Published in final edited form as:

Microcirculation. 2019 July ; 26(5): e12497. doi:10.1111/micc.12497.

Skeletal muscle interstitial O₂ pressures: bridging the gap between the capillary and myocyte

Daniel M. Hirai¹, Trenton D. Colburn¹, Jesse C. Craig¹, Kazuki Hotta², Yutaka Kano², Timothy I. Musch¹, David C. Poole¹

¹Departments of Anatomy & Physiology, Kinesiology; Kansas State University; Manhattan, KS, USA

²Department of Engineering Science, University of Electro-Communications; Tokyo, Japan

Abstract

The oxygen transport pathway from air to mitochondria involves a series of transfer steps within closely integrated systems (pulmonary, cardiovascular and tissue metabolic). Small and finite O₂ stores in most mammalian species require exquisitely controlled changes in O₂ flux rates to support elevated ATP turnover. This is especially true for the contracting skeletal muscle where O₂ requirements may increase two orders of magnitude above rest. This brief review focuses on the mechanistic bases for increased microvascular blood-myocyte O₂ flux ($\dot{V}O_2$) from rest-to-contractions. Fick's law dictates that $\dot{V}O_2$ elevations driven by muscle contractions are produced by commensurate changes in driving force (i.e., O₂ pressure gradients; P_{O_2}) and/or effective diffusing capacity (DO₂). While previous evidence indicates that increased DO₂ helps modulate contracting muscle O₂ flux, up until recently the role of the dynamic P_{O_2} across the capillary wall was unknown. Recent phosphorescence quenching investigations of both microvascular and novel interstitial PO₂ kinetics in health have resolved an important step in the O₂ cascade between the capillary and myocyte. Specifically, the significant transmural P_{O_2} at rest was sustained (but not increased) during submaximal contractions. This supports the contention that the blood-myocyte interface provides a substantial effective resistance to O₂ diffusion and underscores that modulations in erythrocyte hemodynamics and distribution (DO₂) are crucial to preserve the driving force for O₂ flux across the capillary wall (P_{O_2}) during contractions. Investigation of the O₂ transport pathway close to muscle mitochondria is key to identifying disease mechanisms and develop therapeutic approaches to ameliorate dysfunction and exercise intolerance.

Keywords

capillary; diffusion; exercise; microcirculation; oxygen gradients

Introduction

The oxygen transport pathway from atmospheric air to cell mitochondria involves a series of transfer steps within the pulmonary, cardiovascular and muscle metabolic systems (Fig. 1)¹. The rate of O₂ transport through these systems (i.e., convective and diffusive fluxes) is set by the rate at which O₂ accepts electrons within the mitochondria in the process of generating ATP. Unless sleeping or confined to very specific activities (e.g., bed restriction or constant work rate exercise in the research laboratory), humans rarely experience a constant metabolic steady-state^{2,3}. Instead, activities of daily living require multiple transitions from one metabolic rate to another. Because of limited O₂ storage in most mammals, fine-tuned changes in O₂ flux at each step of the pathway are necessary to support alterations in oxidative phosphorylation. This is especially important for the contracting skeletal muscle where O₂ requirements can increase rapidly up to 100-fold above rest⁴.

Under physiological conditions at sea level, the total O₂ stores in the resting human body (70 kg body mass) amount roughly to 1.2 L when considering the aggregate content within the main reservoir compartments as follows: approximately 0.4 L O₂ in the lungs (functional residual capacity = 3 L; fraction of alveolar O₂ = 0.14), 0.6 L O₂ in blood (arterial blood volume = 1.5 L; venous blood volume = 3.5 L; hemoglobin concentration = 15 g/100 ml; arterial saturation = 97%; venous saturation = 40%), and 0.17 L O₂ bound to leg muscle myoglobin (total leg muscle mass = 15 kg; myoglobin concentration = 0.5 mM/kg muscle)^{5,6}. For the typical 70 kg human engaged in vigorous physical activity (e.g., running at an oxygen uptake of 4 L/min), internal O₂ stores could thus support active skeletal muscle oxidative metabolism only during short bursts lasting a few seconds. In the case illustrated above, this period would be less than 20 s even assuming (incorrectly) that the entirety of this O₂ storage could be mobilized exclusively to the leg muscles together with complete hemoglobin and myoglobin desaturation^{7–9}. As such, a well-coordinated increase in and redistribution of O₂ transport along the entire pathway is paramount to support skeletal muscle contractile activity during sustained exercise.

This brief review explores what is generally considered to be the final step in the O₂ pathway from the skeletal muscle microcirculation to mitochondria (Fig. 1, *inset*)^{10,11}. The capillary vascular bed provides the largest surface area for O₂ transport within the skeletal muscle microvascular network thus taking center stage as the principal offloading site during contractions. Specific focus is placed on the movement of O₂ across physical and physiological barriers that separate a) the red blood cells (RBCs) enclosed in capillaries from the interstitium (i.e., barriers surrounding the capillary wall); and b) the interstitium from muscle mitochondria (i.e., sarcolemma and intracellular space) as illustrated by blue arrows in Fig. 1, *inset*. This transport occurs entirely by diffusion (but see also below and Clanton et al.¹² for other putative transport mechanisms) and thus depends on the existence of O₂ pressure (PO₂) gradients between different physiological compartments as described by Fick's law:

$$\dot{V}O_2 = DO_2(\Delta PO_2)$$

where $\dot{V}O_2$ corresponds to the rate of O_2 flux, DO_2 is the diffusing capacity and PO_2 the O_2 partial pressure difference between compartments. Although DO_2 represents a lumped parameter that includes the impediments to blood-myocyte O_2 transport and is determined by a complex interaction between structural and functional factors, there is evidence that DO_2 is dictated primarily by the aggregate number of RBCs within capillaries adjacent to the myocyte at any given moment^{13,14}. Fick's law thus mandates that elevations in O_2 flux ($\dot{V}O_2$) imposed by muscle contractions must be matched by corresponding changes in driving force (PO_2) and/or effective diffusing capacity (DO_2 ; i.e., mainly RBC hemodynamics and distribution).

Until recently, however, investigation of O_2 gradients within the skeletal muscle microcirculatory network has been hindered by technical challenges particularly during the transition from rest to contractions. Although techniques using O_2 microelectrodes, (cryo)microspectrophotometry and proton magnetic resonance spectroscopy (PMRS), for example, have provided an invaluable framework for microcirculatory O_2 transport^{4,15,16}, selective *in vivo* determination of the dynamic PO_2 across the capillary wall can only be performed at present via phosphorescence quenching¹⁷. This has been facilitated by the development of phosphorescent probes which allow the interrogation of O_2 pressures that can be isolated within either the microvascular or interstitial compartments (PO_{2mv} and PO_{2is} , respectively) in resting and contracting skeletal muscle^{18,19}. As discussed in detail below, recent dual-probe studies indicate that the transcapillary PO_2 (i.e., given by the difference between PO_{2mv} and PO_{2is}) found at rest is maintained during the metabolic transient in skeletal muscle²⁰. In accordance with Fick's law, this gradient constitutes the sole driving force for O_2 diffusion across the capillary wall and therefore plays a critical role in supporting increases in $\dot{V}O_2$ during contractions. These recent findings underscore the alterations in capillary hemodynamics necessary to raise perfusive and diffusive O_2 fluxes ($\dot{Q}O_2$ and DO_2 , respectively) in contracting skeletal muscle (i.e., augmented RBC flux, velocity and hematocrit^{21–23}). Importantly, this evaluation of key steps of the O_2 transport pathway at sites close to the mitochondria provides novel insights into the control of skeletal muscle vascular and oxidative function in health and dysfunction in disease.

Skeletal muscle microcirculatory O_2 transport: historical development

The work by the eminent physiologist and Nobel laureate August Krogh in the early 20th century identified the capillary as the principal site of diffusive O_2 delivery within the skeletal muscle microcirculation^{24,25}. Together with the mathematician K. Erlang, Krogh proposed a model for microvascular O_2 transport from a single capillary into a surrounding cylinder of uniform tissue serving as the O_2 sink (i.e., skeletal muscle housing cytochrome *c* oxidase). Based on this representation of the blood-myocyte interface, the Krogh-Erlang model predicts that intracellular PO_2 decreases systematically as a function of distance from the adjacent capillary^{24,25}. This establishes that large PO_2 gradients (PO_2) within the myocyte could produce *anoxic loci* which deprive especially the most distant mitochondria of O_2 . It is noteworthy that the Krogh-Erlang model is predicated on the major premises that a) most capillaries do not support RBC flux in resting skeletal muscle; and that b) initiation of RBC flux in previously non-flowing capillaries during contractions (i.e., the so-called “capillary recruitment” hypothesis) would reduce intramyocyte diffusion distances thereby

facilitating O₂ transport and metabolism. Importantly, acceptance of the “capillary recruitment” concept presumes the existence of pre-capillary sphincters and/or contractile capability of the capillary endothelium regulating RBC and plasma flow through individual capillaries.

