Increased aortic stiffness elevates pulse and mean pressure and compromises endothelial function in Wistar rats

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Increased aortic stiffness elevates pulse and mean pressure and compromises endothelial function in Wistar rats. Am J Physiol Heart Circ Physiol 307: H880–H887, 2014. First published July 18, 2014; doi:10.1152/ajpheart.00265.2014.—An increase in pulse pressure (PP) is highly associated with hypertension. The goal of this study was to determine the effect of increased aortic stiffness by use of a nonconstrictive restraint (glue coating) on aortic surface was created to investigate the change of PP and mean arterial pressure (MAP).

Group I (n = 16) underwent aorta restraint for 4 wk. Group II (n = 12) underwent aortic restraint for 4 wk, followed by restraint removal to evaluate extent of reversibility for additional 4 wk. The aortic and peripheral endothelial function was assessed by Ach-stimulated endothelium-dependent vasodilation. The level of nitrate/nitrite (NOx), endothelin-1 (ET-1), and prostacyclin (PGI2) were measured in the serum and artery tissue. We found that aortic stiffness causes a significant increase in PP and MAP (P < 0.05). The endothelial function was markedly blunted (P < 0.05) in both aorta and small peripheral artery. After removal of the restraint, the impaired endothelium function persisted in the aorta likely due to sustained deterioration of aortic wall, but was partially restored in peripheral artery. The endothelial dysfunction was correlated with a decrease in NOx and PGI2 (P < 0.05) and an increase in ET-1 (P < 0.05). Our results show that aortic stiffening results in widening of PP, which affected endothelium function through changes in synthesis of NOx, ET-1, and PGI2. These findings suggest that increased aortic stiffness may be a cause of increased PP and a precursor to hypertension.

Aortic stiffening has demonstrated an independent predictive value for cardiovascular events such as aging (29), hypertension (4, 13), diabetes (9), myocardial infarction (20), heart failure (6), and stroke (17). The blood pressure (BP) waveform is a composite of steady component [mean arterial pressure (MAP)] and pulsatile component [pulse pressure (PP)] (23).

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Surgical Procedures

Aorta restraint. The animals were anesthetized with intraperitoneal injections of pentobarbital (50 mg/kg). The common carotid artery was cannulated by a catheter (0.7 mm ID), which was connected to a pressure transducer. Heparin (200 U/ml) was used to prevent blood clots in the vessels. Arterial blood pressure and PP were recorded during the procedure. A laparotomy (about 3.0 cm) was performed. The distal abdominal aorta between renal and common iliac artery was carefully exposed, and tissue glue (cyanoacrylate formulation) was coated over a length of the aorta. After the glue was allowed to harden for 5–7 min, a stiff coating formed and covered the anterior and bilateral sides of aorta with an axial length of ~3.0 cm. The external geometry of the abdominal aorta was photographed to obtain the in vivo outer diameter (D) and the axial length (L) of the glue coating on the aorta.

The glue restraint area of aortic surface (A) was computed as

\[ A = \pi D \times L. \]

The sham-operated group underwent an identical surgical procedure, but without application of glue on the aorta (i.e., the same amount of glue was left near the aorta area with no direct contact with the aorta).

Restraint removal. Anesthesia, sterility, and cannulation of the common carotid artery for monitoring of arterial blood pressure and PP were the same as described above. Each of the carotid arteries was cannulated twice at two different locations along the carotid artery. A laparotomy (about 3.0 cm) was performed. The distal abdominal aorta between renal and common iliac artery was carefully exposed. The hardened glue coating around the aorta was carefully dissected and removed.

Terminal study. After blood pressure through the carotid artery catheter was measured, the animal was terminated by administration of an overdose of pentobarbital (150 mg/kg). The thoracic and proximal abdominal aorta (nonrestraint portion above renal artery) and small peripheral arteries (muscular branches of the femoral artery with diameter of 200–300 μm) were excised immediately and used for vessel function, compliance, histology, and Western blot analysis.

