Altered hemodynamics, endothelial function, and protein expression occur with aortic coarctation and persist after repair

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COARCTATION OF THE AORTA (CoA) is one of the most common congenital cardiovascular anomalies and is associated with reduced life expectancy due to sources of morbidity, including hypertension and early-onset coronary disease, despite improved postoperative outcomes (9, 26, 30, 44). Untreated CoA provides mechanical stimuli for vascular remodeling in the form of elevated blood pressure (BP) proximal to the coarctation and potentially adverse distributions of wall shear stress (WSS) throughout the thoracic aorta. Normotensive repaired CoA patients have also demonstrated increased carotid intimal-medial thickness and compromised vascular function that appears to persist upon clinical evaluation 12–20 yr after intervention (11, 72). The mechanism(s) for this compromised vascular function, possibly involving endothelial function or the expression of smooth muscle (SM) proteins affecting structural and functional properties of the aorta, is not presently known in these postrepair patients.

Vascular remodeling describes the response to physiological or pathological stimuli wherein blood vessels undergo compensatory changes in lumen diameter and wall thickness to maintain wall tensile stress or WSS (8, 63). The vascular endothelium is dynamic, serving to sense WSS and regulate vasomotor tone via the release of dilatory substances as well as anti-inflammatory agents to maintain vascular health. Impaired vasorelaxation through endothelial nitric oxide (NO) release, known as “endothelial dysfunction,” is a characteristic effect of hypertension in human and animal models including prior CoA models (16, 38). Along with vasorelaxation, arterial constriction mediated by arterial SM may also play a role in the adaptive response of arteries to hypertension, as alterations in the resting and active contractile force of elastic conduit arteries, such as the thoracic aorta and carotid arteries, may indicate atherogenic changes in stiffness (23). Mounting evidence also suggests these vascular impairments caused by the interplay between altered vasorelaxation and vasoconstriction are a major risk factor in the development of coronary artery disease (40, 48).

In addition to the role of the endothelium alluded to above, the maintenance of arterial structural and the functions described above is critically dependent on the controlled expression of contractile and structural proteins by fully differentiated SM (51, 52). A change from the fully differentiated state (dedifferentiation) of SM has been demonstrated to occur in conditions of vascular injury, and these changes may thus be present in CoA (29, 31). These phenotypic changes may be evident through the expression of SM contractile marker proteins in proximal and distal locations within the descending thoracic aorta (dAo), including SM α-actin, SM and nonmuscle (NM) myosin, and caldesmon. SM α-actin and SM myosin heavy chain are isofoms of contractile proteins and are excellent markers of the differentiation state of SM, whereas caldesmon is an actin-binding and calmodulin-binding protein in SM that may similarly serve as a marker of the differentiation state (52). NM myosin is a motor protein that plays a role in cellular force and translocation through its actin-binding and contractile properties. Although SM myosin is distinguished as an

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NM protein, it is generally present in SM cells and has several functions. Through development, differentiation is thought to occur such that NM myosin expression decreases from ~50% in embryonic stages to <10% in mature animals (19). However, recent research has indicated that NM myosin can uniquely provide a convergence point between external forces and cellular responses that regulate its activation (68), thus providing a possible link between altered mechanical stimuli and the structural and functional changes associated with sources of morbidity commonly observed in CoA. External forces may result in phosphorylation or conformational changes in NM myosin heavy chain that subsequently alter cytoskeleton mobility and filament interaction, ultimately leading to changes in SM migration and adhesion (15). While several reports have documented a mechanosensitive role for NM myosin in cell culture experiments (33, 65), no studies to date have observed the expression of vascular NM myosin in pathophysiological conditions where indexes of hemodynamics and vascular biomechanics can be precisely quantified. Hemodynamics refers to the forces that govern blood flow through the cardiovascular system and are best represented by the indexes of BP and WSS for the present investigation, whereas biomechanics is concerned with the effects produced by these forces exerted on the vasculature that can be quantified through the indexes of cyclic strain, structure, and function.

In addition to contractile proteins, cytoskeletal and extracellular contractile proteins associated with integrins, known as focal adhesions, have been studied to understand their influence on the regulation and contraction of SM (53, 57, 70). Talin is a cytoskeletal focal adhesion protein on the cytosolic side of the membrane, and fibronectin is an extracellular glycoprotein (20, 21). When considered together, expression of these contractile and focal adhesion proteins provides an additional indicator of SM differentiation beyond previous studies, which observed only SM α-actin or SM myosin.

Previous research has characterized changes in pulse BP, aortic capcitance, and WSS occurring due to CoA (24, 35, 36), but the specific mechanisms linking these changes to the long-term morbidity observed in untreated and postrepair patients have not been conclusively identified to date. The objective of this work was to couple subject-specific computational techniques with an established approach in rabbits that mimics untreated CoA as well as vascular morphology typically observed after resection with end-to-end anastomosis to quantify 1) the severity of hemodynamic and vascular biomechanics alterations before and after correction and 2) endothelial function and the phenotypic modulation of vascular SM contractile and focal adhesion proteins along with their relationship to altered vascular structure, function, and stiffness. The data presented here suggest that hemodynamic and biomechanical alterations caused by CoA result in endothelial dysfunction, dedifferentiation of arterial SM, and medial thickening. The mechanical consequences include altered contractile force and resting stiffness and a shift of the SM phenotype from contractile toward “synthetic,” with impaired vasorelaxation via the endothelial NO pathway. Together, these functional and structural alterations may explain the high rates of residual morbidities seen in CoA patients despite BP restoration due to surgical correction.