Although undoubtedly laying the foundation for skeletal muscle microcirculatory O₂ transport and developing crucially the importance of diffusive O₂ transport, the Krogh-Erlang model has been called into question by contemporary theoretical and experimental evidence. Several of its assumptions, including the notion of “capillary recruitment” during the rest-contraction transient, have been theoretically and empirically challenged^{4,26,27}. Modern tools and techniques not subject to the manifest problems of early microcirculatory preparations (e.g., carbon particle aggregation or supra-physiological perfusion pressures as described below) have allowed a closer inspection of the microvascular network and advanced significantly our understanding of the O₂ transport pathway within skeletal muscle. In this regard, it must be noted that some of the misconceptions introduced in the Krogh-Erlang model, especially those arising from the conditions in which data were collected, were acknowledged by Krogh himself^{24,25}. His texts describe some of the extreme measures taken to achieve good muscle perfusion, such as tying off multiple vessels to minimize leakage, utilizing high perfusion pressures of several hundred mmHg (i.e., approximating 1 atm) and, occasionally, waiting multiple days *post-mortem* before conducting experiments. Moreover, the practice of injecting India ink particles to identify flowing capillaries likely clogged those vessels supporting only slow RBC flux. At high experimental perfusion rates, mitigation of this clumping effect in the tissue would raise the probability of more capillaries containing ink and, therefore, erroneously be considered to have been recruited (i.e., support *de novo* RBC flux)^{4,26}. This phenomenon, in and of itself, has been mistakenly interpreted as *prima facie* supportive evidence for the “capillary recruitment” notion.

A key facet of the Krogh-Erlang model lies in the presumption that skeletal muscle capillaries are not passive vessels but, instead, capable of regulating their perfusion via actions of pre-capillary sphincters and/or the capillary wall itself. At least part of the still-widespread belief that pre-capillary sphincters are blood flow and pressure regulators stems from the mistaken view that such structures are universal components of the microcirculation²⁷. While reported as a feature of the mesenteric microcirculation in certain species (as originally described via camera lucida illustrations by Benjamin W. Zweifach²⁸), unequivocal morphological evidence for the presence of pre-capillary sphincters within mammalian skeletal muscle is completely absent^{4,26,27}. In addition, it is now well established that bulk blood flow and pressure regulation largely takes place at the arteriolar level in the peripheral circulation^{29–32}. A completely different picture of muscle microcirculatory control has thus emerged over almost a century of research since the pioneering work by Krogh. Compelling evidence across a wide spectrum of experimental methodologies in both humans and animals indicates that most capillaries support RBC flux in resting muscle and are thus not available to be “recruited” during contractions^{4,26}. Nevertheless, the “capillary recruitment” dogma and its functional consequences (e.g., the purported role of intramyocyte diffusion distances to O₂ flux; see below) continue to be perpetuated by physiological textbooks and a multitude of scientific reports, the vast

majority of which failed to observe the muscle capillary bed or present *prima facie* evidence for “capillary recruitment”. Different views on this issue have sparked intense debates within the past decade^{33–36}.

Another critical aspect of the Krogh-Erlang model is the presumption that intracellular PO₂ decreases proportionally with distance from the capillary. As such, the diffusion distance from the RBC to mitochondria would represent an important impediment to O₂ transport. However, similar to the case of “capillary recruitment” discussed above, contemporary findings have contested this core assumption of the Krogh-Erlang model. Specifically, strong experimental evidence from cryomicrospectrophotometric studies indicates a very low and seemingly uniform intracellular PO₂ in contracting skeletal muscle^{37–39}. These data thus refute the relevance of diffusion distances to capillary-mitochondrial O₂ flux. A landmark study by Russell S. Richardson and colleagues⁸ later confirmed the presence of very low intramyocyte PO₂ values during exercise in humans (spatially averaged values across numerous muscle fibers ranging from ~2–3 to 5 mmHg during submaximal and maximal contractions via PMRS^{9,40}). Additional evidence disputing the importance of capillary-mitochondrial intramyocyte diffusion distances *per se* was obtained by a series of elegant studies by Peter D. Wagner and colleagues^{41,42}. These experiments manipulated chronically intercapillary diffusion distances in the dog gastrocnemius-plantaris complex via leg immobilization or exercise training. Their results demonstrated that muscle DO₂, at $\dot{V}O_{2max}$, could be dissociated from capillary density and intercapillary diffusion distances *per se*. Taken together, experimental evidence from these human and animal studies support the theoretical framework advanced by Federspiel and Popel¹³ and Groebe and Thews¹⁴, which considers that muscle DO₂ is in fact determined by the number of RBCs adjacent to the muscle fiber at any given instant - and not by capillary density or intramyocyte O₂ diffusion distances as proposed by Krogh and Erlang. In this new paradigm, events taking place within the microcirculation (which modulate RBC hemodynamics and distribution) constitute the primary determinants of skeletal muscle DO₂.

Furthermore, given the spatially flat (i.e., relatively uniform) and very low intramyocyte PO₂ as mentioned above^{8,9,37–40}, the greatest apparent resistance to blood-myocyte O₂ flux is now considered to reside within that short distance between the RBC and sarcolemma. Two main mechanisms are responsible for this transport “bottleneck”. First, this diffusion path lacks facilitated O₂ transport due to the absence of a heme protein carrier (e.g., hemoglobin, myoglobin). For this reason, the O₂ pathway segment constituting plasma, capillary wall and interstitium is also known as the carrier-free region (CFR; see Fig. 2)³⁸. In the rat spinotrapezius muscle, the shortest RBC-sarcolemma distance is approximately 1.5 μm .²⁰ Second, given that the same O₂ flux traverses both the capillary endothelium and mitochondrial membrane, high flux density (i.e., flux per unit area) raises the apparent resistance within the CFR due to a) large differences between capillary endothelium and external mitochondrial membrane aggregate surface areas (the latter being up to two orders of magnitude greater than the former); and b) the particulate nature of blood rendering only the capillary surface area in close approximation with the RBC functional for O₂ flux at any given instant^{5,13,14,38,43,44}. As a result, while the Krogh-Erlang model proposed initially a very small PO₂ gradient from the capillary to the myocyte (due to the short diffusion

distance and presumed uniform diffusivity across compartments), contemporary evidence suggests that the PO_2 drop per unit length of diffusion path across the CFR in the rat spinotrapezius is ~7 and 15–20 mmHg/ μ m for low and high intensity contractions, respectively^{14,38,45–47}.

Revision of the Krogh-Erlang model and inevitable transition to a different model of skeletal muscle microcirculatory O_2 transport comes with new and important questions. For instance, if “capillary recruitment” is not obligatory to reduce intramyocyte O_2 distances (and thus counteract substantial intracellular PO_2 gradients invoked originally), the question then becomes as to how O_2 flux ($\dot{V}O_2$) is increased from rest to contractions. As indicated above, elevations in $\dot{V}O_2$ with muscle contractions must be matched by commensurate changes in effective DO_2 and/or PO_2 according to Fick’s law as applied to the microcirculation. Up until very recently, however, only part of that puzzle had been solved. Intravital microscopy studies revealed that, because most capillaries support RBC flux at rest, increases in effective DO_2 within already flowing capillaries during muscle contractions are mediated via elevations in RBC flux, velocity and hematocrit^{21–23}. In healthy young skeletal muscles, these contraction-induced alterations in capillary RBC hemodynamics and distribution augment both convective and diffusive mechanisms for blood-myocyte O_2 transfer within the first contraction cycle²³. Specifically, elevations in RBC flux within capillaries originally demonstrating low flow at rest act to increase the relative contribution of those vessels to blood-myocyte O_2 transport during contractions. Augmented RBC velocity with contractions increases the effective surface area along the length of the capillary available for diffusion; a process termed capillary “longitudinal recruitment”^{4,26}. As such, a relatively greater proportion of the RBC pathlength participates effectively in O_2 transfer during contractions compared to the resting condition (when most of the O_2 transfer might occur in proximity to the arteriolar end of the capillary). In addition, the low resting capillary hematocrit increases towards systemic levels during contractions (from ~15 to potentially 45%) leading to a considerable elevation in the number of RBCs adjacent to the myocyte (thus raising effective DO_2). It is pertinent that this increased capillary hematocrit raises the probability of transverse histological sections transecting RBCs within a given capillary. The faulty assumption that only vessels with transected RBCs supported flow *in vivo* has, however, helped propagate the “capillary recruitment” dogma despite the wealth of evidence to the contrary^{4,23,26,48–52}.

