Aortic Wall Mechanics and Composition in a Transgenic Mouse Model of Marfan Syndrome

Valérie Marque, Pascal Kieffer, Barbara Gayraud, Isabelle Lartaud-Idjouadiene, Francesco Ramirez, Jeffrey Atkinson

Abstract—In Marfan syndrome, mutations of the fibrillin gene (FBN1) lead to aneurysm of the thoracic aorta, making the aortic wall more susceptible to dissection, but the precise sequence of events underlying aneurysm formation is unknown. We used a rodent model of Marfan syndrome, the mgR/mgR mouse (with mgR: hypomorphic FBN1 mutation), which underexpresses FBN1, to distinguish between a defect in the early formation of elastic fibers and the later disruption of elastic fibers. The content of desmosine plus isodesmosine was used as an index of early elastogenesis; disruption of elastic fibers was analyzed by histomorphometry. Because disruption of the medial elastic fibers may produce aortic stiffening, so amplifying the aneurysmal process, we measured thoracoabdominal pulse wave velocity as an indicator of aortic wall stiffness. Both mgR/mgR and wild-type (C57BL/6J–129SV) strains were normotensive, and wall stress was not significantly modified because the increase in internal diameter (0.80 ± 0.06 vs 0.63 ± 0.03 mm in wild type, \( P < 0.05 \)) was accompanied by increased medial cross-sectional area. The aortic wall stiffened (4-fold increase in the elastic modulus–to–wall stress ratio). Desmosine content was not modified (mgR/mgR 432 ± 31 vs wild type 492 ± 42 \( \mu g/\text{mg wet weight}, P > 0.05 \)). Elastic fibers showed severe fragmentation: the percentage of the media occupied by elastic fibers was 18 ± 3% in mgR/mgR mice vs 30 ± 1% in wild-type mice, with the number of elastic segments being 1.9 ± 0.2 vs 1.4 ± 0.1 \( \times 10^{-5}/\text{mm}^2 \) in the wild type (both \( P < 0.05 \)). In conclusion, underexpression of FBN1 in mice leads to severe elastic network fragmentation but no change in cross-linking, together with aortic dilatation. This result suggests that fragmentation of the medial elastic network and not a defect in early elastogenesis is 1 of the determinants of aortic dilatation in Marfan syndrome. (Arterioscler Thromb Vasc Biol. 2001;21:1184-1189.)

Key Words: fibrillin-1 ■ elastic modulus ■ aneurysm ■ desmosines ■ elastic fibers

Marfan syndrome is a dominantly inherited disorder characterized by cardiovascular, skeletal, and ocular abnormalities. The cardiovascular manifestations include aortic root dilatation and dissection that result in rupture of the vessel wall and premature death. Marfan syndrome has been associated with mutations of the gene coding for fibrillin-1 (FBN1), the major constituent of extracellular microfibrils. However, the link between FBN1 deficiency and the development of aneurysm is not yet clearly established.

Early ideas were based on the regulatory role played by microfibrils in the organized deposition of tropoelastin molecules during elastogenesis. It was suggested that FBN1 mutations prevented normal cross-linking (formation of desmosine cross-bridges), leading to disorganized microfibrillar assembly, and that this weakened the mechanical strength of the media. This hypothesis was recently challenged by homologous gene-targeting experiments in the mouse, which indicated that FBN1 microfibrils were predominantly engaged in global tissue homeostasis rather than in elastic matrix assembly. Studies performed on mgR/mgR mice, which are characterized by a hypomorphic mutation of FBN1, suggested that aortic dilatation was due to the failure by the microfibrillar array of the adventitia to sustain wall integrity in the face of hemodynamic stress. The resulting increase in wall stress is associated with localized calcium deposition, macrophage infiltration, and metalloproteinase release in the media, leading to fragmentation of the medial elastic network and aortic dilatation.

The present study is an attempt to distinguish between these 2 mechanisms: a defect in early elastogenesis or later fragmentation of elastic fibers, in mgR/mgR mice underexpressing FBN1. The content of desmosine plus isodesmosine, cross-linking amino acids specific to elastin, was used as an index of early elastogenesis, and fragmentation of elastic fibers was analyzed by histomorphometry.

