Endothelium-derived hyperpolarizing factor contributes to hypoxia-induced skeletal muscle vasodilation in humans

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Spilk S, Herr MD, Sinoway LI, Leuenberger UA. Endothelium-derived hyperpolarizing factor contributes to hypoxia-induced skeletal muscle vasodilation in humans. Am J Physiol Heart Circ Physiol 305: H1639–H1645, 2013. First published September 16, 2013; doi:10.1152/ajpheart.00073.2013.—Systemic hypoxia causes skeletal muscle vasodilation, thereby preserving O₂ delivery to active tissues. Nitric oxide (NO), adenosine, and prostaglandins contribute to this vasodilation, but other factors may also play a role. We tested the hypothesis that regional inhibition of endothelium-derived hyperpolarizing factor with the cytochrome P-450 2C9 antagonist fluconazole, alone or combined with the NO synthase antagonist N⁶⁴-monomethyl-L-arginine (L-NMMA), attenuates hypoxia-induced vasodilation. We compared forearm blood flow (FBF) and skin blood flow before and during brachial artery infusion of fluconazole (0.3 mg/min; trial 1) or fluconazole + L-NMMA (50 mg over 10 min; trial 2) and during systemic hypoxia (10 min, arterial PO₂ ~37 mmHg) in infused (experimental) and control forearms of 12 healthy humans. During normoxia, fluconazole and fluconazole + L-NMMA reduced (P < 0.05) forearm vascular conductance (FVC) by ~10% and ~18%, respectively. During hypoxia and fluconazole (trial 1), FVC increased by 1.76 ± 0.37 and 0.95 ± 0.35 units in control and experimental forearms, respectively (P < 0.05). During hypoxia and fluconazole + L-NMMA (trial 2), FVC increased by 2.32 ± 0.51 and 0.72 ± 0.22 units in control and experimental forearms, respectively (P < 0.05). Similarly, during hypoxia with L-NMMA alone (trial 3; n = 8) FVC increased by 1.51 ± 0.46 and 0.45 ± 0.32 units in control and experimental forearms, respectively (P < 0.05). These effects were not due to altered skin blood flow. We conclude that endothelium-derived hyperpolarizing factor contributes to basal vascular tone and to hypoxia-induced skeletal muscle vasodilation and could be particularly relevant when other vasodilator systems are impaired.

hypoxia; vasodilation; endothelium-derived hyperpolarizing factor; fluconazole; nitric oxide

UNDER BASAL CONDITIONS and during physiological stress, skeletal muscle blood flow is closely matched to metabolic demand. Accordingly, in healthy humans, systemic hypoxia is accompanied by skeletal muscle vasodilation, which serves to maintain O₂ homeostasis (20, 27, 29). Because hypoxia raises sympathetic vasoconstrictor nerve traffic and norepinephrine spillover (14, 31), vasodilation results from release of systemic or local vasodilator factors. In agreement with studies in rodents (22, 24, 26), it has been reported that nitric oxide (NO) (7, 9, 16, 35) and adenosine (15, 18) contribute to hypoxia-induced vasodilation in humans. In addition, some reports (6, 35), but not others (27), support a role for an increase in circulating epinephrine, and one recent report (19) suggests that vasodilator prostaglandins also contribute to the skeletal muscle vasodilation elicited by systemic hypoxia. Thus multiple endogenous vasodilator mechanisms may be engaged to contribute to hypoxia-mediated vasodilation, pointing toward a remarkable redundancy of these vasodilator systems.