METHODS

Experimental protocol. After approval from the Institutional Animal Care and Use Committee, male New Zealand White rabbits (~10 wk old and weighing 0.8–1.2 kg) were randomly designated to undergo proximal dAO CoA, resulting in a ~20-mmHg BP gradient using silk (permanent) or Vicryl (degradable) suture as previously described (43) to mimic untreated CoA and surgically corrected CoA patient populations, respectively. Importantly, the putative guideline suggestive of treatment for CoA in humans is a BP gradient of ≥20 mmHg at rest (55). A control group (nonexperimental) was also established (n = 7 rabbits/group). Rabbits underwent extensive intraoperative monitoring for O₂ saturation, heart and respiratory rate, temperature, and capillary refill time throughout all experimental procedures, which continued for 3–5 h after all surgical and imaging procedures were completed (43).

MRI. Rabbits underwent imaging as previously described (43) with a 3-T Sigma Excite scanner (GE Healthcare, Waukesha, WI) using a water-filled knee coil to avoid damaging resonance angiography (MRA) data for vascular geometry and phase-contrast MRI (PC-MRI) data for the determination of cyclic vascular strain and ascending aortic blood flow for use with computational fluid dynamic (CFD) simulations. Mean and maximum Green-LaGrange strain (E₀ and E₀max, respectively) were calculated as follows: 

\[
E₀ = \frac{1}{2}(D_{\text{current}} - D_{\text{diastolic}})^2 - 1 \text{ and } E₀max = 0.5(D_{\text{systolic}} - D_{\text{diastolic}})^2 - 1, \text{ where } D \text{ is diameter (35).}
\]

The aortic distensibility and pressure-strain elastic modulus were also calculated using PC-MRI data (61). All imaging data was obtained at the conclusion of the experiment, 1 day before the completion of the experiment at 32 wk of age. For corrected and CoA groups, this was ~22 wk after the coarctation was induced.

BP and tissue harvest. Before tissue harvest at 32 wk of age, rabbits were anesthetized for the simultaneous measurement and recording of BP waveforms at the common carotid and femoral arteries via fluid-filled catheters (43). Arteries were then removed from the following four locations after euthanasia by an intravenous overdose of pentobarbital sodium (100 mg/kg): 1) the left common carotid, 2) the dAO above the coarctation (proximal), 3) the dAO downstream of the coarctation (distal), and 4) the femoral artery. Arteries were excised with extreme care to avoid damaging the endothelium or SM tissue and placed in either 10% neutral buffered formalin for histology or cold (4°C) physiological salt solution [PSS; containing (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.6 CaCl₂, 1.2 NaHPO₄, 2.0 MOPS (adjusted to pH 7.4), 0.02 Na₂ EDTA (to chelate heavy metals), and 5.6 g-glucose] in preparation for myograph and immunohistochemical analysis.

CFD simulations. Computational representations of the aorta and arteries of the head and neck were reconstructed from imaging data as previously described (35). Ascending aortic PC-MRI waveforms were then mapped to the inlet face of CFD models using a temporally varying parabolic flow profile. Flow waveforms similarly obtained from the head and neck arteries were used with measured BP data to prescribe outflow boundary conditions. Specifically, to replicate the physiological effect of arterial networks downstream of the CFD model branches, three-element Windkessel model outlet boundary conditions were imposed using a coupled-multidomain method (69). This method provides an intuitive representation of the arterial tree beyond model outlets and can be described by parameters with physiological meaning that were calculated and iteratively adjusted as previously described (37, 47, 62) such that measured BP was replicated. Time-dependent CFD simulations were performed using an in-house stabilized finite-element solver with the embedded commercial linear solver LESLIB (Altair Engineering, Troy, MI) to solve time-dependent Navier-Stokes equations. Vessels were modeled as rigid. Blood was assumed to be a Newtonian fluid with a density of 1.06 g/cm³ and a viscosity of 4 cp consistent with a previous report (74) in rabbits and upon consideration of the shear rates observed in the present investigation. Computational meshes contained ~4 mil-
tion tetrahedral elements, and localized refinement was performed using an adaptive technique to deposit more elements in regions prone to flow disruption (45). Simulations were run for four to six cardiac cycles until the flow rate and BP fields yielded periodic solutions. Results for blood flow velocity, BP, and WSS were visualized using ParaView (Kitware, Clifton Park, NY). Time-averaged WSS (TAWSS) and the oscillatory shear index (OSI) were then calculated (39). Low TAWSS is thought to promote atherogenesis, as is elevated OSI, an index of directional changes in WSS (39, 64). An OSI value of zero indicates that WSS is unidirectional, whereas a value of 0.5 is indicative of bidirectional WSS with a time-averaged value of zero. A previous imaging study (25) found low local TAWSS and elevated OSI values that were statistically different from circumferential averages, motivating the need to report detailed local WSS results in CFD studies. Therefore, circumferential values were extracted near locations corresponding to where histological and myograph analyses were conducted. Spatially equivalent regions were queried for all rabbits using the worst-case CoA rabbits as a guide. Thus, the region denoted as proximal represents the approximate midpoint location between the left subclavian artery and coarctation, whereas the region denoted as distal is in the vicinity of the impact zone created by the impinging velocity jet. Circumferential results at each location were divided into 16 sectors of equal size, and values within each sector were averaged (36) to quantitatively determine the severity of localized hemodynamic alterations due to CoA and correction. Values for TAWSS were also extracted longitudinally along the anatomic right and left luminal surfaces as well as the inner and outer curvatures of the thoracic aorta.

Histology and immunohistochemistry. Histology was conducted as previously described (43). Briefly, fixed arteries were dehydrated, embedded in paraffin wax, sectioned at 5 µm, and stained using Verhoeff-Van Gieson methods to identify internal and external medial borders and elastic fibers for morphometric analysis of artery thickness. The medial area of arteries was also calculated by tracing borders of the internal elastic lamina (IEL) and external elastic lamina and subtracting the respective areas. This approach accounts for possible differences in artery radius and thickness occurring due to fixation at varying BPs (75). All quantification was conducted in triplicate by three investigators blinded to the experimental group.

