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VIEWPOINT

Managing the power grid: how myoglobin can regulate PO_2 and energy distribution in skeletal muscle

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Over two decades ago, investigators using NMR detection of myoglobin (Mb) saturation in human skeletal muscle determined that the mean intracellular P_{O_2} (Pi_{O_2}) quickly drops from ≈34 Torr at rest (27) to 2–5 Torr during intense exercise (22, 28, 29). Pi_{O_2} appears to remain remarkably constant between 60 and 100% of $\dot{V}O_{2peak}$ (28, 29), although some report drops of <2 Torr in this range (22). At the same time, recent studies have reported that the P_{O_2} in the interstitial space, near the muscle fiber surface, also decreases with exercise (14). This means that the pressure drop for O_2 diffusion across the muscle fiber during exercise is not increasing at a time when the flow of O_2 increases by nearly twofold. Assuming the diffusion coefficient for O_2 remains constant, there is no suitable physical model accounting for this phenomenon. It cannot be explained by the reductions in P_{O_2} at the cytochrome c oxidase (CcO) in Mb-containing fibers, because the O_2 flow from Mb to CcO can never exceed the net flow from the fiber surface to Mb [i.e., a "waterfall effect" (31)].

However, recent discoveries in skeletal and heart muscle biology provide insights that point to a likely mechanism involving a leading role for Mb as both an O_2 sensor and a P_{O_2}-dependent catalytic switch for oxidation/reduction reactions of NO and nitrite (NO_2^-). This mechanism, which relies on existence of the mitochondrial reticulum power grid (3, 13, 16), could increase the flow of O_2 with no apparent change in mean pressure drop across the fiber. Rather, the mechanism depends on an effective narrowing of the diffusion distance for O_2 from the muscle fiber surface to a predominant site of electron transport at the subsarcolemmal mitochondria.

Function of the Myoglobin Sensor in a High PO_2 Environment

Cytochrome C oxidase (CcO) in complex IV is exquisitely sensitive to reversible inhibition by nanomolar levels of NO. The sensitivity greatly increases as P_{O_2} is reduced (1, 5). However, in the presence of oxymyoglobin (MbO_2), any available NO reacts rapidly to produce nitrate (NO_3^-) and metmyoglobin [MetMb(Fe^{3+})] (Fig. 1*A*). The rate constant for this reaction (8) is similar to the dissociation constant for O_2 from Mb (11). The resulting oxidation product [MetMb(Fe^{+3})] disappears rapidly by a resident family of metmyoglobin reductases (MMR), some of which are associated with the mitochondrial membrane (2). Skeletal muscles from rodents (25) and humans (23) are net producers of endogenous NO_3^- at rest, with intramuscular concentrations of 80–200 μM, nearly twice the value in plasma. This continual supply of NO_3^- suggests that these reactions are ongoing at rest in muscle.

The NO that is scavenged can come from numerous sources, including from local nitric oxide synthases (NOS1 and NOS3) (17, 18), spillover from the vasculature or from local NO_2^- reactions. Clearly, there is sufficient Mb, being 500–700 μM in human skeletal muscle (4), about twofold higher than heart (32). Mb expression in rat gastrocnemius has been shown to be greatest in fast oxidative, next in slow oxidative, and much lower in fast glycolytic fibers (19). Importantly, recent work of Yamada et al. (35) has shown that the majority of Mb in skeletal muscle resides near or within mitochondria, throughout the reticulum, and several investigators have reported structural binding between myoglobin and mitochondrial cytochromes (10, 35). At high P_{O_2}, the mitochondrial localization of Mb and the rapid reaction rates between NO and MbO_2 would function as a "nitric oxide shield" (Fig. 1*B*), ensuring that any endogenous NO present is scavenged before it can inhibit CcO. Thus, in regions of moderate to high P_{O_2}, e.g., near the subsarcolemma, high rates of CcO activity and $\dot{V}O_2$ are expected. This mechanism would most likely be predominant in Type IIA muscle fibers, based on the observations that they have the strongest expression of all NOS isoforms (26), high concentrations of Mb (19), strong colocalization of Mb with mitochondria (35), and are likely to carry the largest metabolic load during intense aerobic exercise.