Recently, endothelium-derived hyperpolarizing factor (EDHF) has emerged as a NO- and cyclooxygenase-independent endothelial vasodilator that contributes to metabolic blood flow regulation in humans. Among several candidates, including hydrogen peroxide, some EDHF-like compounds are products of cytochrome P-450 metabolism of arachidonic acid and include 11- and 12-eicosatrienoic acid (11- and 12-EET) (1), which act on Ca²⁺-activated K⁺ channels (4, 8, 11) via activation of transient receptor potential vanilloid (TRPV4) channels (2). Antifungal agents such as fluconazole are thought to inhibit EDHF via antagonism of the cytochrome P-450 2C9 isoform and can be administered safely in humans (3, 5, 25). In humans, EDHF is thought to play a role in exercise-induced skeletal muscle vasodilation (13) and may affect basal peripheral arterial tone (25). In general, the role of EDHF-like vasodilator activity is most evident when NO synthase is blocked, prompting speculation that EDHF may be particularly relevant in cardiovascular disease states marked by impaired NO bioavailability (13, 25, 34). In addition, genetic variants of cytochrome P-450 2C9 have recently been recognized, raising the possibility of a genetic predisposition to EDHF-dependent vascular dysfunction in affected individuals (12).

On the basis of these collective findings, we hypothesized that regional administration of the antifungal cytochrome P-450 2C9 inhibitor fluconazole would attenuate forearm skeletal muscle vasodilation during exposure to systemic hypoxia. We further reasoned that this effect would be unmasked by simultaneous inhibition of the NO pathway with the NO synthase inhibitor N⁶⁴-monomethyl-L-arginine (L-NMMA).

METHODS

Subjects. Eighteen healthy, nonsmoking, normotensive subjects (10 women, 8 men; age 27 ± 1 yr, height 172 ± 3 cm, weight 70 ± 2 kg, body mass index 23.5 ± 0.6 kg/m²) who were not taking any medications participated in the study. All subjects provided informed written consent to participate in the studies, which were approved by the Institutional Review Board at The Pennsylvania State University Milton S. Hershey Medical Center. The studies were performed after overnight fasting with the subjects supine, at an ambient temperature of 21–24°C, and having abstained from heavy exercise and caffeinated products for 24 h.

Instrumentation and measurements of heart rate and blood pressure. During instrumentation, blood pressure was measured via an automatic sphygmomanometer (Dinamap, Critikon, FL), and heart rate (HR) and rhythm were monitored via a two-lead surface electrocardiogram. After local anesthesia with ~2 ml of 1% lidocaine, a
of the vasodilator responses in the infused (experimental) and opposed (control) forearms. Pneumatic cuffs were placed on the upper arms (venous occlusion pressure 50 mmHg) (15, 28). The strain gauges were placed ~10 cm distal to the antecubital fossa, and the forearms were elevated ~5 cm above the level of the right atrium. Before FBF measurements, wrist cuffs were inflated to 250 mmHg to exclude blood flow through the hands (15, 28). At each time point, at least six flow curves were obtained and averaged. FBF was expressed as ml·min⁻¹·100 cm⁻² forearm tissue, and forearm vascular conductance (FVC) was calculated as (FBF/MAP) × 100 and expressed in arbitrary units.

Blood flow. Forearm blood flow (FBF) was determined bilaterally by venous occlusion plethysmography (15, 28), allowing comparison of the vasodilator responses in the infused (experimental) and opposite (control) forearms. Pneumatic cuffs were placed on the upper arms (venous occlusion pressure 50 mmHg) (15, 28). The strain gauges were placed ~10 cm distal to the antecubital fossa, and the forearms were elevated ~5 cm above the level of the right atrium. Before FBF measurements, wrist cuffs were inflated to 250 mmHg to exclude blood flow through the hands (15, 28). At each time point, at least six flow curves were obtained and averaged. FBF was expressed as ml·min⁻¹·100 cm⁻² forearm tissue, and forearm vascular conductance (FVC) was calculated as (FBF/MAP) × 100 and expressed in arbitrary units.

To isolate the effects of the interventions on the skeletal muscle vasculature from the effects on blood vessels in the skin, we determined skin blood flow (SBF, units) with laser-Doppler diodes (Laserflow BPM, Vasomedics, St. Paul, MN) positioned over the volar surfaces of both forearms (14). SBF was measured over 1-min periods before each set of FBF measurements. Skin vascular conductance (SVC) was calculated as (SBF/MAP) × 100 and expressed in arbitrary units.