It has also been suggested that pulse pressure is a major determinant of dilatation in patients with Marfan syndrome. Were fragmentation of the medial elastic network to occur in this Marfan model, then the aortic wall would stiffen, as...
previously reported in studies evaluating the impact of elastases\(^7\) or elastocalcinosis.\(^8\) A loss of aortic wall elasticity would lead to increased cyclic stress and pulse pressure, thereby amplifying aortic dilatation.

In the present work, we simultaneously evaluated changes in aortic mechanics and geometry. Elasticity of the aortic wall was estimated from the thoracoabdominal pulse wave velocity at baseline (a pressure-dependent index of wall elasticity) and at steady-state levels of mean arterial pressure after controlled hypotension (the slope of the pulse wave velocity–pressure curve being used as a pressure-independent index of elasticity). Baseline elastic modulus–to–wall stress ratio was used as a pressure- and geometry-independent index of elasticity.

### Methods

#### Animals

Three- to 5-month-old homozygous recombinant mice (mgR/mgR, 3 males and 4 females; Brookdale Center for Development and Molecular Biology, Mount Sinai School of Medicine, New York, NY) and wild-type mice (C57BL/6J–129SV, 5 males and 3 females) were used. Mice were kept under standard conditions and given a standard rodent diet and water ad libitum.

#### Aortic Pulse Wave Velocity in Nonanesthetized Mice

Polyethylene cannulas (intravascular portion 0.61 mm/0.32 mm, outer diameter/inner diameter; extravascular portion 0.96 mm/0.38 mm) were chronically implanted in the mice under pentobarbital anesthesia (80 mg/kg IP) into the descending aorta (1 mm below the carotid ostium), the abdominal aorta (3 mm above the iliac bifurcation), and the abdominal vena cava (3 mm above the iliac bifurcation). Twenty-four hours later, the aortic cannulas of the nonanesthetized, freely moving mice were filled with heparinized (5 U/mL), gas-free, 0.15 mol/L NaCl and connected to low-volume pressure transducers (Baxter, Bentley Laboratories Europe) with 15 cm of polyethylene cannula.

Signal analysis has been described in detail previously.\(^7\)\(^9\) After a 30-minute habituation period, baseline parameters were determined beat-to-beat and averaged over periods of 4 seconds every 30 seconds for 30 minutes, at a sampling rate of 256 Hz. An algorithm detected the maximal and minimal values of each pressure signal and calculated central mean aortic blood pressure (CMABP; mm Hg) from the waveform area, pulse pressure as the diastolic-systolic difference, and heart rate (beats per minute) by counting the number of cycles over the 4-second period.

Pulse wave velocity (cm/s) was calculated as the distance between the 2 cannula tips (measured in situ after postmortem fixation) by sticking a damp cotton thread onto the aorta: 4.6 ± 0.2 cm in mgR/mgR and 4.3 ± 0.2 cm in wild-type mice; \(P = 0.3180\) divided by transit time. Transit times (ms) were measured online for each 4-second period (38 heart beats, 3 to 4 respiratory cycles) by an algorithm that systematically shifted in time the peripheral pressure waveform with respect to the central pressure waveform and then determined the value of the time shift giving the highest correlation.\(^7\) This approach is based on a least-squares analysis of the differences in amplitudes of the central and peripheral pressure signals at a given point in time; the analysis was repeated following increments in the peripheral sampling points and creating intermediate points by linear interpolation. The calculated resolution of the transit time measurement was ±0.039 ms, ie, ±7% error for a wave traveling at 835 cm/s in mgR/mgR mice and ±4% error for a wave traveling at 440 cm/s in wild-type mice. The ratio of pulse wave velocity to the CMABP was used as a pressure-independent index of elasticity.

