Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats

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Frisbee, Jefferson C. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. *Am J Physiol Heart Circ Physiol* 281: H1568–H1574, 2001.—This study determined alterations to hypoxic dilation of isolated skeletal muscle resistance arteries (gracilis arteries; viewed via television microscopy) from obese Zucker rats (OZR) compared with lean Zucker rats (LZR). Hypoxic dilation was reduced in OZR compared with LZR. Endothelium removal and cyclooxygenase inhibition (indomethacin) severely reduced this response in both groups, although nitric oxide synthase inhibition (Nω-nitro-L-arginine methyl ester) reduced dilation in LZR only. Treatment of vessels with a PGH₂-thromboxane A₂ receptor antagonist had no effect on hypoxic dilation in either group. Arterial dilation to arachidonic acid, iloprost, acetylcholine, and sodium nitroprusside was reduced in OZR versus LZR, although dilation to forskolin and aprikalim was unaltered. Treatment of arteries from OZR with oxidative radical scavengers increased dilation to hypoxia and agonists, with no effect on responses in LZR. The restored hypoxic dilation in OZR was abolished by indomethacin. These results suggest that hypoxic dilation of skeletal muscle microvessels from LZR represents the summated effects of prostanoid and nitric oxide release, whereas the impaired response of vessels in OZR may reflect scavenging of PGL₂ by superoxide anion.

skeletal muscle microvessel; type 2 diabetes; hypertension; Zucker rat; hypoxia; superoxide

MATERIALS AND METHODS

*Animals.* All experiments used 13- to 15-wk-old male LZR and OZR. Animals were fed standard chow and drank tap water ad libitum. Rats were housed in an animal care facility at the Medical College of Wisconsin approved by the American Association for the Accreditation of Laboratory Animal Care, and all protocols were approved by the institutional Animal Care and Use Committee at the Medical College of Wisconsin. Rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip), and a carotid artery was cannulated for determination of arterial pressure. In addition to being heavier than LZR [347 ± 10 g body wt; mean arterial pressure (MAP), 119 ± 3.2 mmHg; blood [glucose], 138 ± 15 mg/dl], OZR (613 ± 18 g body wt; MAP, 165 ± 6.4 mmHg; blood [glucose], 486 ± 48 mg/dl) demonstrated significant hypertension and hyperglycemia.

*Preparation of isolated vessels.* Gracilis arteries were surgically dissected from the anesthetized rat as described pre...
viously (8). Arteries were placed in a heated (37°C) chamber that allowed the lumen and exterior of the vessel to be perfused and superfused, respectively, with physiological salt solution (PSS) from separate reservoirs. The PSS used in these experiments was equilibrated with 21% O2-5% CO2-74% N2 and had the following composition (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.18 NaH2PO4, 24 NaHCO3, 0.026 EDTA, and 5.5 glucose. Vessels were cannulated at both ends with glass micropipettes (tip diameter, 100 μm) and secured to the inflow and outflow pipettes using 10-0 nylon sutures. Any side branches were ligated with a single strand teased from a 6-0 silk suture. The inflow pipette was connected to a reservoir perfusion system that allowed the intraluminal pressure and luminal gas concentrations to be controlled. Vessel diameter was measured using television microscopy and an on-screen video micrometer.

Arteries were extended to their in vivo length and equilibrated at 80% of the animal's MAP to approximate the perfusion pressure encountered in vivo (21). Any vessel that did not demonstrate active tone at rest was discarded. Active tone at the equilibration pressure was calculated as follows: (ΔDB/ΔDmax) × 100, where ΔD is the diameter increase from rest in response to Ca2+-free PSS and Dmax is the maximum diameter measured at the equilibration pressure in Ca2+-free PSS.

Removal of the vascular endothelium. The microvessel endothelium was removed via air bolus perfusion (8, 22). Subsequently, PSS perfusion through the lumen was restored, and the artery was allowed to reequilibrate for 30 min. Endothelium denudation procedures were deemed successful when vessel dilator reactivity to 10−6 M acetylcholine was eliminated and when the reactivity of the vessel to the endothelium-independent agonist forskolin (10−7 M) was not altered from responses determined in intact vessels.

Inhibition of cytochrome P-450 4A enzymes. To assess the role of cytochrome P-450 4A-derived arachidonic acid ω-hydroxylation (producing 20-hydroxyeicosatetraenoic acid) and epoxidation (producing epoxyeicosatrienonic acids) in contributing to hypoxic relaxation of arteries, these enzymes were inhibited with 10−9 M 17-octadecynoic acid (17-ODYA) (1, 8).