Expression of the cytosolic and extracellular focal adhesion proteins talin and fibronectin as well as the SM contractile differentiation marker proteins SM α-actin, NM and SM myosin heavy chain, and caldesmon were additionally evaluated using immunohistochemistry. Tissue specimens from the regions described above were cleaned of blood and loose connective tissue, frozen in isopentane cooled in liquid nitrogen, and stored at −80°C. Sections (8 µm) were cut on a Leica CM1900 cryostat, mounted on glass slides, and stored at −20°C until used. Tissues were then treated as previously reported (20). Briefly, frozen sections were fixed with 2% paraformaldehyde for 10 min, permeabilized in 0.5% Triton X-100 for 10 min, and blocked with 5 mg/ml BSA for 1 h. Sections were reacted with primary antibody for 2 h and the appropriate secondary antibody for 2 h, incubated with 4’,6-diamidino-2-phenylindole (DAPI; 0.5 µM) to stain nuclei, and coverslipped. Multiple washes were used after primary and secondary incubation to avoid nonspecific binding. All immunoreacting solutions were made in PBS-Tween with 0.1% BSA to avoid nonspecific binding. Antibodies were obtained from the following sources: SM α-actin (A 2547), SM myosin (F0791), caldesmon (T3287), fibronectin (IST-3), and talin (8D4) from Sigma Chemical (St. Louis, MO), NM myosin (BT-561) from Biomedical Technologies (Stoughton, MA), Cy2 and Cy3 secondary antibodies from Jackson Immuno Research (West Grove, PA), and DAPI (157574) from Molecular Probes/Invitrogen (Eugene, OR).

Stained or immunoreacted sections were observed with an Olympus IX70 microscope using epifluorescence illumination where appropriate. To view the relatively large media of aortic sections, all images were taken with a ×10 air lens. Quantitative analysis was performed using ImageJ. Digital grayscale images for SM α-actin, SM and NM myosin, caldesmon, fibronectin, and talin were collected using similar light intensity and exposure times for all samples with a 16-bit Princeton Instruments camera controlled through a PCI board via IPLab for Windows. For each animal, three regions of interest having equal size and spanning the entire medial wall were randomly chosen. The mean optical density was then determined for each location and averaged, with data expressed as average values of staining intensity. Four to five animals were quantified for each experimental group.

Myograph analysis. Vascular rings (width: 3–4 mm) were sectioned, cleaned of adhering perivascular tissue, mounted on an isometric myograph (Harvard Apparatus, Holliston, MA), maintained in a water-jacketed tissue bath in PSS bubbled continuously with O2 at 37°C, and allowed to equilibrate for at least 1 h. An optimal resting force of 2 g was applied to all sections based on preliminary experiments with this model (43, 58). Arteries underwent K+-PSS contraction followed by three PSS rinses, and this procedure was conducted two to three times until the effective force response of an artery was consistent (27, 59). To quantify endothelial function, aortic rings were subsequently precontracted with 0.2 µM phenylephrine (PE), and endothelium-dependent NO relaxation was determined by cumulative additions of ACh (10⁻⁶–10⁻⁵ M) (3, 4). Samples were washed at least three times with fresh PSS and equilibrated for >1 h before endothelium-independent vasorelaxation to cumulative doses of sodium nitroprusside (SNP; 10⁻⁹–10⁻⁵ M) was determined.

Aortic rings were then again allowed to equilibrate at resting force before being stimulated with cumulative concentrations of PE (10⁻⁹–10⁻⁵ M). The effective force response to each PE concentration was normalized to the tissue’s maximum K+-PSS contractile response, and cumulative dose-response curves were constructed.

Statistical analysis. All data are presented as means ± SE. Statistical evaluation was performed using one-way ANOVA followed by Tukey post hoc analysis. P values of <0.05 were considered significant.

RESULTS

Maximum intensity projections (Fig. 1) of the acquired MRA data confirmed that rabbits undergoing coarctation with silk suture developed a pronounced stenosis after surgery, similar to untreated CoA in humans. Rabbits undergoing coarctation with degradable Vicryl suture initially developed stenosis; however, complete degradation of the suture by 56–70 days returned aortic diameter close to normal with modest residual narrowing present in the suture region, as shown in Fig. 1. These morphological characteristics are similar to human surgical treatment of resection with end-to-end anastomosis, the most common method of surgical treatment for coarctation. Control rabbits represented healthy subjects of similar age and weight.

BP. Rabbits undergoing coarctation with silk suture had a significantly increased mean BP gradient of 20 ± 2.0 mmHg across the stenotic region compared with control rabbits (3.2 ± 1.7 mmHg) and corrected rabbits (2.7 ± 1.3 mmHg, n = 7 rabbits/group for all BP measurements). Representative waveforms (data not shown) revealed increased proximal systolic, mean, and pulse BP but reduced distal pulse BP in CoA rabbits. Corrected rabbits had BP waveforms similar to control rabbits, with systolic, diastolic, and mean BP significantly less than the CoA group at the proximal location. Distal artery BP recordings revealed no significant differences in systolic or mean BP across all groups; however, pulse BP was significantly reduced in the CoA group compared with the control group. Values for each group are shown in Table 1.
**CFD simulations.** Computational simulation results for peak systolic blood flow velocity patterns for all rabbits within each experimental group are shown in Fig. 2. Systolic velocity profiles in control rabbits were generally parabolic, with peak values of ~60 cm/s present throughout the aorta and branches. CoA rabbits showed lower velocity in the aortic arch, dramatic velocity jet due to the coarctation, and a region of poststenotic dilation and tortuosity distal to the coarctation. Corrected rabbits demonstrated a region of increased velocity at the coarctation site, where residual narrowing was present, and reduced velocity magnitude in the distal region, where a moderate dilation was present.

