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REVIEW ARTICLE

Muscle intracellular oxygenation during exercise: optimization for oxygen transport, metabolism, and adaptive change

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Abstract Exercise is the example par excellence of the body functioning as a physiological system. Conventionally we think of the O_2 transport process as a major manifestation of that system linking and integrating pulmonary, cardiovascular, hematological and skeletal muscular contributions to the task of getting O_2 from the air to the mitochondria, and this process has been well described. However, exercise invokes system responses at levels additional to those of macroscopic O_2 transport. One such set of responses appears to center on muscle intracellular PO_2, which falls dramatically from rest to exercise. At rest, it approximates 4 kPa, but during heavy endurance exercise it falls to about 0.4–0.5 kPa, an amazingly low value for a tissue absolutely dependent on the continual supply of O_2 to meet very high energy demands. One wonders why intracellular PO_2 is allowed to fall to such levels. The proposed answer, to be presented in the review, is that a low intramyocyte PO_2 is pivotal in: (a) optimizing oxygen's own physiological transport, and (b) stimulating adaptive gene expression that, after translation, enables greater exercise capacity—all the while maintaining PO_2 at levels sufficient to allow oxidative phosphorylation to operate sufficiently fast enough to support intense muscle contraction. Thus, during exercise, reductions of intracellular PO_2 to less than 1% of that in the atmosphere enables an integrated response that fundamentally and simultaneously optimizes physiological, biochemical and molecular events that support not only the exercise as it happens but the adaptive changes to increase exercise capacity over the longer term.

Keywords Cellular PO_2 · Exercise · Muscle · Magnetic resonance spectroscopy · Oxygen transport · Oxidative phosphorylation

Introduction

The pathway for O_2 from the air we breathe down to the muscle mitochondria has been well described as an *in series* transport system involving the lungs, heart and circulation, blood and the muscle itself (Shephard 1969; Weibel 1984; Wagner 1996). As O_2 molecules travel down this pathway, PO_2 falls at every step, just like voltage down an electric transmission line. From a value of 21.3 kPa in the inhaled air, PO_2 falls to about 13.3 kPa by the time O_2 reaches arterial blood. One of the more remarkable recent discoveries in exercise physiology is that intracellular PO_2 within muscle is nowhere close to this. In fact, it is close to zero during exercise. From values around 4 kPa at rest (Richardson et al. 2006)—not that different from PO_2 in resting muscle venous blood—PO_2 inside the myocyte is found to stabilize at incredibly low values of only 0.4–0.5 kPa during heavy to maximal exercise (Richardson et al. 1995). This needs to be underscored. It amounts to only about 0.5% of an atmosphere, or 1/40th of the concentration of O_2 in the air we breathe. Thus, 39/40th (almost 98%) of the driving force behind O_2 transport has been lost as a cost of transmission.

This remarkably low value is clearly compatible with a metabolic rate higher than for any other tissue—so at once the O_2 requirement is the highest yet the local PO_2 is the lowest, close to zero. This seems like skating on very thin

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biological ice, since if PO_2 fell just another fraction of a kPa, oxidative phosphorylation, energy production, and thus exercise itself, could not be maintained.

However, as Walter B. Cannon said (Cannon 1932), the wisdom of the body generally prevails, and this if nothing else would lead one to suspect there must be good reason for intracellular PO_2 to be so precariously low. The purpose of this review is to suggest good reasons why myocyte PO_2 is so low during exercise, exploring the benefits that this brings. As will be discussed, such a low PO_2 optimizes and supports several of the most fundamental exercise-related phenomena simultaneously: transport of O_2 to mitochondria; structural efficiency of muscle for O_2 transport; biochemical utilization of O_2, and genomic responses to exercise leading to structural and biochemical adaptations. Each of these will be discussed individually. But first, the ways of measuring myocyte PO_2 are briefly reviewed, providing the evidence that during exercise, PO_2 is indeed in the low single digits.