Human PMRS studies examining skeletal muscle DO_2 during contractions have yielded incongruent results. Richardson and colleagues^{8,53} showed that, following an initial fall in myoglobin-associated PO_2 from rest to moderate-intensity knee extension exercise, there is no progressive myoglobin desaturation from moderate to maximal contractions. These data reveal a linear increase in calculated muscle DO_2 with work rate thus placing changes in diffusional O_2 conductance as the primary mechanism elevating O_2 flux with contractions. Conversely, Molé and colleagues⁹ reported progressive myoglobin desaturation with work rate during plantar flexion exercise. This profile has been interpreted, by some, as support for muscle DO_2 remaining nearly constant from rest to contractions thereby not contributing meaningfully to increases in O_2 flux during the metabolic transient. In this latter scenario, augmented PO_2 gradients with exercise intensity would appear to accomplish that goal

instead. It is important to note, however, that such differences in the modulation of contracting muscle O_2 flux between independent studies could arise from the methodological procedures employed by Molé and colleagues⁹. The argument was made that their large PMRS coil increased the likelihood of data acquisition from multiple muscles at the same time that plantar flexion exercise recruited both the gastrocnemius and soleus muscles^{53,54}. It is thus important to consider this signal heterogeneity (in terms of both muscle fiber type and recruitment order) when reconciling conflicting PMRS data. In this context, the scenario emerging from human (PMRS) studies^{8,53} appears to be consistent with that from animal (intravital microscopy) reports^{21–23} indicating substantial increases in muscle DO_2 during.

Therefore, when addressing the question raised above (i.e., pertaining to the mechanisms elevating O_2 flux with contractions), it has become evident that the increase in muscle $\dot{V}O_2$ seen during the rest-contraction transient is mediated, at least in part, by events taking place within already flowing microvascular units which enhance effective DO_2 ^{8,21–23,53}. As predicated by Fick's law, the remaining factor facilitating $\dot{V}O_2$ within the skeletal muscle microvascular network is transcapillary PO_2 . To examine how transcapillary PO_2 allows for increased $\dot{V}O_2$ during the metabolic transient required direct assessment of O_2 pressures within both the microvascular and interstitial spaces (PO_{2mv} and PO_{2is} , respectively). This challenge has been accomplished recently via phosphorescence quenching measurements in the rat spinotrapezius muscle from rest-to-contractions²⁰.

Skeletal muscle PO_2 dynamics: measurements via phosphorescence quenching

While skeletal muscle PO_{2mv} responses have been described in resting and contracting skeletal muscle in health^{17,55–57} and a variety of disease models (e.g., chronic heart failure (CHF), diabetes, emphysema^{58–60}), only recently has the dynamic PO_{2is} profile been reported during transitions in metabolic demand²⁰. PO_{2mv} and PO_{2is} can be evaluated via phosphorescence quenching using the probes Oxyphor G2¹⁸ and G4¹⁹, respectively. Both probes are well-suited for use in biological tissue due to their high solubility in aqueous media (e.g., blood plasma, interstitial fluid) and inability to permeate membranes within skeletal muscle^{18,19,61}. Interrogation of the microvascular space (i.e., PO_{2mv}) has been performed traditionally via phosphorescence quenching using Oxyphor G2^{17,18}. This compound contains palladium-porphyrin cores that bind to biological macromolecules (i.e., mainly plasma albumin) following intra-arterial infusions required for PO_{2mv} measurements. This facilitates uniform distribution of the probe in plasma providing a lumped signal from arterioles, capillaries and venules in proportion to their volumetric contribution within the interrogated field. The G2 signal thus provides a reflection of mean capillary PO_2 ; i.e., the upstream pressure head for diffusive O_2 transport in contracting skeletal muscle^{62,63}. On the other hand, Oxyphor G4 can operate in aqueous environments, independently of albumin, due to its unique polyethylene glycol surface layer. G4 can be delivered directly into the interstitial space via the microinjection technique⁶⁴ for determination of PO_{2is} responses within skeletal muscle. Crucially, evaluation of the dynamic PO_2 associated with these two compartments (i.e., dual probe studies) is necessary

to resolve transmural O₂ gradients ($\Delta PO_{2(t)} = PO_{2mv} - PO_{2is}$) and thus the O₂ driving force for transcapillary O₂ flux from rest-to-muscle contractions.

Skeletal muscle interstitial PO₂.

Previous phosphorescence quenching assessment of muscle PO_{2is} during metabolic transients has produced divergent results (e.g., in absolute resting and contracting values) and, until recently, no formal description of its dynamic profile existed^{64,65}. A recent investigation from our laboratory thus aimed to resolve the temporal profile and determine the model parameters of PO_{2is} on-kinetics in healthy skeletal muscle (Fig. 3, filled symbols in *left panel*)²⁰. In that study, baseline PO_{2is} in the resting spinotrapezius muscle (16 ± 2 mmHg) was lower than reported previously using phosphorescence quenching (~ 28 and 68 mmHg)^{64,65}. The exact bases for this divergence are unclear but could involve methodological differences in probe delivery and/or localization, spatial resolution and phosphorescence quenching analyses. Resting PO_{2is} values found in our study are in close agreement with intracellular PO₂ obtained by others using microelectrodes (~ 14 – 17 mmHg)^{66,67} and cryomicrospectrophotometry (myoglobin-associated PO₂ ~ 10 – 20 mmHg)⁴⁷, but lower than PMRS data (~ 34 mmHg in human quadriceps)⁴⁰. As illustrated in Fig. 3 (*left panel*), during the early contraction transient PO_{2is} fell quasi-exponentially to values approaching intramyocyte PO₂ found in humans performing moderate exercise (i.e., approximately 5 mmHg)^{8,9}. As such, the novel dynamic PO_{2is} profile seems reasonable and well within the expected range of interstitial values based upon estimates of transmural PO₂ gradients from rest-to-contractions^{14,38,45–47} and previously-reported microvascular and intramyocyte PO₂ data^{8,9,63}. Furthermore, the recent PO_{2is} kinetic profile obtained in the rat spinotrapezius during the contraction transient²⁰ resembles closely that of arterial-venous O₂ content difference^{68,69}, deoxygenated hemoglobin concentration⁷⁰, PO_{2mv}⁵⁵ and intracellular PO₂⁷¹.

Skeletal muscle transcapillary PO₂.

Previous dual-probe phosphorescence quenching studies of both PO_{2mv} and PO_{2is} had examined exclusively resting (i.e., non-contracting) muscle. Using different preparations such as the rat cremaster, hamster cheek pouch and mouse thigh muscle, the resting transmural gradient has been found to be either very small (~ 1 mmHg around $45 \mu\text{m}$ diameter arterioles and <1.5 mmHg across the capillary endothelium^{17,56}) or relatively large (~ 15 – 20 mmHg around 40 – $130 \mu\text{m}$ diameter arterioles⁵⁷). Although these discrepancies could relate, at least in part, to distinct muscles and/or experimental procedures, it is critical to acknowledge that non-existent or very small transmural PO₂ imply equilibrium between microvascular blood and interstitium. As dictated by Fick's law, this scenario negates appreciable O₂ diffusion between compartments and is, therefore, incompatible with the observed O₂ unloading from the RBC along the length of the muscle capillary network (i.e., longitudinal PO₂ gradients^{16,72}).

As mentioned above, we have recently employed both G2 and G4 probes in the rat spinotrapezius muscle from rest-to-submaximal contractions²⁰. Acquisition of both PO_{2mv} and PO_{2is} data within the same animals (Fig. 3, *left panel*) allowed, for the first time, the determination of individual transcapillary PO₂ kinetics during the metabolic transition (see

Fig. 3, *right panel* for mean responses). This study revealed that a relatively large transcapillary PO_2 observed at rest was maintained throughout the contraction protocol (resting baseline: 19 ± 3 ; contracting steady-state: 17 ± 2 mmHg; $P > 0.05$). These findings align partially with those of Shibata et al.⁵⁷, who observed large transmural gradients in the resting cremaster muscle (~ 15 – 20 mmHg) but at substantially higher microvascular and interstitial PO_2 values (~ 47 – 75 and 29 – 51 mmHg, respectively). In our study²⁰, the transmural O_2 flux across the CFR in the resting spinotrapezius muscle was calculated (via Fick's law and tissue permeability $K = 3.17 \times 10^{-10}$ ml O_2 cm^{-1} s^{-1} mmHg $^{-1}$) to be $\sim 4.2 \times 10^{-5}$ ml O_2 cm^{-2} s^{-1} , which falls within the physiological range predicted previously by Vadapalli et al.⁷³. Moreover, close inspection of the spinotrapezius muscle via transmission electron microscopy paired with PO_2mv and PO_2is measurements unveiled that the PO_2 across the CFR during moderate-intensity contractions (~ 12 mmHg/ μm)²⁰ matches closely with that estimated previously (~ 7 to 15 – 20 mmHg/ μm from low to high intensity muscle contractions)^{14,38,45–47}. Presence of a significant transcapillary PO_2 gradient from rest to contractions thus supports that the CFR is the site of substantial resistance to O_2 diffusion within skeletal muscle^{10,13,14,38}. Together with the growing body of evidence challenging fundamental elements of the Krogh-Erlang model, these data expose the complex interplay between distinct diffusional compartments regulating microcirculatory O_2 transport not recognized originally.