#### Pulse Wave Velocity–Pressure Curves During Pharmacological Hypotension in Nonanesthetized Mice

After baseline hemodynamic measurements were made, CMABP was reduced in a stepwise fashion (10 mm Hg per step) to half its initial value by progressively increasing the infusion rate of a sodium nitroprusside solution (2.3 mmol/L in 10 mmol/L phosphate buffer, pH 7.4 at 25°C; Sigma Chemical Co).\(^9\) At each stabilized step, 15 measurements of aortic blood pressure and transit time were performed and averaged. By the end of infusion, animals had received a volume <8% of their total blood volume; the mean dose of sodium nitroprusside in mgR/mgR and wild-type mice was 979 ± 167 and 442 ± 117 mmol · kg\(^{-1}\) · min\(^{-1}\), respectively (\(P\) for the group, 0.0223 by 1-way ANOVA). For each mouse, pulse wave velocity (\(y\)) was expressed as a function of CMABP (\(x\)) by using an exponential model: \(y = b - ae^{-x}\). Slopes (\(a\)) and intercepts (\(b\)) of the pulse wave velocity–pressure curves were treated as independent, parametric variables and averaged. Slopes were used as a pressure-independent index of elasticity.

#### Descending Thoracic Aorta Geometry, Wall Stress, Elastic Modulus, and Elastic Fiber Network Fragmentation

At the end of the hypotension protocol, the sodium nitroprusside infusion was stopped; the mice were humanely killed with a sodium pentobarbital overdose and perfused for 15 minutes at their baseline CMABP with 10% formal containing phosphate-buffered saline. A 0.5-cm-long sample of the proximal descending thoracic aorta (downstream from the aortic arch) was excised, immersed in 10% formal, dehydrated in graded ethanol solutions, and embedded in paraffin. Sections (10 \(\mu\)m thick) stained with Weigert’s solution were used for measurement of internal diameter and medial thickness and for determination of the degree of elastic network fragmentation (see below). Morphometric analysis was performed using the Saisam® algorithm (Microvision Instruments); each section was examined twice in a blinded fashion. Medial cross-sectional area (mm\(^2\)) was calculated as \(\pi(D_i^2 - D_h^2)\), where \(D_i\) and \(D_h\) are the outer and inner diameters (mm), with \(D_i\) delimited by the internal elastic lamina, such that \(D_i = D_h + 2h\), where \(h\) is media thickness defined as the distance between internal and external elastic laminae (mm).

Elastic modulus and circumferential wall stress (10\(^6\) dyn/cm\(^2\)) were calculated from the Moens-Korteweg or Lamé equations, which correct pulse wave velocity (PWV; elastic modulus) or CMABP (wall stress) by internal diameter (\(D_i\)) and medial thickness (\(h\)): elastic modulus = (\(PWV^2\)) · (\(D_i\) · \(p\)) and wall stress = (CMABP · \(D_i\))/2h, where PWV was measured in nonanesthetized mice (cm/s), \(D_i\) = internal diameter (as above); \(cm\), \(h\) = medial thickness (cm), \(p\) = blood density (1.05 g/mL), and CMABP = (dyn/cm\(^2\)).

The elastic modulus–to–wall stress ratio was used as an index of wall elasticity, which is independent of intravascular pressure and aortic morphology. Elastic network fragmentation was evaluated by measuring the number of medial elastic segments per square millimeter and the percentage of medial surface occupied by elastic fibers (excluding the external and internal laminae).

#### Calcium, Elastin Cross-Linking, and Collagen Contents of the Aorta

A second 1-cm-long sample of the thoracic aorta was excised and the wall calcium content determined by atomic absorption spectrophotometry (AA10, Varian Ltd) after mineralization and HNO\(_3\) digestion.\(^10\) The remaining abdominal end of the aorta was removed, weighed, and hydrolyzed in HCl (6 mol/L, 24 hours at 105°C). Protein content (mg/g wet weight) was determined by the dinitrofluorobenzene reaction by using a value of 92 for the molecular weight of an amino acid.\(^11\) Collagen content (mg/g wet weight) was determined by the chloramine T and paranitrofenylbenzaldehyde reaction as hydroxyproline content multiplied by 7.46.\(^11\) Desmosine and isodesmosine contents were determined by capillary zone electrophoresis.\(^12\)

#### Statistics

Values are given as mean ± SEM. Because 2-way ANOVA (sex, age) gave probability levels >0.05 for age, sex, and age×sex, we pooled the results from different age groups and both sexes. Differences between groups were evaluated by 1-way ANOVA plus the Bonfer-
TABLE 1. Body Weight, Baseline Heart Rate, Central Aortic Blood Pressures, and Pulse Wave Velocity in Nonanesthetized mgR/mgR and Wild-Type Mice