Evidence that muscle intracellular PO_2 is only 0.4–0.5 kPa during exercise

First, what is the definition of "intracellular PO_2"? This in itself is a problem, as we do not know whether, even during constant-load exercise, PO_2 within a single myocyte is uniform in time or space, or whether different subcellular regions may be associated with different PO_2 values, which may or may not vary significantly over time. It has been a huge accomplishment just to be able to measure, non-invasively [by magnetic resonance spectroscopy (MRS), Jue and Anderson 1990; Wang et al. 1990], a PO_2 that is clearly intracellular (and not "contaminated" by PO_2 values of the perfusing blood) in intact exercising humans. However, as spatial and temporal resolutions are so limited, we are forced to express intramyocyte PO_2 as an average over many fibers and many seconds. Moreover, this PO_2 is clearly that associated with the molecule myoglobin (Mb), since the signal actually seen by MRS reflects Mb desaturation (Jue and Anderson 1990; Wang et al. 1990). This PO_2 may or may not be the same as that within mitochondria. However, since this PO_2 is, as stated, so low at only 0.4–0.5 kPa, and since Mb is likely to be upstream of the mitochondria in terms of O_2 transport, yet in very close physical proximity, mitochondrial PO_2 is likely only slightly lower. But that remains speculative.

There are several methods that have been used over the years to determine PO_2 in tissues. Measuring PO_2 in muscle during exercise poses a special set of challenges—there is often considerable fat and skin between any external probe and the muscle; exercising muscle is moving, both macroscopically as in limb movements during running or cycling and microscopically as fibers alternate between contraction and relaxation. Techniques that may be usable only at rest and/or which due to their invasive nature require anesthesia cannot be used in intact humans.

One method used extensively in muscle employs oxygen microelectrodes (Kessler and Lubbers 1966). This technique can provide an entire distribution of local PO_2 values, but has inherent limitations. The electrode tip may be intravascular, interstitial or intracellular, and must cause local damage that itself may influence the PO_2 in its catchment area, more so during muscle movement/contraction. This damage will likely affect local blood flow and thus local PO_2. It is hard to know if the tip is positioned near to small arterioles and/or venules with the data reflecting those larger vessels compared to electrodes that happen to be inserted some distance from such vessels. It is also hard to know if the electrode tip is inside a myocyte or in the interstitial space. Data collected using electrodes has revealed a wide regional variance in resting PO_2 from near zero to near arterial. That variance could reflect innate biological variation in the regional ratio of metabolism to blood flow, or tissue damage, or variance in electrode positioning, and in humans, it is impossible to know which of these is contributory.

Another technique is near-infrared spectroscopy or NIRS (Jobsis 1977). NIRS uses light signals in the near-infrared spectrum that reflect the oxygenation status of both hemoglobin (Hb) in blood and Mb within muscle cells. The contributions from Hb and Mb cannot be separated, yielding numbers that reflect some unknown weighted average of intracellular and extracellular values. NIRS is a completely non-invasive approach with high temporal and quite good spatial resolution, and uses signals from natural Hb and Mb so that no tracer compound needs to be injected. However, in muscle, the data obtained are often difficult to interpret because the single oxygenation number provided reflects not just Mb saturation with O_2 but also Hb saturation. There may also be a contribution from mitochondrial cytochromes. Moreover, the signal from Hb reflects unknown relative contributions from arterioles, capillaries and venules. Thus, NIRS is not considered a satisfactory tool for this particular application—that is, measuring intracellular oxygenation separately from that in the blood.

Yet another method, O_2 phosphorescence quenching, is based on the rate of decay of phosphorescence when exogenous, porphyrin-based molecules are injected intravascularly and excited to phosphoresce (Vanderkooi and Wilson 1986; Vanderkooi et al. 1987). This decay rate is PO_2 dependent. A porphyrin-based molecule is injected intravenously where it binds to plasma proteins, and thus this method indicates oxygenation state within the vasculature. It does not reflect PO_2 within the myocyte, and the phosphor is not suitable for human use.