That transcapillary PO_2 is preserved during the metabolic transient is remarkable given the elevations in $\dot{\text{V}}\text{O}_2$ imposed by muscle contractions and the prevailing high flux density across the CFR. It thus seems that the rate of blood-myocyte O_2 transfer and its physiological determinants are important regulated variables in the skeletal muscle microcirculation as insightfully predicted nearly 5 decades ago by Brian R. Duling^{74,75}. This PO_2 gradient constitutes the exclusive driving force for O_2 flux across the capillary wall as per Fick's law, thereby playing a critical role in supporting increased oxidative phosphorylation during contractions. A major implication of these findings is that, in healthy skeletal muscle, transmural PO_2 preservation during contractions is contingent upon quick and robust alterations in capillary RBC hemodynamics and distribution^{21–23}. This, in turn, underscores the interdependence between microcirculatory perfusive and diffusive conductances which ultimately set muscle O_2 extraction and $\dot{\text{V}}\text{O}_2$ ⁷⁶:

$$\% \text{O}_2 \text{ extraction} = 1 - e^{-\text{DO}_2 / \beta \dot{Q}_m}$$

where DO_2 is muscle diffusing capacity as defined above, β is the slope of the O_2 dissociation curve in the physiological range, and $\dot{Q}_m$ is muscle blood flow (mainly RBC flux when considering the microcirculation). As β is unlikely to be altered appreciably even by diverse conditions such as chronic disease or exercise training, $\% \text{O}_2$ extraction and thus PO_2mv will depend mainly on the $\text{DO}_2/\dot{Q}_m$ ratio. This relationship demonstrates how impairments in capillary hemodynamic control in disease states (e.g., CHF, diabetes and emphysema^{77–79}) reduce muscle PO_2mv during contractions^{58–60} and thus the upstream O_2 diffusion pressure for transcapillary flux (i.e., $\Delta \text{PO}_{2(t)} = \text{PO}_2mv - \text{PO}_2is$). The main functional consequence is a reduction in O_2 flux across the capillary wall unless commensurate

lowering of PO_2 (and, by extension, intramyocyte PO_2) takes place. It must be noted, however, that there is relatively little room for compensatory reductions in intramyocyte PO_2 given the very low values attained during both submaximal and maximal contractions in healthy skeletal muscle (~2–3 to 5 mmHg myoglobin PO_2)^{8,9}. Additionally, even small decrements in intramyocyte PO_2 during contractions can have major metabolic consequences and threaten mitochondrial respiration^{8,80}. Substantial reductions in transcapillary PO_2 could thus have pernicious consequences for oxidative metabolism and exercise tolerance.

Bridging the gap between the capillary and myocyte

The series of PO_2 gradients along the O_2 cascade from atmospheric air to skeletal muscle furnishes valuable information on the transport of O_2 in health and disease. Integration of the recent *in vivo* rat spinotrapezius PO_{2mv} and PO_{2is} data²⁰ to those derived from previous reports^{6,8,47,66} offers a more complete picture of this O_2 cascade. As illustrated in Fig. 4, an additional step bridging the gap between the capillary and myocyte has been resolved at rest and during contractions. Analysis of the series of PO_2 drops within skeletal muscle suggests a relatively small PO_2 across the sarcolemma (i.e., between the interstitial and intramyocyte spaces), thereby placing the greatest (but not the exclusive) resistance to diffusive O_2 flux at the microvascular-interstitium interface (i.e., CFR) in accordance with previous considerations and some predictions^{10,13,14,38}. Understanding the mechanisms underlying each PO_2 gradient can thus hold the key to identifying O_2 transport impairments associated with disease or enhancements resulting from exercise training and/or other therapeutic intervention.

The anatomical arrangement of the last three compartments (i.e., microvascular, interstitial and intracellular) comprising the O_2 transport pathway within skeletal muscle is shown in Fig. 2. This cross-sectional electron micrograph of the rat spinotrapezius shows a RBC enclosed in a capillary, the interstitial space and adjacent myocyte. As mentioned above, blood-myocyte O_2 transfer occurs entirely via diffusion (although the interesting question has been posed as to whether aquaporins can act as “ O_2 channels” and facilitate transmembrane O_2 flux¹²). Once within the muscle microcirculation, the O_2 journey towards the mitochondria starts with its dissociation from hemoglobin packaged in RBCs. Next, O_2 must diffuse out of the RBC before transiting the plasma and capillary wall to reach the interstitial space. This short diffusion distance traversing capillary plasma, endothelium and interstitial space is devoid of an O_2 carrier (i.e., CFR) and poses the greatest resistance to diffusive O_2 flux within skeletal muscle (hence the relatively large PO_2 drop between mean capillary and interstitial values in Fig. 4)^{10,13,14,20,38}. Subsequently, O_2 has to diffuse through the sarcolemma into the myocyte to ultimately arrive at its site of reduction in the mitochondria.

As reviewed by Clanton, Hogan and Gladden¹², there is substantial disagreement as to whether plasma membranes constitute a major barrier for O_2 diffusion. Resolution of this issue has important implications for intracellular O_2 transport and distribution. If the sarcolemma does not pose considerable resistance to O_2 flux into the myocyte, then the bulk of the intracellular resistance would be expected to reside within the sarcoplasmic domain.

There is also the intriguing possibility that lipid membranes could serve as O₂ storage sites and facilitate intracellular O₂ distribution via intramembrane lateral movement (see Fig. 4 in the review by Clanton, Hogan and Gladden¹²). Alternatively, a scenario where the sarcolemma hinders O₂ flux into the myocyte would benefit from parallel O₂ transport mechanisms such as aquaporins (“O₂ channels”), which putatively may assist overall diffusion through biological membranes, is intriguing.

Once inside the myocyte O₂ has to diffuse towards and ultimately into the mitochondria to serve as the final electron acceptor in the respiratory chain. Intracellular O₂ movement is traditionally considered to be facilitated by myoglobin particularly during muscle contractions^{5,12,38,81}. In the resting muscle, most myoglobin O₂ binding sites are saturated^{8,9} thus minimizing the potential for facilitated diffusion. This leads to the establishment of “functionally carrier-depleted regions” (FCDR) within the myocyte³⁸. Lowering of intracellular PO₂ during contractions^{8,9} reduces myoglobin O₂ saturation and consequently the thickness of the FCD. These events are thought to enhance intracellular DO₂ and the transport of O₂ to mitochondria within the myocyte. Nonetheless, the role of myoglobin in facilitated O₂ diffusion has been challenged and remains equivocal. Myoglobin knockout mice demonstrate no apparent decline in exercise tolerance during treadmill running along with no differences in isolated muscle fatigue in either normoxia or hypoxia⁸². The caveat here is that these transgenic animals experience high in utero mortality with the survivors displaying multiple central and peripheral adaptations (e.g., increased cardiac and skeletal muscle capillary density, elevated coronary reserve and flow, and increased nitric oxide-mediated vasodilation in certain locomotor muscles) that appear to compensate for the lack of myoglobin^{83,84}. In addition, discrepancy regarding the magnitude of intracellular diffusivity of myoglobin raises questions regarding its contribution to overall O₂ flux within contracting skeletal muscle^{5,12,81}. Myoglobin is also capable of scavenging nitric oxide and reactive oxygen species^{5,12,81} adding further complexity to its regulation of O₂ delivery-utilization matching.

To finally gain admittance into the mitochondria O₂ must diffuse through the mitochondrial membrane. This structure provides by far the greatest aggregate surface area in the O₂ diffusion pathway from hemoglobin to cytochromes^{5,37,44}. Based largely on this morphometric feature and mathematical models⁸⁵, PO₂ gradients across the mitochondrial membrane are expected to be considerably lower compared to those observed upstream across the capillary wall and sarcolemma during steady-state O₂ flux. In accordance with this notion, experimental evidence from frozen skeletal muscle (cryomicrospectrophotometry^{37,44}) and isolated mitochondria (dual luminescence probes; one bound to the membrane and the other in solution⁸⁶) indicate very shallow perimitochondrial PO₂. This gradient is thus often assumed to be negligible such that the PO₂ experienced by cytochrome oxidase is considered to reflect closely that of volume-averaged sarcoplasmic (or myoglobin) PO₂.^{5,37,44}

Perspectives and future directions

Determination of skeletal muscle PO₂*mv* and PO₂*is* responses via phosphorescence quenching allows resolution of transcapillary PO₂ during the rest-contraction transient

(Fig. 3)²⁰. This pressure gradient is necessary for O₂ diffusion across the capillary wall from the microvascular to interstitial space. Preservation of transcapillary PO₂ in rodent skeletal muscle in our recent study²⁰ corroborates human PMRS data from Richardson and colleagues^{8,53} (i.e., blood-myocyte PO₂) thus underscoring the instrumental role of increased DO₂ in modulating O₂ flux rates during contractions. Whether transcapillary

PO₂ is maintained during the metabolic transition in conditions that impair O₂ delivery-utilization matching such as aging and chronic diseases remains to be determined. Given the microvascular hemodynamic^{77–79,87} and oxygenation^{58–60,88} deficits typical of these conditions, it is conceivable that lowering of transcapillary PO₂ could contribute mechanistically to exercise intolerance thereby constituting an important therapeutic target.