Control rabbits had fairly consistent distributions of TAWSS, particularly in the dAo, ranging from 15–20 dyn/cm² (Fig. 3). CoA rabbits showed marked differences, including regions of low TAWSS proximal to the coarctation and extremely high TAWSS at the location where the velocity jet due to the coarctation impinged on the posterior portion of the dAo distally. The corrected group revealed a small region of increased TAWSS at the site of residual narrowing and reduced TAWSS distal to the coarctation.

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**Table 1. BP measurements obtained at carotid (proximal) and femoral (distal) locations**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>CoA</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>71 ± 3</td>
<td>99 ± 7†</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Mean BP</td>
<td>64 ± 4</td>
<td>87 ± 8†</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>58 ± 4</td>
<td>74 ± 10</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>Pulse Pressure</td>
<td>13 ± 1</td>
<td>25 ± 4†</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; †n = 7 rabbits/group. BP, blood pressure. *Coarctation of aorta (CoA) group significantly different the control group (P < 0.05); †CoA group significantly different from the corrected group (P < 0.05).

Spatial distributions of OSI for all rabbits within each experimental group are shown in Fig. 4. Values generally ranged from 0.20 to 0.25 in the aortic arch and distal dAo of control rabbits. In contrast, CoA rabbits had elevated OSI immediately distal to the coarctation and in the tortuous region with values of 0.40 to 0.50. These trends were similarly present in corrected rabbits, but to a lesser extent.

**Local TAWSS quantification.** TAWSS results unwrapped about the inner curvature of the dAo are shown in Fig. 5. In the region of circumferential quantification proximal to the coarctation, CoA rabbits had significantly reduced TAWSS compared with control rabbits along the outer wall, but no differences were observed for the corrected group. In the distal region of quantification, CoA TAWSS was significantly elevated at the site where the velocity jet impinged in the outer left luminal surface (100–130 dyn/cm²) greater than control rabbits. In contrast, corrected rabbits showed significantly reduced TAWSS in the inner right luminal surface distal to the coarctation compared with control rabbits at this location (corrected rabbits: 3.3–5.4 dyn/cm² vs. control rabbits: 13.8–17.5 dyn/cm²).

The longitudinal plots of TAWSS 1–10 diameters downstream of the left subclavian artery shown in Fig. 5, right, further demonstrated the regional differences between groups. CoA rabbits showed drastically increased TAWSS (700–780 dyn/cm²) at 2–2.5 diameters, corresponding to the location where the lumen radius was reduced due to the suture. CoA rabbits also displayed significantly reduced TAWSS proximal to the suture at 1.0–1.5 diameters along the right and outer luminal surfaces (CoA rabbits: 4.6–7.9 dyn/cm² vs. control rabbits: 15.4–31.7 dyn/cm²) as well as significantly reduced TAWSS from ~3–6 diameters along the right, outer, and left luminal surfaces (CoA rabbits: 1.1–8.7 dyn/cm² vs. control rabbits: 12.4–21.4 dyn/cm²). The stenotic velocity jet resulting from the coarctation caused increased TAWSS in a rotational manner at 5.5–7 diameters along the outer luminal surface, 6.5–8 diameters along the left luminal surface, and 8–9.5 diameters along the inner luminal surface (CoA rabbits: 110.9–136.6 dyn/cm² vs. control rabbits: 18.5–21.1 dyn/cm²). Cor-

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**Fig. 1.** Representative maximum intensity projections of magnetic resonance angiography data from each experimental group. All images were obtained at the conclusion of the experiment, 1 day before the completion of the experiment at 32 wk of age. For corrected and coarctation of the aorta (CoA) groups, this was ~22 wk after the coarctation was induced.
rected rabbits also showed a reduction in TAWSS proximal to the suture at 1.0–1.5 diameters in the outer luminal surface (corrected rabbits: 13.9–16.2 dyn/cm² vs. control rabbits: 29.0–31.7 dyn/cm²), similar to the CoA group, as well as reduced TAWSS in the right, outer, and left luminal surfaces at 3.5–4.5 diameters. Corrected rabbits also revealed reduced TAWSS along the inner and right luminal surfaces from 5.5–7.5 diameters, in agreement with circumferential data, and at 9.5–10 diameters in the outer luminal surface.

**Histology and immunohistochemistry.** Figure 6 shows representative Verhoeff-Van Gieson-stained images at each region, where the hatched portions of the bars in Fig. 6, right, indicate the amount of medial thickness containing disorganized or fragmented elastic lamellae not present in the control group. In both left common carotid artery and proximal dAo regions, total medial thickness was significantly increased with pronounced elastin fragmentation in the CoA and corrected groups compared with the control group. These differences in medial thickness were not present distal to the coarctation site. Similarly, medial area quantified in the proximal dAo was significantly greater for CoA and corrected rabbits compared with control rabbits, but there were no differences between groups in the distal dAo (proximal dAo: 5.70 ± 0.74 mm² in CoA rabbits, and 3.96 ± 0.18 mm² in control rabbits; distal dAo: 1.97 ± 0.07 mm² in control rabbits, 2.37 ± 0.15 mm² in CoA rabbits, and 2.41 ± 0.23 mm² in corrected rabbits).

NM myosin staining intensity was significantly increased in proximal dAo arteries of both CoA and corrected rabbits compared with control rabbits (CoA and corrected rabbits: 87.0 ± 6.4 and 83.2 ± 3.6, respectively, vs. control rabbits: 37.6 ± 2.6; Fig. 7). These differences were not present in the distal dAo region. No significant differences were observed in fluorophore intensity radially across the media. The use of histogram distributions to further detect any spatial trends in the distribution of NM myosin also did not yield any significant differences. SM myosin staining intensity of proximal arteries was significantly reduced in CoA and corrected groups compared with the control group (CoA and corrected groups: 51.6 ± 4.4 and 57.7 ± 5.8, respectively, vs. control group: 118 ± 11.2), whereas no significant differences in SM myosin intensity were present distally. Caldesmon (data not shown) intensity was significantly reduced in the proximal arteries of CoA rabbits (51.5 ± 4.0 in CoA rabbits vs. 116 ± 6.3 in control rabbits), whereas no differences were present distally. There were also no statistical differences in talin, fibronectin (data not shown), or SM α-actin staining intensity across groups.