Still another approach uses electron paramagnetic resonance (EPR) (Subczynski et al. 1986). Here, a paramagnetic particle is inserted into the tissue region of interest, and EPR signals are recorded reflecting local PO_2. EPR is a somewhat less invasive approach, but also requires some intervention with insertion of paramagnetic agents to enhance measurement, the effects of which are also hard to account for. This method also yields PO_2 distributions within muscle showing high variance.

Each of these methods gives a number for PO_2 that reflects different (combinations of) intracellular and extracellular compartments. Some are tainted by inevitable tissue damage; others are not. Some cannot be used in humans; others can.

Applicable only in animal studies that are done under anesthesia and which are terminal, Mb saturation has been measured directly in muscle spectrophotometrically (Gayeski and Honig 1986, 1988). This is done by surgically exposing the muscle of interest in situ, electrically stimulating it to contract via its motor nerve, and rapidly freezing the muscle while it is contracting. The muscle is then sectioned transversely, and the Mb saturation of the frozen cut surface measured. Using this strategy, Mb saturation has been found to be 50% or less, indicating single digit PO_2 values within the contracting myocyte (because the P_{50} of Mb is about 0.5 kPa). While this appears to be a reliable laboratory method in experienced hands, it is clearly a terminal approach that is unsuitable for humans.

Magnetic resonance spectroscopy (MRS) is a completely non-invasive tool that can measure intracellular O_2 levels in muscle, and which, with some limitations, can be used in human exercise. It turns out that the F8 histidine molecule in Mb contains a proton that resonates at about 72 ppm relative to water when Mb is deoxygenated, but not when Mb is oxygenated (Jue and Anderson 1990; Wang et al. 1990). There is no signal from Hb, which thus makes this ideal for isolating intracellular muscle oxygenation. This technique directly measures the degree of Mb desaturation in the field of interrogation. There are significant limitations of this approach. They include a volume many ml in size, thus resulting an average signal over hundreds of fibers. Also, temporal resolution is not high and several seconds of data must be averaged because the signal/noise ratio is inherently low. A special magnet is usually required—one large enough to accommodate a human subject exercising the quadriceps if that is the muscle of interest. A special non-magnetic ergometer that exercises the quadriceps using a leg kick action is usually employed, although calf or even smaller-muscle (e.g. thenar) exercise can also be studied, and requires a smaller magnet. For quadriceps studies, the thigh needs to be immobilized to enable the MRS signal to be recorded, and thus only the leg below the knee moves. By inflating a cuff around the resting upper thigh to above arterial blood pressure for several minutes, complete ischemia allows full Mb desaturation to be recorded, providing a calibration signal. When the cuff is deflated, the signal disappears, indicating that resting muscle Mb is essentially fully saturated. This cuff procedure is illustrated in Fig. 1. From zero signal prior to cuff inflation, full signal is seen at about 4 min. The peak is stable thereafter (taken as evidence for complete desaturation) until the cuff is released to re-establish blood flow and restore Mb oxygenation.

When this approach is used in a normal subject, the results are as in Fig. 2. The abscissa here is time, showing a 600-s period of exercise bounded by resting data both before and after. Each point is a 30-s bin of MRS spectral data. Despite considerable noise, it is clear that Mb saturation is ~100% at rest, falling to essentially 50% during exercise, similar to values observed using spectrophotometry (Gayeski and Honig 1988). In this particular example, exercise intensity was progressively increased from moderate to maximal over time, and interestingly, the Mb

