Catalase overexpression in aortic smooth muscle prevents pathological mechanical changes underlying abdominal aortic aneurysm formation

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Maiellaro-Rafferty K, Weiss D, Joseph G, Wan W, Gleason RL, Taylor WR. Catalase overexpression in aortic smooth muscle prevents pathological mechanical changes underlying abdominal aortic aneurysm formation. Am J Physiol Heart Circ Physiol 301: H355–H362, 2011. First published May 6, 2011; doi:10.1152/ajpheart.00040.2011.—The causality of the associations between cellular and mechanical mechanisms of abdominal aortic aneurysm (AAA) formation has not been completely defined. Because reactive oxygen species are established mediators of AAA growth and remodeling, our objective was to investigate oxidative-stress-induced alterations in aortic biomechanics and microstructure during subclinical AAA development. We investigated the mechanisms of AAA in an angiotensin II (ANG II) infusion model of AAA in apolipoprotein E-deficient (apoE−/−) mice that overexpress catalase in vascular smooth muscle cells (apoE−/−/xTgSMC-Cat). At baseline, aortas from apoE−/−/xTgSMC-Cat exhibited increased stiffness and the microstructure was characterized by 50% more collagen content and less elastin fragmentation. ANG II treatment for 7 days in apoE−/− mice altered the transmural distribution of suprarenal aortic circumferential strain (quantified by opening angle, which increased from 130 ± 1° at baseline to 198 ± 8° after 7 days of ANG II treatment) without obvious changes in the aortic microstructure. No differences in aortic mechanical behavior or suprarenal opening angle were observed in apoE−/−/xTgSMC-Cat after 7 days of ANG II treatment. These data suggest that at the earliest stages of AAA development H2O2 is functionally important and is involved in the control of local variations in remodeling across the vessel wall. They further suggest that reduced elastin integrity at baseline may predispose the abdominal aorta to aneurysmal mechanical remodeling.

little evidence that links ROS directly to the biomechanical changes involved in AAA development.

ROS have been detected at all stages of aneurysm development (13, 29, 30) and are established mediators of extracellular matrix (ECM) degradation and remodeling (16, 34). AAA formation proceeds from localized remodeling and vessel dilation attributable to degeneration of elastin and alterations in collagen proteins within the aortic wall. Both events potentially reduce the compliance and tensile strength of aneurysmal aortas (19, 32, 41). Aneurysm formation is limited by inhibition of proteolytic enzymes, such as matrix metalloproteinases (MMPs; Ref. 26), and in animal models deficient in MMPs (Refs. 2, 20, 23, 37). These data suggest that proteolytic activity is necessary for AAA formation and that synergistic factors, such as spatial vulnerability to mechanical changes, are also relevant in the early stages of AAA formation.

We were motivated by the observation in a previous study from our group that transgenic apolipoprotein E-deficient (apoE−/−) mice with vascular smooth muscle cell (SMC)-specific overexpression of the human catalase gene (apoE−/−/xTgSMC-Cat) were completely protected from AAA formation when treated with angiotensin II (ANG II; Ref. 28). ANG II infusion into apoE−/− mice has been shown by several groups to promote AAA formation (7, 8). We utilized this model to investigate H2O2-mediated alteration of aortic mechanics in the earliest stages of AAA formation, because previous studies in this model (45) demonstrated that aorta from WT mice showed significant ANG II-mediated aortic ROS production after 14 days, an event that was blunted in the TgSMC-Cat mice. The working hypothesis was that H2O2 is mechanistically and spatially linked to the biomechanical remodeling that promotes AAA. The data obtained in this study are the first to measure early changes in mechanical behavior in a mouse model of ANG II-induced AAA and demonstrate that local H2O2 is associated with the development of early alterations in local circumferential strain across the aortic wall.