**Myograph analysis.** Arterial relaxation curves in response to the endothelium-dependent agonist ACh and the endothelium-independent agonist SNP are shown in Fig. 8 for proximal and distal locations. Proximal to the coarctation, control rabbits showed intact endothelial function by demonstrating ACh relaxation to ~80% of precontracted force. In contrast, both CoA and corrected rabbits showed significantly impaired ACh
relaxation (e.g., $-3 \pm 11\%$ and $27 \pm 9\%$, respectively), with CoA rabbits showing marked impairment at peak ACh values. In the distal region, differences between corrected and control groups were absent, with relaxations of $86 \pm 2\%$ and $78 \pm 3\%$, respectively. In contrast, CoA rabbits continued to show significantly impaired ACh relaxation distally, with a peak relaxation of $-18 \pm 11\%$ of precontracted force. Endothelium-independent relaxation in the proximal dAo reached $90\%$ of precontracted values for control rabbits. A small but significant reduction in relaxation for CoA compared with control rabbits was present at intermediate doses of SNP [log(7.5 to 6.5)]; however, peak SNP relaxation was not significantly different between all groups. Corrected rabbits showed similar SNP relaxation responses compared with control rabbits regardless of dose. In the distal dAo, corrected and control rabbits continued to show similar SNP relaxation responses, whereas CoA rabbits had significantly greater relaxation compared with the corrected group at intermediate doses [log(7 to 4.5)] and control rabbits at peak doses ($98 \pm 1\%$ in CoA rabbits vs. $90 \pm 1\%$ in control rabbits).

The PE force response at proximal and distal dAo locations is also shown in Fig. 8. In the proximal dAo, control rabbits showed a normalized effective force response peaking at $1.13 \pm 0.08$, whereas both CoA and corrected rabbits peaked at only $0.94 \pm 0.04$ and $0.92 \pm 0.03$, respectively, and showed significantly diminished force at several concentrations. Peak $K^+$-PSS contraction results are shown in Table 2, and proximal aortic rings demonstrated significantly reduced force in corrected and CoA rabbits compared with control rabbits, similar to PE contraction trends. In the distal dAo, control rabbits demonstrated a peak contractility of $1.04 \pm 0.05$, and the CoA force response was statistically unchanged throughout. Interestingly, the corrected force response remained significantly reduced compared with control rabbits, with a peak effective response of only $0.88 \pm 0.06$. $K^+$-PSS force from distal aortic rings was similar between corrected and control groups, whereas $K^+$-PSS force in the CoA group was significantly increased (Table 2).

**Cyclic strain.** CoA and corrected rabbits demonstrated significantly reduced mean and maximum strain as well as distensibility compared with control rabbits, with CoA rabbits showing the greatest reductions compared with corrected rabbits (Table 3). The pressure-strain elastic modulus was significantly increased in CoA rabbits ($47.2 \pm 4.66 \times 10^3$ N/m$^2$) compared with both control ($8.46 \pm 1.05 \times 10^3$ N/m$^2$) and corrected ($15.6 \pm 1.39 \times 10^3$ N/m$^2$) rabbits, indicating increased stiffness in CoA rabbits. Ascending aortic diameter in CoA rabbits was significantly greater than in control rabbits: 8.98 $\pm$ 0.39 vs. 7.53 $\pm$ 0.23 mm. Corrected rabbits

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**Fig. 3.** Spatial distributions of time-averaged wall shear stress (TAWSS) for 32-wk-old male New Zealand White rabbits. Results from control rabbits (top) are shown relative to CoA (middle) or corrected (bottom) rabbits at 10–12 wk of age. Each number (1–7) refers to an individual rabbit, and the numbering of rabbits is consistent in Figs. 2, 4, and 5. Arrows on the models in rabbit 1 indicate where circumferential TAWSS was obtained for the plots shown in Fig. 5. **AJP-Heart Circ Physiol** • doi:10.1152/ajpheart.00420.2012 • www.ajpheart.org
showed a slight increase in mean diameter (8.14 ± 0.20 mm), but this did not reach significance.

**DISCUSSION**

The objective of this work was to use an experimental model of CoA and correction to quantify alterations in hemodynamic and vascular biomechanics indexes and the associated expression of key SM contractile and focal adhesion proteins along with their relationship to vascular remodeling, endothelial dysfunction, and stiffness. There were three important findings, as discussed in more detail below: 1) in addition to the mechanical stimuli of elevated BP, CoA induces significant alterations in WSS that persist despite the total alleviation of the BP gradient across the coarctation; 2) endothelial dysfunction exists in both CoA and corrected rabbits; and 3) proximal NM myosin expression is increased and SM myosin expression is decreased in both CoA and corrected rabbits at locations where there is vascular remodeling with altered function and stiffness.