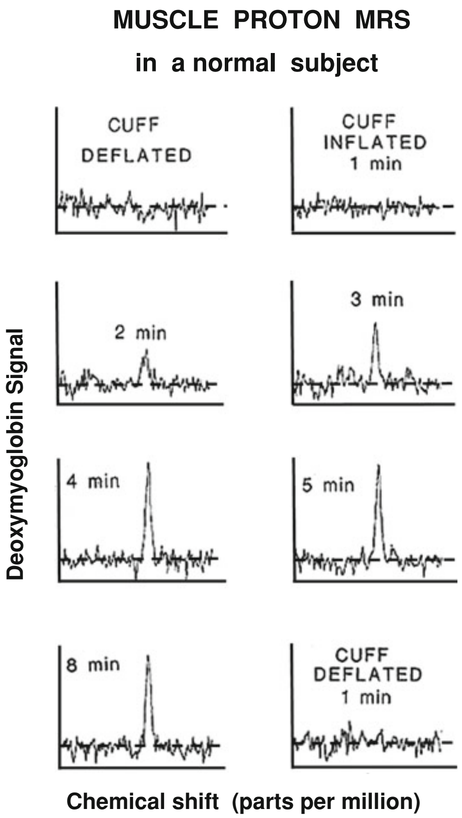

Fig. 1 Magnetic resonance spectrometric (MRS) signal for myoglobin O_2 desaturation over the resting mid-thigh prior to, during, and after 8 min of inflation of an upper thigh cuff to above arterial pressure to abolish blood flow. No discernible deoxygenation is seen at rest, but within 2 min of cuff inflation, deoxygenation is apparent, becoming maximal (presumably anoxic) by 4 min. Abscissa is chemical shift (ppm), with the signal seen at 72 ppm, and ordinate is signal strength. From data reported by Richardson et al. (1995)

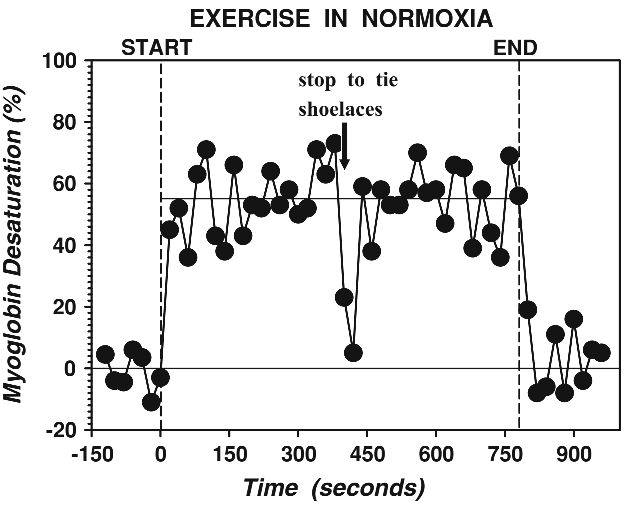

Fig. 2 Percentage myoglobin O_2 desaturation in the thigh of a normal subject at rest, during, and after leg-extension exercise. Each point represents 30 s of data collection. While noise is substantial, it is evident that exercise reduces myoglobin O_2 saturation from close to 100% at rest to about 50%. In addition, the response is rapid as indicated by the return to resting values in less than 1 min of exercise interruption at about 400 s. From data reported by Richardson et al. (1995)

saturation remained constant. Because saturation was 50%, the PO_2 associated with Mb inside the myocyte must have equaled the value of the P_{50} of Mb—that is, ~0.5 kPa.

Mathematical modeling of the radial PO_2 profile away from capillaries within contracting muscle has been performed (Groebe and Thews 1988), and the predicted average intracellular PO_2 is in the same range. Thus, between calculations, MRS and direct spectroscopy of muscle, there is essential agreement that during exercise intracellular PO_2 in muscle—at least the PO_2 associated with Mb—is just 0.4–0.5 kPa.

Low PO_2, optimization of O_2 transport, and muscle structure/function implications

Maximizing diffusion

The preceding discussion of MRS as a tool for measuring muscle PO_2 during exercise focused on the knee extensors as the test muscle group. Using this set of muscles has the additional major advantage of allowing oxygenation of muscle venous blood to be measured via a femoral venous catheter. When this is done in normal subjects using identical exercise paradigms as employed during MRS, femoral venous PO_2 is most commonly found to be 2.5–4 kPa during heavy to maximal exercise (Pirnay et al. 1972; Roca et al. 1989).