METHODS

Animals. Transgenic mice with smooth muscle-specific overexpression of catalase (apoE−/−/xTgSMC-Cat) and littermate control (apoE−/−) were used in this study. ANG II (0.75 mg·kg−1·day−1) was dissolved in sterile saline and delivered subcutaneously via osmotic minipumps (Alzet, Cupertino, CA) for 7 days. Mice were fed either a standard chow diet (Certified Rodent Chow 5001; Purina) or a high-fat diet (atherogenic diet; Research Diets, New Brunswick, NJ). The generation and phenotypic characterization of the apoE−/−/xTgSMC-Cat mice have been performed previously (45). All procedures were approved by the Emory University Institutional Animal Care and Use Commit-

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Aortic preparation and isolated vessel perfusion setup. Descending aortas, from the sixth intercostal arteries to the left renal artery, were cleared of connective tissue, and the overall loaded length \( l \) was measured. Each vessel was excised and placed in sterile culture medium [DMEM (Sigma-Aldrich, St. Louis, MO) with HEPES at pH 7.4 containing 10 \( \mu \)M sodium nitroprusside] and the unloaded axial length \( L \) and unloaded suprarenal diameter \( D \) were measured. The intercostal arteries were individually tied off with suture, and the aortas were cannulated and placed in a vessel isolation chamber [Living Systems (LSI), Burlington, VT]. The vessels were extended to the in vivo axial stretch \( \lambda_s = l/L \) and bathed in sterile culture medium held at 37°C. The chamber was mounted on a Nikon DIAPHOT 200 inverted microscope and the vessels were equilibrated for 30 min. Transmural pressure \( (P) \) was ramped from 0 to 130 mmHg at 0.5 mmHg/s using a perfusion pump and pressure controller (LSI). Outer aortic diameter \( (d) \) was measured with a video dimension analyzer (LSI).

Data analysis. Thus, from recorded values \((P, d, \text{and} \lambda_s)\), fixed length transmural pressure vs. aortic outer diameter \((P-d)\) data were plotted for apoE\(^{-/-}\), apoE\(^{-/-}\)*TgSMC-Cat, and apoE\(^{-/-}\) + ANG II to compare the global mechanical response across groups. Local pressure-dependent compliance \( C_P \) was calculated in 20 mmHg intervals as the derivative of the \( P-d \) data, where \( C_P = (P + 10 \text{ mmHg} - \frac{r_p}{10} \text{ mmHg})/20 \text{ mmHg} \).

Because the diameter-pressure response depends on both material properties and geometry, it is difficult to quantify differences in the material properties between different vessels from these plots or compliance measures; e.g., vessels made of identical material, but with different thicknesses will exhibit different diameter-pressure curves and different values of compliance (the thicker vessel will be stiffer). Stress-strain plots, however, only depend on material properties; thus differences in the stress-strain response indicate differences in the material that constitutes the vessel. The mean circumferential stress, \( \sigma_0 \), and mid-wall circumferential Green strain, \( E_0 \) plots for each group were generated from these mechanical data as

\[
\sigma_0 = P a / h \quad \text{and} \quad E_0 = (\lambda_s^2 - 1) / 2
\]

where \( a \) is the inner radius, \( h \) is the vessel wall thickness, and \( \lambda_s \) is the mid-wall circumferential stretch, all in the loaded configuration and calculated from experimentally recorded measures \( P, d, \text{and} \lambda_s \). The unloaded inner radius, \( A \), outer radius, \( B \), and thickness, \( H \) (intima to outer adventitia), were measured from histology. Assuming material incompressibility, \( a = \sqrt{b^2 - (B^2 - A^2)\lambda_s} \) (where \( b = d/2 \) was the outer radius), \( h = b - a \), and \( \lambda_s = (a + b)(A + B) \).