Blood gas analysis. Before and at the end of each hypoxia trial, blood samples (0.5 ml) were drawn through the brachial artery catheter and analyzed with a blood gas analyzer (Rapidlab 865, Bayer).

Experimental protocol. After instrumentation and a rest period to establish basal conditions, one group of subjects underwent two sequential trials of hypoxia, first during regional administration of fluconazole alone and then during coadministration of fluconazole and the NO synthase antagonist L-NMMA into the experimental forearm. The untreated opposite forearm served as a control.

In trial 1, after baseline measurements of FBF, SBF, and blood pressure during baseline, the facemask was positioned and checked for air leaks. After acclimatization and establishment of stable normocapnic breathing, hemodynamic, blood flow, and ventilatory measurements were made, and an arterial blood gas was obtained (normoxic baseline). Fluconazole (0.3 mg/min for 20 min) was then infused intra-arterially into the experimental forearm, and measurements were repeated at 5 and 10 min of the drug infusion. The facemask was then connected to the hypoxic gas (fraction of inspired \(O_2 = 0.1\)) for 10 min. Hemodynamic, blood flow, and ventilatory measurements were repeated at 5 and 10 min of hypoxia. At the end of hypoxia, an arterial blood gas was collected, and the subjects returned to room air.

In trial 2, the hypoxia protocol was performed under identical conditions, except L-NMMA alone was infused into the experimental forearm (5 mg/min for 10 min).

Drugs. Fluconazole (Hospira, Lake Forest, IL) was reconstituted in normal saline and infused at 0.3 mg/min (~1 \(\mu\)mol/min, 0.5 ml/min), at continuous infusion rates of 0.4–1.6 \(\mu\)mol/min (for 5–8 min at various levels), fluconazole has been shown to attenuate flow-mediated dilation (3) and reduce basal blood flow in the radial artery (25). L-NMMA (Clinalfa) was reconstituted in normal saline and infused at 5 mg/min (0.5 ml/min) intra-arterially for 10 min, as reported previously (35). Because of the prolonged duration of action of L-NMMA, a continuous infusion was not necessary (35). The drug infusions were administered with a precision pump (Harvard, Biolab).

Data analysis and statistics. Data were collected via a MacLab system (ADInstruments, Castle Hill, Australia) at a sampling rate of 200 Hz and analyzed offline. Because the 5- and 10-min FBF and SBF values during the drug infusions and during hypoxia, respectively, did not differ \(P =\) not significant (NS), the two data points for each condition were averaged. Hemodynamic, ventilatory, and arterial blood gas data between normoxia and hypoxia were compared by paired \(t\)-tests. The FBF and SBF data were examined by two-way analysis of variance for repeated measures testing for effects of

**Fig. 1.** Schematic of experimental protocols for trials 1 and 2. Protocol for trial 3 was identical to that of trial 2, except \(N^\text{O}\)-monomethyl-L-arginine (L-NMMA) alone was administered.
condition (normoxia vs. hypoxia) and pharmacological blockade (experimental vs. control forearm). Where a main effect was noted, contrasts were constructed within the analysis of variance model to test for simple effects, with correction of $P$ values for multiple comparisons by Bonferroni’s method. Absolute and percent changes of FBF and FVC in response to hypoxia in the experimental and control forearms were compared with paired t-tests. Values are means ± SE. The level of significance was set at $P < 0.05$.

RESULTS

Effects of systemic hypoxia on blood pressure, HR, ventilatory parameters, and arterial blood gases. The effects of breathing the hypoxic gas (fraction of inspired $O_2 = 0.1$) on hemodynamic and ventilatory parameters and on arterial blood gases in the three trials are shown in Table 1. All three trials resulted in comparable hemodynamic and ventilatory effects and severity of hypoxia.