<table>
<thead>
<tr>
<th></th>
<th>mgR/mgR</th>
<th>Wild Type</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26±1</td>
<td>27±1</td>
<td>0.4624</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>588±13</td>
<td>565±30</td>
<td>0.5095</td>
</tr>
<tr>
<td>Central aortic blood pressures, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>107±5</td>
<td>107±3</td>
<td>0.9280</td>
</tr>
<tr>
<td>Systolic</td>
<td>131±5</td>
<td>124±3</td>
<td>0.2399</td>
</tr>
<tr>
<td>Diastolic</td>
<td>88±4</td>
<td>91±4</td>
<td>0.5936</td>
</tr>
<tr>
<td>Pulse</td>
<td>43±3</td>
<td>34±3</td>
<td>0.0459</td>
</tr>
<tr>
<td>Pulse wave velocity, cm/s</td>
<td>835±148</td>
<td>440±32</td>
<td>0.0110</td>
</tr>
<tr>
<td>Pulse wave velocity/central mean aortic blood pressure, cm/s · mm Hg⁻¹</td>
<td>8.1±1.7</td>
<td>4.1±0.3</td>
<td>0.0239</td>
</tr>
</tbody>
</table>

Ronin test. A value of P<0.05 was chosen as being indicative of statistical significance.

Results

Body Weight, Heart Rate, CMABP, and Pulse Wave Velocity

Body weight and baseline heart rate were similar in mgR/mgR and wild-type mice (Table 1). Pulse pressure was higher in mgR/mgR mice (26%, P<0.05), as were baseline pulse wave velocity (+90%, P<0.05) and pulse wave velocity/CMABP (+98%, P<0.05; Table 1). During pharmacological hypotension, the slope relating pulse wave velocity to CMABP was doubled in mgR/mgR mice (Figure 1; also see Table 2).

Descending Thoracic Aorta Geometry, Wall Stress, Elastic Modulus, Elastic Network Fragmentation, and Wall Composition

Internal diameter (27%, P<0.05) and medial cross-sectional area (36%, P<0.05) were greater in mgR/mgR mice (outward hypertrophic remodeling). Wall stress was not significantly different in mgR/mgR mice, but there were substantial increases in elastic modulus (4-fold, P<0.05) and the elastic modulus–to–wall stress ratio (4-fold, P<0.05) (Table 3). The elastic network was highly fragmented in mgR/mgR mice (Figure 2), as revealed by a substantial increase in the number of medial elastic segments (+75%) and a decrease in the percentage of medial surface occupied by elastic fibers (−40%; Table 3).

There were significant, negative linear relations between dilatation (ie, internal diameter, a dependent variable) and the percentage of medial surface occupied by elastic fibers (an independent variable; slope = −2.7±0.2 mm, r² = 0.982, P = 0.0010; intercept = 1.3±0.1 mm, P = 0.0001) as well as between wall stiffness (ie, elastic modulus, a dependent variable) and the percentage of medial surface occupied by elastic fibers (an independent variable; slope = −161±33 10⁶ dyn/cm², r² = 0.887, P = 0.0167; intercept = 49±6 10⁶ dyn/cm², P = 0.0047) in mgR/mgR but not in wild-type mice. Desmosine content was similar in mgR/mgR and wild-type mice (Table 3), as were protein, collagen, and calcium contents. There was no significant correlation between aortic dilatation and wall desmosine content (P>0.05; results not shown).

Discussion

Our results show that in a mouse model of Marfan syndrome, which underexpresses FBN1, the desmosine content of the aortic wall does not change, but the elastic network is extremely fragmented, suggesting that FBN1 mutations do not modify the normal cross-linking involved in early elastogenesis but instead lead to later elastic network fragmentation. Concomitantly, the aorta dilates, the wall stiffens (as shown by an increase in the elastic modulus–to–wall stress ratio, the baseline pulse wave velocity–to–CMABP ratio, and the pulse wave velocity–to–CMABP slope obtained during pharmacological hypotension), and pulse pressure increases.

Defects in Early Elastogenesis or Later Fragmentation of the Medial Elastic Network?

