Oxidative Stress

Endothelial Dysfunction and Enhanced Contractility in Microvessels From Ovariectomized Rats
Roles of Oxidative Stress and Perivascular Adipose Tissue

Dan Wang, Cheng Wang, Xie Wu, Wei Zheng, Kathryn Sandberg, Hong Ji, William J. Welch, Christopher S. Wilcox

Abstract—Ovarian hormone loss increases reactive oxidative species, endothelial dysfunction, and cardiovascular disease. Because perivascular adipose tissue (PVAT) regulates endothelial function, we hypothesized that reactive oxidative species in PVAT mediate adverse microvascular effects of ovarian hormone deficiency. Rats were ovariectomized or sham operated and given vehicle or tempol for 6 weeks. Mesenteric resistance arterioles from ovariectomized compared with sham-operated rats had dysfunctional responses to acetylcholine (ACh) including decreased ACh-induced endothelium-dependent relaxation (50±6% versus 72±2%) and endothelium-dependent relaxation factor (17±4% versus 37±2%) and increased endothelium-dependent contracting factor (27±5% versus 9±3%). OVX rat mesenteric arterioles had increased contractions to the thromboxane/prostanoid receptor agonist U-46619 (58±3% versus 40±5%) and increased reactive oxidative species (tempo-9-AC fluorescence) with U-46619 (0.65±0.17 versus 0.14±0.06 Δ unit) or ACh (0.49±0.09 versus 0.09±0.05 Δ unit) and increased p22<sup>phox</sup> protein expression (0.89±0.05 versus 0.18±0.04 Δ unit), whereas nitric oxide activity (DAF-FM [4-amino-5-methylamino-2,7'-difluorofluorescein diacetate] fluorescence) with ACh was reduced (0.39±0.1 versus 0.70±0.10 Δ unit). No differences were found in endothelium-dependent hyperpolarizing factor or contractile responses to phenylephrine. PVAT restored ACh-induced relaxation, endothelium-dependent relaxation factor, and nitric oxide only in sham-operated rats. Tempol prevented ovariectomy-induced endothelial dysfunction and restored the enhancing effects of PVAT on ACh-induced relaxation, endothelium-dependent relaxation factor, and nitric oxide in ovariectomized rat vessels, but both tempol and PVAT were required to normalize the enhanced U-46619 contractions after ovariectomy. In conclusion, ovariectomy redirects endothelial responses from relaxation to contraction by reducing vascular nitric oxide, augmenting thromboxane/prostanoid receptor signaling, and attenuating the vasodilatory effects of PVAT, all of which were dependent on reactive oxidative species. (Hypertension. 2014;63:1063-1069.)

Key Words: menopause • nitric oxide • ovariectomy • reactive oxygen species • tempol • thromboxane prostanoid receptors

Ovarian hormone loss in women is associated with increased vascular reactive oxygen species (ROS), reduced endothelial nitric oxide (NO) synthase expression and NO, increased endothelial dysfunction, and risk of cardiovascular disease (CVD). Similar findings have been reported in ovariectomized animal models. Endothelial dysfunction entails both impaired relaxation and an endothelium-dependent contracting factor (EDCF) generated by ROS. The EDCF is a prostaglandin or thromboxane product of cyclooxygenase and thromboxane A<sub>2</sub> synthase that vasoconstricts vascular smooth muscle cells (VSMCs) by activating thromboxane/prostanoid receptors (TP-Rs). Ovariectomy leads to a cyclooxygenase-dependent EDCF response in rat mesenteric arteries and in pig coronary arteries. Furthermore, endothelial dysfunction in cutaneous blood vessels of postmenopausal women was mediated by cyclooxygenase-2. However, the effects of ovariectomy on ROS and TP-R signaling in microvessels remain poorly understood.

Obesity is an independent CVD risk factor whose incidence is increased in women with ovarian hormone loss or animal models of ovarian hormone deficiency. Perivascular adipose tissue (PVAT) improves vascular function through generation of NO, hydrogen peroxide, hydrogen sulphide, and poorly characterized adipokine pathways. ROS inhibit these protective effects of PVAT in hypertensive models and contribute to endothelial dysfunction in animal models and in patients with obesity and the metabolic syndrome.

