Skeletal Muscle Vasodilation During Systemic Hypoxia in Humans

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Abstract: In humans, the net effect of acute systemic hypoxia in quiescent skeletal muscle is vasodilation despite significant reflex increases in muscle sympathetic vasoconstrictor nerve activity. This vasodilation increases tissue perfusion and oxygen delivery to maintain tissue oxygen consumption. Although several mechanisms may be involved, we recently tested the roles of two endothelial-derived substances during conditions of sympathoadrenal blockade to isolate local vascular control mechanisms: nitric oxide (NO) and prostaglandins (PGs). Our findings indicate that (1) NO normally plays a role in regulating vascular tone during hypoxia independent of the PG pathway; (2) PGs do not normally contribute to vascular tone during hypoxia, however do impact vascular tone when NO is inhibited; (3) NO and PGs are not independently obligatory to observe hypoxic vasodilation when assessed as a response from rest to steady-state hypoxia; and (4) combined NO and PG inhibition abolishes hypoxic vasodilation in human skeletal muscle. When the stimulus is exacerbated via combined submaximal rhythmic exercise and systemic hypoxia to cause further red blood cell (RBC) deoxygenation, skeletal muscle blood flow is augmented compared with normoxic exercise via local dilator mechanisms to maintain oxygen delivery to the active tissue. Data obtained in a follow-up study indicate that combined NO and PG inhibition during hypoxic exercise blunts the augmented vasodilation and hyperemia compared with control (normoxic) conditions ~50%, however in contrast to hypoxia alone, the response is not abolished implicating other local substances are involved. Factors associated with greater RBC deoxygenation such as ATP release and/or nitrite reduction to NO likely play a role in regulating this response.
In conscious humans and experimental animals, acute exposure to systemic hypoxia evokes autonomic reflex responses and alterations in the synthesis of a variety of vasoactive substances within the local tissue, blood vessels, and red blood cells, all of which contribute to the control of vascular tone (Marshall, 1999). With respect to the skeletal muscle circulation, the net effect of these changes is vasodilation that is graded with the degree of systemic hypoxia (2, 24, 39) despite concurrent sympathetic activation as evidenced by increases in muscle sympathetic nerve activity (54, 82). This vasodilation increases tissue perfusion and oxygen delivery to maintain tissue oxygen consumption (18). Although many studies have been performed in experimental animals \textit{in vivo} as well as in isolated blood vessels \textit{in vitro}, the focus of this review will be on our understanding of vascular control mechanisms from studies conducted in conscious human subjects utilizing local (vs systemic) pharmacological approaches as highlighted in an invited presentation at the 19th International Hypoxia Symposium at the Chateau Lake Louise within the session entitled “Gasotransmitters, Adenosine and Vasodilation to Counteract Hypoxia.” Additionally, the majority of mechanistic studies in humans have been performed in the forearm muscle circulation, and as such, the physiology discussed reflects vascular control of a small muscle mass when cardiac pumping capacity is not limited. Finally, although there are many proposed theories and excellent reviews on similar topics, this is a brief (non-exhaustive) review that reflects the opinion of the author and presents alternative interpretations to existing data as well as ideas regarding future directions.

\textbf{Skeletal Muscle Vasodilation During Systemic Hypoxia in Quiescent Tissue}

\textit{Sympathoadrenal Control of Vascular Tone: norepinephrine-mediated vasoconstriction}
Acute systemic hypoxia in humans leads to chemoreflex (and perhaps baroreflex) - mediated increases in sympathetic nervous systemic activity as evidenced by a marked increase in muscle sympathetic nerve discharge via direct neural recordings (40, 54, 82). Despite this, circulating norepinephrine (NE) is not typically elevated due to greater neurotransmitter clearance during hypoxia (54). Because MSNA is high and net vasodilation (vs vasoconstriction) is observed, it was originally suspected that systemic hypoxia reduces NE responsiveness in resting skeletal muscle, analogous to what occurs in contracting skeletal muscle (76, 94). However, we directly tested this hypothesis and found that post-junctional \( \alpha \)-adrenergic receptor responsiveness is not blunted in resting (quiescent) skeletal muscle during systemic hypoxia (24), a finding that was replicated by Wilkins et al. (98). These findings are also consistent with the observation that hypoxic vasodilation is restrained by elevations in sympathetic vasoconstriction, such that acute blockade of \( \alpha \)-adrenergic receptors results in an augmented local dilatory response (96). From a teleological perspective, this sympathetic “restraint” of hypoxic vasodilation may be important for appropriate blood pressure regulation as several vascular beds including skeletal muscle (e.g. coronary, cerebral) vasodilate in response to systemic hypoxia.