**Trial 1:** effects of fluconazole and hypoxia on total FBF and SBF and on FVC and SVC. The responses of FBF, FVC, SBF, and SVC to regional infusion of fluconazole before and during systemic hypoxia were measured in experimental and control forearms of 12 male and 7 female subjects (Table 2, Figs. 2–4). Analysis of variance for FBF and FVC demonstrated main effects of condition ($F = 18.6$, $P < 0.0001$; and $F = 16.8$, $P < 0.0001$), respectively) and statistical interactions of condition as a function of the forearm ($F = 3.1$, $P = 0.056$; and $F = 4.2$, $P < 0.05$, respectively). Post hoc testing revealed that, in the experimental forearm, fluconazole reduced FVC by 8% in the control forearm and the degree of its attenuation ($P < 0.05$), and had no effect on SVC in either forearm ($P = NS$). In the forearm infused with fluconazole, FVC decreased in six of seven female and four of five male subjects.

In the control forearm, hypoxia elicited a robust increase in FBF and FVC ($P < 0.05$) but no change in SBF or SVC ($P = NS$), suggesting vasodilation in skeletal muscle, but not in skin. Compared with the control forearm, the hypoxia-induced increases in FBF and FVC were attenuated in the experimental forearm infused with fluconazole (56 ± 14% vs. 27 ± 9%, and 55 ± 13% vs. 26 ± 8%, respectively, $P < 0.05$ for both). The hypoxia-induced change in FVC in the control and experimental (infused) forearms in trial 1 is shown in Fig. 5.

When the vascular responses for female and male subjects were analyzed separately, in the experimental forearm, fluconazole attenuated the hypoxia-induced increase in FVC in all seven female subjects and in three of five male subjects.

**Trial 2:** effects of fluconazole + l-NMMA and hypoxia on total FBF, FVC, SBF, and SVC. The responses of FBF, FVC, SBF, and SVC to regional infusion of fluconazole + l-NMMA before and during systemic hypoxia in 12 subjects are shown in Table 2 and Figs. 2–4. Analysis of variance for FBF and FVC demonstrated main effects of condition ($F = 13.1$, $P < 0.0001$; and $F = 13.7$, $P < 0.0001$, respectively) and statistical interactions of condition as a function of the forearm ($F = 8.3$, $P < 0.001$; and $F = 8.5$, $P < 0.001$, respectively). Post hoc testing revealed that compared with the control forearm, in the experimental forearm, fluconazole + l-NMMA reduced FVC and SVC during normoxia ($P < 0.05$ for both), whereas the decrease of FBF was only a trend ($P = 0.08$).

Compared with the control forearm, the hypoxia-induced increases in FBF and FVC were attenuated in the experimental forearm infused with fluconazole + l-NMMA (61 ± 11 vs. 21 ± 7%, and 64 ± 11 vs. 23 ± 7%, respectively, $P < 0.05$ for both). SBF and SVC were not affected ($P = NS$). The change in FVC in response to hypoxia in the control and experimental (infused) forearms in trial 2 is shown in Fig. 5.

When the vascular responses for female and male subjects were analyzed separately, in the experimental forearm, fluconazole + l-NMMA attenuated the hypoxia-induced increase in FVC in all female subjects and in three of five male subjects.

The magnitude of the hypoxia-induced forearm vasodilation in the control forearm and the degree of its attenuation

### Table 1. Effects of systemic hypoxia on hemodynamics, ventilatory parameters, and arterial blood gases

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>Hypoxia</th>
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<tr>
<td>MAP, mmHg</td>
<td>85 ± 3</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>66 ± 3</td>
<td>83 ± 3*</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>8.2 ± 0.4</td>
<td>10.2 ± 0.3*</td>
</tr>
<tr>
<td>End-tidal CO$_2$, mmHg</td>
<td>39 ± 1</td>
<td>34 ± 1*</td>
</tr>
<tr>
<td>Arterial $O_2$ saturation, %</td>
<td>98 ± 0.2</td>
<td>78 ± 1*</td>
</tr>
<tr>
<td>PO$_2$, mmHg</td>
<td>108 ± 3</td>
<td>37 ± 1*</td>
</tr>
<tr>
<td>PCO$_2$, mmHg</td>
<td>38 ± 1</td>
<td>30 ± 1*</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.01</td>
<td>7.47 ± 0.01*</td>
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</tbody>
</table>