Setting aside the difficult problem of potential heterogeneity of muscle blood flow with respect to its metabolic demand, the numbers are remarkable: arterial PO_2 is about 13 kPa, femoral venous PO_2 is 2.5–4 kPa, yet intracellular PO_2 is just 0.4–0.5 kPa. Using simple numerical analysis, the average PO_2 within the vasculature, when arterial is 13 and venous is 2.5–4 kPa, is calculated to be about 5–7 kPa. Thus, on average, mean capillary PO_2 is tenfold higher than intracellular PO_2. There is therefore a very large gradient in PO_2 from the microvasculature to the mitochondria (Richardson et al. 1995).

At first sight, this might be interpreted as a problem. Such a gradient implies either a problem with diffusive transport of O_2 from vessels to mitochondria or serious heterogeneity of perfusion with respect to metabolic demand (which is also being set aside for the present). A more constructive view of this gradient, however, suggests that for a given muscle O_2 diffusing capacity (dictated by capillary and fiber anatomy), O_2 transport between vessels and mitochondria is maximized by this gradient. This can be understood from the Fick law of diffusion, which in its simplest form looks like this:

$$\dot{V}O_2 = \mathrm{Dm} \times [P_{\mathrm{cap}}O_2 - P_{\mathrm{mito}}O_2] \qquad (1)$$

Here $\dot{V}O_2$ is amount of O_2 transported per unit time between vessels and mitochondria; Dm is the diffusing capacity of the muscle for O_2—that is, how much O_2 can be transported by diffusion per unit PO_2 difference between the vessels and the mitochondria; $P_{\mathrm{cap}}O_2$ is the mean capillary PO_2 as defined above; $P_{\mathrm{mito}}O_2$ is the mitochondrial PO_2.

The bottom line is that a given $\dot{V}O_2$ can be attained by a large diffusing capacity (Dm) coupled with a small PO_2 gradient $[P_{\mathrm{cap}}O_2 - P_{\mathrm{mito}}O_2]$, or a small diffusing capacity coupled with a large PO_2 gradient. The latter strategy minimizes the need for both capillaries and/or small fiber diameters to achieve a given $\dot{V}O_2$.

Intracellular PO_2, compared to average capillary PO_2, is, as discussed above, nearly zero. Thus, almost the entire possible PO_2 gradient (in the above examples 5–7 minus only 0.4–0.5 kPa) is in place to help transport O_2, and thus minimize the need for building more capillaries and/or greater numbers of smaller fibers.

In summary, a very low mitochondrial PO_2 maximizes diffusive transport of O_2 from muscle microvascular red cells to the mitochondria for a given muscle fiber and capillary structure.

Minimizing heterogeneity of perfusion to metabolic demand

There is an entirely separate O_2 transport "bonus" likely attributable to the low intracellular PO_2. A low PO_2 in a region of the muscle circulation has been known for many years to result in vasodilatation of the associated vascular bed (Ross et al. 1962; Carrier et al. 1964; Sullivan and Johnson 1981). Should PO_2 in some small region of muscle fall because blood flow is low in relation to the local metabolic demand, local vasodilatation may occur, which reduces local vascular resistance. This acts as autoregulation in an attempt to restore local perfusion, and is thought to be mediated by vasodilators such as adenosine (Berne 1963) and possibly nitric oxide responding to the low local PO_2 (Allen et al. 2009).

To the extent that this innate mechanism works, blood flow can at least in part be matched to metabolic demand within different regions of a muscle. Were PO_2 never to be low, such a self-correcting mechanism for the presence of heterogeneity would not likely exist (although were that the case, heterogeneity might not matter).