**Sympathoadrenal Control of Vascular Tone: epinephrine-mediated vasodilation**

In addition to elevations in muscle sympathetic nerve activity, circulating epinephrine is typically (78, 96-98) but not always (3, 79) increased presumably as a result of elevated neural activity to the adrenal medulla (72), or perhaps a direct effect of hypoxia on the gland. The role of circulating epinephrine and subsequent stimulation of \( \beta \)-adrenergic receptors in contributing to hypoxic vasodilation in the human forearm was originally studied in the late 1960’s (79) and
more recently by Dr. Joyner and colleagues (96), with the results being equivocal. It should be
noted here that most studies on this topic have reduced hemoglobin (Hb) saturations to ~75-80%,
with corresponding arterial partial pressure of oxygen (PO$_2$) to ~40-45 mmHg. Under such
experimental conditions, Richardson et al. (79) demonstrated that local blockade of $\beta$-adrenergic
receptors had a non-significant (<10%) effect on forearm blood flow and vascular resistance. In
contrast, Blauw et al. (3) demonstrated that local blockade of $\beta$-receptors not only blunted the
local vasodilation in the forearm, but resulted in a net vasoconstrictor response presumably due
to “unopposed” sympathetic vasoconstriction (as $\alpha$-adrenergic receptors were not blocked in this
study). Interestingly, this was observed in the absence of significant elevations in circulating
epinephrine.

A series of studies from Dr. Joyner and colleagues have also implicated a role for
epinephrine, suggesting that $\beta$-receptor stimulation mediates up to ~50% of hypoxic vasodilation
(96, 97), however some caution is warranted when interpreting their findings. Specifically, the
role of $\beta$-mediated vasodilation was always studied when $\alpha$-adrenergic receptors were inhibited
(96, 97). Although this approach is important for evaluating vasodilating mechanisms
independent of sympathetic vasoconstriction, local non-selective $\alpha$-blockade can increase NE
release from sympathetic nerve endings via inhibition of pre-junctional $\alpha_2$-adrenergic receptors
leading to stimulation of $\beta$-receptors independent of circulating epinephrine (30, 84).
Importantly, this effect could be enhanced during systemic hypoxia when sympathetic nerve
discharge is elevated, leading to a potential overestimation of the contribution of $\beta$-adrenergic
receptors to the net dilatory responses. Recent data from our laboratory in young healthy
humans indicate that, despite a significant increase in plasma epinephrine, hypoxic vasodilation
is only modestly blunted (~10%) during blockade of $\beta$-receptors (without concomitant $\alpha$-
receptor blockade) (78), consistent with observations in the 1960’s. Taken together, the collective data indicate that β-receptor activation can participate in hypoxic vasodilation in humans, however evidence also exists that this may not be obligatory to observe the normal dilatory response.

Local Control of Vascular Tone: endothelial-derived nitric oxide and prostaglandins

The endothelial autocoids nitric oxide (NO) and prostaglandins (PGs) have received attention for potentially mediating hypoxic vasodilation in experimental animals (75), isolated vessel preparations, as well as in humans in vivo (4). This most likely reflects the observations that an intact healthy endothelium may be necessary to observe hypoxic vasodilation (8, 31, 58, 73), and early studies clearly demonstrate release of NO and PGs from the endothelium when hypoxic. Blitzer et al. (4) were the first to directly test whether endothelial-derived NO was involved in hypoxic vasodilation in the human forearm. Utilizing intra-brachial infusions of L-NMMA to inhibit NO synthase (NOS), they observed a significant attenuation of local vasodilation during hypoxia. An important control experiment revealed that pre-constriction of the forearm vasculature with the α1-agonist phenylephrine (to reduce vascular tone similar to what occurs with L-NMMA infusions) did not impact the normal hypoxic dilatory response. These data indicate that NO actively participated in hypoxic vasodilation and that the effect of NOS inhibition was not simply due to a reduction in basal vascular tone.