Values are means ± SE. Baseline vs. hypoxia, *P < 0.05 vs. baseline (normoxia).
achieved by the regional drug infusions in the experimental forearm were similar in the two trials \((P = \text{NS})\).

**Trial 3:** effects of \(\text{L-NMMA}\) alone and of hypoxia on total FBF, SBF, FVC, and SVC. The responses of FBF and FVC to regional infusion of \(\text{L-NMMA}\) alone before and during systemic hypoxia in eight male and four female subjects are shown in Table 3. Analysis of variance for FBF and FVC demonstrated main effects of condition \((F = 9.9, P < 0.001; \text{and } F = 11.5, P < 0.001, \text{respectively})\) and statistical interactions of condition as a function of the forearm \((F = 5.6, P < 0.01; \text{and } F = 7.3, P < 0.01, \text{respectively})\). Post hoc testing revealed that, in the experimental forearm, \(\text{L-NMMA}\) reduced FBF and FVC during normoxia \((-34 \pm 7 \text{ and } 36 \pm 6\%\), respectively, \(P < 0.05 \text{ for both})\). Compared with the control forearm, in the experimental forearm \(\text{L-NMMA}\) attenuated the increases in FBF and FVC during hypoxia \((-31 \pm 10 \% \text{ vs. } +12 \pm 9\%\), and \(+33 \pm 8 \% \text{ vs. } +16 \pm 8\%\), respectively, \(P < 0.05 \text{ for both})\). The effects of \(\text{L-NMMA}\) alone and of hypoxia on SBF and SVC mirrored the effects in trial 2 (data not shown).

**DISCUSSION**

The principal new findings of this study are twofold. 1) During normoxia, regional infusion of the cytochrome \(P-450\) 2C9 antagonist fluconazole resulted in mild forearm vasoconstriction. 2) Compared with the control forearm, infusion of fluconazole alone, coadministration of fluconazole and the NO synthase antagonist \(\text{L-NMMA}\), or infusion of \(\text{L-NMMA}\) alone substantially attenuated the vasodilation induced by moderate systemic hypoxia. However, in this small study, the attenuation of hypoxia-mediated forearm vasodilation achieved by fluconazole \(+\text{L-NMMA}\) was not significantly greater than that achieved by fluconazole alone. Since, by inhibition of cytochrome \(P-450\) 2C9, fluconazole is thought to inhibit EDHF, these data indirectly suggest that an EDHF-like metabolite of this enzyme contributes to basal forearm vascular tone and plays a role in the vasodilation elicited by moderate systemic hypoxia, even when the NO pathway is intact.

To our knowledge, this is the first report that demonstrates, via pharmacological blockade of cytochrome \(P-450\) 2C9 with fluconazole, a role for an EDHF-like compound in the skeletal muscle vasodilation induced by systemic hypoxia in humans. The present findings extend results from a number of prior investigations in animal models and in humans that examined various vasodilator systems in the regulation of skeletal muscle blood flow during systemic hypoxia. In a series of studies, Marshall and collaborators demonstrated that adenosine, NO, and vasodilator prostaglandins each contribute to the skeletal...
Fig. 4. Change in forearm vascular conductance in response to infusions of fluconazole alone (trial 1) and fluconazole + L-NMMA (trial 2) during normoxia. Open bars, control (uninfused) forearm; filled bars, experimental (drug-infused) forearm. Values are means ± SE. *P < 0.05 vs. control.