Early hypotheses explaining why an FBN1 deficiency leads to aneurysm in Marfan patients were based on the regulatory role played by microfibrils in the organized deposition of tropoelastin molecules during elastogenesis. It was suggested that FBN1 mutations prevented the normal formation of desmosine cross-bridges and that this weakened the mechanical strength of the media. Elastin expression and its organization into insoluble polymers after formation of desmosine cross-bridges are largely confined to the perinatal period. In mice hemizygous for the elastin gene (ELN⁻¹), a transgenic model for alterations of this early period of elastic fiber formation, there is a paradoxical increase in the number of elastic lamellae, and therefore, arterial compliance at physiological pressures remains normal. The authors suggested that reduced elastin mRNA expression, together with

![Figure 1. Pulse wave velocity (y)–pressure (x) curves (average of the individual exponential relations y=b·e^ax obtained during sodium nitroprusside infusion) in nonanesthetized mgR/mgR (filled circles) and wild-type (open circles) mice. Slopes, intercepts, and probability values are shown in Table 2.](image-url)
Medial Elastic Network Fragmentation and Aortic Stiffening

The increase in pulse wave velocity and the slope of the relation between pulse wave velocity and distending intraluminal pressure or wall stress suggest that elastic fiber fragmentation leads to increased wall stiffness in mgR/mgR mice. This hypothesis depends on the veracity of pulse wave velocity as an index of wall stiffness. Although the method that we used for the measurement of aortic pulse wave transit time has been extensively verified in the rat, this use of this technique in the mouse could be complicated by several factors.

First, the frequency response of the cannula plus transducer system is lower in the mouse than in the rat. Although this characteristic will modify the harmonic composition of the waveform, it will presumably be of less importance because our algorithm compares whole waveforms rather than in other systems, which compare specific points on the waveform (wavefront or the “foot” of the initial diastolic-systolic pulse). Furthermore, because identical cannulas were used at the central and peripheral sites, distortion of the 2 signals will be the same. In a separate experiment performed on anesthetized adult OF1 mice, differences in transit time measured with polyethylene cannulas (n = 3) or Millar Mikro-Tip pressure transducers (1.4 f, SPR-671, Millar Instruments; n = 3) were not significantly different (Mikro-tip transducers: 0.084 ± 0.002 ms/mm Hg, n = 78 observations; cannulas: 0.086 ± 0.001 ms/mm Hg, n = 75 observations; P = 0.4086).

A second factor that may be of concern is the higher heart rate of the mouse compared with that of the rat. Here again, this difference will change the harmonics of the waveform and could theoretically modify wave transmission. However, because baseline heart rate and reflex tachycardia after hypotension (results not shown) were not statistically different in the 2 groups, this factor should not be important.

A third factor is the smaller aortic length in the mouse than in the rat, which may increase the error of measurement of pulse wave transit time and require a compensatory increase in sample rate. However, our algorithm creates intermediate points by linear interpolation, thus allowing sample resolution to be increased 10-fold. In the present experiment at 256 Hz, the percent error for measurement of transit time was 4% to 7%. In a separate experiment, transit times measured at 256 or 1024 Hz were not different (anesthetized adult OF1 mice, n = 5, polyethylene cannulas, 100 to 110 mm Hg: 12.6 ± 1.2 ms at 256 Hz and 11.7 ± 0.3 ms at 1024 Hz, P > 0.05; at 130 to 140 mm Hg: 5.8 ± 0.8 ms at 256 Hz and 4.6 ± 0.3 ms at 1024 Hz, P > 0.05). On the basis of these methodological experiments, we conclude that the 4-fold increase in wall stiffness in mgR/mgR mice is not artifactual.

Although aortic stiffening in mgR/mgR mice is probably primarily related to medial elastic network fragmentation, other factors have to be considered. Fibrillin is involved in calcium fixation, and it is known that diffuse calcification of elastocalcinosis is associated with a severe and statistically significant fall in desmosine content (~50%). Overall, these observations suggest that changes in absolute amounts of desmosine (even if they were statistically significant) are not highly involved in the functional abnormalities of the aorta in mgR/mgR mice.

thinning and reduced extensibility of elastic lamella during gestation, stimulated the synthesis of new elastic lamellae. Were this early period of elastogenesis to be affected in our mgR/mgR model (by means of a defect in desmosine cross-linking?), then a similar compensatory increase in elastic lamellae synthesis might also occur, thereby maintaining aortic wall elastic properties. This is not the case, suggesting that in the mgR/mgR mouse, fragmentation of the elastic fibers occurs later in life when lamellar structure is already established.