Subsequent to this study, Weisbrod et al. (96) questioned whether this NO-mediated dilation was due to β-adrenergic receptor stimulation via circulating epinephrine, as experimental evidence in the human forearm indicates that a significant portion of β-mediated dilation is NO-dependent (20). In this study, infusion of L-NMMA did not further attenuate local hypoxic
vasodilation beyond what was observed during β-blockade, and when viewed in the context of prior reports, led to the conclusion that the NO component of hypoxic vasodilation was downstream of β-receptor stimulation.

Although this is one plausible interpretation of their data, many studies in several experimental preparations have demonstrated a significant interplay or “cross-talk” between NO and PGs in the regulation of vascular tone. For example, it has been clearly demonstrated that isolated endothelial cells or resistance vessels subjected to acute and chronic NOS inhibition demonstrate an augmented shear stress-induced increase in the synthesis of vasodilating PGs (e.g. prostacyclin) which acts to maintain normal vasodilation (70, 92). Further, in some models of exercise hyperemia, single inhibition of either NO or PGs do not impact muscle blood flow responses to contracting muscle in humans whereas combined inhibition can reduce exercise hyperemia ~20-30% (5, 22, 61, 86). Thus, our laboratory questioned whether this interplay was operative during systemic hypoxia in humans and could possibly explain the lack of NOS inhibition on hypoxic vasodilation during β-blockade in the study by Weisbrod et al. (96). In other words, we questioned whether PGs compensated for the acute inhibition and “loss” of NO dependent signaling and thus maintain a “normal” hypoxic vasodilator response. Importantly, prior to our studies on this topic, no studies had been designed to determine whether PGs were involved in hypoxic vasodilation in human skeletal muscle, despite evidence from very detailed studies in experimental animals (75).

To gain further insight into the roles of NO and PGs in hypoxic vasodilation, we designed a complex study to understand the independent and potential interactive roles of these endothelial-derived substances in humans (57). Prior to any experimental trials, we administered both phentolamine (α-receptor antagonist) and propranolol (β-receptor antagonist) to eliminate
sympathoadrenal influences on vascular tone, thus isolating local vascular control mechanisms.

By design, our study allowed us to determine (1) the independent and interactive roles of NO and PGs to vascular control during systemic hypoxia, (2) the independent roles of NO and PGs to hypoxic vasodilation (i.e. from baseline to steady-state hypoxia), and (3) the combined effect of NO and PGs on hypoxic vasodilation. One group of subjects received the NOS inhibitor L-NAME first, another group of subjects received the cyclooxygenase (COX) inhibitor ketorolac first (to inhibit PG synthesis). As shown in Figure 1, our results indicate that (1) NO normally plays a role in regulating vascular tone during hypoxia independent of the PG pathway; (2) PGs do not normally contribute to vascular tone during hypoxia, however do impact vascular tone when NO is inhibited; (3) NO and PGs are not independently obligatory to observe hypoxic vasodilation when assessed as a response from rest to steady-state hypoxia; and (4) combined NO and PG inhibition abolishes hypoxic vasodilation in human skeletal muscle under these conditions (57). Our findings also indicate that stimulation of NO under these conditions of systemic hypoxia in quiescent skeletal muscle is not a result of PG synthesis (67).