Endothelial Function

An isovolumic myograph recently developed by our group was used to evaluate the endothelium-dependent vasorelaxation (19). Briefly, the segments from thoracic aorta and small peripheral artery were cannulated on both ends in a physiological bath with HEPES physiologic saline solution (HEPES-PSS, concentration in mmol/l: 142 NaCl, 4.7 KCl, 2.7 sodium HEPES, 3 HEPES acid, 0.15 NaHPO4, 1.17 MgSO4, 2.79 CaCl2, and 5.5 glucose, solution gassed by 95% O2 plus 5% CO2 and stretched to a set length. The branches on the artery were ligated to prevent leakage, and the artery was then allowed to incubate in 37°C bath over 1 to 1.5 h. The pressure and external diameter were measured with pressure transducer (Mikro-Tip SPR-524; Millar Instruments) and digital diameter tracking (DiamTrak v3+; Australia), respectively. The vessel segment was maximally preconstricted with phenylephrine (PE, an agonist of α-adrenoceptor) by a series of doses (10^{-10} to 10^{-5} mol/l in the PSS) to reach maximal constriction, and then relaxed with ACh by a series of doses: 10^{-10} to 10^{-5} mol/l. The endothelium-independent relaxation to verify the sensitivity of vascular smooth muscle to NO was induced with a series of doses of sodium nitroprusside (SNP): 10^{-10} to 10^{-5} mol/l. The percent decrease in tension was calculated by the equation:

\[ \%{\text{Relaxation}} = \frac{(T_d - T_l)(T_{max} - T_l)}{100}, \]

where, \( T_d \), \( T_l \), and \( T_{max} \) are the tension at every dose of vasodilators (\( T_d \)), physiological level (\( T_l \)), and maximum tension (\( T_{max} \)) at the sub-maximal concentration of phenylephrine, respectively.

Arterial Compliance

The segments from thoracic aorta and small peripheral artery were cannulated on both ends and fully relaxed in Ca^{2+} free HEPES-PSS. The arterial segment was preconditioned with five cyclic changes in pressure from 0 to 150 mmHg. The pressure was then increased in 30-mmHg steps from 30 to 150 mmHg in a staircase manner. The passive pressure (P)-diameter (D) was recorded. The lumen cross-sectional area (CSA) was computed from the lumen diameter, D as \( D = (4CSA/\pi)^{1/2} \). The volume compliance (CV) of the artery was determined by the slope of the pressure-volume relationship; i.e., \( CV = \Delta V/\Delta P \). The normalized CSA compliance (C_{CSA}) was determined similarly as \( C_{CSA} = \Delta CSA/(\Delta P \cdot CSA) \) at the physiological pressure.

Histological Preparations

The segments from proximal abdominal aorta (nonrestraint portion) and small peripheral artery were fixed with 10% formalin in phosphate buffer for at least 6 h. The vessel was then embedded in paraffin and cross-sectioned at 4 μm thickness. Sections were stained with Verhoeff Van Gieson (VvG; Sigma-Aldrich) method to identify internal and external medial borders and elastic fibers. Stained sections were observed and imaged with a Nikon light microscope (Eclipse E600; Japan) connected to a digital camera. ImageJ was used for the quantitative analysis of elastin content and vessel medial thickness.

Serum NOx, ET-1, and PGI2 Level

Blood samples were collected in EDTA tubes before aorta restraint, at 4 wk of aortic restraint, and at 4 wk after restraint removal. After centrifugation at 5,000 rpm and 4°C for 15 min, serum was immediately separated and stored at ~80°C until analysis. The endogenous NO synthesis was evaluated as the concentrations of nitrate and nitrite (NOx, the final products of NO in serum) by colorimetric assay kit (Cayman Chemical). The circulating level of ET-1 in serum was measured by ELISA kit (R&D System, MN). Because PGI2 is unstable, its metabolite 6-keto-PGF1α was measured to reflect molecular level by EIA kit (Cayman Chemical).