What is not yet clear is how effective a mechanism this is for keeping local ratios of perfusion to metabolic demand uniform. To begin to answer this question, Richardson et al. (2001) used MRS methods to assess regional muscle perfusion and corresponding metabolic rate simultaneously in normal calf muscle during exercise. While the metabolic indices used are indirect estimates of local $\dot{V}O_2$, the data suggested a small degree of perfusion/metabolism heterogeneity that would have very little influence on overall O_2 availability. Similarly, Alders et al. (2004) combined carbon tracer markers of metabolism with microsphere measurement of blood flow to demonstrate heterogeneity in the left ventricle of the heart. Techniques such as these will need refinement to improve spatial and temporal resolution before heterogeneity can be reliably measured.

Biochemical implications of a low intracellular PO_2

The discussion to this point has focused on the low intracellular PO_2 in contracting muscle optimizing both diffusive transport of O_2 and also invoking a local negative feedback system to limit perfusion/metabolism heterogeneity. It thus becomes important to discuss whether the low PO_2 has an effect on the production of adenosine triphosphate (ATP) to fuel muscle contraction.

The mitochondria in the cell use O_2 in oxidative phosphorylation to produce ATP according to a well-known biochemical reaction that is summarized as follows:

$$3\mathrm{ADP} + 3\mathrm{Pi} + 1/2\mathrm{O}_2 + \mathrm{NADH} + H^+ \\ \rightarrow 3\mathrm{ATP} + \mathrm{NAD}^+ + \mathrm{H}_2\mathrm{O}.$$

The abbreviations are as follows: ADP, adenosine diphosphate; Pi, inorganic phosphate; NADH, nicotine adenine dinucleotide, reduced state; H^+, hydrogen ion; NAD^+, nicotine adenine dinucleotide, oxidized state.

The key point for the present discussion is to note that O_2 appears as a reactant on the left side of the reaction. Thus, invoking the law of mass action, the local PO_2 can influence the speed of this reaction—that is, affecting O_2 consumption ($\dot{V}O_2$) itself. Figure 3, adapted from work by Wilson et al. (1977) shows that this is indeed seen when isolated mitochondria are studied in vitro. At low PO_2 values, PO_2 strongly affects the rate of reaction, while at higher PO_2 values, a maximal velocity is reached that becomes independent of PO_2. This reflects classical Michaelis–Menten chemical kinetic theory. While the data in this figure were obtained over 30 years ago, more recent work has confirmed this pattern.

The critical observation is that maximal rate of reaction requires a PO_2 of only about 0.3 kPa. While in vitro studies like this may not formally reproduce in vivo conditions, there is no reason to suspect a very different quantitative relationship between PO_2 and $\dot{V}O_2$ in vivo. The conclusion that is reached is that a PO_2 of just 0.4–0.5 kPa does not greatly compromise the rate of oxidative phosphorylation and thus does not substantially limit $\dot{V}O_2$. Hence, allowing PO_2 to fall as low (to serve the tissues in other ways, as mentioned both above and below) appears not to greatly limit metabolism during exercise.

A relatively straightforward approach can be used to test this conclusion in normal human subjects. One can vary inspired O_2 fraction (FIO_2)—both increasing and decreasing it. When FIO_2 is raised so that subjects are breathing more O_2, trained, fit humans can be shown to increase $\dot{V}O_{2\,\mathrm{max}}$ slightly (Powers et al. 1989; Richardson et al. 1999). This finding implies that the Mb-associated PO_2 of 0.4–0.5 kPa seen when these subjects exercise breathing