Exacerbating the Stimulus: Hypoxic Exercise in Humans

A common experimental approach to understand hypoxic vascular control in human skeletal muscle is to combine manipulations of systemic oxygen levels with muscle contractions to further create mismatches between oxygen delivery and demand, leading to greater red blood cell (RBC) deoxygenation. As recently reviewed by my colleagues Drs. Casey and Joyner (10, 46), the elevation in skeletal muscle blood flow during submaximal hypoxic exercise compared with that during normoxic exercise is due to local vasodilation. This “augmented” or “compensatory” vasodilation occurs to effectively match oxygen delivery (via elevated blood
flow) to oxygen demand of the contracting muscle in the face of lower arterial oxygen content 
(CaO2) (10, 17, 50, 80, 83). In addition to the effects of muscle contraction as an added 
complexity to vascular control, these combined stimuli evoke greater local deoxygenation of 
RBCs, and the absolute change in muscle blood flow during hypoxic exercise is greater than the 
sum of hypoxia or exercise alone (10).

Sympathoadrenal Control of Vascular Tone

The role of sympathetic α-adrenergic vasoconstriction and β-mediated vasodilation 
during combined mild intensity exercise and systemic hypoxia is quite similar to that observed in 
quiescent muscle. Muscle blood flow and vascular conductance are significantly greater than 
during normoxic exercise despite concomitant elevations in sympathetic outflow (41, 42, 88, 97). 
Because post-junctional α-responsiveness is intact under these conditions (98), elevations in 
sympathetic activity result in a greater sympathetic “restraint” compared with normoxic 
conditions, and this sympathetic restraint acts to limit the magnitude of vasodilation (13, 97). 
β-receptor stimulation appears to play a role in vasodilation during mild intensity hypoxic exercise 
(9, 97), but again, this data needs to be interpreted with caution as all studies were performed 
after local α-blockade (see previous section for discussion).

Local Control of Vascular Tone: endothelial-derived NO and PGs

In normoxia, there is evidence to suggest that (1) NO or PGs are not obligatory for 
exercise hyperemia (5, 61, 86, 87), (2) NO independently contributes ∼20% to exercise 
hyperemia whereas the independent PG contribution is modest and transient (86), and (3) NO 
and PGs act synergistically in the regulation of vascular tone and thus muscle blood flow during
exercise in humans (61, 62, 86). When exercise during systemic hypoxia was performed at higher intensities, the skeletal muscle vasodilation observed was clearly independent of β-receptors and thus it appears that there is a greater reliance on local dilatory signaling mechanisms (9, 97). Accordingly, Casey and colleagues have performed a series of investigations on this topic and have made a number of key observations [for review see ref (11)]. The most consistent finding from these studies was that acute inhibition of NO synthesis significantly reduced the augmented hyperemia and vasodilation during hypoxic exercise in humans, and close inspection of this data indicates that NO (presumably from the endothelium) contributes ~60% if not more to the entire response (9, 12). This was observed with or without local β-blockade, and thus a stimulus beyond epinephrine (or norepinephrine) acting on β-receptors augmented NO synthesis under these conditions (9). These studies were performed under normal conditions when sympathetic vasoconstriction was intact, and thus these observations reflect an interaction between NO-mediated vasodilatation and sympathetic α-adrenergic receptor vasoconstriction. Given that sympathetic restraint of muscle blood flow during hypoxic exercise is greater than that during normoxia (97), this intact sympathetic vasoconstriction may be masking other vasodilating pathways operative under these conditions.

Although this is one way to study hypoxic exercise hyperemia, as a follow-up to our studies in quiescent muscle, we were interested in performing similar studies utilizing pharmacological inhibition of both α- and β-adrenergic receptors to eliminate sympathoadrenal influences on vascular tone, and thus isolate local control mechanisms. Given our recent findings under these experimental conditions that combined inhibition of NO and vasodilating PGs abolished hypoxic vasodilation in resting muscle, whereas single inhibition of NO or PGs alone did not impact the response from rest to steady-state hypoxia (57), we questioned whether
these two endothelium-derived substances played a significant role in augmenting the
vasodilatory (and hyperemic) response during hypoxic exercise. We also questioned this, given
the observations during forearm exercise that combined NO and PG inhibition does not impact
the normal dilatory and hyperemic response to handgrip exercise (86). In this study, as opposed
to our prior experiments during both normoxic exercise (86) and systemic hypoxia at rest (57),
we did not inhibit NO or PGs after achieving steady-state hyperemia. The data from these
experiments demonstrate that, consistent with prior findings, combined inhibition of NO and PGs
(1) does not impact the normal local vasodilator responses to normoxic handgrip exercise and (2)
abolished hypoxic vasodilation to resting (quiescent) skeletal muscle (Figure 2). Further, our
data indicate that combined inhibition of NO and PGs significantly blunts the augmented local
dilator response by ~50% to moderate intensity hypoxic exercise (Figure 2), however local
vasodilation and muscle blood flow was still augmented compared with control conditions (18).
Thus, it is clear that additional mechanisms beyond endothelial NO and PGs can influence
vascular tone and participate in the augmented dilatory and blood flow response to hypoxic
exercise. Our current understanding of the remaining signaling pathways(s) in humans remain
incomplete, and perhaps future work should incorporate studies in which sympathetic $\alpha$-
adrenergic vasoconstriction is both intact and experimentally removed to gain more integrative
insight into this regulation.