Artery eNOS, ET-1, and PGIS Expression

Western blot was used to analyze the protein expression of eNOS, ET-1, and PGIS in arterial tissue. The thoracic aorta and small peripheral arteries were homogenized in a lysis buffer and then incubated on ice for 1 h. The sample was centrifuged at 1,000 g for 15 min at 1°C and the supernatant was collected. The total protein was measured by BCA kit (Bio-Rad Laboratories). Equal amounts of protein (100 μg) were loaded and electrophoresed in 4–20% SDS-PAGE gel and transferred onto a polyvinylidene difluoride membrane. After being blocked for 2 h in 6% dried milk in TBS-Tween buffer, the membrane was incubated overnight at 4°C with specific primary antibody (1:300 dilution in blocking buffer) for ET-1 provided by BD Transduction Laboratory, CA; 1:200 dilution for ET-1 and PGIS provided by Santa Cruz Biotechnology). The membrane was then rinsed and incubated with horseradish peroxidase-conjugated secondary antibody for 2 h (1:5,000 dilution in blocking buffer, Santa Cruz Biotechnology). The specific protein was detected by enhanced chemiluminescence (ECL, Amersham Biosciences, NJ) and evaluated by densitometry (Sigma Scan, systat Software, CA). All samples from each group were simultaneously probed with anti-β-actin, a mouse monoclonal antibody (primary antibody 1:1,000 dilution in blocking buffer, Santa Cruz Biotechnology) to correct for sample loading. Positive control protein was obtained from cultured human whole cell lysate provided by Santa Cruz Biotechnology. The final value for eNOS, ET-1, and PGIS proteins densitometry was computed as the ratio of protein density to β-actin density.

Statistical Analysis

Results were shown as means ± SD or means ± SE as specified, and significance of the differences between two groups was evaluated by either one-way ANOVA or t-test. Significant differences between
the dose-dependent groups for endothelial function testing were determined by two-way ANOVA. The results were considered statistically significant when \( P < 0.05 \) (2-tailed).

**RESULTS**

All the Wistar rats (3 groups) subjected to surgical aortic restraint and restraint removal survived until the termination date. Four weeks of aorta restraint had no significant influence on the body weight of the experimental group as compared with the sham. The age and the mean body weight for the experimental and sham-operated groups are shown in Table 1.

**Blood Pressure**

Before surgical intervention, a comparison of PP, MAP, systolic blood pressure (SBP), and diastolic blood pressure (DBP) between experimental and sham-operated groups did not show statistically significant differences. Table 1 lists PP, MAP, SBP, and DBP before and after aorta restraint and restraint removal for the experimental and sham-operated groups. No difference in PP, SBP, DBP, and MAP was seen in sham-operated group before and after restraint and restraint removal. After 4 wk of restraint, the PP, SBP, and MAP were significantly increased in experimental compared with pre-intervention level of experimental and sham-operated group (\( P < 0.01 \)). Although PP with restraint removal was decreased by 10% in the experimental group as compared with restraint (\( P = 0.02 \)), the value was still significantly higher than that in sham (\( P < 0.01 \)). The SBP and MAP after 4 wk of restraint removal tended to decrease, but the change was not statistically significant as compared with restraint and sham-operated groups. The DBP was practically unchanged after restraint and restraint removal as compared with pre-intervention level of experimental and sham. Figure 1 shows the relationship between PP change (percentage) and the restraint area of aorta covered by glue. The percent change in PP was calculated as: 

\[
\% \text{PP change} = \left( \frac{\text{PP after restraint} - \text{PP before restraint}}{\text{PP before restraint}} \right) \times 100.
\]

The change in PP after restraint increased linearly with an increase in the area of aortic restraint, and the data were fitted with a linear least-square fit \( (R^2 = 0.70, P < 0.01) \).

**Arterial Compliance**

Figure 2 shows the normalized CSA compliance in thoracic aorta and small peripheral artery after aorta restraint and restraint removal. The normalized CSA compliance of aorta was significantly decreased after restraint and restraint removal in experimental compared with the sham-operated group \( (P < 0.05) \). For the small peripheral artery, the CSA compliance after restraint was significantly lower in experimental than that in sham \( (P < 0.05) \), but it was restored to the sham level after 4 wk of restraint removal.

**Histological Sections**

Figure 3A shows representative VvG stained images of aorta (a nonrestraint segment from abdominal aorta) and small peripheral artery after aorta restraint and restraint removal. The aorta restraint caused severe elastin fragmentation, defined as focal fragmentation of elastic lamellae in aortic media. The quantitative analysis showed that the total medial thickness of aorta was significantly increased \( (P < 0.05) \) with a pronounced