This conclusion (later fragmentation of elastic fibers leading to functional abnormalities of the aorta, dilatation, and stiffening) is based on histological analysis and on the lack of difference in desmosine content between mgR/mgR and wild-type mice. Because the sensitivity and reproducibility of the method used to measure desmosine content (capillary electrophoresis) are very high (1 ng and <5%, respectively), the possibility cannot be excluded that the small differences observed in desmosine content between groups (432 ± 31 vs 492 ± 42 ng/mg, or ~14%) would reach statistical significance with larger numbers of animals; however, the difference will probably remain tenuous. In another model of stiffening of the aortic wall (the VDN rat model) with the same degree of wall stiffening as that observed in the mgR/mgR mouse, fragmentation of elastic fibers (induced by
The arterial wall makes it stiffer. In our experiment, the total calcium content of the descending aorta was not increased in mgR/mgR mice, thus excluding diffuse calcification as an important determinant of aortic stiffening in this model. However, this does not exclude a role for focal aortic wall calcification in the inflammation process observed in the early stages of aortic aneurysmal dissection. Collagen content did not change in the aortic wall of mgR/mgR mice, thus excluding fibrosis as a mechanism of aortic stiffening. Aortic smooth muscle tone, which may modify wall stiffness, cannot be entirely ruled out, because the induced hypotension in mgR/mgR mice required a vasodilator dose twice as high as that required in wild-type mice. In the rat, however, we have previously shown that aortic vasomotion is of minor importance in the determination of aortic wall mechanics.

Whether aortic stiffening per se participates in aortic dilatation remains to be elucidated, and several aspects need to be investigated and discussed. First, the method for determining aortic dimensions by fixation and dehydration per se may induce tissue shrinkage. However, because fixation was performed in situ and before the aorta was isolated and dissected free from the surrounding tissue, shrinkage was probably minimal and uniform within samples and likely did not dramatically influence the calculated values for mechanical parameters. Second, some authors have concluded that aortic dilatation and wall stiffness evolve independently, whereas others have shown that the increase in central pulse pressure (resulting from stiffening of the aortic wall) is a major determinant of aortic diameter in Marfan syndrome. Central pulse pressure increased in mgR/mgR mice; although all of the determinants of pulse pressure were not measured in the present study—and it would be appropriate to normalize the measured pulse pressures for differences in stroke volume—this increase in pulse pressure is more dependent on the 4-fold increase in aortic wall stiffness than on any increase in stroke volume. This suggests that, as in Marfan syndrome, the fatiguing effect of cyclic wall stress is more important than that of steady stress for elastic fiber fragmentation and aortic dilatation in mgR/mgR mice. In Marfan patients, elastic fiber degradation is most prominent in the cardiac valves and thoracic aorta, where elastic fibers may be subjected to the most severe mechanical stresses. Finally, the latter authors showed that elastic fiber fragmentation occurred in association with an upregulation of the synthesis of metalloproteinases and probably with an increased susceptibility to metalloproteinase activity of the elastin formed in the presence of the defect in FBN1. Therefore, after inflammation and macrophage infiltration of the aortic wall, as has been shown in mgR/mgR mice, a stress-dependent and/or stress-independent activation of metalloproteinases may also participate in elastolysis.

In conclusion, underexpression of FBN1 in mice leads to severe elastic network fragmentation, with no change in elastin cross-linking. Subsequently, the aorta dilates and the wall stiffens. These results suggest that medial elastic fiber fragmentation and not defects in early elastogenesis is the major determinant of aortic dilatation and stiffening in Marfan syndrome.

When attempting to extrapolate the above data to humans, it must be borne in mind that there are dramatic differences in aortic geometry, heart rate, pressures, and time course. We observed marked dilatation in the mgR/mgR mouse with no decrease in medial thickness or wall collagen content. Because vascular wall integrity depends on collagen, which is mainly located in the adventitia, it has been suggested that aneurysm occurs in humans once destruction of adventitial collagen occurs (after medial necrosis). Further studies on the evolution in time of the relative roles of the media and adventitia are required.

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