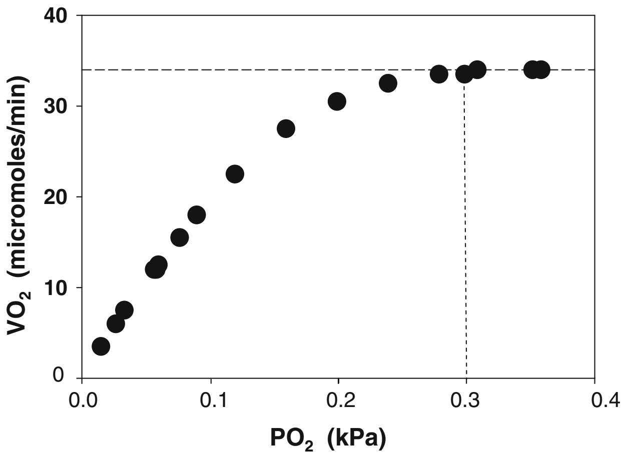

Fig. 3 O_2 consumption ($\dot{V}O_2$) of isolated mitochondria as a function of ambient PO_2. Data are replotted from Wilson et al. (1977). $\dot{V}O_2$ is linearly dependent on PO_2 below 0.15 kPa, and is decreasingly affected by higher values, becoming independent of PO_2 above about 0.3 kPa (*dashed lines*)

room air does not lead to a mitochondrial PO_2 quite high enough to maximally drive oxidative phosphorylation. On the other hand, sedentary subjects appear not to be able to raise $\dot{V}O_{2\,\max}$ to anywhere near the same degree as when FIO_2 is raised (Cardus et al. 1998), and this outcome suggests that when breathing room air, intracellular PO_2 is sufficient to maximally drive ATP generation in such subjects. What is thought to explain this is that sedentary subjects have measurably lower activity of metabolic enzymes than do fit subjects, thus allowing oxidative phosphorylation to run maximally at a PO_2 of 0.4–0.5 kPa.

In all normal subjects, but especially in those who are the fittest, ascent to altitude, which reduces inspired PO_2, results in substantial, acutely reversible, losses in maximal $\dot{V}O_2$. Clearly in this situation, intracellular PO_2 is not high enough to maintain $\dot{V}O_{2\,\max}$ at sea level values. In the most extreme circumstances, on the summit of Mt. Everest (8,848 m above sea level) or its equivalent in an altitude chamber, $\dot{V}O_{2\,\max}$ is only slightly higher than resting $\dot{V}O_2$. (Cymerman et al. 1989).

There is, however, an interesting adaptation found in high altitude natives of the South American Andes. In normal natives living at about 4,000 m above sea level, breathing 55% O_2 resulted in no increase in $\dot{V}O_{2\,\max}$ above that found on the same day breathing ambient air (Wagner et al. 2002). Even acclimatized lowlanders will normalize $\dot{V}O_{2\,\max}$ when exercising breathing 100% O_2 at altitude (Pronk et al. 2003) eliminating the roughly 30% loss of $\dot{V}O_{2\,\max}$ associated with an altitude of 4,000 m. This finding suggests that, like sedentary subjects at sea level, metabolic capacity in Andean high altitude natives has been reduced so that it runs maximally even in hypoxia. One can argue that this conserves energy that would be otherwise required to maintain higher metabolic capacity than can be utilized in the absence of enough O_2.

In summary, the low intracellular PO_2 seems to be sufficient to maximally drive energy metabolism during exercise in sedentary subjects at sea level and also in high altitude natives at their residential altitudes, but is not quite sufficient for this in trained athletes. Nonetheless, despite this apparent insufficiency, the best human athletes are capable of a maximal $\dot{V}O_{2\,\max}$ of more than 80 ml/min/kg.