What is the Stimulus for Local Vasodilation?

There is clear experimental evidence that NO and PGs play a major role in hypoxic
vasodilation in quiescent skeletal muscle (4, 57), and also contribute significantly to the
augmented vasodilation and hyperemia to contracting skeletal muscle during systemic hypoxia
These are important mechanistic studies particularly in humans, but what is the stimulus for eNOS and COX to produce these autocoids? Arguments have been made that the hypoxic “sensor” is located in the endothelium, vascular smooth muscle cells, and the red blood cells. While there is evidence to support all of these theories, classic human physiology studies dating back to the 1990’s, and more thereafter using a variety of experimental manipulations, demonstrated that skeletal muscle blood flow and vascular conductance are more related to changes in CaO₂, not simple changes in PO₂ (36-38, 51, 80). This is important to understand given that studies utilizing systemic hypoxia or systemic hypoxia in combination with exercise often manipulate both CaO₂ and PO₂. Thus, whatever is stimulating NO and PG synthesis and the remaining unexplained factors regulating vascular tone to active hypoxic muscle must be sensitive to changes in CaO₂. This idea is further highlighted by the lack of effect of combined NO and PG inhibition on normoxic exercise hyperemia, whereas there is a significant effect during both hypoxia at rest and during hypoxic exercise (18). Given that changes in CaO₂ are directly related to reductions in the oxygenation of Hb, the RBC may be implicated as a potential sensor of the hypoxic stimulus and modulate vascular tone, blood flow, and oxygen delivery accordingly (more discussion below).