Fig. 5. Change in forearm vascular conductance in response to infusions of fluconazole alone (trial 1) and fluconazole + L-NMMA (trial 2) during systemic hypoxia. Open bars, control (uninfused) forearm; filled bars, experimental (drug-infused) forearm. Values are means ± SE. *P < 0.05 vs. control.

Table 3. Effects of L-NMMA before and during systemic hypoxia on total FBF and FVC in infused and opposite forearms

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>L-NMMA</th>
<th>Hypoxia</th>
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<tbody>
<tr>
<td></td>
<td>Trial 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>4.99 ± 0.77</td>
<td>2.98 ± 0.27</td>
<td>3.50 ± 0.42†</td>
</tr>
<tr>
<td>Control</td>
<td>4.42 ± 0.50</td>
<td>4.46 ± 0.65</td>
<td>5.67 ± 0.80*</td>
</tr>
<tr>
<td>FVC, units</td>
<td>5.90 ± 0.90</td>
<td>3.41 ± 0.29</td>
<td>3.86 ± 0.48†</td>
</tr>
<tr>
<td>Experimental</td>
<td>5.52 ± 0.57</td>
<td>5.05 ± 0.65</td>
<td>6.56 ± 0.91*</td>
</tr>
<tr>
<td>Control</td>
<td>5.23 ± 0.57</td>
<td>5.05 ± 0.65</td>
<td>6.56 ± 0.91*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. L-NMMA. †P < 0.05 vs. control. ‡P < 0.05 vs. baseline.

that, in healthy humans, EDHF contributes importantly to O₂ homeostasis in skeletal muscle. However, in this study, an inhibitory effect of fluconazole on the expected skeletal muscle vasodilation during hypoxia was seen, even in the absence of NO blockade. This reinforces the concept of a remarkable redundancy of metabolic vasodilator systems that are capable of serving to maintain O₂ homeostasis during metabolic stress, such as local or systemic hypoxia (20, 21).

In the present study, under normoxic conditions, fluconazole alone resulted in mild forearm vasoconstriction. Because SVC was unaffected by fluconazole, this suggests that EDHF affects resting vascular tone in skeletal muscle of the forearm. This finding contrasts with one prior report (5) but agrees with another (25) and implies that this effect is likely small. However, whether this effect extends to larger (e.g., postural) muscle groups in the leg is not known.

On the basis of findings in rodents, it has been suggested that the role of EDHF in regulating vascular tone may be more prominent in female than male subjects (17, 32). While our study was not designed to examine potential sex-related differences in EDHF activity, our data showing a more consistent effect of fluconazole in female subjects support the notion that, in female subjects, EDHF may play a more prominent role in regulating skeletal muscle vascular tone under normoxic basal conditions and during hypoxic stress. Whereas common genetic variants of cytochrome P-450 2C9 are known to affect drug metabolism (e.g., warfarin) (36), their impact on blood flow regulation during physiological stress is not known. Furthermore, evidence that such genetic variants affect the risk of atherosclerosis and its complications is lacking (36).

In agreement with prior studies (14, 15, 23, 27, 35), moderate systemic hypoxia was associated with robust forearm vasodilation in noncutaneous tissues. Although modest vasodilation has been reported during normocapnic hypoxia in nonacral skin (33), in the present study a small vasodilator effect of hypoxia in skin could have been masked by mild vasoconstriction due to the associated mild hypocapnia. The hypoxia-induced vasodilation in skeletal muscle was attenuated significantly in the forearm undergoing regional infusion with fluconazole, fluconazole + L-NMMA, and L-NMMA alone. Importantly, the effects of fluconazole alone or fluconazole + L-NMMA on skin vascular tone do not explain the drug effects noted during hypoxia. For example, while fluconazole was associated with a mild decrease in FBF and a concomitant small decrease in total FVC, SBF and SVC were not affected by the drug during normoxia or hypoxia. Similarly, whereas
diminishing the need for further vasodilation when arterial O2 signal that arises in skeletal muscle during hypoxia, thus blood flow (23) and, thereby, may attenuate the metabolic error. However, oxia should be examined during
been suggested that vasodilator mechanisms engaged by hypoxia-induced skeletal muscle vasodilation during hypoxia, it has counteracted the metabolic vasodilation during hypoxia, thereby also exposing the endothelium of afferent vessels to hypoxic stress. Accordingly, the sites of production of vasodilator factors and their concentration gradients across the tissue gap from vessel to muscle cell are likely different.