We tested the hypothesis that ovariectomy impairs PVAT-induced microvascular NO-dependent relaxation and enhances endothelium-dependent contractions through ROS and enhanced TP-R signaling. We reduced ROS using tempol which is a redox cycling nitroxide and activated TP-Rs with
the stable agonist U-46619. These experiments are clinically significant because restoration of endothelial function and NO and abrogation of EDCF and enhanced TP-R signaling could attenuate the enhanced CVD risk after ovarian hormone deficiency, but it is unclear whether the microvessels and the surrounding PVAT should be the preferred therapeutic target.

Methods

Animal Preparation and Protocols
Female Sprague-Dawley rats (200–220 g; Taconics Lab, Germantown, NY) maintained on tap water and standard chow (Na+ 0.3 g · 100 g–1) under conditions approved by the Institutional Animal Care and Use Committee of Georgetown University underwent ovariectomy or sham surgery (SHAM) and received oral tempol (2 mmol·L–1) or vehicle in the drinking water for 6 weeks. After euthanasia by exsanguination, the second branch mesenteric resistance arterioles were isolated with the surrounding PVAT intact (PVAT+) or removed (PVAT–) and mounted in a 4-chamber myograph or snap frozen and stored at –80°C for analysis of p22phox protein.

Vascular ACh-Induced Endothelial Responses, NO, ROS Activities, and Contractions
The media and lumen cross-sectional areas of mesenteric arterioles were measured. For the first series, mesenteric arterioles were pre-constricted with 10–4 mol·L–1 norepinephrine to study: ACH-induced relaxation (endothelium-dependent relaxation [EDR]), endothelium-dependent relaxation factor (EDRF; change in EDR after 10–6 mol·L–1 L-NG-nitroarginine methylster), endothelium-dependent hyperpolarizing factor (EDHF; change in EDRF after 10–5 mol·L–1 l-arginine), and endothelium-independent responses (relaxation to sodium nitroprusside). To study ACH-induced NO, arterioles were loaded with 5×10–5 mol·L–1 DAF-FM (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate). The change of fluorescence (ΔF/F0) with 10–5 mol·L–1 ACH quantified vascular NO.

For the second series, EDCF was measured in arterioles under spontaneous tone with relaxation pathways blocked by L-NG-nitroarginine met hylster+aminopentachloro and contracted with ACH. Other vessels prepared similarly were loaded with tempo-9-AC and the change of fluorescence with 10–5 mol·L–1 ACH determined to quantify vascular ROS. For the third series, vessels were contracted with phenylephrine or U-46619. ROS generation with 10–6 mol·L–1 U-46619 was quantitated by tempo-9-AC fluorescence.

Expression of p22phox
The protein expression of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase regulatory subunit p22phox in lysates of mesenteric arterioles was measured by Western blot and normalized to β-actin.

Statistics
Data are expressed as mean±SEM calculated from 6 rats per group. The concentration–response relationships were analyzed by 2-way repeated measures ANOVA to assess the effects of ovariectomy, PVAT, and the interaction (effects of PVAT on responses to ovariectomy) followed, where appropriate, by Bonferroni tests. Separate analyses were undertaken in the groups given tempol. Significance was defined as P<0.05.

Results

Body Weight, Vascular Structure, and p22phox Protein Expression
Ovariectomy increased body weight by 16%, whereas tempol reduced it by a similar degree (Table 1). Ovariectomy almost doubled the mesenteric artery media area without changing the lumen area, resulting in a doubling of the ratio of media to lumen. The p22phox protein expression was increased by 400% in ovariectomized rat vessels (Figure 1). These effects of ovariectomy were prevented by tempol.

Effects of Ovariectomy and PVAT on Endothelial Relaxation and Generation of NO
The maximum ACh-induced relaxation and EDRF responses in preconstricted mesenteric arterioles from ovariectomized rats were reduced by 30±3% and 53±3% (P<0.05), respectively (Figure 2A and 2B), but the EDHF and endothelium-independent responses were maintained (Table 2). Vascular NO activity with ACH was reduced in mesenteric arterioles from ovariectomized rats by ≈2-fold (P<0.001; Table 2; Figure 3A).