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**Table 1. Age, body weight, and blood pressure for experimental and sham-operated groups before and after aortic restraint and restraint removal**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>After Restraint, 4 wk</th>
<th>After Removal, 4 wk</th>
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<tbody>
<tr>
<td>Age, wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp</td>
<td>13.2 ± 0.7</td>
<td>17.6 ± 1.0</td>
<td>21.5 ± 1.1</td>
</tr>
<tr>
<td>Sham</td>
<td>13.6 ± 0.5</td>
<td>17.9 ± 0.6</td>
<td>22.0 ± 0.8</td>
</tr>
<tr>
<td>Weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp</td>
<td>423.1 ± 26.7</td>
<td>495.5 ± 30.8</td>
<td>551.1 ± 46.1</td>
</tr>
<tr>
<td>Sham</td>
<td>428.4 ± 35.3</td>
<td>498.3 ± 26.4</td>
<td>560.2 ± 32.4</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp</td>
<td>98.9 ± 10.1</td>
<td>107.3 ± 12.2*</td>
<td>103.5 ± 9.0</td>
</tr>
<tr>
<td>Sham</td>
<td>99.4 ± 9.3</td>
<td>98.9 ± 10.1</td>
<td>101.3 ± 10.9</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp</td>
<td>117.9 ± 11.4</td>
<td>132.6 ± 14.4*</td>
<td>126.2 ± 11.9</td>
</tr>
<tr>
<td>Sham</td>
<td>117.4 ± 8.9</td>
<td>116.6 ± 9.6</td>
<td>119.7 ± 10.4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
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<td></td>
<td></td>
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<tr>
<td>Exp</td>
<td>89.5 ± 9.8</td>
<td>94.6 ± 11.5</td>
<td>92.2 ± 14.0</td>
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<tr>
<td>Sham</td>
<td>90.2 ± 9.1</td>
<td>90.0 ± 10.6</td>
<td>92.1 ± 9.7</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
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</tr>
<tr>
<td>Exp</td>
<td>28.4 ± 4.5</td>
<td>37.6 ± 5.7*</td>
<td>33.9 ± 5.2*#</td>
</tr>
<tr>
<td>Sham</td>
<td>27.2 ± 3.8</td>
<td>26.5 ± 4.7</td>
<td>27.4 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. Exp, experimental group; sham, sham-operated group. *\( P < 0.05 \), when compared with baseline and sham; #\( P < 0.05 \), when compared with restraint.
decrease in elastin contents ($P < 0.05$) in restraint and restraint removal groups compared with sham as shown in Fig. 3, B and C. For the small peripheral artery, VvG staining revealed that elastin is mainly restricted to a fenestrated internal elastic lamina and external elastic lamina (Fig. 3A). No difference in the medial thickness and elastin contents was observed between restraint and restraint removal and sham-operated groups (Fig. 3, B and C).

**Endothelial Function**

Endothelial function was evaluated by ex-vivo PE precontractile endothelium-dependent vasorelaxation. In the segments from thoracic aorta and small peripheral artery, the vascular contraction in response to PE was not significantly different in the experimental and sham-operated groups (data not shown). Figure 4 shows endothelial function represented by endothelium-dependent vasorelaxation in response to ACh after restraint and restraint removal. The 4 wk of aorta restraint caused a compromised endothelium-dependent relaxation compared with the sham ($P < 0.05$) in both aorta (Fig. 4A) and small peripheral artery (Fig. 4B). In aorta, restraint removal group continued to show a decrease in endothelium-dependent relaxation ($P < 0.05$) as compared with sham. The endothelial dysfunction for the small peripheral artery, however, was partially improved after restraint removal as compared with the restraint group, which is not significantly different from sham. Endothelium-independent relaxation to SNP did not show significant difference before and after restraint (data not shown) for both aorta and small peripheral artery.

**Serum NOx, ET-1, and PGI2 Level**

Figure 5 shows the serum NOx, ET-1, and PGI2 metabolite (6-keto-PGF1α) level after aorta restraint and restraint removal for experimental and sham-operated groups. The NOx level was significantly decreased in experimental group after 4 wk of restraint as compared with baseline ($P < 0.05$). With restraint removal, the NOx level tended to increase in the experimental group, albeit it was not significantly different from restraint group (Fig. 5A). The serum ET-1 level after restraint was found to be higher in the experimental group compared with baseline of experimental and sham groups ($P < 0.05$), which was not significantly different from restraint removal group (Fig. 5B). In the experimental group, the serum PGI2 metabolite level was significantly decreased after aortic restraint as compared with baseline of experimental and sham-operated group ($P < 0.05$). After restraint removal, the PGI2 level increased as compared with that of restraint group, albeit it did not reach statistical significance (Fig. 5C).