Measurement of aortic opening angle and strain distribution. Suprarenal aortic opening angles, \( \Phi \), were measured following a published protocol (17) to quantify the distribution of circumferential strain across the aortic wall. Opening angles were measured in aortas not used for mechanical testing. Using the approach of Chuong and Fung (6), the distribution of the circumferential component of Green strain across the vessel wall is

\[
E_0(r) = \frac{\pi r}{(\pi - \Phi)R}
\]

where \( r \in [a, b] \) is an arbitrary position in the aorta wall in a loaded configuration, \( R \in (R_i, R_o) \) is the corresponding location in the stress-free configuration and \( R_i \) and \( R_o \) are the inner and outer radii in the stress-free configuration, respectively. Note that the incompressibility constraint requires that

\[
r = \sqrt{\frac{\pi}{\pi - \Phi}(R^2 - R_i^2) + a^2}
\]

(2)

Following Rachev (33), changes in axial length and wall thickness between the unloaded configuration and the radially cut, stress-free configuration are typically small and may be neglected. For this case, for a given opening angle measurement, \( R_i \) and \( R_o \) may be calculated as

\[
R_i = \frac{P(B + A)}{2(\pi - \Phi)} - H \quad \text{and} \quad R_o = \frac{\pi (B + A)}{2(\pi - \Phi)} - H
\]

Thus, given the unloaded radius and thickness, the opening angle, and loaded outer diameter, the stress free inner and outer radius can be approximated. For all locations across the vessel wall \( (R \) in the stress-free location), from the inner wall location \( R_i \) to the outer wall location \( R_o \), the radial location in the loaded configuration \( r \) may be calculated via Eq. 2 and the circumferential Green strain may be calculated via Eq. 1. The distribution of \( E_0 \) vs. radius can then be plotted.

Total aortic collagen content. To quantify aortic collagen content, total aortic collagen per dry weight was measured by hydroxyproline assay as described by Woessner (43). Briefly, aortic tissue freed from fat was weighed and dehydrated in a speed vacuum for 2.5 h, and the dry weight was recorded. The dry tissue was digested in 500 \( \mu \)l 0.25 M sodium phosphate buffer with 0.0125 g protease K/g wet weight and then hydrolyzed into amino acid components in 6 N HCl at 120°C for 14 h. Fifty microliters of each sample and hydroxyproline standards were oxidized with Chloramine-T (Sigma). Oxidation was terminated with perchloric acid solution. A colorometric reaction was performed with \( p \)-dimethylaminobenzaldehyde at 60°C, and the plate was read at 540 nm. Total collagen content per dry weight was calculated on the assumption that collagen is 13% hydroxyproline.

Histological analysis. Histological analysis was performed on the bisected aortic rings used for opening angle measurement, which were snap frozen in optimal cutting temperature medium after each experiment. Five-micrometer sections from each sample were stained with Verhoff Geison Elastic stain (Sigma-Aldrich) for thickness measurement and quantification of elastin fragmentation or with picrosirius red (staining performed on intact aortic rings) to analyze collagen (3). Dimension analysis was performed using Image J software (NIH, Bethesda, MD). Elastin fragmentation was expressed as the number of elastin breaks per medial area, which was measured as the area between the inner and outer elastic laminae.

Statistical analysis. Data are presented as means \( \pm SE \). Statistical analyses were performed using GraphPad Prism software. The pressure-diameter data were analyzed by ANOVA to examine the effect of mouse background on aortic compliance, and Bonferroni post tests were used for post hoc analyses. All other analysis between groups was performed with Mann-Whitney test. Analysis of covariance and an F-test to compare slopes were performed on the mean circumferential strain vs. radial data. \( P < 0.05 \) was considered significant.