The presence of PVAT around mesenteric arterioles from SHAM rats increased their ACH-induced relaxation and EDRF response by 20±4% and 29±3%, respectively (P<0.05; Figure 2A and 2B), and increased their vascular NO activity with ACH by 41±4% (P<0.001; Figure 3A) without changing their EDHF or endothelium-independent responses (Table 2). However, these effects were lost in ovariectomized rat vessels.

EDCF contractions were increased by 200% in vessels from ovariectomized rats (Figure 2C), accompanied by a 500% (P<0.001) increase in vascular ROS activity (Table 2; Figure 3C). PVAT reduced the EDCF responses in ovariectomized rats (P<0.01) without reducing the associated vascular ROS generation.

Contractions with phenylephrine were unchanged in mesenteric arterioles from ovariectomized rats, but contractions with U-46619 were increased by 45% (P<0.05; Table 2; Figure 4B), accompanied by a 350% increase in vascular ROS (P<0.001; Figure 3E). PVAT moderated the contractions with U-46619 in both SHAM and ovariectomized rat vessels and moderated the accompanying ROS generation in vessels from ovariectomized rats. Nevertheless, both the vascular contractions and the ROS generation with U-46619 in ovariectomized rat vessels with PVAT remained >3-fold higher than in vessels from SHAM rats, despite the moderating effects of PVAT (Table 2).

Table 1. Effect of Ovariectomy and Tempol on Body Weight and MRA Structure and p22phox Expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHAM</th>
<th>O VX</th>
<th>SHAM+Tempol</th>
<th>O VX+Tempol</th>
<th>By ANOVA, Effect of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>251±11</td>
<td>287±9</td>
<td>219±5</td>
<td>246±6</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>MRA media area (M), μm²</td>
<td>48±5</td>
<td>91±7†</td>
<td>51±5</td>
<td>48±6</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>MRA lumen area (L), μm²</td>
<td>187±12</td>
<td>170±6</td>
<td>178±14</td>
<td>193±5</td>
<td>NS</td>
</tr>
<tr>
<td>MRA M/L ratio (cross-section area), μm²/μm²</td>
<td>1.54±0.29</td>
<td>3.43±0.38*</td>
<td>1.53±0.24</td>
<td>1.63±0.19</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>MRA p22phox expression (relative to β-actin)</td>
<td>0.18±0.04</td>
<td>0.89±0.05†</td>
<td>0.28±0.11</td>
<td>0.26±0.14</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean±SEM value (n=6 per group). Compared with SHAM: *P<0.05; †P<0.01. MRA indicates mesenteric arteriole; NS, nonsignificant; and O VX, ovariectomized.
Effects of Tempol on Endothelial Function, Contractility, and Generation of NO and ROS

Tempol did not affect the vascular responses or NO generation of vessels from SHAM rats. However, it improved these responses in ovariectomized rat vessels and prevented the enhanced EDCF responses (Table 2; Figure 2F) and ROS generation (Table 2; Figure 3D). However, although tempol prevented the enhanced vascular ROS generation with U-46619 in ovariec-tomized rats given tempol was 17±4% with PVAT which was similar to the value of 10±2% in vessels with PVAT from SHAM rats (P=NS). Thus tempol+PVAT normalized the enhanced contraction to U-46619 in vessels from ovariectomized rats. Again, the effects of PVAT to moderate U-46619 contraction after tempol occurred independent of ROS which was effectively suppressed by tempol in vessels without PVAT. Thus, neither PVAT nor tempol alone was sufficient to prevent enhanced contractions to U-46619, but the contractions were normalized by their combination. This suggests that ovariectomy enhanced contractions to U-46619 by independent effects of enhanced ROS and diminished blunting by PVAT. Thus, a primary effect of tempol on ovariectomized rats was to restore normal signaling from PVAT which blunted the response to U-46619 in the adjacent blood vessels.