**Artery eNOS, ET-1, and PGIS Expression**

Figure 6 shows Western blot and quantitative analysis of eNOS, ET-1, and PGIS protein expressions in thoracic aorta and small peripheral artery for the experimental and sham-operated groups. The protein band at 140 kDa for eNOS, 24 kDa for ET-1, and 52 kDa for PGIS was detected. The anti-β-actin antibody reacted with a 42 kDa protein corresponding to the size of β-actin. The eNOS expression in aorta and peripheral artery was significantly lower in restraint group as compared with sham ($P < 0.05$). After 4 wk of restraint removal, the eNOS expression in aorta tended to increase as compared with the restraint group, albeit it remained significantly lower than that in the sham-operated group ($P < 0.05$). The ET-1 protein expression was significantly increased after 4 wk of restraint compared with the sham-operated group ($P < 0.05$). After restraint removal, the ET-1 expression significantly decreased in peripheral artery, which was not statistically different from sham. The aorta ET-1 expression tended to decrease after restraint removal, but the change was not statistically significant compared with restraint and sham-operated groups. For PGIS protein expressions, a significant decrease in aorta was observed in the restraint group as compared with the sham ($P < 0.05$). Moreover, the PGIS expression in aorta remained lower in the 4 wk after restraint removal ($P < 0.05$). The PGIS expression of peripheral artery was significantly decreased after 4 wk of restraint as compared with sham ($P < 0.05$), and it was restored to normal level after restraint removal.

**DISCUSSION**

The major findings of the study were as follows: 1) four weeks of aortic restraint causes a significant increase in PP and MAP; 2) a significant decrease in arterial compliance was confirmed in both aorta and peripheral artery in response to aortic stiffening; 3) a dose-response relation was observed between the surface area of aortic restraint and the degree of PP change; 4) endothelial dysfunction occurred in both aorta and peripheral artery following aorta restraint, but was partially recovered in the peripheral artery with restraint removal; 5) aortic stiffening results in a decrease in NOx and eNOS and PGII2 (and PGIS) and an increase in ET-1 level in blood and tissue.

**Blood Pressure**

There is general consensus that PP provides a measure of arterial stiffness and is an important risk factor for cardiovascular diseases (2, 3, 11). In this study, 4 wk of aortic restraint with glue coating caused a significant increase in PP (33%, $P < 0.001$), SBP (12%, $P < 0.01$), and MAP (8.8%, $P = 0.03$), but had no significant influence on DBP (Table 1). These data suggest that SBP, rather than DBP, is the major determinant of the PP increase in this aortic stiffening model. Furthermore, the PP change was positively correlated to the restraint area of aortic surface (Fig. 1), which indicates a dose-response relation between the degree of aortic stiffness and PP. To provide further support for the cause-and-effect relation, we evaluated the reversibility of the aortic stiffness by removal of glue coating from the aorta. We found that PP remained higher in comparison with sham due to the incomplete reversibility of aortic compliance. The restraint induces persistent deterioration of structural (medial thickening) and elastic properties (obvious elastin fragmentation and reduced elastin content) on the medial layer of the nonrestraint aortic segment as shown in the histological sections (Fig. 3).

A bidirectional relation between arterial stiffness and hypertension has been recently proposed (18). A recent study by Weissbrod et al. (32) showed that arterial stiffness precedes hypertension in an animal model of diet-induced obesity and normalization of the obese state leads to return of arterial...
stiffness and blood pressure to normal. It is reported that an increase in PP may trigger rarefaction (as does obesity), remodeling, and increased tone in the microcirculation, which may secondarily increase MAP (14). Our current findings suggest that aortic stiffening for 4 wk through a restraint applied locally without the systemic effects (e.g., such as in obesity) results in a persistent and marked increase in PP. Although the MAP was only increased by 8.8%, it is likely that the severity of hypertension may increase with greater degree of aortic restraint or longer term studies. Future studies should include telemetry to monitor blood pressure in freely moving animals for longer durations of aortic restraint.