RESULTS

Overexpression of catalase alters aortic mechanical behavior. To determine whether AAA protection may derive from endogenous differences in mean systolic blood pressure and descending aortic mechanical behavior, unloaded and loaded dimensions (Table 1) and \( P-d \) response were measured in apoE\(^{-/-}\) and apoE\(^{-/-}\)*TgSMC-Cat mice. Mean systolic blood pressure for each group was the same (96 \( \pm \) 9.3 vs. 102 \( \pm \) 10 mmHg; \( P = NS \)). At baseline, descending aortas from apoE\(^{-/-}\)*TgSMC-Cat mice demonstrated reduced in vivo axial stretch, \( \lambda_s \), compared with aortas from apoE\(^{-/-}\) mice (Table 1; 1.26 \( \pm \) 0.008 vs. 1.48 \( \pm \) 0.005, respectively), indicating altered axial
loading. The P-d data (Fig. 1A) of both groups exhibited biphasic behavior. The apoE^+/-xTg<sup>SMC-Cat</sup> P-d curve was shifted downward from apoE^+/-, indicating that apoE^+/-xTg<sup>SMC-Cat</sup> aortas had smaller diameters and were less distensible. Pressure-dependent compliance (C<sub>p</sub>) curves (Fig. 1B) further demonstrated that apoE^+/-xTg<sup>SMC-Cat</sup> aortas were stiffer than apoE^+/- aortas at mid-range pressure. Mean circumferential stress-strain (δ<sub>θ</sub> - E<sub>θ</sub>) curves (Fig. 1C) of each group were nonlinear and exhibited increased stiffening at high strain. The δ<sub>θ</sub> - E<sub>θ</sub> curves indicate that apoE^+/-xTg<sup>SMC-Cat</sup> aortas had higher material stiffness compared with apoE^+/- aortas at baseline.

Opening angles were measured to quantify variations in strain across the wall of the suprarenal aorta at the site of AAA formation. At baseline, apoE^+/- and apoE^+/-xTg<sup>SMC-Cat</sup> aortic rings exhibited comparable opening angles (Table 1), with magnitudes in agreement with published values from apoE^+/- mice (18). Mean circumferential strain E<sub>θ</sub> across the wall was nearly uniform for both groups (Fig. 1D), although the strain magnitude of apoE^+/- aortas was higher than apoE^+/-xTg<sup>SMC-Cat</sup> aortas. Thus, at baseline, the apoE^+/-xTg<sup>SMC-Cat</sup> abdominal aortas had different axial strain, diameter-pressure behavior, mean circumferential strain magnitude, and material properties compared with the apoE^+/- aortas. Furthermore, both groups exhibited comparable opening angle and nearly uniform distribution of circumferential strain across the vessel wall.

Overexpression of catalase alters aortic microstructure. To investigate the structural basis for the mechanical differences between the two groups, matrix composition of the aortic walls was examined. At baseline, collagen content in the apoE^+/- group was 50% lower than the apoE^+/-xTg<sup>SMC-Cat</sup> group (Fig. 2A). Measurement of unloaded wall thickness (Table 1), adventitial thickness (Fig. 2B), and picrosirius red staining of untreated aortic cross sections imaged under polarized light (Fig. 2, C and D) confirmed that apoE^+/- aortas were thinner and had less adventitial collagen than apoE^+/-xTg<sup>SMC-Cat</sup> aortas. Elastin fragmentation was significantly greater in