Inhibition of prostanooid and NO production. To assess the role of prostanooid or NO release from the microvessel endothelium in regulating hypoxic dilation of isolated arteries, the cyclooxygenase inhibitor indomethacin (10−6 M) or the NO synthase inhibitor Nω-nitro-l-arginine methyl ester (l-NAME; 10−4 M) was added to the vessel bath to inhibit the production of prostanooids and NO, respectively (7, 8).

Blockade of PGH2-thromboxane A2 receptors. To determine the contribution of activation of PGH2-thromboxane A2 (TXA2) receptors in contributing to altered hypoxic dilation of microvessels from OZR versus LZR, dilator responses of microvessels from both groups were assessed in response to hypoxia and to iloprost (10−9 g/ml) under control conditions and after application of the PGH2-TXA2 receptor antagonist SQ-29548 (10−5 M) (15).

Dilator agonists and hypoxia. In a separate series of experiments, dilator responses of isolated gracilis arteries from LZR and OZR were assessed in response to challenge with 1) the PGH2 analog iloprost (10−10–10−9 g/ml), 2) arachidonic acid (10−8–10−6 M), 3) acetylcholine (10−9–10−6 M), 4) the adenylate cyclase activator forskolin (10−13–10−7 M), 5) the endothelium-independent NO donor sodium nitroprusside (10−9–10−6 M), 6) the ATP-sensitive K+ channel agonist aprikalim (10−9–10−6 M), and 7) Ca2+-free PSS containing 10−3 M adenosine to produce maximal dilation and determine the maximum diameter of the microvessel.

Scavenging of oxidative free radicals. Previous studies (3, 17) have demonstrated that with the development of obesity, hypertension, and diabetes, oxidant stress levels in tissues of OZR are increased. With the use of the established dihydroethidine microfluorography assay (3, 11, 25), Friske and Stepp (9) demonstrated that vascular oxidant stress levels are substantially increased in arteries of OZR versus LZR. Results from this study also demonstrated that this increased oxidant tone can be normalized to levels that are not distinguishable from those in LZR after treatment of vessels with polyethylene glycol-superoxide dismutase (PEG-SOD) and catalase (9). To determine the extent to which increased oxidant stress levels might contribute to alterations in hypoxic dilation of skeletal muscle microvessels between LZR and OZR, vessels from both groups were treated with the free radical scavengers PEG-SOD (200 U/ml, Sigma) and catalase (80 U/ml, Sigma). Treatment of tissues with these substances has been previously demonstrated to significantly lower oxidant stress levels (12, 20).

Experiment protocols. In the present study, arterial responses to hypoxia were determined at the equilibration pressure for each microvessel (95 ± 5 mmHg for LZR and 131 ± 6 mmHg for OZR). For each vessel, the O2 content of the equilibration gas for the PSS was varied between 21% and 0% O2 (5% CO2-balance N2 for each). In the present experiments, this would cause a reduction in PSS Po2 from ~140 mmHg (under 21% O2) to ~35 mmHg (under 0% O2) (7).

Determination of mediators of hypoxic dilation of skeletal muscle resistance arteries. Initial experiments determined whether a difference in hypoxic dilation of isolated microvessels exists between LZR and OZR and the relative contribution of vascular endothelium-dependent and -independent processes regulating this response. Hypoxia-induced alterations in vascular tone were assessed in vessels under four conditions: control, after application of 17-ODYA, after endothelium removal, and after imposition of both treatments.

The second series of experiments determined the contribution of endothelium-derived vasoactive metabolites, specifically, NO, prostanoids, and cytochrome P-450 metabolites of arachidonic acid (i.e., epoxyeicosatrienonic acid) in regulating the dilatation of skeletal muscle microvessels to reduced Po2. After the response of vessels to hypoxia was determined, arteries were treated with l-NAME, indomethacin, or 17-ODYA. Vessels were also subjected to combinations of the inhibitors to more accurately elucidate the contribution of