**Arterial Compliance**

Compliance of arteries expresses the relationship between arterial volume responses to change in pressure. Reduced arterial elasticity, measured in terms of vascular compliance, is an early sign of vascular damage and can be used as a surrogate marker of arterial function (8). In the present study, we observed a significant decrease in compliance of aorta and small peripheral artery in response to aortic stiffening, suggesting an impaired arterial elasticity (Fig. 2). Moreover, we found the compliance of peripheral artery to be restored to normal value after restraint removal, whereas it remained lower in the aorta as compared with sham. These results imply that the increased aortic stiffness at the site of restraint placement causes an increase in PP, which may further stiffen the rest of the aorta and dynamically extend to peripheral arteries. It has been reported that the principal structural modification of the vessel wall is hypertrophy of the medial layer in subjects with hypertension (16). We speculate that 4 wk of restraint induces slowly reversible aortic stiffening due to structural deteriora-

**Fig. 3.** *A*: representative Verhoeff Van Gieson (VvG) staining of sections from proximal abdominal aorta (nonrestraint part) and small peripheral artery in aorta restraint and restraint removal groups as compared with spatially equivalent locations from sham-operated group. The medial layer of aorta containing fragmented lamellae devoid of darkly stained elastin was seen in restraint and restraint removal groups. Magnification, ×200. *B*: medial wall thickness and elastin content (C) of aorta and small peripheral artery for sham, aorta restraint, and restraint removal group. Data correspond to means ± SD. *P* < 0.05, restraint (∏ = 11) or restraint removal group (∏ = 10) compared with sham-operated group (∏ = 8).
tion of vessel wall (medial thickening as shown in Fig. 3), accounting for the sustained low aortic compliance after restraint removal. In small peripheral arteries, however, no substantial structural changes of vessel wall were seen despite a transient decrease in arterial compliance. It is possible that adaptive mechanisms may be involved in the mechanical and morphological changes of peripheral arteries during the onset of hypertension. Accordingly, the peripheral compliance was recovered with the restraint removal.

**Endothelial Dysfunction**

Dysfunction of the vascular endothelium has been associated with high blood pressure and arterial stiffening (24, 27). An animal study by Ryan et al. (26) exhibited a correlation between PP elevation and endothelial dysfunction assessed by ACh reactivity in intact carotid arteries of rabbits. Similarly, our data show the endothelium-dependent relaxations to ACh were markedly blunted with an increase in PP and MAP due to acute aortic stiffening (Fig. 4). Although PP remained elevated following restraint removal, the decrease in MAP partially restored the endothelium function in peripheral artery (Fig. 4B). In contrast, the thoracic aortic segment after restraint removal continued to show significantly impaired ACh relaxation (Fig. 4A), which is consistent with the persistent deleterious changes in aortic vascular structure following restraint removal (Fig. 3). Our findings provide direct evidence that aortic stiffening increases PP and MAP and leads to compromised endothelial function of aorta and peripheral artery, which may progress to hypertension. The separate potential roles of PP and MAP in endothelial dysfunction remain to be determined in the future. In addition, we found that the smooth muscle contraction under PE was unchanged before and after restraint, suggesting that the expression and signal transduction of adrenergic receptors is maintained during 4 wk of aorta restraint. The comparable NO donor (SNP) induced endothelium-independent vasorelaxation suggests that 4 wk of aortic restraint has no influence on vascular smooth muscle relaxation in response to NO. This result implies that the impaired response to ACh may be attributed to functional alterations of the endothelium rather than vascular smooth muscle.

**Endothelium-Derived Vasoactive Factors**

Endothelial cells release both relaxing and contracting factors that modulate vascular smooth muscle tone and contribute to the pathophysiology of essential hypertension.

![Fig. 4. Endothelial function of thoracic aorta (A) and peripheral artery (B) represented by endothelium-dependent vasorelaxation in response to ACh after restraint and restraint removal. Data correspond to means ± SE. *P < 0.05, statistical difference of the dose-dependent curve when restraint (n = 12) or restraint removal group (n = 9) compared with sham-operated group (n = 8).](image)