Genomic adaptive responses and a low PO_2

The dramatic fall in intracellular PO_2 during exercise begs the question of whether directly or indirectly, the low PO_2 plays a role in genomic activation. In recent years, it has been shown that a large number of genes become upregulated when PO_2 falls. Most of these are governed by hypoxia inducible factor (HIF) (Semenza 1999, 2010). This family of transcription factors is normally degraded very rapidly and therefore has limited effects in normal, normoxic conditions. However, in hypoxia, HIF degradation is decelerated and HIF protein levels rise within hypoxic cells. HIF then binds to the hypoxic response element present in the promoter of many genes, enhancing their transactivation to increase corresponding protein synthesis and subsequent physiological effect. Rather than reproduce here the extensive list of genes governed by HIF, the reader is referred to the above Semenza reviews. Many of the genes are highly relevant for muscle, and in particular for supporting exercise capacity. As an example, vascular endothelial growth factor (VEGF) is one such gene, and it is now known that not only does exercise stimulate its upregulation, but also that this is enhanced by imposing acute hypoxia during exercise (Breen et al. 1996). Electrical stimulation of muscle contraction substantially raises HIF protein levels along with VEGF message and protein (Tang et al. 2004). This observation suggests that the drop in PO_2 during exercise is indeed sufficient to stabilize HIF and initiate events that may underlie some of the adaptive responses to exercise training.

In this way, the low PO_2 during exercise has direct effects on adaptive gene activation. It is also possible that the low PO_2 can indirectly lead to activation of genes involved in adaptation. For example, exercise has been shown to increase generation of reactive O_2 species within muscle (Clanton 2007; Zuo and Clanton 2005; Powers et al. 2010) and is also associated with release of several inflammatory mediators such as interleukin-6 (IL6), tumor necrosis factor (TNFα) and interleukin 1-beta (IL1β) (Suzuki et al. 2002). These effects could be stimulated in part by the low PO_2 (Zuo and Clanton 2005) although other mechanisms could also be involved. Separately, hypoxia, such as that seen at altitude, is also pro-inflammatory, although the origins of this inflammatory response may lie in alveolar macrophages, not distal tissues such as muscle (Gonzalez and Wood 2010). Overall, these findings allow one to postulate that the low PO_2 seen during exercise incites the muscle inflammatory response, and this may contribute to genomic responses resulting in adaptation enabling greater exercise capacity.

This is because several genes involved in muscle adaptation to training can be affected by inflammatory molecules in that there are binding sites for such molecules in their promoter. For example, it is known that reactive O_2 species can signal activation of VEGF (Ushio-Fukai and Alexander 2004), a gene apparently required for both capillary maintenance (Olfert et al. 2009) and training-induced angiogenesis (Olfert et al. 2010) and that the VEGF promoter has IL6 binding sites as well (Cohen et al. 1996). It is too soon to be sure about such mechanisms, but it is possible that several parallel consequences of the low

*P*O$_2$ seen in muscle during exercise act in concert to turn on key genes responsible for the training response. This complex research area will likely evolve rapidly in the coming years.

Summary

This review has brought together a considerable body of evidence to show that the normally high resting muscle intracellular *P*O$_2$ of about 4 kPa falls dramatically during heavy and maximal exercise, to levels of only 0.4–0.5 kPa (1/40th of the *P*O$_2$ in inspired air). That muscles can achieve such extraordinary levels of metabolic activity and energy generation when one of its key substrates, O$_2$, is present at such low concentrations, is remarkable, and hardly coincidental. The very low *P*O$_2$ appears to be sufficient to maximally drive oxidative phosphorylation in all but trained athletes, and results in several biological benefits to the organism. These include optimizing O$_2$ availability to the mitochondria through both diffusive and convective transport pathways. For diffusion, making use of the full *P*O$_2$ gradient between red cells and mitochondria maximizes O$_2$ transport for a given muscle geometry, while locally reduced *P*O$_2$ values resulting from low perfusion in relation to metabolic demand stimulate a negative feedback system to raise perfusion and improve O$_2$ availability. The other major apparent benefit of the low *P*O$_2$ is as a signal to induce, directly and/or indirectly, increased expression of many genes required to generate the adaptive response to muscle training—a response which results in higher metabolic capacity to use O$_2$ as well as in greater availability of O$_2$ through capillary angiogenesis. Important questions for future research in this area revolve around the roles for hypoxia and inflammation in the adaptive response to repeated exercise—both the structural adaptation of muscle fibers and capillary supply, and the functional adaptations involved in enhanced acute responses to exercise.

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