### Table 1. Resting diameters of isolated gracilis arteries of LZR and OZR under experimental conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>LZR</th>
<th>OZR</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>122 ± 4.1</td>
<td>119 ± 3.0</td>
</tr>
<tr>
<td>Endothelium removed</td>
<td>124 ± 5.8</td>
<td>120 ± 2.9</td>
</tr>
<tr>
<td>+17-ODYA (10 μM)</td>
<td>130 ± 3.6</td>
<td>122 ± 3.1</td>
</tr>
<tr>
<td>+l-NAME (100 μM)</td>
<td>117 ± 2.7</td>
<td>112 ± 3.4</td>
</tr>
<tr>
<td>+Indomethacin (1 μM)</td>
<td>118 ± 3.9</td>
<td>113 ± 2.2</td>
</tr>
<tr>
<td>+PEG-SOD (200 U/ml) and catalase</td>
<td>126 ± 3.6</td>
<td>127 ± 2.5*</td>
</tr>
<tr>
<td>Maximum diameter, μM</td>
<td>199 ± 6.2*</td>
<td>175 ± 4.8*</td>
</tr>
<tr>
<td>Active tone, %</td>
<td>38.7 ± 3.0</td>
<td>32.5 ± 3.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Resting diameters are in micrometers. LZR and OZR, lean and obese Zucker rats, respectively; 17-ODYA, 17-octadecynoic acid; l-NAME, Nω-nitro-l-arginine methyl ester; PEG-SOD, polyethylene glycol-superoxide dismutase. *P < 0.05 vs. control value within that group.
the individual pathways to hypoxic dilation of gracilis arteries of LZR and OZR.

**Determination of vascular responses to dilator agonists.** Subsequent experiments determined vascular responses to dilator agonists associated with the pathways of hypoxic dilation determined in the above experiments. After the hypoxic dilation of vessels was determined, microvessel reactivity was evaluated following application of arachidonic acid, acetylcholine, iloprost, forskolin, sodium nitroprusside, and aprikalim, as described above.

**Determination of vascular responses to agonists and hypoxia after treatment with PEG-SOD and catalase.** For these experiments, the response of isolated microvessels from LZR and OZR to arachidonic acid, acetylcholine, iloprost, sodium nitroprusside, and hypoxia was determined before and after treatment of the vessel with the oxidative free radical scavengers PEG-SOD and catalase or catalase alone, as described above.

**Role of intraluminal pressure in mediating dilator responses.** Given that the evaluation of the dilator reactivity of gracilis arteries from LZR and OZR was performed at different intraluminal equilibration pressures, additional data were collected to address the possibility that the elevated intraluminal pressure employed for OZR may have contributed to alterations in dilator reactivity in these vessels. For these experiments, the dilator reactivity of isolated vessels of OZR in response to $10^{-6}$ M acetylcholine, $10^{-6}$ M sodium nitroprusside, and hypoxia was evaluated at the intraluminal equilibration pressure employed for both LZR (95 mmHg) and OZR (131 mmHg).

**Data and statistical analyses.** For all concentration-response curves, vascular reactivity data were fit with the following regression equation

$$y = \alpha + \beta \log(x) \tag{I}$$

where $y$ is the dilator response (increase in vessel diameter in response to challenge with a specific agonist), $\alpha$ is an intercept term, and $x$ is the agonist concentration. For all regression equations, $r^2 > 0.85$. For this analysis, the $\beta$ (slope) coefficient represents the change in microvessel diameter for a logarithmic change in agonist concentration.

All data are presented as means $\pm$ SE. Differences in $\beta$-coefficients and in the responses of isolated vessels to hypoxia were determined using ANOVA, with Tukey’s post hoc test and Student’s $t$-test where appropriate. For all analyses, $P < 0.05$ was considered to be statistically significant.

**RESULTS**

Under control conditions, resting diameter and active tone of isolated microvessels from LZR and OZR were not different. However, the maximum microvessel diameter, determined during superfusion with Ca$^{2+}$-free PSS, was significantly lower in OZR versus LZR (Table 1).

**Mediators of hypoxic dilation.** Data describing hypoxic dilation of microvessels from LZR and OZR and the effects of endothelium denudation are presented in Fig. 1. Gracilis arteries from OZR demonstrated a significant impairment in vessel dilation to reduced PO2 compared with responses in LZR. Removal of the microvessel endothelium with air bolus perfusion abolished the dilator reactivity of vessels of LZR to hypoxia, whereas this response was severely inhibited in vessels of OZR.