![Fig. 5. Serum nitrate/nitrite (NOx; A), endothelin-1 (ET-1; B), and 6-keto-PGF1α (C) levels after aorta restraint and restraint removal for experimental and sham-operated group. Data correspond to means ± SE. *P < 0.05, experimental group (n = 26) compared with sham-operated group (n = 8).](image)
Fig. 6. Western blot and quantitative analysis of endothelial nitric oxide synthase (eNOS), ET-1, and PGI synthases (PGIS) protein expression in aorta (A) and peripheral artery (B) for sham, aorta restraint, and restraint removal group. Data correspond to means ± SD. *P < 0.05, restraint (n = 16) or restraint removal group (n = 12) compared with sham-operated group (n = 8). (24). NO is the major relaxing factor synthesized in arterial endothelium by eNOS, which is expressed constitutively in endothelial cell (22). Diminished eNOS expression or activity has been proposed as an important mechanism leading to reduced NO availability and endothelial dysfunction in hypertensive animals (12). Our direct measurement of serum NOx (aqueous oxidation products of NO) after 4 wk of aortic restraint showed that aortic stiffening significantly reduces the NOx production (Fig. 5A). Moreover, the down-regulated synthesis of NOx was partially recovered with restraint removal. Similar to the changes of NOx, the eNOS expression of aortic and small peripheral arterial tissue revealed a significant decrease in response to aortic stiffening. Four weeks of restraint removal reversed the eNOS expression in peripheral artery, but not in aorta (Fig. 6). These results imply that the impaired release of endothelial NO is likely due to reduced levels of eNOS which may contribute to aortic stiffening induced endothelial dysfunction.

It is well accepted that increased production of vasoconstrictor factors are involved in reduced endothelium-dependent relaxations. ET-1 is the most potent mammalian vasoconstric-

tor peptide produced by endothelial cells in pulmonary and systemic circulations (31). In the present study, we found that the serum ET-1 level in aorta and peripheral artery ET-1 expression were significantly increased after aorta restraint (Figs. 5B and 6). With restraint removal, the ET-1 production in serum and aorta tissue tended to decrease, albeit it did not reach statistical significance. In peripheral artery with the partial recovery of endothelial function following restraint removal; however, ET-1 expression was restored to the same level of the sham. In fact, increased levels of ET-1 in blood and lung tissue have been confirmed in the course of pulmonary hypertension (5). Our results suggest that the upregulated synthesis of ET-1 in blood and vessels is associated with endothelial dysfunction as a result of aortic stiffening, which may play a role in the pathogenesis of hypertension.

Endothelium-dependent vasodilation is not only modulated by NO but also by PGI2. PGI2 is synthesized predominantly by endothelial cells which inhibits platelet aggregation and has a vasodilating effect (7). PGIS, a catalyst of PGI2 synthesis from prostaglandin H2, is widely found in vascular endothelial and smooth muscle cells (28). In woman with pregnancy-induced hypertension and some patients with essential hypertension, plasma PG12 synthesis has been evaluated by measuring PGI1α and has proved to be greatly reduced (15). In the current study, the serum level of 6-keto-PGF1α, a PGI2 metabolite, was found to decrease with an increase in PP following aortic restraint (Fig. 5C). After restraint removal, although an increasing trend in serum PGI2 level was observed, the change was not significantly different from the sham. In accordance with the change in serum PGI2, aortic restraint significantly decreases the PGIS protein expression in aortic and peripheral arterial tissue (Fig. 6). After 4 wk of restraint removal, the PGIS expression remained lower in aorta coupled with unchanged endothelial dysfunction, but returned to normal in peripheral artery coupled with improved endothelium function. This result is in agreement with the report by Tuder et al. (30), who examined the lungs of patients with pulmonary hypertension and found a decreased expression of the PGIS in the vessels. Our findings suggest that the endothelial dysfunction induced by aortic stiffening and PP elevation may be responsible for the marked decrease of PGI2 (and PGIS) and NOx (and eNOS) and the increase of ET-1 synthesis in blood and vessels, which may in turn amplify hypertension.

Conclusion

The current study demonstrates that an increase in PP induced by aortic stiffening results in deleterious alterations in aortic vascular structure and endothelial function, which in turn, may further widen the increased PP and worsen arterial stiffening via the imbalance of NOx, ET-1, and PGI2 synthesis in the course of hypertension. These findings support the concept that increased aortic stiffness may be a cause of PP elevation and a precursor to hypertension. Future studies on the effects of NO donors, ET-1 inhibitors, and other antihypertensive drugs on the reversibility of aortic stiffening and PP elevation are necessary to provide additional insights for the development of therapeutic strategies to combat arterial stiffness and associated hypertension.

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

AUTHOR CONTRIBUTIONS

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