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**Table 1. Mechanical and geometry results of aortas from apoE^+/- and apoE^+/-xTg<sup>SMC-Cat</sup> mice with and without ANG II treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>ANG II</th>
<th>λ&lt;sub&gt;s&lt;/sub&gt;</th>
<th>d, mm</th>
<th>B, mm</th>
<th>H, μm</th>
<th>O.A., °</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE^+/-</td>
<td>−</td>
<td>1.48 ± 0.005</td>
<td>1.37 ± 0.03</td>
<td>0.343 ± 0.02</td>
<td>89 ± 2.1</td>
<td>130 ± 1</td>
</tr>
<tr>
<td>apoE^+/-xTg&lt;sup&gt;SMC-Cat&lt;/sup&gt;</td>
<td>+</td>
<td>1.38 ± 0.04*</td>
<td>1.39 ± 0.08</td>
<td>0.342 ± 0.06</td>
<td>89 ± 1.9</td>
<td>198 ± 8*</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>1.26 ± 0.008*</td>
<td>1.16 ± 0.01*†</td>
<td>0.347 ± 0.005</td>
<td>98 ± 2.1†</td>
<td>130 ± 8†</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.23 ± 0.05*</td>
<td>1.12 ± 0.02*</td>
<td>0.37 ± 0.02</td>
<td>88 ± 1.62</td>
<td>130 ± 5†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Stretch ratio and loaded diameter data were acquired during isolated aortic inflation. Unloaded morphology was acquired from histological analysis of bisected aortic rings used in opening angle (O.A.) measurement. apoE^+/-, apolipoprotein E-deficient mice; apoE^+/-xTg<sup>SMC-Cat</sup>, apoE^+/- mice overexpress catalase in vascular smooth muscle cells; λ<sub>s</sub>, axial stretch ratio (l/L), where l is loaded length and L is unloaded length; d, ex vivo loaded diameter; B, unloaded outer radius; H, unloaded thickness. *P < 0.05 vs. apoE^+/-, †P < 0.05 vs. apoE^+/- + ANG II, ‡P < 0.05 vs. apoE^+/-xTg<sup>SMC-Cat</sup>.
Fig. 2. Morphology of apoE−/− and apoE−/−xTgSMC-Cat aortas at baseline. A: total collagen content per aortic dry weight decreased by 41% in apoE−/− compared with apoE−/−xTgSMC-Cat. B: apoE−/− adventitia thickness was 32% less than apoE−/−xTgSMC-Cat. C and D: representative images of the aortic wall stained with picrosirius red and imaged under polarized light. ApoE−/− image shows red and green illumination indicating presence of type I and type III collagen, respectively. ApoE−/−xTgSMC-Cat aortic cross section showed intense red illumination indicating a greater abundance of type I collagen. E: elastin fragmentation in the abdominal aortic wall was greater in apoE−/− compared with apoE−/−xTgSMC-Cat aortas. Elastin fragmentation was expressed as the number of breaks in elastic laminae per medial area. Medial area was defined as the area enclosed by the inner and outer elastic laminae measured from aortic sections. *P < 0.05; n = 5 for apoE−/−; n = 3 for apoE−/−xTgSMC-Cat.

aortas from apoE−/− mice compared with apoE−/−xTgSMC-Cat (Fig. 2E), indicating less functional elastin in apoE−/− aortas. Thus, at baseline, both the collagen content and elastin integrity of apoE−/− aortas were reduced from apoE−/−xTgSMC-Cat aortas.

ANG II infusion alters the circumferential strain distribution of aortas from apoE−/− mice but does not affect aortic microstructure. To determine if H2O2 is causally associated with the modulation of aortic mechanics during AAA progression, apoE−/− mice were treated with ANG II and an atherogenic diet for 7 days. This time point captured the earliest stages of ANG II-mediated AAA formation. The P-d behavior and compliance were unchanged in aortas from ANG II-treated apoE−/− mice compared with untreated apoE−/− mice (Fig. 3, A and B), and axial stretch was reduced (Table 1). The aortic mean circumferential stress-strain (E_b) behavior (Fig. 3C) in both groups was comparable at low and mid-range strain, but ANG II-treated aortas reached greater strain values. Abdominal aortic opening angle increased significantly with ANG II treatment (Table 1 and Fig. 4A). In addition, after a 7-day ANG II treatment, mean circumferential wall strain change with radius E_c(r), which was fairly uniform across the vessel wall at baseline, was higher at the outer vessel wall compared with the inner wall (Fig. 3D). Note also that the circumferential strain at the inner wall was similar before and after ANG II treatment. These data indicate that short-term ANG II treatment alters the opening angle and circumferential strain distribution in the apoE−/− abdominal aorta.

To determine if catalase overexpression in apoE−/−xTgSMC-Cat aortas protected against ANG II-induced changes in wall strain, blood pressure and opening angle were measured after treatment with ANG II for 7 days. ANG II infusion elevated mean systolic blood pressure equally in both apoE−/− and apoE−/−xTgSMC-Cat mice (150 ± 13 vs. 147 ± 10 mmHg; P = NS). Importantly, catalase overexpression blunted the ANG II-induced changes in opening angle (Table 1) and in mean circumferential strain distribution (Fig. 3D).