Figure 2 presents data describing hypoxic dilation of skeletal muscle microvessels from LZR and OZR after inhibition of NO synthase, cyclooxygenase, or both. In LZR, treatment of vessels with L-NAME or indomethacin significantly reduced hypoxic dilation of vessels from control values, whereas treatment of vessels with both inhibitors abolished this response (Fig. 2A). In contrast, treatment of vessels from OZR with L-NAME had no effect on hypoxic dilation, although application of indomethacin significantly reduced the dilator reactivity of these vessels to hypoxia (Fig. 2B).
versus LZR. In contrast, arterial dilation to these agonists was reduced in OZR. Iloprost were significantly reduced compared with val-
dilator reactivity of microvessels from OZR to arachi-
dinator responses of these vessels to forksolin and 
agonists employed in the present study are summa-
6 AA, arachidonic acid; ACh, acetylcholine; SNP, sodium nitroprus-
side. *P < 0.05 vs. LZR.

Treatment of intact vessels from LZR or OZR or endothelium-denuded vessels of LZR with 17-ODYA did not alter the dilation of vessels to reduced PO2 from responses determined in untreated vessels. However, after endothelium denudation, the small residual dilata-
tion of vessels from OZR in response to reduced PO2 (3.6 ± 1.1 μm) was eliminated by subsequent applica-
tion of 17-ODYA (−0.3 ± 0.3 μm).

In response to application of the PGH2-Txa2 receptor 
antagonist, microvessel dilation to hypoxia or to il-
prost was not altered in either group from values 
determined under control conditions. In LZR, micro-
vessels dilated by 18.5 ± 3.9 and 27.5 ± 3.7 μm in 
response to hypoxia and iloprost, respectively. After 
application of SQ-29548, vessels dilated by 16.9 ± 3.1 
and 28.2 ± 4.8 μm to these same stimuli. In OZR, 
under control conditions, vessels dilated by 11.2 ± 2.5 
μm in response to hypoxia and 13.4 ± 3.6 μm after 
application of iloprost. During blockade of PGH2-Txa2 
receptors, microvessels of OZR dilated by 9.4 ± 3.4 and 
15.4 ± 4.1 μm to hypoxia and iloprost, respectively.

Reactivity to dilator agonists. Data describing the 
dilator responses of skeletal muscle microvessels to the 
agonists employed in the present study are summa-
rized in Table 2. β (slope) coefficients describing the 
dilator reactivity of microvessels from OZR to arachi-
donic acid, acetylcholine, sodium nitroprusside, and 
iloprost were significantly reduced compared with val-
ues determined in LZR, indicating that skeletal muscle 
arterial dilation to these agonists was reduced in OZR 
versus LZR. In contrast, β-coefficients describing dil-
tor responses of these vessels to forksolin and 
aprikalim were not different between groups, indicat-
ing a comparable level of dilator reactivity to direct 
adenylate cyclase and ATP-sensitive K+ channel acti-
vation, respectively, in skeletal muscle resistance ar-
terioles of LZR and OZR. In vessels of LZR, treatment of 
isolated arteries with L-NAME completely abolished 
the reactivity of the vessels to 10−6 M acetylcholine 
(control vessel dilation, 21.3 ± 3.5 μm; l-NAME-
treated vessel dilation, 2.5 ± 3.1 μm). Similarly, the 
small dilation of vessels of OZR in response to acetyl-
choline (6.4 ± 2.5 μm) was abolished after treatment of 
vessels with l-NAME (−1.5 ± 1.8 μm).

Table 3 presents data describing the effects of treat-
ment of skeletal muscle microvessels from LZR and 
OZR with PEG-SOD and catalase on dilator responses 
to arachidonic acid, acetylcholine, sodium nitroprus-
side, and iloprost. In LZR, application of the free 
radical scavengers did not significantly alter β-coefficients 
describing the concentration-response curves of vessels 
any to the agonists. In contrast, treatment of vessels of 
OZR with PEG-SOD and catalase significantly in-
creased β-coefficients describing the vessel concentra-
tion-response curves to each of the agonists, indicating 
a significant increase in the dilator reactivity of skele-
tal muscle microvessels to these agonists after treat-
ment with the oxidative free radical scavengers. In 
additional studies where catalase alone was applied to 
the isolated vessels, the dilator responses of gracilis 
arteries from both LZR and OZR to the employed 
agonists was not significantly altered from levels de-
termined in untreated vessels (Table 3).

