponds with reported in vivo measures of increased stiffness and pulse wave velocity (PWV) previously reported in LPK (40) and VDN (55) models as well as SHRs (39). Previous studies in young or young adult SHRs have shown no changes in tensile parameters, although changes were subsequently seen in old SHRs (8, 32). It may be that there are critical changes between 18 and 24 wk of age in SHRs that may correspond to changes in aortic stiffness seen in both in vivo and ex vivo testing. Alternatively, established increases in the sympathetic drive (24) and sympathetic innervation density (21) or decreased aortic endothelial function (27) of SHRs compared with normotensive control rats and its impact on aortic smooth muscle tone may result in an increase in the functional but not structural stiffness of the SHR aorta.
Within the present study, no in vivo assessment of arterial stiffness was made. Whether or not changes in abdominal aortic stiffness impacts on whole aortic PWV, an independent predictor of cardiovascular disease in humans, is an area that warrants further investigation.

The findings of lower thoracic aorta stiffness ($E_{low}$) in LPK compared with control rats and greater breaking strain in both LPK and VDN rats compared with control rats are against the hypothesis that calcification causes increased stiffness. The changes in the thoracic aorta may reflect a compensatory change in the face of accelerated vascular stiffening of the abdominal aorta. Arterial regional differences in response to a cardiovascular challenge are not without precedent. Boutouyrie et al. (10) showed that in vivo strain in the radial artery does not change in a normotensive population but increases with age in hypertensive patients. The carotid artery shows a different response, with in vivo strain decreasing with age in both normotensive and hypertensive subjects (10).

Aortic remodeling and vascular hypertrophy were evident in LPK and VDN rats. The LPK thoracic and abdominal aorta showed parallel vascular changes, including increased media thickness, media CSA, and nuclear density and a decreased elastin-to-collagen ratio. In VDN rats, the thoracic aorta demonstrated a greater degree of remodeling than the abdominal regions compared with control rats. The thoracic aorta displayed greater media CSA, higher nuclear density, and a lower elastin-to-collagen ratio. The elastic lamellae population was upregulated in the thoracic but not abdominal aorta of both LPK and VDN rats, supporting the proposition of a compensatory adaptation of the thoracic aortic region, which could have contributed to the lesser stiffness at low strain.

Increased calcium deposition was evident in both the thoracic and abdominal aorta of LPK rats and the abdominal aorta of VDN rats. Calcinification in the thoracic aorta of VDN rats, while three times that of control rats, was not significantly greater ($P = 0.22$). Additionally, the size of the deposits appeared much larger in the VDN model. Calcification appeared to be mostly confined to the media of the arterial wall, thus displaying Mönckeberg’s type of calcification (57). This form of calcification occurs preferentially on the internal elastic lamina and is likely involved in the destruction and fragmentation of the elastic network of the vessel. Gross disruption of the elastic fibers could facilitate the calcification process (16). Calcium deposition can lead to proteolysis of elastic fibers/elastocalcinosis and hence loss of arterial compliance (57). Both LPK and VDN rats demonstrated markedly greater numbers of fractures in the elastic lamellae of the thoracic and abdominal aorta compared with control rats. The increase in aorta lamellae elastin breaks correlated significantly with altered calcium deposition in the aortic wall, consistent with the notion that these breaks could serve to facilitate calcium deposition or, in turn, be a consequence of calcification.

While there is still discussion on the precise mechanism of vascular calcification in chronic kidney disease (38), recent studies have implied a role of elastin degradation to the stiffening of the aorta (51), with calcification being promoted through disruption of elastin integrity (2). These mechanisms may differ when considering calcification of focalized plaque-centered atherosclerotic calcification (1, 2) compared with the disperse vascular arteriosclerotic calcification seen in the models used in this study, which typify the calcification seen in chronic kidney disease (31) and aging with the associated increase in aortic stiffness (34).

Specifically, two types of medial calcification have been described (36): 1) a slowly progressive form with thin medial calcification and little or no luminal narrowing, coherent with that observed in VDN rats, and 2) a malignant, progressive form in which large medial calcification displaces the internal elastic lamina toward the lumen, even penetrating into the intima and causes luminal narrowing. The LPK aortic calcification showed features midway between the two forms, as evidenced by patchy calcification of the media with a marked displacement of the elastic lamellae but no penetration of the intima, as seen with arteriosclerosis (57).