Further down in the O₂ cascade at the interstitium-myocyte interface, it is necessary to recognize that the dynamic PO₂*is* represents the ultimate driving force for O₂ transport into the muscle cell (i.e., transsarcolemmal O₂ flux). This is particularly evident when considering that intracellular PO₂ falls to very low levels during submaximal and maximal contractions (~2–3 to 5 mmHg)^{8,9}. Due to its strategic position between the microvascular and intracellular domains, the interstitium is subjected to the impact of events taking place in both of those compartments. These include processes modulating capillary RBC hemodynamics and distribution (upstream; O₂ delivery mechanisms), as well as oxidative phosphorylation (downstream; O₂ utilization mechanisms). It is also tempting to speculate that dynamic, rhythmic contractions could facilitate interstitial O₂ diffusion via mechanical effects (e.g., altering the shape/distribution of the CFR). In this context, the interstitium may thus be considered to take center stage in skeletal muscle function and, ultimately, exercise tolerance.

Using phosphorescence quenching measurements of PO₂*is* kinetics, follow-up studies from our laboratory have identified sex differences in the responsiveness to the vasodilator nitric oxide (upstream influences)⁸⁹. Ongoing efforts examine how muscle fiber type and oxidative capacity modulate PO₂*is* kinetics during the rest-contraction transient (downstream influences). The impact of aging and chronic diseases such as CHF are also the subject of great interest. Whether these conditions affect PO₂*is* responses in a similar fashion to that seen in the microvascular space (as illustrated in Fig. 5, *top panel*)^{58–60,88} is unknown. It is likely that skeletal muscle PO₂*is* kinetics are not only affected by potential reductions in PO₂*mv* with age and/or disease, but also alterations intrinsic to the interstitial space. For example, fluid retention and congestion are hallmarks of cardiovascular diseases such as CHF and hypertension. Tissue edema and the increased interstitial volume that ensues might hinder O₂ diffusion into skeletal muscle at rest and during contractions. This opens the possibility that, in this scenario, diuretic treatment used commonly for the management of volume overload may also improve diffusive O₂ transport within and across the interstitium. If that hypothesis is ratified, then manipulation of PO₂*is* responses via pharmacological and non-pharmacological interventions could prove beneficial in restoring pathologically-impaired muscle contractile performance.

As illustrated in Fig. 5 (*top panel*), in addition to the typical reduction in PO₂*mv* during contractions, skeletal muscle from rats with CHF may display a more irregular, “jagged” temporal profile. Interestingly, this erratic PO₂*mv* trait is not exclusive to the CHF muscle

but may also be found in other conditions such as diabetes and aging^{60,90}. Given this commonality, it is conceivable that such PO_2mv fluctuations reflect a reduced ability to adjust rapidly and consistently muscle O_2 delivery-to-utilization and minimize the physiological error signal in those conditions. Greater model residuals are thus obtained from mathematical modelling as a result of this aberrant response when compared to the healthy young muscle (Fig. 5, *bottom panel*). Notwithstanding substantial differences in compartmental volumes and O_2 pressure regulation^{1,4,10,12,15,16,20,47}, it is unclear at this time whether such behavior is translated from the microvascular to the interstitial space in aged/diseased muscle and, if so, its potential implications to exercise tolerance. Furthermore, it is unknown how structural, functional and hemodynamic heterogeneity within microvascular networks^{91–94} interact to modulate skeletal muscle PO_2is responses. The RBC itself plays an important role in matching local O_2 delivery-to-utilization via O_2 -dependent release of the vasodilator ATP^{95,96} thereby likely impacting interstitial PO_2 .

Assessment of interstitial oxygenation via phosphorescence quenching has yielded other important advances in our understanding of skeletal muscle function and oxidative metabolism. Pittman and colleagues have employed muscle PO_2is measurements to evaluate the viability of hemoglobin-based oxygen carriers and their effects on microcirculatory function⁹⁷. Moreover, combining phosphorescence quenching microscopy with local blood flow arrest (via pneumatic tissue compression) permitted evaluation of resting and contracting muscle O_2 utilization as well as the O_2 dependence of respiration^{65,98–100}. The latter not only describes the range of O_2 pressures maintaining metabolic homeostasis and regulating oxidative phosphorylation, but also defines the potential for the mitochondria to serve as a oxygen sensor for systemic and/or local O_2 delivery-utilization matching (and thus modulate cellular and tissue function accordingly)^{12,101,102}. As reviewed recently by David F. Wilson¹⁰³, it is important to note that the rate of ATP synthesis (and consequently O_2 consumption) by mitochondria *in vivo* is modulated by several independent variables of which PO_2 is only one. Others include the fraction of reduced cytochrome *c* (f_{red}), energy state ($[ATP]/[ADP][Pi]$) and pH^{104,105}. Within this framework, redox ($\uparrow f_{red}$) and metabolic ($\downarrow [ATP]/[ADP][Pi]$) compensation for decrements in intracellular PO_2 can preserve the ATP synthesis rate to match that of consumption at least to a certain extent^{104,105}. However, to date the PO_2 range at which these compensations can operate before maximal mitochondrial respiration becomes limited by O_2 (as demarcated by what has been termed critical PO_2) remains controversial^{12,101,102,106}. This issue has been examined in multiple settings and experimental paradigms including isolated mitochondria, as well as in *in vitro* and *in vivo* skeletal muscle. In general, critical PO_2 and the O_2 dependence of respiration (i.e., the intracellular PO_2 range eliciting redox and metabolic compensation to PO_2 changes) have been reported to vary widely and range from approximately <1–5 mmHg to as high as 30 mmHg, respectively^{44,99,106–111}.

Summary and conclusions

Technological advances in the ability to interrogate the skeletal muscle microcirculation have forced a new model of capillary function to emerge^{4,26}. This paradigm-shift in our understanding of microcirculatory O_2 transport was made possible following integration of evidence from theoretical and experimental (both human and animal) studies. Recently, the

presence of relatively large transmural PO_2 gradients was detected in the resting and contracting skeletal muscle microcirculation via phosphorescence quenching²⁰. Together with intravital microscopy^{21–23} and PMRS^{8,53} evidence, these contemporary findings help elucidate the mechanistic bases for increased O_2 flux ($\dot{V}O_2$) during transitions in metabolic demand. Specifically, modulations in capillary RBC hemodynamics and distribution (which determine effective DO_2) are vital to preserve the main driving force for O_2 flux across the capillary wall (i.e., transcapillary PO_2) during muscle contractions. These responses provide the dynamic O_2 flux and $\dot{V}O_2$ kinetics emblematic of muscle mitochondrial metabolism across the metabolic transients associated with contractile activity.

Acknowledgements

We thank Mal Rooks Hoover for expert assistance with graphical illustrations.

Funding: This work was supported in part by a Post-Doctoral Fellowship from the College of Human Ecology, Kansas State University; and National Heart, Lung and Blood Institute Grant HL-2-108328.

Abbreviations

PO_2	oxygen partial pressure gradient
β	slope of the O_2 dissociation curve in the physiological range
CHF	chronic heart failure
CFR	carrier-free region
DO_2	oxygen diffusing capacity
FCDR	functionally carrier-depleted region
f_{red}	fraction of reduced cytochrome <i>c</i>
PMRS	proton magnetic resonance spectroscopy
PO_2	oxygen partial pressure
PO_{2is}	interstitial oxygen partial pressure
PO_{2mv}	microvascular oxygen partial pressure
Q_m	muscle blood flow
RBC	red blood cell
TEM	transmission electron microscopy
$\dot{V}O_2$	oxygen flux

References

1. Weibel ER. The Pathway for Oxygen: Structure and Function in the Mammalian Respiratory System Harvard University Press; 1984.