Discussion

This study confirms previous findings that ovariectomy or ovarian hormone deficiency lead to endothelial dysfunction and enhanced vascular oxidative stress. The main new findings are that defects in EDR in ovariectomized rat vessels were specific for EDRF because they did not affect tempol administered to ovariectomized rats without PVAT restored endothelium-dependent relaxations and EDRF and attenuated EDCF responses (Table 2; Figure 2D–2F). However, the enhanced contractions to U-46619 were not significantly moderated by tempol (Figure 4D), despite prevention of the enhanced generation of ROS. This indicated that there was a ROS-independent vascular effect of ovariectomy to enhance U-46619 contractions.

PVAT reduced the contractions to U-46619 in ovariec-tomized rat vessels by 40% (Table 2; Figure 4B), but after tempol, PVAT reduced the contractions by 70% (Table 2; Figure 4D). The U-46619 contractions in vessels from ovariec-tomized rats given tempol was 17±4% with PVAT which was similar to the value of 10±2% in vessels with PVAT from SHAM rats (P=NS). Thus tempol+PVAT normalized the enhanced contraction to U-46619 in vessels from ovariectomized rats. Again, the effects of PVAT to moderate U-46619 contraction after tempol occurred independent of ROS which was effectively suppressed by tempol in vessels without PVAT. Thus, neither PVAT nor tempol alone was sufficient to prevent enhanced contractions to U-46619, but the contractions were normalized by their combination. This suggests that ovariectomy enhanced contractions to U-46619 by independent effects of enhanced ROS and diminished blunting by PVAT. Thus, a primary effect of tempol on ovariectomized rat vessels was to restore normal signaling from PVAT which blunted the response to U-46619 in the adjacent blood vessels.

Figure 1. Effect of ovariectomy and tempol on p22phox protein expression. Means±SEM values normalized to β-actin in mesenteric arterioles from sham-operated (SHAM; open bar) or ovariectomized (OVX; hatched bar) rats after 6 weeks of vehicle or tempol treatment (n=6 per group). Top. Representative Western blot used to quantify p22phox expression. MRA indicates mesenteric arteriole.

Figure 2. Effect of perivascular adipose tissue and ovariectomy on acetylcholine (Ach)-induced relaxation, endothelium-dependent relaxation factor (EDRF), and endothelium-dependent contracting factor (EDCF) responses after 6 weeks of vehicle or tempol administration. Means±SEM (n=6 per group) values for Ach-induced relaxation (A and D), EDRF (B and E), and EDCF (C and F) in mesenteric arterioles with perivascular adipose tissue (PVAT; solid symbol) or without PVAT (open symbol) from sham-operated (SHAM; circles) or ovariectomized (OVX; squares) rats after 6 weeks of vehicle (A–C) or tempol (D–F) treatment. Comparing PVAT+ vs – in same treatment groups (*P<0.05; **P<0.01). Comparing SHAM vs OVX in same treatment groups (†††P<0.005). NS indicates non significant.
EDHF and were accompanied by reduced NO generation. Ovariectomized rat vessels had an enhanced expression of p22\(^{phox}\), which is a critical regulatory component of NADPH oxidase. Indeed, these vessels developed enhanced ACh- and U-46619-induced ROS generation and contractions.

The main new findings related to PVAT signaling are summarized in Figure 5. The presence of PVAT surrounding the mesenteric arteries from SHAM rats enhanced their EDRF responses and NO generation and diminished their contractions to U-46619 substantially without modifying the modest vascular ROS generation or contractions to phenylephrine. In contrast, PVAT surrounding ovariectomized rat vessels failed to enhance EDRF responses or to restore NO generation. Furthermore, PVAT was less effective in moderating the contractions to U-46619 after ovariectomy and failed to prevent the associated ROS generation.

As in prior studies,\(^{3,18,19}\) tempol did not affect relaxation or contraction responses or NO generation of SHAM vessels, implying that ROS have little effect on normal vascular function. The main new findings related to tempol in ovariectomized rat vessels are that it prevented all of the endothelial dysfunction and diminished NO generation and prevented the augmented EDCF contractions and ACh-induced ROS generation. Moreover, tempol given to ovariectomized rats restored the effects of PVAT to enhance endothelial function. In contrast, tempol failed to prevent the enhanced contractions to U-46619, despite preventing the excessive ROS generation.\(^{26}\)

In fact, a combination of PVAT and tempol was required to reduce U-46619 contractions in ovariectomized rat vessels to SHAM levels.