On the basis of prior reports (13, 25, 34), we considered the possibility that the effect of fluconazole might only become apparent after inhibition of the NO pathway. However, our data demonstrate that fluconazole alone affected basal vascular tone, as well as the ability of skeletal muscle vessels to dilate during systemic hypoxia. Although the attenuation of hypoxia-induced vasodilation was slightly larger during combined NO and EDHF blockade than during EDHF inhibition with fluconazole alone (and was also seen prominently during NO blockade alone), possibly as a result of the small study sample, this difference did not reach statistical significance. On the basis of the data variability, a much larger study sample would be required to answer this question definitively.

Because hypoxia is known to raise sympathetic vasoconstrictor nerve activity (14, 30) and is therefore expected to counteract the metabolic vasodilation during hypoxia, it has been suggested that vasodilator mechanisms engaged by hypoxia should be examined during α-adrenergic blockade (35). However, α-adrenergic blockade markedly raises basal muscle blood flow (23) and, thereby, may attenuate the metabolic error signal that arises in skeletal muscle during hypoxia, thus diminishing the need for further vasodilation when arterial O2 content is reduced.

Several limitations of our study should be addressed. We did not directly test whether, at the dose used in our studies, fluconazole inhibits EDHF-like products of the cytochrome P-450 2C9 pathway and whether such an effect was due to interference with hyperpolarization or, alternatively, through nonspecific effects of the drug. However, the infusion rate employed in our study is similar to that estimated to produce local concentrations of fluconazole severalfold higher than those required to inhibit EDHF production in vivo (3) and was shown to inhibit metabolic vasodilation (3, 5, 25). Furthermore, the mild vasoconstriction during the fluconazole infusion and normoxia and its inhibitory effect on the vasodilation induced by hypoxia strongly support the conclusion that this drug inhibits an important vasodilator mechanism in skeletal muscle. Our studies were done in the absence of inhibition of prostaglandins, and compensatory effects of altered prostaglandin production during hypoxia could have partly masked the apparent effects of inhibition of EDHF and NO. It has also been suggested that hypoxia-induced skeletal muscle vasodilation in humans may in part be mediated by stimulation of β-adrenergic receptors (6, 35). However, plasma epinephrine has been shown to rise during systemic hypoxia in some (35), but not in other (6, 27), studies and may depend on the severity of hypoxia (30). In addition, such an effect would not account for the differences in the two experimental trials between the two forearms.

In conclusion, the present data expand our understanding of hypoxia-induced skeletal muscle vasodilation in humans and suggest that an endothelial, but NO- and cyclooxygenase-independent, vasodilator system with EDHF-like properties that can be inhibited with the antifungal agent fluconazole is capable of contributing to the vasodilation needed to match blood flow with O2 demand during moderate systemic hypoxia. In healthy humans, this vasodilator mechanism is operative even when NO synthesis is intact but may have heightened importance when other vasodilator systems are impaired.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
S.S., M.D.H., and U.A.L. performed the experiments; S.S. and U.A.L. analyzed the data; S.S. and U.A.L. drafted the manuscript; S.S., M.D.H., L.I.S., and U.A.L. approved the final version of the manuscript; L.I.S. and U.A.L. interpreted the results of the experiments; L.I.S. and U.A.L. edited and revised the manuscript; U.A.L. is responsible for conception and design of the research; U.A.L. prepared the figures.

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