2. Rossiter HB. Exercise: Kinetic considerations for gas exchange. *Compr Physiol*. 2011;1(1):203–244. doi:10.1002/cphy.c090010 [PubMed: 23737170]
3. Poole DC, Jones AM. Oxygen uptake kinetics. *Compr Physiol*. 2012;2(2):933–996. doi:10.1002/cphy.c100072 [PubMed: 23798293]
4. Poole DC, Copp SW, Hirai DM, Musch TI. Dynamics of muscle microcirculatory and blood-myocyte O₂ flux during contractions. *Acta Physiol* 2011;202(3):293–310. doi:10.1111/j.1748-1716.2010.02246.x
5. Wittenberg JB, Wittenberg BA. Myoglobin function reassessed. *J Exp Biol*. 2003;206(Pt 12):2011–2020. [PubMed: 12756283]
6. West JB. *Respiratory Physiology: The Essentials*. Lippincott Williams & Wilkins; 2012.
7. Roca J, Hogan MC, Story D, et al. Evidence for tissue diffusion limitation of VO₂max in normal humans. *J Appl Physiol*. 1989;67(1):291–299. doi:10.1152/jappl.1989.67.1.291 [PubMed: 2759955]
8. Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD. Myoglobin O₂ desaturation during exercise. Evidence of limited O₂ transport. *J Clin Invest*. 1995;96(4):1916–1926. doi:10.1172/JCI118237 [PubMed: 7560083]
9. Molé PA, Chung Y, Tran TK, Sailasuta N, Hurd R, Jue T. Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. *Am J Physiol*. 1999;277(1 Pt 2):R173–180. [PubMed: 10409271]
10. Wagner PD. Diffusive resistance to O₂ transport in muscle. *Acta Physiol Scand*. 2000;168(4):609–614. doi:10.1046/j.1365-201x.2000.00712.x [PubMed: 10759597]
11. Poole DC, Ferreira LF, Behnke BJ, Barstow TJ, Jones AM. The final frontier: oxygen flux into muscle at exercise onset. *Exerc Sport Sci Rev*. 2007;35(4):166–173. doi:10.1097/jes.0b013e318156e4ac [PubMed: 17921784]
12. Clanton TL, Hogan MC, Gladden LB. Regulation of cellular gas exchange, oxygen sensing, and metabolic control. *Compr Physiol* 2013;3(3):1135–1190. doi:10.1002/cphy.c120030 [PubMed: 23897683]
13. Federspiel WJ, Popel AS. A theoretical analysis of the effect of the particulate nature of blood on oxygen release in capillaries. *Microvasc Res*. 1986;32(2):164–189. [PubMed: 3762425]
14. Groebe K, Thews G. Calculated intra- and extracellular PO₂ gradients in heavily working red muscle. *Am J Physiol*. 1990;259(1 Pt 2):H84–92. [PubMed: 2375415]
15. Tsai AG, Johnson PC, Intaglietta M. Oxygen gradients in the microcirculation. *Physiol Rev*. 2003;83(3):933–963. doi:10.1152/physrev.00034.2002 [PubMed: 12843412]
16. Pittman RN. Oxygen gradients in the microcirculation. *Acta Physiol*. 2011;202(3):311–322. doi:10.1111/j.1748-1716.2010.02232.x
17. Wilson DF, Lee WMF, Makonnen S, Finikova O, Apreleva S, Vinogradov SA. Oxygen pressures in the interstitial space and their relationship to those in the blood plasma in resting skeletal muscle. *J Appl Physiol*. 2006;101(6):1648–1656. doi:10.1152/jappphysiol.00394.2006 [PubMed: 16888050]
18. Dunphy I, Vinogradov SA, Wilson DF. Oxyphor R2 and G2: phosphors for measuring oxygen by oxygen-dependent quenching of phosphorescence. *Anal Biochem*. 2002;310(2):191–198. [PubMed: 12423638]
19. Esipova TV, Karagodov A, Miller J, Wilson DF, Busch TM, Vinogradov SA. Two new “protected” oxyphors for biological oximetry: properties and application in tumor imaging. *Anal Chem*. 2011;83(22):8756–8765. doi:10.1021/ac2022234 [PubMed: 21961699]
20. Hirai DM, Craig JC, Colburn TD, et al. Skeletal muscle microvascular and interstitial PO₂ from rest to contractions. *J Physiol*. 2018;596(5):869–883. doi:10.1113/JP275170 [PubMed: 29288568]
21. Klitzman B, Duling BR. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. *Am J Physiol*. 1979;237(4):H481–490. [PubMed: 495734]
22. Hudlická O, Zweifach BW, Tyler KR. Capillary recruitment and flow velocity in skeletal muscle after contractions. *Microvasc Res*. 1982;23(2):201–213. [PubMed: 7099017]
23. Kindig CA, Richardson TE, Poole DC. Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer. *J Appl Physiol*. 2002;92(6):2513–2520. doi:10.1152/jappphysiol.01222.2001 [PubMed: 12015367]

24. Krogh A The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *J Physiol.* 1919;52(6):409–415. [PubMed: 16993405]
25. Krogh A The supply of oxygen to the tissues and the regulation of the capillary circulation. *J Physiol.* 1919;52(6):457–474. [PubMed: 16993410]
26. Poole DC, Copp SW, Ferguson SK, Musch TI. Skeletal muscle capillary function: contemporary observations and novel hypotheses. *Exp Physiol.* 2013;98(12):1645–1658. doi:10.1113/expphysiol.2013.073874 [PubMed: 23995101]
27. Sakai T, Hosoyamada Y. Are the precapillary sphincters and metarterioles universal components of the microcirculation? An historical review. *J Physiol Sci.* 2013;63(5):319–331. doi:10.1007/s12576-013-0274-7 [PubMed: 23824465]
28. Zweifach BW, Shorr E, Black MM. The influence of the adrenal cortex on behavior of terminal vascular bed. *Ann N Y Acad Sci.* 1953;56(4):626–633. [PubMed: 13208053]
29. Rowell LB. *Human Cardiovascular Control* Oxford University Press; 1993.
30. Clifford PS, Hellsten Y. Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol.* 2004;97(1):393–403. doi:10.1152/japplphysiol.00179.2004 [PubMed: 15220322]
31. Laughlin MH, Davis MJ, Secher NH, et al. Peripheral circulation. *Compr Physiol.* 2012;2(1):321–447. doi:10.1002/cphy.c100048 [PubMed: 23728977]
32. Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev.* 2015;95(2):549–601. doi:10.1152/physrev.00035.2013 [PubMed: 25834232]
33. Clark MG, Rattigan S, Barrett EJ, Vincent MA. Point: There is capillary recruitment in active skeletal muscle during exercise. *J Appl Physiol.* 2008;104(3):889–891. doi:10.1152/japplphysiol.00779.2007 [PubMed: 17656624]
34. Poole DC, Brown MD, Hudlicka O. Counterpoint: There is not capillary recruitment in active skeletal muscle during exercise. *J Appl Physiol.* 2008;104(3):891–893; discussion 893–894. doi:10.1152/japplphysiol.00779.2007a [PubMed: 18326874]
35. Barrett EJ, Keske MA, Rattigan S, Eringa EC. CrossTalk proposal: De novo capillary recruitment in healthy muscle is necessary. *J Physiol.* 2014;592(23):5129–5131. doi:10.1113/jphysiol.2014.282137 [PubMed: 25448178]
36. Poole DC. CrossTalk opposing view: De novo capillary recruitment in healthy muscle is not necessary to explain physiological outcomes. *J Physiol.* 2014;592(23):5133–5135. doi:10.1113/jphysiol.2014.282145 [PubMed: 25448179]
37. Gayeski TE, Honig CR. O₂ gradients from sarcolemma to cell interior in red muscle at maximal VO₂. *Am J Physiol.* 1986;251(4 Pt 2):H789–799. [PubMed: 3766756]
38. Honig CR, Gayeski TE. Resistance to O₂ diffusion in anemic red muscle: roles of flux density and cell PO₂. *Am J Physiol.* 1993;265(3 Pt 2):H868–875. [PubMed: 8214121]
39. Voter WA, Gayeski TE. Determination of myoglobin saturation of frozen specimens using a reflecting cryospectrophotometer. *Am J Physiol.* 1995;269(4 Pt 2):H1328–1341. [PubMed: 7485565]
40. Richardson RS, Duteil S, Wary C, Wray DW, Hoff J, Carlier PG. Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol.* 2006;571(Pt 2):415–424. doi:10.1113/jphysiol.2005.102327 [PubMed: 16396926]
41. Bebout DE, Hogan MC, Hempleman SC, Wagner PD. Effects of training and immobilization on VO₂ and DO₂ in dog gastrocnemius muscle in situ. *J Appl Physiol.* 1993;74(4):1697–1703. [PubMed: 8514685]
42. Hepple RT, Hogan MC, Stary C, Bebout DE, Mathieu-Costello O, Wagner PD. Structural basis of muscle O₂ diffusing capacity: evidence from muscle function in situ. *J Appl Physiol.* 2000;88(2):560–566. [PubMed: 10658023]
43. Golub AS, Pittman RN. Erythrocyte-associated transients in PO₂ revealed in capillaries of rat mesentery. *Am J Physiol Heart Circ Physiol.* 2005;288(6):H2735–2743. doi:10.1152/ajpheart.00711.2004 [PubMed: 15695557]