Function of the Myoglobin Sensor at Low P_{O_2}

At low P_{O_2}, deoxyMb no longer functions as a NO scavenger, but rather as a NO producer. The "switch" happens as Pi_{O_2} approaches the P_{50} for O_2 binding to Mb, or ≈3 Torr (30; Fig. 1*C*). Within this P_{O_2} range, skeletal muscles are capable of converting NO_3^- to NO_2^- by resident enzymes that belong to a family of xanthine oxidoreductases (XOR) (Fig. 1*A*). The responsible reaction pathways were pioneered in studies on myocardium, where production of NO from NO_2^- in ischemia provides a significant source of NO (9, 12, 33). In noncontracting, normoxic conditions, these reactions are slow, e.g., the ratio of $[NO_2^-]/[NO_3^-]$ is 0.005 in muscle (24). This slow conversion is due, in part, to lack of reducing substrates and other conditions necessary for effective NO_3^- reduction. However, in working muscle, the reaction is accelerated when xanthine and hypoxanthine become available through ATP degradation and when pH is decreasing (21). When pH drops from 7.5 to 6.5, NO_3^- conversion to NO_2^- nearly doubles (24).

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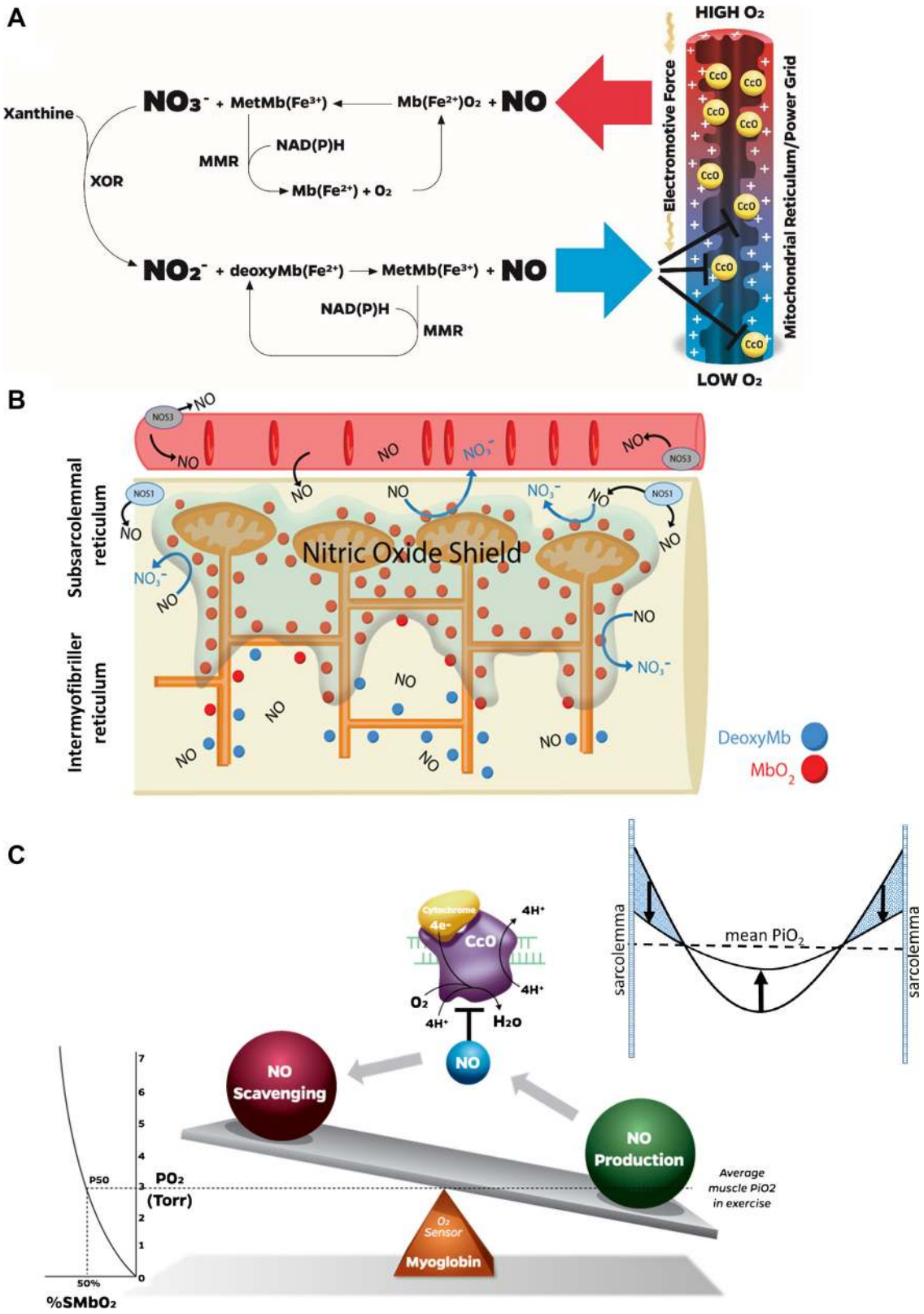