The effects of treatment of vessels from LZR and 
OZR with PEG-SOD and catalase on hypoxic dilation 
are presented in Fig. 3. In LZR, application of both free 
radical scavengers did not alter hypoxic dilation of 
skeletal muscle microvessels from levels under control 
conditions (Fig. 3A). In contrast, treatment of vessels of 
OZR with the scavengers significantly increased dil-
ator reactivity to hypoxia from levels in untreated 
vessels (Fig. 3B). However, compared with the hypoxic 
dilation of isolated vessels of OZR under control condi-
tions (8.7 ± 2.2 μm), application of catalase alone had 
no significant effect on vessel responses to reduced PO2 
(9.4 ± 2.8 μm). Subsequent application of L-NAME to 
vessels of OZR that had been treated with PEG-SOD 
and catalase had no effect on the enhanced dilator 
response to reduced PO2. However, application of indo-
methacin to vessels of OZR after treatment with the

Table 2. β (slope) coefficients from regression equations of skeletal muscle microvessels of LZR and OZR in response to agonists

<table>
<thead>
<tr>
<th>Agonists</th>
<th>LZR</th>
<th>OZR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, μM/log M</td>
<td>2.53 ± 0.19</td>
<td>1.50 ± 0.25*</td>
</tr>
<tr>
<td>ACh, μM/log M</td>
<td>2.84 ± 0.28</td>
<td>0.54 ± 0.08*</td>
</tr>
<tr>
<td>SNP, μM/log M</td>
<td>5.42 ± 0.42</td>
<td>1.93 ± 0.26</td>
</tr>
<tr>
<td>Iloprost, μM/log g/ml</td>
<td>1.84 ± 0.17</td>
<td>0.70 ± 0.13*</td>
</tr>
<tr>
<td>Forskolin, μM/log M</td>
<td>2.74 ± 0.21</td>
<td>2.59 ± 0.27</td>
</tr>
<tr>
<td>Aprikalim, μM/log M</td>
<td>4.42 ± 0.46</td>
<td>4.17 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE. β (slope) coefficients are from the regression equations describing concentration-response curves (see Eq. 1). AA, arachidonic acid; ACh, acetylcholine; SNP, sodium nitroprusside. *P < 0.05 vs. LZR.

Table 3. β (slope) coefficients from regression equations of skeletal muscle microvessels of LZR and OZR in response to agonists under control conditions and after treatment with PEG-SOD and catalase

<table>
<thead>
<tr>
<th></th>
<th>LZR</th>
<th>OZR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PEG-SOD + Catalase</td>
</tr>
<tr>
<td>AA, μM/log M</td>
<td>2.68 ± 0.24</td>
<td>2.80 ± 0.29</td>
</tr>
<tr>
<td>ACh, μM/log M</td>
<td>2.72 ± 0.29</td>
<td>3.18 ± 0.34</td>
</tr>
<tr>
<td>SNP, μM/log M</td>
<td>6.49 ± 0.96</td>
<td>6.58 ± 0.75</td>
</tr>
<tr>
<td>Iloprost, μM/log g/ml</td>
<td>2.17 ± 0.19</td>
<td>2.25 ± 0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE. β (slope) coefficients are from the regression equations describing concentration-response curves (see Eq. 1). *P < 0.05 vs. control.
oxidative free radical scavengers completely abolished hypoxic dilation.

The effects of elevated intraluminal equilibration pressure on the dilator reactivity of isolated skeletal muscle resistance arteries from OZR are presented in Fig. 4. When the intraluminal pressure employed for vessels of OZR (131 ± 6 mmHg) was reduced to that used for vessels of LZR (95 ± 5 mmHg), the dilator responses of vessels to acetylcholine, sodium nitroprusside, and reduced PO2 were not significantly different from those determined at the normal equilibration pressure for OZR.

DISCUSSION

The primary observation of the present study was that hypoxic dilation of gracilis arteries of OZR was impaired relative to LZR (Fig. 1). This impaired reactivity to hypoxia, a novel observation of the present study, is in general agreement with existing observations of impaired endothelium-dependent dilation of intestinal and mesenteric microvessels (13, 35, 37) and skeletal muscle arterioles of OZR (9). Interestingly, in OZR, a significant reactivity of skeletal muscle microvessels to hypoxia was retained. The persistent dilator response to reduced PO2 despite the development of hypertension is in agreement with a recent study (8) by the author investigating the effects of Dahl hypertension but contrasts with a previous study (21) investigating the reduced renal mass model of hypertension. These observations suggest that substantial heterogeneity exists regarding the regulation of microvessel tone with hypoxia across different models of hypertension.