**TAWSS alterations in untreated and corrected CoA.** The present results confirm and extend preliminarily results documented in our previous work (43) by showing that significant alterations in TAWSS occur in both untreated and corrected CoA using a novel rabbit model. Low TAWSS occurs in a rotating pattern down the dAo of healthy adults (25, 32), and a study (73) has shown a correlation in these sites with atherosclerotic plaques. In the present investigation, CFD was used to quantify local hemodynamics circumferentially and longitudinally in untreated and corrected CoA rabbits compared with control rabbits using MRI and BP data. Untreated CoA rabbits demonstrated reduced TAWSS proximal to the coarctation and significantly elevated TAWSS distally due to the velocity jet. Results from the corrected rabbits demonstrated reduced TAWSS proximal to the suture and furthermore that the velocity jet may have persistent effects on tortuosity, with reduced TAWSS occurring in a rotational pattern along the distal wall due to the influence of the jet. These alterations to TAWSS caused by CoA that persist upon correction may thus indicate an increased risk of atherosclerotic plaque formation in the dAo of these subjects. While the general patterns of WSS and OSI in this study correspond well with available data in humans (35, 36), the use of a representative rabbit model allows the observation of CoA-induced alterations independent of any confounding factors or heterogeneity often present in clinical populations.

**Endothelial dysfunction in untreated and corrected CoA.** Proximal dAo arteries from untreated and corrected rabbits exhibited endothelial dysfunction, as ACh relaxation curves from both groups showed significant impairments, whereas the peak SNP relaxation response (endothelium independent) was unchanged. Distal arteries of the CoA group continued to show endothelial dysfunction, but no differences between corrected
and control groups were present. While endothelial dysfunction in the proximal arteries of untreated CoA rabbits is in agreement with spontaneously hypertensive animal models (16, 28, 38), the data presented from corrected rabbits are the first to demonstrate endothelial dysfunction in coarctation despite the restoration of normal BP using a chronic animal model mimicking treatment. Importantly, researchers investigating tissue specimens for viability often consider rabbit arteries exhibiting ≥50% relaxation to be nonfunctional (41), and maximum ACh relaxation from CoA and corrected rabbits in the present study was 17% and 32%, respectively, underscoring the severity of this dysfunction. These results are consistent with recent clinical evaluations of normotensive postrepair patients who demonstrated reduced forearm vasodi-

Fig. 5. Left: local quantification conducted using unwrapped TAWSS results. Numbers (1–7) next to unwrapped images refer to an individual rabbit, and the numbering of rabbits is consistent in Figs. 2–4. The locations of circumferential quantification are indicated by dashed lines and correspond to arrows on models in rabbit 1 in Fig. 3. These locations represent regions in the proximal and distal descending thoracic aorta where histological and myograph arteries were obtained. Middle: the two images show the convention used to define luminal surfaces by their outer or inner curvatures (top image) and anatomic left or right luminal surfaces (bottom image) and provide a key for the division of circumferential TAWSS plots into 16 equal sectors along these surfaces. The plots show ensemble-averaged circumferential TAWSS results (proximal and distal plots), with the distal TAWSS plot additionally magnified to elucidate differences between control and corrected values (distal zoomed plot). Right: ensemble-averaged longitudinal TAWSS plots along the outer, anatomic right, anatomic left, and inner luminal surfaces for the collection of rabbits in each group. Please note the breaks in the ordinate axes of longitudinal plots that were necessary to accommodate elevated TAWSS values in the coarctation. *CoA different from control rabbits; †corrected rabbits different from control rabbits; §CoA rabbits different from corrected rabbits (all \( P < 0.05 \)).
ulation during reactive hyperemia compared with age-matched control patients (11, 12). Atherosclerosis, particularly in coronary artery disease, is associated with endothelial dysfunction as a result of reduced endothelial NO release (17, 18, 66), and, indeed, high rates of early-onset coronary artery disease are a primary form of morbidity leading to reduced life expectancy in surgically treated CoA. Whether the etiology of endothelial dysfunction observed here is a result of ROS or increased expression of inflammatory or adhesion molecules is currently unclear, as studies have reported evidence of both phenomena (5, 12, 67).

The finding of endothelial impairment in distal arteries of CoA rabbits may be attributable to the presence of high velocity and WSS in these regions. Previous research (42) has observed remodeling and fragmentation of the IEL under increased blood flow rates. Thus, sustained conditions of increased WSS in the CoA group may serve to alter the endothelial environment and subsequently NO release. IEL fragmentation due to high WSS may also explain the slight increase in the distal dAo SNP relaxation response at several concentrations, as NO may diffuse more readily into medial SM to cause relaxation.

Altered protein expression occurs with vascular remodeling, aortic stiffening, and reduced active contractile responses. Analysis of phenotypic SM modulation, manifested by immunoreactivity of contractile and focal adhesion proteins, revealed increased NM myosin and decreased SM myosin expression in CoA and corrected rabbits proximally, whereas SM α-actin was unchanged. Phenotypic modulation occurring via decreased SM α-actin has been reported in several studies of hypertension and atherosclerosis (13, 52). However, few of these studies to date have observed the SM response in the...
Fig. 7. Left: representative micrographs of immunohistochemical staining of the proximal (top) and distal (bottom) aorta with nonmuscle (NM) myosin, smooth muscle (SM) α-actin, and SM myosin. Right: quantified staining intensity (means ± SE, pixel counts) for each location and group. *Significantly different from the control group (P < 0.05).
large arteries, and previous work with animal models of coarctation has demonstrated altered expression of SM contractile proteins without significant depression of SM α-actin (29, 31). It was reasoned in these previous studies that medial SM may be “multifunctional” and that loss of SM α-actin is not an absolute prerequisite for phenotypic changes. No changes were observed in the focal adhesion-associated proteins fibronectin and talin, suggesting that these extracellular and cytoskeletal (adherens junctions) proteins may not play a key role in remodeling in response to CoA.