To determine if ANG II promoted matrix changes that altered opening angle and circumferential strain in aortas from apoE−/− mice, total collagen protein and elastin fragmentation were measured in aortas from ANG II-treated apoE−/− and apoE−/−xTgSMC-Cat mice. Total collagen content (Fig. 4B) and adventitial thickness (Fig. 4C) in the ANG II-treated apoE−/−xTgSMC-Cat group decreased to the same level as aortas from apoE−/− mice. ANG II treatment had no effect on elastin fragmentation, which remained higher in apoE−/− aortas compared with apoE−/−xTgSMC-Cat aortas (Fig. 4D). Thus the data suggest that ANG II-induced increases in apoE−/− mean circumferential wall strain and opening angle were not attributed to changes in collagen content or elastin fragmentation measured in the aortic ECM.
and material properties of apoE vulnerability to AAA formation. At baseline, the mechanical to reduce the stiffness of the rat abdominal aorta (31), elastin functional elastin. As elastic laminae rupture has been shown to entail stiffness, contained less adventitial collagen, and had less degradation of medial elastin and collagen affected an opening angle decrease. ANG II-treated apoE mice showed reduced circumferential stress. D: mean circumferential Green strain \( E_g \) values, where aortas from treated apoE mice showed reduced circumferential stress. Thus these data support an association between ROS production and early aneurysmal remodeling and mechanical adaptation.

AAA formation results from local aortic mechanical failure, but whether the spatial vulnerability of aneurysmal dilation is dominated by external hemodynamic forces or by local aortic weakness is not fully understood. Our study suggests that aortic microstructure in apoE\(^{-/-}\) mice may confer mechanical vulnerability to AAA formation. At baseline, the mechanical and material properties of apoE\(^{-/-}\) aortas were different from apoE\(^{-/-}\) xTg\(^{SMC-Cat}\) aortas, in that apoE\(^{-/-}\) aortas had greater circumferential strain magnitude, i.e., had reduced circumferential stiffness, contained less adventitial collagen, and had less functional elastin. As elastic laminae rupture has been shown to reduce the stiffness of the rat abdominal aorta (31), elastin fragmentation in apoE\(^{-/-}\) aortas likely contributes to low stiffness compared with apoE\(^{-/-}\) xTg\(^{SMC-Cat}\). The critical role of elastin to vessel stability is well established in AAA, as emphasized by other models of AAA development that directly degrade elastin fibers to cause aortic dilation (1). In this case, the basal increase in elastin fragmentation in apoE\(^{-/-}\) aortas may be due to the advanced age (16–18 wk) of the mice, as loss of elastin fiber structural integrity with aging is well established (35). Thus, given the baseline differences in elastin fragmentation between aortas from apoE\(^{-/-}\) and apoE\(^{-/-}\) xTg\(^{SMC-Cat}\) mice, reduced elastin integrity may be a predisposing factor in the ANG II-infusion model of AAA.

ANG II infusion stimulates the progression of AAA in apoE\(^{-/-}\) mice, and short-term infusion allowed examination of early aneurysmal changes in the descending aorta and in the local abdominal aorta where AAA develop. Acute ANG II treatment promoted increased aortic opening angle and redistribution of mean circumferential strain across the abdominal aortic wall, despite conservation of global pressure-diameter behavior. Opening angle is a manifestation of residual stresses in an unloaded aortic ring. Residual stress is thought to be a consequence of achieving uniform stress distribution across the medial thickness and, likely a different, uniform stress distribution across the adventitial thickness under physiologic loading. Opening angle is affected by multiple factors, including changes in geometry (e.g., radius-to-thickness ratio) and changes in material properties, which arise through changes in the content and organization of cells and ECM. Evidence that opening angles are larger in aortic rings containing intimal atheromas compared with autologous nonatherosclerotic regions (40) suggests that opening angle enlargement is indicative of preferential growth and/or increased compressive forces in the vessel interior (11). The converse, however, has been demonstrated by Greenwald et al. (14), wherein enzymatic degradation of medial elastin and collagen affected an opening angle decrease. ANG II-treated apoE\(^{-/-}\) aortas in our study, however, did not have atheromas or significant changes in elastin integrity or collagen content. A possible difference...
between the characterization by Greenwald et al. and our study is that, in our model, degraded elastin fibers remained embedded within an intact medial collagen. Because other factors not measured here, such as collagen undulation and recruitment or ECM cross-linking, may underlie the microstructural basis for opening angle changes, a mechanical evaluation of strain distribution across the abdominal vessel was performed.