In contrast, incubation of canine coronary arteries with bradykinin released an adipokine that reduced vascular ROS, yet inhibited vasodilation. Another apparently paradoxical effect of tempol was to reduce bradykinin-induced relaxations. There are important differences in the functions of PVAT between vessels and experimental circumstances.\(^{26}\) After ovariectomy, tempol enhanced both the intrinsic relaxation to ACh (in vessels without PVAT) and the relaxations mediated by PVAT while reducing ROS generation consistent with many other reports that tempol moderated ROS and contractility and enhanced relaxation during oxidative stress.\(^{18}\)

Of interest was the finding that ovariectomy increased weight gain similar to the menopause.\(^{10}\) However, tempol reduced the weight gain in both ovariectomized and SHAM rats. Tempol given to fat-fed mice also prevented weight gain,\(^{27}\) which was attributed to alteration in the microbiome and signaling via intestinal farnesoid X receptors.

Thus, oxidative stress in microvessels from ovariectomized rats redirected endothelial function from NO-dependent vaso-relaxation to ROS-dependent vasoconstriction. About one half of these changes related to effects of ROS mediated by PVAT. The endothelial dysfunction is analogous to the effects of prolonged angiotensin II infusion in rabbits\(^{19}\) and rats,\(^{5}\) which also

### Table 2. Effect of Ovariectomy and PVAT on Maximum Vascular Reactivity, NO, and ROS in Mesenteric Arterioles

<table>
<thead>
<tr>
<th>Responses</th>
<th>SHAM</th>
<th>PVAT</th>
<th>OXV</th>
<th>PVAT</th>
<th>By ANOVA, Effect of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVAT–</td>
<td>PVAT+</td>
<td>PVAT–</td>
<td>PVAT+</td>
<td>OVX</td>
</tr>
<tr>
<td>After vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh, %</td>
<td>71.9±2.1</td>
<td>89.5±4.4</td>
<td>50.1±6.2</td>
<td>56.7±6.4</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>EDRF, %</td>
<td>37.1±3.5</td>
<td>52.0±3.3</td>
<td>17.0±1.3</td>
<td>19.8±5.6</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>EDHF, %</td>
<td>27.9±5.2</td>
<td>31.2±2.4</td>
<td>25.3±4.9</td>
<td>29.3±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>SNP, %</td>
<td>96.3±2.1</td>
<td>98.1±4.1</td>
<td>93.6±2.8</td>
<td>91.6±3.8</td>
<td>NS</td>
</tr>
<tr>
<td>EDCF, %</td>
<td>8.7±2.9</td>
<td>7.5±2.7</td>
<td>26.7±2.9</td>
<td>17.4±2.7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>PE, %</td>
<td>67.0±5.3</td>
<td>55.7±8.1</td>
<td>65.6±6.1</td>
<td>55.9±9.3</td>
<td>NS</td>
</tr>
<tr>
<td>U46619, %</td>
<td>39.6±5.7</td>
<td>10.0±2.4</td>
<td>57.5±3.4</td>
<td>35.4±8.5</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ACh-NO, Δ unit</td>
<td>0.09±0.07</td>
<td>1.17±0.07</td>
<td>0.39±0.08</td>
<td>0.55±0.11</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>EDCF-ROS, Δ unit</td>
<td>0.14±0.06</td>
<td>0.11±0.05</td>
<td>0.65±0.17</td>
<td>0.46±0.14</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>After tempol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh, %</td>
<td>71.1±2.6</td>
<td>86.5±2.1</td>
<td>72.5±1.8</td>
<td>87.0±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>EDRF, %</td>
<td>31.3±4.2</td>
<td>51.2±4.2</td>
<td>30.7±4.0</td>
<td>51.4±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>EDHF, %</td>
<td>33.6±1.6</td>
<td>27.4±2.5</td>
<td>36.1±2.4</td>
<td>30.1±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>EDHF, %</td>
<td>6.7±2.1</td>
<td>5.1±1.8</td>
<td>11.5±3.1</td>
<td>9.4±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>PE, %</td>
<td>66.5±4.9</td>
<td>47.9±6.9</td>
<td>76.9±7.3</td>
<td>61.2±6.8</td>
<td>NS</td>
</tr>
<tr>
<td>U46619, %</td>
<td>37.9±3.9</td>
<td>8.9±3.6</td>
<td>51.9±5.1</td>
<td>16.8±3.8</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ACh-NO, Δ unit</td>
<td>0.67±0.09</td>
<td>1.14±0.10</td>
<td>0.62±0.11</td>
<td>0.96±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>EDCF-ROS, Δ unit</td>
<td>0.08±0.04</td>
<td>0.06±0.03</td>
<td>0.12±0.06</td>
<td>0.09±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>U46619-ROS, Δ unit</td>
<td>0.13±0.07</td>
<td>0.10±0.06</td>
<td>0.27±0.12</td>
<td>0.24±0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data were obtained from experiments described in Figures 2, 3 and 4. Compared with SHAM, \(P<0.05\); \(\dagger P<0.01\); \(\ddagger P<0.001\). Compared with PVAT, \(\ast P<0.05\); \(\ast\ast P<0.01\); \(\ast\ast\ast P<0.001\). ACh indicates acetylcholine; EDCF, endothelium-dependent contracting factor; EDHF, endothelium-dependent hyperpolarizing factor; EDCF, endothelium-dependent relaxation factor; NO, nitric oxide; NS, nonsignificant; OVX, ovariectomized; PE, phenylephrine; PVAT, perivascular adipose tissue; ROS, reactive oxidative species; and SNP, sodium nitroprusside.
enhanced p22phox expression. EDCF requires vascular ROS generation after endothelial activation by shear stress, ACh, angiotensin II, or endothelin-1. Vascular ROS generate vasoconstrictor prostaglandins and thromboxane A2 from endothelial cyclooxygenase-1 or -2 and thromboxane A2 synthase that activate TP-Rs on adjacent VSMCs to mediate EDCF responses. A second effect of vascular ROS is to enhance TP-R responsiveness of VSMCs by reducing the recycling of TP-Rs. The enhanced TP-R responsiveness of mesenteric arterioles from ovariectomized rats confirms a prior study.