Adenosine has long been suggested to be involved in skeletal muscle vascular control, particularly during mismatches in oxygen delivery and demand such as occurs during hypoxia, exercise, and ischemia. Despite the number of investigations on this topic, very limited data exist with respect to plasma adenosine concentrations during hypoxia, most likely reflecting the extremely rapid catabolism of this purine and half-life in the blood (64). A few studies have shown adenosine concentrations increase in plasma during systemic hypoxia (25, 85), and it appears interstitial adenosine increases as well (56). The source of adenosine in plasma is
unclear but may be related to deoxygenated RBC adenosine triphosphate (ATP) release (see
below) and subsequent hydrolysis to adenosine or direct release from endothelial cells, and
interstitial adenosine reflects release from skeletal muscle. Interestingly, adenosine-mediated
vasodilation is significantly reduced via inhibition of NO and PG synthesis (23, 62, 68),
consistent with a role for these autocoids in vascular control during systemic hypoxia in
quiescent and active skeletal muscle (12, 18, 57). The first study in humans to determine
whether adenosine was involved in hypoxic vasodilation was performed by Leuenberger et al.
(55), and they demonstrated that acute adenosine receptor inhibition via intra-brachial
aminophylline abolished forearm vasodilation during systemic hypoxia. However, more recent
data from Casey et al. provide compelling evidence that adenosine is not obligatory to observe
hypoxic vasodilation in quiescent skeletal muscle, nor is it involved in the augmented
vasodilatory response during hypoxic handgrip exercise (12, 13). This latter observation has been
supported during knee extensor exercise (43). The exact reason for the discrepant findings,
particularly in resting muscle, is unclear, but one possibility is that the “off-target” effects
aminophylline (e.g. non-selective phosphodiesterase inhibition) can increase resting muscle
blood flow (and thus local oxygenation) and potentially impact the response to the hypoxic
stimulus. Additionally, significant alterations in resting vascular tone may impact data
quantification and interpretation; this has not been an issue where selective pharmacology for the
adenosine receptors have been utilized in animal studies (6). Finally, aminophylline only
inhibits ~50% of adenosine-mediated dilation in humans (49). Thus, although questions exist
regarding the role of adenosine, experimental considerations noted above preclude the ability to
state definitively that this substance is not involved in hypoxic vascular control in humans.
Studies dating back to the 1950’s and 1960’s also implicated another purine, ATP, in the control of skeletal muscle vascular tone (26, 29). Building upon original observations by Bergfeld and Forrester that isolated RBCs release ATP during hypoxia and hypercapnia (1), Drs. Ellsworth and Sprague and collaborators have proposed that the RBC not only delivers oxygen, but acts as an oxygen sensor. In this context, they have elegantly demonstrated that (1) ATP is released from RBCs during hypoxia and this is graded with the level of Hb deoxygenation (not PO$_2$) (44); (2) there is a clear oxygen-sensitive intracellular signaling cascade leading to ATP release from pannexin-1 channels (90); and (3) hypoxic vasodilation in isolated skeletal muscle arterioles requires the presence of RBCs and ATP release (21, 89) [also see ref (28) for an excellent review]. Consistent with this, more recent studies in humans from our laboratory and others have shown elevations in venous plasma ATP draining hypoxic and active skeletal muscle (Figure 3A and B) (37, 48, 63), although it should be noted that similar challenges exist when measuring this purine in circulation as its half-life is estimated to be <1 second (63), and thus concentrations at the level of the microcirculation are likely much greater than reported. Similar to adenosine, some studies have demonstrated that NO and PGs mediate a portion of ATP-induced dilation in humans (17, 60), whereas other studies have argued against this (15, 81, 95). 

*In vitro* studies have demonstrated that ATP-mediated vasodilation is endothelium-dependent (7, 74) and activates endothelial small and intermediate $K_{Ca}$ channels (99). Consistent with a downstream effect of activating these endothelial $K_{Ca}$ channels (27), our laboratory has recently demonstrated that ~50% of ATP-mediated vasodilation occurs via activation of inwardly rectifying-potassium (K$_{IR}$) channels (Figure 3C). To date, direct mechanistic data regarding ATP and P$_{2y}$ receptor stimulation in mediating hypoxic vasodilation in quiescent or active skeletal muscle in humans is limited due to the lack of pharmacology to inhibit the receptors. However,
given recent pharmacological approaches we have adopted to inhibit downstream signaling of intravascular ATP in humans, studies incorporating inhibition of K$_{IR}$ channels (in addition to NOS and COX inhibition) and determination of the remaining unexplained hypoxic vasodilation during hypoxic exercise could provide further insight into the role of ATP in vascular control under these conditions.

Beyond ATP release, there are two other prevailing theories regarding RBCs acting as oxygen sensors, serving as a source of NO bioavailability (potentially independent of eNOS) particularly in hypoxic environments. The original theory proposed by Stamler and colleagues states that hemoglobin becomes S-nitros(yl)ated on a specific and conserved cysteine residue on the β–chain (β93Cys) as erythrocytes become oxygenated in the lungs (forming S-nitrosohemoglobin; SNO-Hb); this NO is transmitted out of the RBC upon deoxygenation evoking local vasodilation (45, 91). More recently, Drs. Gladwin and Patel and collaborators have demonstrated that Hb can function as a nitrite reductase, whereby low oxygen results in bioactivation of nitrite via deoxyhemoglobin and stimulation of blood flow via NO-mediated dilation and restoration of oxygen delivery (14, 32, 33). Of note related to our studies and others on hypoxic exercise, deep venous PO$_2$ is very close to the P50 of the oxygen dissociation curve of Hb where this reductase mechanism is optimized (32), making this an attractive mechanism to contribute to hypoxic vasodilation under these conditions.