After ANG II treatment, mean circumferential strain distribution in the apoE<sup>−/−</sup> aorta outer wall adjusted to higher strain. Physiologic strain redistribution toward the adventitia has been explored as a protective mechanism to offload stress from the intima during hypertension (42) and may underlie the changes in mean strain observed here, especially given that ANG II infusion induced hypertensive blood pressure in apoE<sup>−/−</sup> mice. However, ANG II treatment raised blood pressure in apoE<sup>−/−</sup>xTg<sup>SMC-Cat</sup> mice as well without affecting opening angle. Importantly, the key finding in our study was the dramatic change in local circumferential mechanics in the absence of overt ECM changes, as measured by collagen content, wall thickness, and elastin fragmentation, in the apoE<sup>−/−</sup> abdominal aorta after ANG II infusion. It should be noted that other ultrastructural alterations in ECM cross-linking or cellular composition not measured here may ultimately underlie the biomechanical events observed. Dysfunctional elastin and collagen cross-linking in lysyl oxidase-deficient mice promotes AAA (25), and deficiency of the cystein protease inhibitor cystatin C is linked to AAA formation in humans (22). Aneurysm microstructure is also complicated by infiltrating inflammatory cells (10), reduced proteoglycans (38), and defects in collagen microarchitecture, as demonstrated by Lindeman et al. (21). Given the absence of ANG II-induced mechanical changes in mice with overexpression of catalase before the development of AAA, the current data suggest that there are additional ROS-associated changes in the vessel wall that remain to be elucidated.

SMC-specific overexpression of catalase in apoE<sup>−/−</sup> mice allowed us to determine if H<sub>2</sub>O<sub>2</sub> is a critical molecular mediator in the subclinical mechanism of AAA pathogenesis. Aortas from these mice had thicker walls, greater functional elastin, and increased collagen content, which clearly accounted for the increase in mechanical stiffness detected at baseline compared with the apoE<sup>−/−</sup> group. However, after ANG II treatment, the collagen content and adventitial thickness of both apoE<sup>−/−</sup> and apoE<sup>−/−</sup>xTg<sup>SMC-Cat</sup> aortas were equivalent. Thus, given that overexpression of catalase-blunted ANG II-mediated changes in opening angle and mean circumferential strain distribution across the aorta, our data suggest that H<sub>2</sub>O<sub>2</sub> is functionally important and is involved in the control of local variations in remodeling across the vessel wall. ROS are shown to be significantly upregulated in established AAA (13, 29, 30), promoting ECM degradation by upregulating MMP activity via the NAD(P)H oxidase (16, 34). Our data establish H<sub>2</sub>O<sub>2</sub> as a mediator of subclinical AAA and support the pivotal role of ROS at all stages of AAA pathogenesis. Antioxidant studies in animal models also support the link between H<sub>2</sub>O<sub>2</sub> and AAA. Catalase deficiency was associated with AAA formation in a study utilizing the elastase infusion model (15), supporting our finding that catalase participates in mitigating AAA formation. Indeed, our investigation showed a trend for increased procollagen type I.
mRNA expression in apoE−/−xTgSMC-Cat aortas. We note that in the transgenic model of SMC-specific catalase overexpression studied here, we cannot exclude the possibility that catalase was a “sink” for freely diffusible H₂O₂ secreted by other cell types within the vessel wall.

In conclusion, the present study suggests that the earliest local mechanical changes in ANG II-mediated AAA development are associated with the local production of ROS. The ANG II-mediated increase in abdominal aorta opening angle was blunted by overexpression of catalase, suggesting that H₂O₂ is a pivotal molecular signal in the pathogenesis of AAA. Furthermore, our data suggest that H₂O₂ initially impacts ANG II-mediated increase in abdominal aorta opening angle.

**DISCLOSURES**

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

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