Although calcification was not quantified in SHRs in this study, it has previously been shown that aortic calcification increases with age in SHRs (26). Calcium deposition per mass of aortic tissue does not differ from normotensive control animals at ages of 3, 9, and 15 mo (32), although greater calcium content at 6 mo of age has been previously shown (39).

Cardiac parameters were not measured in this study; however, they have been characterized for these animal models elsewhere. VDN rats display increased left ventricular end-diastolic pressure, decreased pressure-volume area, decreased stroke volume, and left ventricular hypertrophy compared with control rats (23, 41). Both SHRs (44) and LPK rats (45) also showed cardiac hypertrophy compared with control rats. Cardiac hypertrophy is an important factor that is associated with aortic vascular remodeling (17), and future longitudinal analysis.

Fig. 5. Relationship between elastin breaks and calcium density in the thoracic (A) and abdominal (B) aorta of Lewis, LPK, and VDN rats. The plots show a positive correlation in both aortic segments.
of ventricular-vascular coupling may provide further information on the source of the differences in aortic remodeling shown in the present study between VDN rats, LPK rats, and SHRs.

Other potential mechanisms may underlie our findings. For example, advanced glycation end products (AGEs) are known to cross-link proteins, such as vascular collagen, and are a possible cause of increased vascular stiffness, being associated with aortic stiffness in hypertensive humans (35), vascular wall changes with aging in rats (11), and calcification (58). This warrants future studies investigating regional differences in the concentration or activity of AGEs within the aorta to determine if they are a contributing factor to the stiffness parameters described in this study within the thoracic and abdominal aorta within the different models. If shown to be differentially elevated, they may present novel therapeutic targets for improving aortic structure and function.

The adventitia can also contribute to the mechanical strength of arteries, although more so at applied loads above physiological pressures (50). For example, it contributes to the \( E_{\text{high}} \) but not \( E_{\text{low}} \) parameters in WKY and SHR strains (19). Our study focused on medial characteristics; however, physical separation and tensile testing of the adventitia from the media of the aortic ring, such as that conducted by Han et al. (19), would quantify whether mechanical differences at high stress are due to adventitial or medial changes. The adventitia also plays a signaling role causing medial smooth muscle hypertrophy (30) and can regulate vascular inflammation (54), which can ultimately affect arterial stiffness. The adventitia, therefore, is an additional mechanistic factor to be considered in the heterogeneity of aortic biomechanics.

This study showed that the hypertensive LPK and normo-tensive VDN aorta exhibited a regional stiffening profile that skewed away from the natural stiffening pattern observed under the control conditions, whereas the genetic model of hypertension did not. These findings suggest that vascular calcification as a mechanism triggering vascular wall changes is independent of the set point of the resting blood pressure. Other factors are likely to be influential, given that the two models gave two different aortic stiffness profiles. The SHR aorta, on the other hand, is mechanically adapted despite the high level of wall stress, with evidence of a thickened wall (8) but, in agreement with the findings in this study, no difference in vessel stiffness (9).

This study highlights the regional specificity of vascular changes in the large arteries. Further studies are required to investigate whether these regional changes have positive or negative impacts on cardiovascular function because while stiffening of the abdominal region may have a negative impact on blood pressure and ventricular load, it may be that positive remodeling of the thoracic aorta offers a protective effect. This study suggests that current clinical tests, such as carotid-femoral PWV measurement, should be assessed to determine whether they are able to adequately detect the regional changes in large artery stiffness.

Aortic stiffness is an important determinant of the overall cardiovascular health status, hence necessitating the need to understand how aortic function is altered during disease states. This study provides insights into the importance of regional aortic function and structure and indicates that different pathophysiological insults to the large arteries alter regional aortic function in different ways. These findings show that the aorta cannot be viewed as a uniform structure, and studies and measurements of the aorta should be made across several regions to detect differences across the aortic profile.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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