Evidence for these reactions being active in exercising muscle comes from studies in rats, where substantial conversion of muscle NO_3^- to NO_2^- is evident only following intense and prolonged exercise (24). In conditions of low Mb saturation, the NO_2^- produced reacts with deoxyMb to form deoxymetMbNO(Fe^{+3}) (Fig. 1*A*), which then dissociates to free NO and deoxymetMb(Fe^{+3}). When pH drops from 7.0 to 6.0 the rate of NO production from NO_2^- via Mb reactions increases by ~12-fold (21).

Because Mb is concentrated near mitochondria (35) and the reaction of NO with CcO is rapid, this regulatory step could happen in a microenvironment where no measurable elevations in NO would be detected. The small elevations in NO near CcO could easily function to inhibit respiration, and the gain of this controller would be elevated by marked increases CcO sensitivity to NO at low P_{O_2} (1, 5). The overall effect is that as P_{O_2} approaches the P_{50} for Mb, NO becomes elevated, CcO inhibited, and $\dot{V}_{O_2}$ suppressed, thus preserving local Pi_{O_2}. This process could also function to protect mitochondria from extreme reductions in Pi_{O_2} at high metabolic rate that could result in mitochondrial damage (15, 20).

Function of Myoglobin within the Mitochondrial Power Grid

Recently, Glancy et al. (13) demonstrated that the mitochondrial reticulum, described earlier by Bakeeva et al. (3) and later by Brooks and colleagues (16), is contiguous between the subsarcollemal and intermyofibrillar mitochondria in normal muscle, forming a network. They have also confirmed that this comprises a kind of "power grid" by which the H^+-electrochemical gradient is rapidly shared across the reticulum. If one region becomes depolarized, charge created and stored in other regions will re-establish the gradient so that local ATP production can continue via ATP synthase, despite lack of available O_2.

During intense exercise, it is likely that there are regions of the inner muscle core that experience O_2 depletion. Although gradients during exercise are difficult to confirm (6) measurements from frozen muscle near $\dot{V}_{O_{2peak}}$ suggest that relatively large gradients are common in individual fibers (7, 34). However, when Pi_{O_2} is in the range of 2–5 Torr, nearly unmeasurable gradients of <0.5 Torr would greatly impact these reactions. As illustrated in Fig. 1c, Mb operates in this P_{O_2} range as a sensor around a set point defined by its P_{50}. If P_{O_2} drops below the set point, proportionally more O_2Mb molecules convert to deoxyMb, less endogenous NO is scavenged, and more NO produced by NO_2^- conversion. As local NO increases, CcO activity slows down, reducing $\dot{V}_{O_2}$ and returning local Pi_{O_2} toward the set point. Conversely, as Pi_{O_2} begins to rise above the set point, MbO_2 scavenges more NO, allowing CcO to operate at higher O_2. This, in turn, reduces Pi_{O_2} closer to the set point. Any radial P_{O_2} gradient across the muscle fiber would thus be attenuated, while ensuring that areas of the highest P_{O_2} are available to sustain the mitochondrial H^+-electrochemical potential.