Previous work has shown NM myosin to have mechanosensitive properties that allow it to respond to changes in mechanical stimuli through its expression and activation. Increased application of cyclic strain reduces NM myosin expression (54), and more recent studies (6, 7) have demonstrated NM myosin activation to be increased in cell cultures with greater rigidity. The mechanosensitive response of NM myosin is thought to involve changes to its actin-linking and contractile functions, which subsequently affect SM cell migration and adhesion. In the present study, mechanical stimuli in the form of increased systolic, mean, and pulse BP appear to result in increased NM myosin expression with decreased SM myosin and thus a shift in phenotype from contractile to synthetic SM isoform expression. This dedifferentiation may lead to medial thickening and elastin fragmentation at locations near the site of coarctation (proximal dAo). This response appears to be part of a compensatory mechanism to restore tensile stress to homeostatic levels by increasing wall thickness and may occur through SM proliferation, hypertrophy, and increased extracellular matrix deposition. Previous models of coarctation-induced hypertension have demonstrated vascular remodeling to occur as far upstream as the coronary arteries but not the cerebral vasculature (60). In the present study, medial thickening was observed upstream of the CoA in the carotid arteries, but immunohistochemistry data were not available to link these structural changes with SM phenotypic alterations. Medial thickening in the corrected group despite BP alleviation is of particular interest as it demonstrates the establishment of vascular remodeling processes that persist after the removal of the mechanical stimuli and is in agreement with postrepair clinical

Table 2. Peak $K^+$-physiological salt solution contractions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>CoA</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>13.31 ± 1.12</td>
<td>8.27 ± 0.36*</td>
<td>8.28 ± 0.14\†</td>
</tr>
<tr>
<td>Distal</td>
<td>11.05 ± 1.19</td>
<td>17.03 ± 1.09\‡</td>
<td>11.73 ± 0.46</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 4$ rabbits/group. \*CoA group significantly different from the control group ($P < 0.05$); †corrected group significantly different from the control group ($P < 0.05$); ‡CoA group significantly different from the corrected group ($P < 0.05$).

Table 3. Strain parameters delineated from phase-contrast MRI imaging data

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>CoA</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $E_{\text{iso}}$</td>
<td>0.138 ± 0.013</td>
<td>0.044 ± 0.004\‡</td>
<td>0.068 ± 0.004\†</td>
</tr>
<tr>
<td>Maximum $E_{\text{iso}}$</td>
<td>0.274 ± 0.025</td>
<td>0.082 ± 0.005\‡</td>
<td>0.156 ± 0.003\‡</td>
</tr>
<tr>
<td>Elastic modulus, N/m²</td>
<td>8.46 ± 1.05e³</td>
<td>47.2 ± 4.66e³\‡</td>
<td>15.6 ± 1.39e³</td>
</tr>
<tr>
<td>Mean diameter, mm</td>
<td>7.531 ± 0.225</td>
<td>8.976 ± 0.393*</td>
<td>8.137 ± 0.195</td>
</tr>
<tr>
<td>Aortic distensibility</td>
<td>0.038 ± 0.005</td>
<td>0.008 ± 0.002\‡</td>
<td>0.019 ± 0.002\‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 7$ rabbits/group. $E_{\text{iso}}$, Green-LaGrange strain. \*CoA group significantly different from the control group ($P < 0.05$); †corrected group significantly different from the control group ($P < 0.05$); ‡CoA group significantly different from the corrected group ($P < 0.05$).
reports (71, 72) of increased carotid intima-media thickness in human patients.

Vascular remodeling due to NM and SM myosin expression changes may cause proximal arterial stiffening, manifested through indexes of reduced distensibility and strain in untreated and corrected CoA. While strain indexes in the untreated group are also likely due to the downstream coarctation, the findings of reduced strain and distensibility in the corrected group indicates that resting mechanical properties are altered despite elevated BP alleviation and is consistent with a previous study (50) suggesting that altered vascular properties persist despite treatment. Changes in resting mechanical properties are typically reflected by alterations to collagen, elastin, and medial thickness (75), and aortic stiffening causes increased cardiac afterload and decreased coronary perfusion (46). The present results indicate that significant vascular SM dedifferentiation is present in these regions, and thus persistent vascular alterations may be caused by persistent vascular remodeling and extracellular matrix deposition, which increase medial thickness at the expense of arterial stiffening.

Myograph results demonstrated reduced active contractile tone through diminished PE force response in proximal arteries of both untreated and corrected rabbits. These findings are somewhat paradoxical as vascular remodeling observed in these proximal arteries would be expected to result in increased vessel contractility (increased contractile units in parallel increase force); however, these findings may be attributable to the shift in SM phenotype from contractile to synthetic, as evidenced by NM and SM myosin, which may cause the increase resting properties and stiffness observed. It appears that the shift of SM to NM myosin has a larger effect on force than the increase in wall thickness, as average PE force decreases. To maintain the vessel diameter, previous work (10) using hypertensive rat arterioles has shown that increasing resting force decreases the capacity for an active force response. Although studied less frequently than resistance vessels, elastic conduit arteries demonstrate an ability to adapt SM tone in response to WSS and BP to control luminal diameter. It is possible that if NM and SM myosin expression changes and vascular remodeling increase wall stiffness in response to CoA-induced mechanical stimuli, active tension of proximal aortas in untreated and treated groups must be limited to avoid excessive vasoconstriction. Since maximum active tension in the CoA and corrected groups was reduced compared with the control group in response to PE and K⁺-PSS, it is likely that active tension is reduced in the presence of increased aortic stiffness and thus does not contribute to increased total force. In the distal aorta, a reduced active force response occurred in untreated rabbits without a transient increase in BP or myosin expression changes and is thus not strictly consistent with proximal results. Since K⁺-PSS force generated in distal aortic rings did not change significantly from that in the control group, we hypothesize that the decreased PE response is not due to a decreased ability of the SM to contract. The presence of significantly reduced TAWSS in corrected rabbits at this region from CFD results may explain this reduced force, as significant alterations in WSS may affect SM contractility (as discussed below). The fact that K⁺-PSS contraction in distal CoA aortic rings was significantly greater than both control and corrected aortic rings at this region may be related to the fact that NO relaxation in the CoA distal aorta was significantly impaired. Since K⁺-PSS force results from both corrected and CoA distal aortic rings were not consistent with the trends observed in PE force results at this region, there may be a combination of factors that contribute to these changes, and further studies will be required to fully address these phenomena.