Figure 3. A–F. Effects of perivascular adipose tissue and ovariectomy on nitric oxide (NO) and reactive oxygen species (ROS) generation in mesenteric resistant arterioles with 10^{-4} mol L^{-1} of acetylcholine or 10^{-6} mol L^{-1} of U-46619. Comparing sham-operated (SHAM) vs ovariectomized (OVX) in same treatment groups (*P<0.05; **P<0.01; ***P<0.005). Comparing tempol vs vehicle in same treatment groups (‡P<0.05; ‡‡P<0.01; ‡‡‡P<0.005).

Figure 4. A–D. Effect of perivascular adipose tissue and ovariectomy on contractions to phenylephrine and U-46619 in mesenteric arterioles with perivascular adipose tissue (PVAT; solid symbols) or without PVAT (open symbols) from sham-operated (SHAM; circles) or ovariectomized (OVX; squares) rats after 6 weeks of vehicle or tempol. Comparing PVAT+ vs – in same treatment groups (*P<0.05; **P<0.01). Comparing SHAM vs OVX in same treatment groups (‡P<0.05). NS indicates non significant.
ROS in PVAT surrounding ovariectomized rat vessels impaired the adipokine signaling that normally enhanced NO generation and EDRF responses or that ROS in blood vessels prevented the signaling effects of the adipokine. Thus, ROS in PVAT were implicated in both enhancing the EDCF responses and reducing the EDRF/NO responses in vessels from ovariectomized rats.

This study has some limitations. It was confined to mesenteric arterioles. However, their function parallels that of renal afferent arterioles and systemic vessels. We did not test the effects of ovarian hormone replacement in ovariectomized rats. However, prior studies have reported that concomitant 17β-estradiol replacement at the time of ovariectomy prevents endothelial dysfunction and enhances TP-R signaling. The adipokines released from PVAT were not identified. However, these studies suggest that it was not hydrogen sulfide that activates vascular ATP-dependent K+ channels and leads to hyperpolarization of VSMCs because we did not detect any effect of PVAT on EDHF responses.