This regulatory behavior of Mb is likely to be subtle. Even at P_{O_2} of 10 Torr (e.g., near the sarcolemma), ≈20% of the Mb is desaturated and could contribute to NO formation, while the remaining 80% is simultaneously absorbing NO. Such a gradation of response, above and below the set point, encompasses the engineering characteristics of a "proportional controller" set to optimize Pi_{O_2} at a set point and minimize overshoot during transients in P_{O_2}.

The net effect is that as exercise intensity increases, regional P_{O_2} will remain more uniform across the fiber with little or no loss in the rate of local ATP production in any location (Fig. 1*C*, *inset*). In addition, when a greater load of $\dot{V}_{O_2}$ is carried by the mitochondrial reticulum just below the sarcolemma, the trans-membrane gradient for O_2 diffusion will be increased. This effectively reduces the diffusion path for O_2. In this way, "mean" muscle fiber Pi_{O_2} remains unchanged (Fig. 1*C*, *inset*), although the rate of metabolism and O_2 flux is drastically elevated.

The concept of regional separation of mitochondrial metabolic function, radially across the fiber is also supported by measurements of the distribution of metabolic enzymes (13). Subsarcolemmal mitochondria contain significantly higher concentrations of complex IV (CcO), but complex V (ATP synthase) is found throughout the fiber. In this setting, myoglobin's role is to coordinate the effectiveness of the power grid to continue to generate new ATP throughout the fiber, optimizing which regions of the reticulum are supporting the proton gradient and assigning these to areas of highest P_{O_2}.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

T.L.C. conceived and designed research; T.L.C. interpreted results of experiments; T.L.C. prepared figures; T.L.C. drafted manuscript; T.L.C. edited and revised manuscript; T.L.C. approved final version of manuscript.

Fig. 1. *A*: reactions of myoglobin (Mb) with nitric oxide (NO). Two contrasting reactions of oxymyoglobin (MbO_2) and deoxymyoglobin (deoxyMb). At high P_{O_2} (*top* region), MbO_2 reacts rapidly with NO to form nitrate (NO_3^-), which can be secreted into the circulation. At low P_{O_2} (*bottom* region), deoxyMb reacts with nitrite (NO_2^-) to produce NO. On the far right is an idealized long segment of the mitochondrial reticulum, in which at low P_{O_2}, NO inhibits cytochrome c oxidase (CcO). At high P_{O_2}, the CcO is ungoverned and able to operate at full capacity, as required. It can therefore provide electrical power in the form of H^+ gradient throughout areas of the mitochondrial reticulum exposed to low P_{O_2}. *B*: regional distribution of NO regulated by Mb. Within the intact mitochondrial network, the rapid reaction of MbO_2 with NO, very near the sarcolemma, functions as a protective shield, essentially eliminating the inhibitory effects of NO on CcO activity. Cytosolic NO in active skeletal muscle can arise from NOS1 or NOS3; it can diffuse from NOS3 in the vasculature or from the NO_2^- reduction reactions. *C*: Mb-NO regulatory controller: Mb behaves as a proportional O_2 sensor/switching system, directing regional oxygen consumption based on O_2 availability. The "sensor" is Mb, the "effector" is NO, and the "molecular target" is CcO. The set point for the sensor is ~3 Torr, the P_{50} for MbO_2. *Inset:* overall effects of minimizing Pi_{O_2} across the fiber, resulting in lower P_{O_2} at the subsarcollemal microenvironment, thus reducing the effective mean diffusion distance for O_2.

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