The present results should be interpreted within the constraints of several potential limitations. This work focused on the expression of several key SM contractile and focal adhesion proteins associated with coarctation-induced vascular remodeling and functional impairment. Previous work (13) has demonstrated that fibronectin plays a regulatory role in flow-induced vascular remodeling, and it is thus somewhat surprising that spatial locations of reduced TAWSS, such as the proximal dAo in CoA and distal dAo in corrected rabbits, did not show increased expression of fibronectin. This is perhaps explained by the degree to which WSS was reduced by ligation in these prior experiments (~25 dyn/cm² decrease), whereas the present study observed relative reductions in TAWSS of ~10–13 dyn/cm².

Observed differences in active force across experimental groups are thought to be a result of changes in resting and active components of SM contraction, independent of intracellular Ca²⁺ regulation. However, it is conceivable that observations may be a result of Ca²⁺-dependent pathways, including cGMP kinase and L-type channels, or Ca²⁺-independent G protein-coupling, all of which serve to alter contractility in response to sustained hemodynamic stimuli (1, 2, 14, 56). Future studies using inhibitors for Ca²⁺ channels (verapamil) and agonist activation (phenolamine) are feasible using the current methods and will allow the determination of Ca²⁺-dependent regulation in contractile force response in untreated and corrected CoA.

The mean Reynold’s number in the ascending aorta of the rabbits in the present study ranged from ~260–300, whereas a previous study (36) investigating CoA in humans found a mean Reynold’s number of ~1,100–1,500. While flow may be generally laminar in both cases, these differences suggest the flow regime of rabbits is viscous dominated, unlike the inertially dominated flow present in humans. An important implication of these differing regimes is that turbulence and secondary flows are not likely to occur in the rabbit aorta.

Simulations were performed using a rigid wall assumption since the version of CFD software used does not account for variable compliance in the thoracic aorta present under control or CoA conditions, detailed local material properties were not quantified, and to reduce computational expense. We (35) have previously observed that cyclic strain is reduced in treated CoA compared with control patients, a finding also represented with the present rabbit model of CoA. Hence, while the incorporation of variable local vessel compliance could provide more realistic values for indexes of WSS, it is unlikely that deformable CFD results would lead to appreciable differences in the findings presented here given the elevated stiffness observed in CoA and corrected rabbits.

Analysis of CFD model morphology shows that pronounced tortuosity can develop distal to the coarctation. Anecdotally, we have noticed that the severity of this tortuosity appears to be related to the location within the proximal aorta where the CoA is induced and whether a resulting velocity jet directly impacts the distal posterior luminal surface and is then redirected down.
the dAo or is dissipated within the center of the downstream aortic flow domain. Interestingly, this tortuosity is not typically observed in humans with CoA. Turbulence has previously been linked to poststenotic dilation (34, 49, 76). Hence, it is possible that the viscous-dominated regime in the thoracic aorta of the rabbit may limit poststenotic dilation that could occur in humans and ameliorate the stimulus for tortuosity introduced by a high velocity jet. However, this hypothesis remains to be tested.

Product literature for the Vicryl suture used with corrected rabbits (Polyglactin 910, Ethicon Novartis) indicates that this suture is completely absorbed after 8–10 wk. While the exact timing of when the coarctation is alleviated in vivo was not precisely determined, the product literature provides an initial strength of ~15 lbf and a known strength retention curve based on the number of days since suture implementation. An analysis using this retention curve with our previous CFD results and knowledge of rabbit aortic diameters indicates that the force exerted on the suture used to create the coarctation by integrating the distribution of tractions within this region (22) exceeds the strength indicated by the manufacturer after ~21 days. Importantly, this information suggests that the approach of inducing CoA with dissolvable Vicryl suture used for the present investigation provides the stimulus of altered hemodynamic from CoA for 3 wk before restoring BP to normal for >4 mo (~6 human years) before the experimental end point.

Conclusions. This study is the first to provide a possible explanation involving persistent endothelial dysfunction and phenotypic changes in vascular SM via altered protein expression as mechanisms for cardiovascular disease. These changes may be useful as surrogates for endothelial dysfunction in subsequent cardiovascular disease as they remain altered despite successful repair of aortic coarctation.

CoA appears to cause increased BP and tensile stress as well as reduced TAWSS in proximal arteries, which results in SMC phenotypic changes, as evidenced by altered NM and SM myosin expression. This dedifferentiation may result in increased medial thickness and stiffness, which reduces the need for an active force response. Furthermore, the increased tensile stress and reduced TAWSS cause proximal endothelial dysfunction in CoA. Distally TAWSS is markedly increased by the stenotic velocity jet, which causes endothelial dysfunction and increased SM (endothelium independent) relaxation. These results are in partial agreement with previous evidence showing that high WSS results in fragmentation and disruptions of the IEL, which may also explain altered mechanical function (42).

Despite almost complete reversal of the stimuli for vascular alterations and CoA-induced morbidity in corrected rabbits (no change in BP or TAWSS in the proximal arteries), SM cell dedifferentiation persists and is coincident with increased mean pressure gradient and stenosis of the IEL, which may also explain altered mechanical function (42).

Taken together, these results provided further evidence that the ramifications of CoA go far beyond removal of the stenosis and restoration of a favorable BP gradient, as hemodynamic indexes of morbidity persist under these current treatment guidelines. Considering these alterations persist upon correction, it is likely that current interventions for CoA could benefit by addressing these phenomena. Specifically, the data in the present study point to the possibility of inhibitors in the NM myosin activation pathway that may have therapeutic applications in CoA.

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DISCLOSURES

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

REFERENCES