Other Potential Contributing Mechanisms

Another recent idea regarding NO-mediated vascular control, independent of RBCs, has been proposed by Golub and Pittman where it is suggested that interstitial NO acts as an error signal whose magnitude is related to the mismatch in oxygen and glucose delivery and their
consumption. In this model, increases in blood flow during hypoxia or muscle contractions occurs via the lowering of the NO inhibitor superoxide (O$_2^-$) into the interstitial space, allowing endothelial-derived NO to diffuse to the vascular smooth muscle cells and evoke vasodilation (34, 35). This is an interesting theory as it challenges the conventional “metabolic” theory of blood flow regulation, and postulates that the system is designed to remove an inhibitor of local vasodilation (in this case, O$_2^-$), versus the accumulation or production of various “metabolites.” However, if this were the sole mechanism matching blood flow and oxygen delivery to metabolic demand as postulated, it is unclear how inhibition of NOS alone in our studies failed to impact hypoxic vasodilation in quiescent muscle (57) as well as the onset and steady-state response to normoxic exercise (18, 19, 86). Although a very provocative theory, there is clearly more complexity to vascular control during active muscle contractions as mechanical deformation of blood vessels during contractions (47), and the release of K$^+$ ions from active muscle (16, 19) evoke feedforward vasodilation and these signals among others would need to be integrated into this model with NO in a manner that leads to the overall matching of blood flow and oxygen delivery to metabolic demand.

In addition to the gas NO, other “gasotransmitters” have been recognized as playing a role in many responses to acute hypoxia, including vasodilation. Although beyond the scope of this review, carbon monoxide (CO) and hydrogen sulfide (H$_2$S) have received attention. With respect to the control of skeletal muscle vascular tone during hypoxia, it is unlikely that CO plays a role in the vasodilatory response as hypoxia inhibits heme oxygenase-2 activity and reduces CO generation (59). Although not demonstrated in humans, H$_2$S has been proposed as an oxygen sensor [for review see ref (69)] whereby low oxygen can stimulate cystathioinine $\gamma$-lyase (CSE) in peripheral tissues to increase H$_2$S production (71) and hyperpolarize the
endothelium and smooth muscle cells to evoke vasodilation (65). Recent data in the human cutaneous microvasculature support a functional vasodilatory role of H₂S (53), and future studies should be aimed at extending these observations to skeletal muscle and other vascular beds in response to systemic and/or local hypoxia. Figure 4 summarizes potential vascular control mechanisms involving the red blood cell during hypoxic conditions in quiescent and contracting human skeletal muscle.

**Summary and Future Directions**

Acute systemic hypoxia in humans leads to a net vasodilation in quiescent skeletal muscle that is graded with the level of hypoxia. This vasodilation serves to increase blood flow to maintain oxygen delivery when CaO₂ content is reduced. When exercise is combined with hypoxia which leads to greater RBC deoxygenation, a similar phenomenon is observed in that the normal dilatory response to exercise is augmented which in turn maintains oxygen delivery via elevations in blood flow to the contracting tissue. In both quiescent and active skeletal muscle, these responses act to maintain tissue oxygen consumption. A number of studies designed to partition out various pathways involved in the response have demonstrated that elevated sympathetic outflow and NE release evokes vasoconstriction that “restrains” resistance vessels and limits the net vasodilation in both resting and active muscle, that circulating epinephrine and β-receptor stimulation may be involved in the vasodilation at rest and during mild intensity exercise, and that endothelial-derived NO and PGs are stimulated during hypoxia in resting and contracting muscle. Other factors related to the oxygenation status of Hb likely play a role under these conditions during hypoxic exercise, and accumulating evidence indicates that RBC ATP release and nitrite reduction to NO may be involved.
The purpose of this review was to highlight our basic understanding of blood flow and oxygen delivery control to hypoxic skeletal muscle in healthy humans. Accumulating evidence indicates that hypoxic vasodilation is impaired in older healthy adults (52, 78), heart failure patients (66), and obstructive sleep apnea (77), populations clearly at elevated risk for ischemic coronary and cerebrovascular disease. Future studies will require furthering our basic understanding of the role of purines (adenosine and ATP), RBC-NO mediated dilation, and H₂S in regulating blood flow and oxygen delivery during hypoxia in humans. Further, although the function of transient receptor potential cation (TRP) channels are multifactorial, recent evidence implicates TRPA1 channels in oxygen sensing (93) and represents an interesting area of future investigation. Translation of these basic physiology studies to understanding hypoxic vascular control in various pathological states could provide ideas on how to improve tissue blood flow and oxygen delivery in at-risk patient populations.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: F.A.D. conception and design of research; interpreted results of experiments, drafted manuscript; approved final version of manuscript.
References:


Figure Legends

Figure 1. Hypoxic vascular control by nitric oxide and prostaglandins in quiescent skeletal muscle. NO synthase (NOS) inhibition reduces forearm vascular tone during steady-state hypoxia independent of cyclooxygenase (COX) inhibition (A), whereas COX inhibition only reduces vascular tone after inhibition of NO (B). NO inhibition (C) or PG inhibition (D) alone does not impact vasodilation from rest to steady-state hypoxia, however combined NO/PG inhibition abolishes hypoxic vasodilation in humans (Copyright 2011 The Physiological Society. Used with permission). * P<0.05 vs zero; † P < 0.05 vs independent COX inhibition (A); † † P < 0.05 vs control and single inhibition (B).

Figure 2. Effect of combined nitric oxide and prostaglandin inhibition on skeletal muscle vascular control at rest and during hypoxic exercise. Forearm blood flow (A) and vascular conductance (B) are significantly augmented during hypoxic exercise compared with normoxia. Combined NO and PG inhibition reduces this augmented response, but forearm hemodynamics are still greater compared with normoxia indicating other operative mechanisms are involved in the responses. Quantification of the impact of combined inhibition of NO and PGs on the augmented hypoxic exercise hyperemia indicates these autacoids explain ~50% of the response (Copyright 2011 The Physiological Society. Used with permission). *P<0.05 vs control condition; † P < 0.05 vs Rest 2; § P < 0.05 vs normoxic exercise (within condition; A and B). *P<0.05 vs normoxia/rest (A and B). ‡ P<0.05 vs 5 and 15% MVC (B). * P<0.05 vs control (C).

Figure 3. Intravascular ATP and mechanism of vasodilation in human skeletal muscle. Venous plasma [ATP] draining forearm skeletal muscle increases during systemic hypoxia (A) and graded exercise (B) in young healthy humans (Copyright 2012 The American Heart Association. Used with permission). Low dose barium chloride (BaCl2) to inhibit inwardly rectifying potassium (K_{IR}) channels significantly blunts ATP-mediated vasodilation in the human forearm (Copyright 2012 The Physiological Society. Used with permission). * P<0.05 vs normoxia/rest (A and B). ‡ P<0.05 vs 5 and 15% MVC (B). * P<0.05 vs control (C).

Figure 4. Potential mediators of local hypoxic vascular control in quiescent and contracting human skeletal muscle. NO and PG synthesis (from eNOS and COX, respectively) may be elevated by intravascular adenosine binding to purinergic 1 (P1) receptors and/or intravascular ATP released from red blood cells and binding to P_{2y} receptors. Nitrite may be reduced to NO via deoxygenated hemoglobin independent of eNOS. Intravascular ATP may also activate endothelial sK_{ca} and iK_{ca}, hyperpolarizing endothelial cells which can conduct to adjacent cells via gap junctions (GJ). This would result in elevated interstitial K^{+} and activation of (K_{IR}) channels resulting in smooth muscle cell hyperpolarization and “spreading” or “conducted” vasodilation. NO = nitric oxide; PGs = prostaglandins; eNOS = endothelial nitric oxide synthase; COX = cyclooxygenase; GJ = gap junction; MEGJ = myoendothelial gap junction; sK_{ca} = small calcium activated K^{+} channels; iK_{ca} = intermediate calcium activated K^{+} channels; VGCC = voltage-gated calcium channels.