44. Gayeski TE, Connett RJ, Honig CR. Minimum intracellular PO₂ for maximum cytochrome turnover in red muscle in situ. *Am J Physiol.* 1987;252(5 Pt 2):H906–915. doi:10.1152/ajpheart.1987.252.5.H906 [PubMed: 3578540]
45. Popel AS, Pittman RN, Ellsworth ML. Rate of oxygen loss from arterioles is an order of magnitude higher than expected. *Am J Physiol.* 1989;256(3 Pt 2):H921–924. [PubMed: 2923247]
46. Groebe K A versatile model of steady state O₂ supply to tissue. Application to skeletal muscle. *Biophys J.* 1990;57(3):485–498. doi:10.1016/S0006-3495(90)82565-0 [PubMed: 2306498]
47. Honig CR, Gayeski TE, Groebe K. Myoglobin and oxygen gradients In: *The Lung: Scientific Foundations*; Crystal RG, West JB, Weibel ER, Barnes P (Eds). Philadelphia, PA: Lippincott-Raven Publishers; 1997:1925–1933.
48. Anderson SI, Hudlicka O, Brown MD. Capillary red blood cell flow and activation of white blood cells in chronic muscle ischemia in the rat. *Am J Physiol.* 1997;272(6 Pt 2):H2757–2764. doi:10.1152/ajpheart.1997.272.6.H2757 [PubMed: 9227555]
49. Burton KS, Johnson PC. Reactive hyperemia in individual capillaries of skeletal muscle. *Am J Physiol.* 1972;223(3):517–524. doi:10.1152/ajplegacy.1972.223.3.517 [PubMed: 5055308]
50. Damon DH, Duling BR. Distribution of capillary blood flow in the microcirculation of the hamster: an in vivo study using epifluorescent microscopy. *Microvasc Res.* 1984;27(1):81–95. [PubMed: 6708826]
51. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary O₂ extraction in sepsis. *Am J Physiol Heart Circ Physiol.* 2002;282(1):H156–164. doi:10.1152/ajpheart.2002.282.1.H156 [PubMed: 11748059]
52. Oude Vrielink HH, Slaaf DW, Tangelde GJ, Reneman RS. Does capillary recruitment exist in young rabbit skeletal muscle? *Int J Microcirc Clin Exp.* 1987;6(4):321–332. [PubMed: 3429142]
53. Richardson RS, Newcomer SC, Noyszewski EA. Skeletal muscle intracellular PO₂ assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol.* 2001;91(6):2679–2685. doi:10.1152/jappl.2001.91.6.2679 [PubMed: 11717234]
54. Allen PS, Matheson GO, Zhu G, et al. Simultaneous ³¹P MRS of the soleus and gastrocnemius in Sherpas during graded calf muscle exercise. *Am J Physiol.* 1997;273(3 Pt 2):R999–1007. doi:10.1152/ajpregu.1997.273.3.R999 [PubMed: 9321879]
55. Behnke BJ, Barstow TJ, Kindig CA, McDonough P, Musch TI, Poole DC. Dynamics of oxygen uptake following exercise onset in rat skeletal muscle. *Respir Physiol Neurobiol.* 2002;133(3):229–239. [PubMed: 12425970]
56. Carvalho H, Pittman RN. Longitudinal and radial gradients of PO₂ in the hamster cheek pouch microcirculation. *Microcirculation.* 2008;15(3):215–224. doi:10.1080/10739680701616175 [PubMed: 18386217]
57. Shibata M, Ichioka S, Ando J, Kamiya A. Microvascular and interstitial PO₂ measurements in rat skeletal muscle by phosphorescence quenching. *J Appl Physiol.* 2001;91(1):321–327. [PubMed: 11408447]
58. Poole DC, Kindig CA, Behnke BJ. Effects of emphysema on diaphragm microvascular oxygen pressure. *Am J Respir Crit Care Med.* 2001;163(5):1081–1086. doi:10.1164/ajrccm.163.5.2008065 [PubMed: 11316639]
59. Diederich ER, Behnke BJ, McDonough P, et al. Dynamics of microvascular oxygen partial pressure in contracting skeletal muscle of rats with chronic heart failure. *Cardiovasc Res.* 2002;56(3):479–486. [PubMed: 12445889]
60. Padilla DJ, McDonough P, Behnke BJ, et al. Effects of Type II diabetes on muscle microvascular oxygen pressures. *Respir Physiol Neurobiol.* 2007;156(2):187–195. doi:10.1016/j.resp.2006.08.008 [PubMed: 17015044]
61. Poole DC, Behnke BJ, McDonough P, McAllister RM, Wilson DF. Measurement of muscle microvascular oxygen pressures: compartmentalization of phosphorescent probe. *Microcirculation.* 2004;11(4):317–326. doi:10.1080/10739680490437487 [PubMed: 15280071]
62. Poole DC, Wagner PD, Wilson DF. Diaphragm microvascular plasma PO₂ measured in vivo. *J Appl Physiol.* 1995;79(6):2050–2057. [PubMed: 8847273]

63. Behnke BJ, Kindig CA, Musch TI, Koga S, Poole DC. Dynamics of microvascular oxygen pressure across the rest-exercise transition in rat skeletal muscle. *Respir Physiol* 2001;126(1):53–63. [PubMed: 11311310]
64. Smith LM, Golub AS, Pittman RN. Interstitial PO₂ determination by phosphorescence quenching microscopy. *Microcirculation*. 2002;9(5):389–395. doi:10.1038/sj.mn.7800147 [PubMed: 12375176]
65. Nugent WH, Song BK, Pittman RN, Golub AS. Simultaneous sampling of tissue oxygenation and oxygen consumption in skeletal muscle. *Microvasc Res*. 2016;105:15–22. doi:10.1016/j.mvr.2015.12.007 [PubMed: 26683232]
66. Whalen WJ, Nair P, Buerk D, Thuning CA. Tissue PO₂ in normal and denervated cat skeletal muscle. *Am J Physiol*. 1974;227(6):1221–1225. [PubMed: 4440766]
67. Gorczynski RJ, Duling BR. Role of oxygen in arteriolar functional vasodilation in hamster striated muscle. *Am J Physiol*. 1978;235(5):H505–515. [PubMed: 727273]
68. Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD. Muscle O₂ uptake kinetics in humans: implications for metabolic control. *J Appl Physiol*. 1996;80(3):988–998. [PubMed: 8964765]
69. Grassi B, Hogan MC, Greenhaff PL, et al. Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *J Physiol*. 2002;538(Pt 1):195–207. [PubMed: 11773328]
70. Grassi B, Pogliaghi S, Rampichini S, et al. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. *J Appl Physiol*. 2003;95(1):149–158. doi:10.1152/japplphysiol.00695.2002 [PubMed: 12611769]
71. Hogan MC. Fall in intracellular PO₂ at the onset of contractions in *Xenopus* single skeletal muscle fibers. *J Appl Physiol*. 2001;90(5):1871–1876. [PubMed: 11299280]
72. Duling BR, Berne RM. Longitudinal gradients in periarteriolar oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow. *Circ Res*. 1970;27(5):669–678. [PubMed: 5486243]
73. Vadapalli A, Pittman RN, Popel AS. Estimating oxygen transport resistance of the microvascular wall. *Am J Physiol Heart Circ Physiol*. 2000;279(2):H657–671. [PubMed: 10924065]
74. Duling BR. Microvascular responses to alterations in oxygen tension. *Circ Res*. 1972;31(4):481–489. [PubMed: 5075370]
75. Duling BR. Changes in microvascular diameter and oxygen tension induced by carbon dioxide. *Circ Res*. 1973;32(3):370–376. [PubMed: 4691342]
76. Roca J, Agusti AG, Alonso A, et al. Effects of training on muscle O₂ transport at VO₂max. *J Appl Physiol*. 1992;73(3):1067–1076. [PubMed: 1400019]
77. Poole DC, Mathieu-Costello O. Effect of pulmonary emphysema on diaphragm capillary geometry. *J Appl Physiol*. 1997;82(2):599–606. [PubMed: 9049743]
78. Richardson TE, Kindig CA, Musch TI, Poole DC. Effects of chronic heart failure on skeletal muscle capillary hemodynamics at rest and during contractions. *J Appl Physiol*. 2003;95(3):1055–1062. doi:10.1152/japplphysiol.00308.2003 [PubMed: 12740313]
79. Padilla DJ, McDonough P, Behnke BJ, et al. Effects of Type II diabetes on capillary hemodynamics in skeletal muscle. *Am J Physiol Heart Circ Physiol*. 2006;291(5):H2439–2444. doi:10.1152/ajpheart.00290.2006 [PubMed: 16844923]
80. Richardson RS, Leigh JS, Wagner PD, Noyszewski EA. Cellular PO₂ as a determinant of maximal mitochondrial O₂ consumption in trained human skeletal muscle. *J Appl Physiol*. 1999;87(1):325–331. [PubMed: 10409591]
81. Ordway GA, Garry DJ. Myoglobin: an essential hemoprotein in striated muscle. *J Exp Biol*. 2004;207(Pt 20):3441–3446. doi:10.1242/jeb.01172 [PubMed: 15339940]
82. Garry DJ, Ordway GA, Lorenz JN, et al. Mice without myoglobin. *Nature*. 1998;395(6705):905–908. doi:10.1038/27681 [PubMed: 9804424]
83. Gödecke A, Flögel U, Zanger K, et al. Disruption of myoglobin in mice induces multiple compensatory mechanisms. *Proc Natl Acad Sci U S A*. 1999;96(18):10495–10500. [PubMed: 10468637]