The results of the present study suggest that the release of NO and/or prostanoids from the vascular endothelium are responsible for hypoxic dilation of skeletal muscle microvessels from LZR and OZR. While these data are in agreement with previous studies (7, 8, 16, 24, 30) indicating that hypoxic dilation in normotensive rats is mediated by the release of these substances, the present results contrast with a recent study (8) suggesting an endothelium-independent cytochrome P-450 \(\omega\)-hydroxylase-dependent component contributing to this response in gracilis arteries of hypertensive Dahl rats. The results of the present study support only a minimal role for cytochrome P-450 enzymes in mediating hypoxic dilation in skeletal muscle microvessels of Zucker rats. It is important to note, however, that the present study employed only one level of hypoxia for the isolated vessels. Additional studies, with more mild or more severe levels of hypoxia, could elucidate additional contributing mechanisms underlying the dilation of skeletal muscle microvessels to reduced PO2.

Hypoxic dilation of skeletal muscle resistance arteries of LZR appeared to be wholly dependent on NO and prostanoid release from the vascular endothelium (Fig. 2A). In contrast, the dilation of gracilis arteries of OZR in response to reduced PO2 was predominantly prostanoid dependent, with a reduced role for NO synthesis and/or release in mediating this response (Fig. 2B). On the basis of data presented in Fig. 2, it is apparent that an impairment of NO or prostanoid signaling pathways or both underlies the compromised dilation of skeletal muscle microvessels of OZR to hypoxia.

Previous studies have suggested that an impaired hypoxic dilation of vessels from hypertensive animals could be due to either a release of vasoconstrictor...
metabolites via cyclooxygenase in addition to release of PGI2 (i.e., PGH2 and TxA2, see Refs. 4 and 31) or to an inappropriate activation of PGH2-TxA2 receptors by PGI2 (23). Furthermore, a recent study (32) has indicated that blockade of PGH2-TxA2 receptors in the cheek pouch of spontaneously hypertensive hamsters restores microvessel dilator reactivity to acetylcholine and vasoactive intestinal peptide. However, the results of the present study suggest that these processes do not contribute to the impaired hypoxic dilation of gracilis arteries of OZR, because application of the PGH2-TxA2 receptor antagonist SQ-29548 did not alter hypoxia- or iloprost-induced responses of vessels from LZR or OZR.

It has been suggested that tissue oxidant stress levels are elevated in OZR, compromising dilator responses of microvessels to NO-dependent stimuli (3, 17). Furthermore, the author (9) has previously demonstrated that vascular superoxide anion levels were increased in OZR versus LZR and that treatment of vessels with PEG-SOD and catalase reduces vascular oxidant stress in OZR to levels that are not distinguishable from LZR. As presented in Table 3, vascular oxidant stress levels in OZR were correlated with dilator reactivity of skeletal muscle microvessels to NO- and PGI2-dependent dilator agonists. When compared with the agonist-induced dilator responses of vessels from LZR, these data suggest that reduced vascular smooth muscle reactivity to NO and PGI2 in OZR may reflect increased scavenging of these signaling molecules by oxidative radicals and their conversion to peroxynitrate (34) and isoprostanes (6, 17–19, 28), respectively. However, the results of the present study suggest that the primary source of the oxidant stress-induced impairment in dilator reactivity in OZR was due to superoxide anion and not hydrogen peroxide, because treatment of vessels from OZR with catalase alone had no significant effect on the dilation of vessels from these rats to either reduced PO2 or to the employed agonists.

After normalization of vascular oxidant stress in OZR, hypoxic dilation of skeletal muscle microvessels was increased compared with responses determined under control conditions (Fig. 3). Subsequent application of L-NAME to vessels of OZR treated with PEG-SOD and catalase did not alter hypoxia- or iloprost-induced responses of vessels from LZR or OZR.

Summary and conclusions. The results of the present study indicate that hypoxic dilation of skeletal muscle microvessels from OZR is impaired compared with responses in microvessels from LZR. In LZR, hypoxic dilation is mediated by the combined effects of NO and prostaglandin release from the vascular endothelium, whereas in OZR, microvessel dilation to reduced PO2 is largely dependent on endothelium-dependent prostanoid release. Normalization of tissue oxidant stress improves dilator responses of microvessels from OZR to NO- and PGI2-dependent dilator agonists and restores the prostanoid-dependent component of hypoxic dilation. These data may represent the first observations suggesting impairment of a PGI2-dependent vasodilator response due to an inactivation of endothelium-derived prostacyclin by superoxide anion.

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