**Perspective**

TP-R signaling not only mediates EDCF responses but also contributes to hypertension, vascular remodeling, renal vasoconstriction, oxidative stress, and platelet aggregation, as observed during angiotensin1 or Goldblatt renovascular hypertension.24,35 Thus, enhanced TP-R signaling may contribute to the microvascular remodeling, endothelial dysfunction, and oxidative stress observed in ovariectomized rat vessels and also to the loss of protection from CVD observed in women with ovarian hormone deficiency. The finding that microvessels from ovariectomized rats have defects in endothelial relaxation, an EDCF response, and enhanced TP-R signaling that were mediated by adverse ROS-dependent signaling in the vessels and the surrounding PVAT suggests that full restoration of the beneficial signaling from PVAT would be a valuable therapeutic approach for reducing CVD risk in women with ovarian hormone deficiency.

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**Disclosures**

None.

**References**

K, Rösen R, Böhm M, Nickenie G. Endothelial dysfunction and oxida-
tive stress during estrogen deficiency in spontaneously hypertensive rats. 
T, Wilcox CS. Impaired endothelial function and microvascular asymmet-
rical dimethylarginine in angiotensin II-infused rats: effects of tempol. 
6. Davidge ST, Zhang Y. Estrogen replacement suppresses a prostaglandin H 
synthese-dependent vasoconstrictor in rat mesenteric arteries. Circ Res. 
7. Thompson LP, Weiner CP. Long-term estradiol replacement decreases 
contractility of guinea pig coronary arteries to the thromboxane mimetic U46619. 
8. Calkin AC, Sudhir K, Honisett S, Williams MR, Dawood T, Komesaroff 
PA. Rapid potentiation of endothelium-dependent vasodilation by es-
tradiol in postmenopausal women is mediated via cyclooxygenase 2. J Clin 
Endocrinol Metab. 2002;87:5072–5075.
9. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lans F, McQueen M, 
infarction in 52 countries (the INTERHEART study): case-control study. 
Behav. 2009;97:199–204.
11. Brown LM, Gent L, Davis K, Clegg DJ. Metabolic impact of sex hor-
12. Gao YJ, Lu C, Su LY, Sharma AM, Lee RM. Modulation of vascular func-
tion by perivascular adipose tissue: the role of endothelium and hydrogen 
14. Vacek TP, Gillespie W, Tyagi N, Vacek JC, Tyagi SC. Hydrogen sulfide 
protects against vascular remodeling from endothelial damage. Amino Acids. 
2010;39:1161–1169.
15. Galvánez-Prieto B, Dubrovsk G, Cano MV, Delgado M, Aranguez I, 
González MC, Ruiz-Gayo M, Gollasch M, Fernández-Alfonso MS. A reduc-
tion in the amount and anti-contractile effect of periadventitial meso-
vascular endothelial nitric-oxide synthase. Am J Physiol Regul Integr Comp 
nitric oxide synthase uncoupling and perivascular adipose oxidative stress 
and inflammation contribute to vascular dysfunction in a rodent model of 
17. Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, 
Jeziorska M, Laing I, Pemberton PW, Malik RA, Heagerty AM. Local inflammation and hypoxia abolish the protective anticontractile 
effects of oxidative stress in blood vessel and adverse adipokine signaling 
18. Wang et al. PVAT and ROS After OVX 1069

Novelty and Significance

What Is New?

- Microvascular endothelial dysfunction and reduced nitric oxide in this 
  model of ovarian hormone deficiency derives from reactive oxidative 
  species and impaired relaxant signaling from perivascular adipose tis-
ue. Correction of oxidative stress and presence of perivascular adipose 
tissue are both required to normalize the greatly enhanced thromboxane/ 
prostanoid receptor responses.

What Is Relevant?

- Correction of vascular reactive oxidative species alone is insufficient to 
  correct microvascular dysfunction in full. Vascular thromboxane/pro-
nitoid receptors and perivascular adipose tissue are novel targets to 
  correct menopausal microvascular dysfunction.

Summary

Microvascular endothelial dysfunction and enhanced thromboxane/ 
prostanoid receptor signaling after ovariectomy originate from in-
teractive effects of oxidative stress in blood vessel and adverse 
adipokine signaling from perivascular adipose tissue.

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