84. Grange RW, Meeson A, Chin E, et al. Functional and molecular adaptations in skeletal muscle of myoglobin-mutant mice. *Am J Physiol Cell Physiol*. 2001;281(5):C1487–1494. doi:10.1152/ajpcell.2001.281.5.C1487 [PubMed: 11600411]
85. Clark A, Clark PA. Local oxygen gradients near isolated mitochondria. *Biophys J*. 1985;48(6):931–938. doi:10.1016/S0006-3495(85)83856-X [PubMed: 4092070]
86. Vanderkooi JM, Wright WW, Erecinska M. Oxygen gradients in mitochondria examined with delayed luminescence from excited-state triplet probes. *Biochemistry (Mosc)* 1990;29(22):5332–5338.
87. Copp SW, Ferreira LF, Herspring KF, Musch TI, Poole DC. The effects of aging on capillary hemodynamics in contracting rat spinotrapezius muscle. *Microvasc Res*. 2009;77(2):113–119. doi:10.1016/j.mvr.2008.11.001 [PubMed: 19094997]
88. Behnke BJ, Delp MD, Dougherty PJ, Musch TI, Poole DC. Effects of aging on microvascular oxygen pressures in rat skeletal muscle. *Respir Physiol Neurobiol*. 2005;146(2–3):259–268. doi:10.1016/j.resp.2004.12.009 [PubMed: 15766914]
89. Craig JC, Colburn TD, Hirai DM, Schettler MJ, Musch TI, Poole DC. Sex and nitric oxide bioavailability interact to modulate interstitial PO₂ in healthy rat skeletal muscle. *J Appl Physiol*. 1 2018. doi:10.1152/jappphysiol.01022.2017
90. Behnke BJ, Delp MD, Poole DC, Musch TI. Aging potentiates the effect of congestive heart failure on muscle microvascular oxygenation. *J Appl Physiol*. 2007;103(5):1757–1763. doi:10.1152/jappphysiol.00487.2007 [PubMed: 17761789]
91. Duling BR, Damon DH. An examination of the measurement of flow heterogeneity in striated muscle. *Circ Res*. 1987;60(1):1–13. [PubMed: 3552283]
92. Segal SS. Regulation of blood flow in the microcirculation. *Microcirc N Y N* 1994. 2005;12(1):33–45. doi:10.1080/10739680590895028
93. Pries AR, Secomb TW. Blood Flow in Microvascular Networks In: Terjung R, ed. *Comprehensive Physiology*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2011 3–36. doi:10.1002/cphy.cp020401
94. Davis MJ, Hill MA, Kuo L. Local Regulation of Microvascular Perfusion In: *Handbook of Physiology: Microcirculation*, 2nd ed, ed. Tuma RF, Duran WN, Ley K. San Diego, CA: Elsevier, 2008:161–284. doi:10.1002/cphy.cp020406
95. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH. The erythrocyte as a regulator of vascular tone. *Am J Physiol*. 1995;269(6 Pt 2):H2155–2161. doi:10.1152/ajpheart.1995.269.6.H2155 [PubMed: 8594927]
96. Ellsworth ML, Ellis CG, Sprague RS. Role of erythrocyte-released ATP in the regulation of microvascular oxygen supply in skeletal muscle. *Acta Physiol Oxf Engl*. 2016;216(3):265–276. doi:10.1111/apha.12596
97. Song BK, Nugent WH, Moon-Massat PF, Pittman RN. Effects of a hemoglobin-based oxygen carrier (HBOC-201) and derivatives with altered oxygen affinity and viscosity on systemic and microcirculatory variables in a top-load rat model. *Microvasc Res* 2014;95:124–130. doi:10.1016/j.mvr.2014.07.005 [PubMed: 25046829]
98. Golub AS, Tevald MA, Pittman RN. Phosphorescence quenching microrespirometry of skeletal muscle in situ. *Am J Physiol Heart Circ Physiol*. 2011;300(1):H135–143. doi:10.1152/ajpheart.00626.2010 [PubMed: 20971766]
99. Golub AS, Pittman RN. Oxygen dependence of respiration in rat spinotrapezius muscle in situ. *Am J Physiol Heart Circ Physiol*. 2012;303(1):H47–56. doi:10.1152/ajpheart.00131.2012 [PubMed: 22523254]
100. Golub AS, Dodhy SC, Pittman RN. Oxygen dependency of respiration in the rat spinotrapezius muscle contracting at 0.5 to 8 twitches per second. *J Appl Physiol*. 3 2018. doi:10.1152/jappphysiol.01136.2016
101. Connett RJ, Honig CR, Gayeski TE, Brooks GA. Defining hypoxia: a systems view of VO₂, glycolysis, energetics, and intracellular PO₂. *J Appl Physiol*. 1990;68(3):833–842. doi:10.1152/jappl.1990.68.3.833 [PubMed: 2187852]
102. Wilson DF. Quantifying the role of oxygen pressure in tissue function. *Am J Physiol Heart Circ Physiol*. 2008;294(1):H11–13. doi:10.1152/ajpheart.01293.2007 [PubMed: 17993593]

103. Wilson DF. Oxidative phosphorylation: regulation and role in cellular and tissue metabolism. *J Physiol.* 2017;595(23):7023–7038. doi:10.1113/JP273839 [PubMed: 29023737]
104. Wilson DF, Harrison DK, Vinogradov A. Mitochondrial cytochrome c oxidase and control of energy metabolism: measurements in suspensions of isolated mitochondria. *J Appl Physiol.* 2014;117(12):1424–1430. doi:10.1152/jappphysiol.00736.2014 [PubMed: 25324517]
105. Wilson DF, Vinogradov SA. Mitochondrial cytochrome c oxidase: mechanism of action and role in regulating oxidative phosphorylation. *J Appl Physiol.* 2014;117(12):1431–1439. doi:10.1152/jappphysiol.00737.2014 [PubMed: 25324518]
106. Scandurra FM, Gnaiger E. Cell respiration under hypoxia: facts and artefacts in mitochondrial oxygen kinetics. *Adv Exp Med Biol.* 2010;662:7–25. doi:10.1007/978-1-4419-1241-1_2 [PubMed: 20204766]
107. Chance B Early reduction of cytochrome c in hypoxia. *FEBS Lett.* 1988;226(2):343–346. [PubMed: 2828107]
108. Wilson DF, Rumsey WL, Green TJ, Vanderkooi JM. The oxygen dependence of mitochondrial oxidative phosphorylation measured by a new optical method for measuring oxygen concentration. *J Biol Chem.* 1988;263(6):2712–2718. [PubMed: 2830260]
109. Wilson DF, Harrison DK, Vinogradov SA. Oxygen, pH, and mitochondrial oxidative phosphorylation. *J Appl Physiol.* 2012;113(12):1838–1845. doi:10.1152/jappphysiol.01160.2012 [PubMed: 23104697]
110. Richmond KN, Shonat RD, Lynch RM, Johnson PC. Critical PO_2 of skeletal muscle in vivo. *Am J Physiol.* 1999;277(5 Pt 2):H1831–1840. [PubMed: 10564137]
111. Lanza IR, Tevald MA, Befroy DE, Kent-Braun JA. Intracellular energetics and critical PO_2 in resting ischemic human skeletal muscle in vivo. *Am J Physiol Regul Integr Comp Physiol.* 2010;299(5):R1415–1422. doi:10.1152/ajpregu.00225.2010 [PubMed: 20811007]

Figure 1. Schematic illustration of the oxygen transport pathway with its major components (pulmonary, cardiovascular and muscle metabolic systems). The *inset* depicts skeletal muscle fibers with adjacent capillaries and flowing red blood cells. *Blue arrows* denote the last step in the O₂ transport pathway from the skeletal muscle microcirculation to mitochondria.

Figure 2.

The oxygen transport pathway within the skeletal muscle microcirculation. This cross-sectional transmission electron microscopy (TEM) image of the rat spinotrapezius muscle shows the short diffusion distance from the red blood cell (*RBC*) surface to the sarcolemma (*s*) known as the carrier-free region (*arrow*). Note the thin plasma layer (*p*) between the RBC and capillary wall (*w*). The endothelial surface layer is not detectable with this technique. *is*, interstitial space; *f*, muscle fiber; *m*, mitochondrion. TEM magnification: 8000x. Scale bar: 1 μm .

Figure 3.

Skeletal muscle microvascular and interstitial oxygen pressures (PO_{2mv} and PO_{2is} , respectively) from rest to contractions (*left panel*). Note that the pronounced transmural oxygen pressure gradient ($\Delta PO_{2(t)} = PO_{2mv} - PO_{2is}$) observed at rest was maintained during submaximal muscle contractions (*right panel*). Time zero denotes the onset of muscle contractions. Values are mean $\pm$ SE. Adapted from Hirai et al.²⁰.

Figure 4.

The oxygen cascade from air to skeletal muscle at sea level. *Solid* and *broken lines* depict resting and contracting conditions, respectively. Inspired air, alveolar and arterial values at sea level (barometric pressure: 760 mmHg; water vapor pressure at 37°C: 47 mmHg) from West⁶. Mean muscle capillary and interstitial values from Hirai et al.²⁰ (contracting PO₂ corresponds to nadir values). Resting muscle intracellular values from Whalen et al.⁶⁶, Gorczynski and Duling⁶⁷ and Honig et al.⁴⁷. Contracting muscle intracellular values from Richardson et al.⁸. Note the PO₂ gradient between the microvascular and interstitial compartments resolved recently via dual-probe phosphorescence quenching in healthy skeletal muscle from rest to contractions²⁰. *RBC*, red blood cell; *IS*, interstitial space. Adapted from West⁶ and Richardson et al.⁸.

Figure 5.

Top panel: skeletal muscle microvascular oxygen pressures (PO_2mv , symbols) and their model fits (solid lines) from rest to contractions in representative healthy and chronic heart failure (CHF) rats. *Bottom panel:* PO_2mv residuals obtained from exponential model fits. Note the lower PO_2mv values during the metabolic transient in CHF and far greater instability of PO_2mv . This profile provides information regarding O_2 delivery-to-utilization matching and its derangement in pathology. Time zero denotes the onset of muscle contractions.