The Journal of Physiology

LETTER TO THE EDITOR

Rethinking oxygen handling in exercising muscle: From myoglobin deficiency to a CO shield

Introduction

Progress in physiology does not always depend on discovering new facts, but often on re-interpreting established ones in a new conceptual light. Few examples illustrate this better than the evolving story of myoglobin (Mb). Since Millikan's early description (Millikan, 1939) of Mb as a 'short-term O_2 store' in 1939, the protein has been classically regarded as a facilitator of intracellular oxygen diffusion during muscle contraction (Wittenberg & Wittenberg, 1961). This view, however, is gradually giving way to a more physiologically intricate mechanism.

A decisive turning point in this reappraisal stems from experimental work recently published in *The Journal of Physiology*, which demonstrated that Mb-deficient ($Mb^{-/-}$) mice display impaired maximal oxygen uptake ($\dot{V}_{O_2max}$), reduced exercise tolerance, altered substrate utilisation and disturbed systemic nitric oxide (NO•) homeostasis (Zoladz et al., 2024). These findings not only consolidate the role of Mb as a critical regulator of oxygen flux but also provide the impetus for the present Opinion piece, highlighting the need to reinterpret the physiological role of Mb within broader frameworks of oxygen diffusion, mitochondrial reticulum organisation and transcriptional adaptation.

A recent comprehensive review has reappraised Mb, highlighting not only its multifunctionality in oxygen transport and NO• metabolism but also its broader roles in redox signalling and cellular adaptation (Adepu et al., 2024). Within the physiological range of intracellular oxygen tension in skeletal muscle fibres (~10–1 mmHg, Poole et al., 2022), Mb acts as a condition-sensitive enzyme. At the higher end of this physioxic spectrum (~5–10 mmHg, as measured in interstitial regions), Mb removes NO• through dioxygenase activity, whilst at the lower end (~2–3 mmHg within myofibrillar regions) and under reduced pH during muscle contractions, it reduces nitrite to NO• (Clanton, 2019). This duality positions Mb not merely as a buffer, but also as a sensor and regulator of cellular redox and oxygen flux.

Here, we propose integrating the role of Mb in oxygen and NO• dynamics with the activity of hemeoxygenase-1 (HO-1), an inducible heme-degrading enzyme whose products – biliverdin, carbon monoxide (CO) and Fe^{2+} – play key roles in O_2 metabolism and signalling (Maines, 1997). We advance the view that the Mb–HO-1 axis acts as a regulatory module for oxygen homeostasis and physiological adaptation to exercise.

From sheer abundance to subtle control

The structural resolution of Mb by Kendrew et al. (1960) confirmed its evolutionary conservation and its high expression in oxidative muscle. In human skeletal muscle, Mb concentrations of 0.3–0.7 mM – demonstrated by microspectrophotometry and histochemistry (Peters et al., 1994; van Beek-Harmsen et al., 2004) – remain far above those of most regulatory proteins at micro- to nanomolar levels. Such sustained abundance argues strongly that Mb cannot be physiologically dispensable for exercise performance – a notion that early knockout reports tentatively entertained (Garry et al., 1998).

Building on this, the article recently published in *The Journal of Physiology* demonstrated that $Mb^{-/-}$ mice display an ~37% reduction in absolute $\dot{V}_{O_2max}$, earlier lactate accumulation and shorter time to exhaustion (Zoladz et al., 2024). These findings directly contradict the earlier suggestion that Mb might be dispensable for exercise tolerance and instead underscore its critical role in meeting high oxygen demands. Importantly, the same study also further highlighted the involvement of Mb in nitric oxide handling, reporting disturbed systemic nitrite/nitrate balance in $Mb^{-/-}$ mice during exercise.

This dovetails with a defining property of Mb: its responsiveness to oxygen tension. At P_{O_2} values close to its P_{50} (~2–3 mmHg), Mb may simultaneously release oxygen, reduce nitrite to NO• or scavenge NO• through dioxygenase activity (Adepu et al, 2024; Poole et al., 2022). Mb actions also vary with fibre location: subsarcolemmal regions, with relatively higher P_{O_2}, favour NO• scavenging, whilst deeper myofibrillar regions, closer to contractile elements, are more prone to nitrite reduction. This spatial and functional complexity underlies the concept of an 'NO• shield', whereby Mb regulates oxygen consumption by modulating cytochrome c oxidase (COX) activity through NO• availability (Clanton, 2019).

Classic models predicted steep P_{O_2} drops from capillaries to mitochondria, with 'anoxic corners' in contracting fibres. Recent evidence contradicts this: interstitial and intracellular P_{O_2} remain low but stable at 3–10 and 1–5 mmHg, respectively, even under intense contraction (Keeley & Mann, 2019; Poole et al., 2022). These data reveal relatively flat gradients and efficient oxygenation without widespread anoxia. The mitochondrial reticulum (MR) – an electrically and structurally integrated network – enables deep fibre oxygenation with minimal P_{O_2} differences (Glancy & Balaban, 2021). In this context, Mb's role shifts from transporter to regulator, fine-tuning flux within the MR through NO• modulation. By producing or scavenging NO•, Mb modulates COX activity, preventing collapse of respiration at low P_{O_2} or relieving inhibition when oxygen is sufficient (Adepu et al, 2024). This layered regulation contributes to the stability of the intrafibre microatmosphere.

The overlooked partner: HO-1 and its transcriptional control

Such insights place Mb at the centre of oxygen and NO• regulation within the muscle fibre. Yet, its abundance and activity also imply vulnerability: oxidation, turnover or fibre damage inevitably release free heme (Nath et al., 1992). This heme cannot accumulate without consequence, as it is both cytotoxic and a potent signalling molecule (Ryter & Tyrrell, 2000). Here, the inducible enzyme HO-1 becomes a critical partner. First characterised by Maines (1997), HO-1 degrades haem into biliverdin, CO and Fe^{2+}. Beyond preventing haem toxicity, these products exert signalling functions: biliverdin/bilirubin act as redox buffers, whilst CO influences mitochondrial respiration and vascular tone (Alves de Souza et al 2021; Ryter et al., 2006). In this way, the Mb–HO-1 axis emerges as a plausible regulatory module

 DOI: 10.1113/JP290460

for sustaining the delicate microatmosphere of exercising muscle fibres.

HO-1 expression correlates with Mb content, fibre type and mitochondrial density (Pecorella et al., 2015; Vesely et al., 1999). Exercise-induced Mb oxidation and turnover, together with redox imbalance, act as potent stimuli for HO-1 upregulation (Alves de Souza et al 2021; Muthusamy et al., 2012). In skeletal muscle, HO-1 deficiency leads to impaired haem clearance, mitochondrial dysfunction, fibre-type shifts towards glycolytic profiles and poor adaptation to training (Alves de Souza et al 2021). These maladaptations parallel those seen with Mb deficiency and link HO-1 to the regulation of COX, another hemoprotein central to O_2 flux.

Crosstalk and systems integration

Together, these findings suggest a regulatory crosstalk between Mb and HO-1. Mb stability and turnover determine the rate of heme release; HO-1 catabolises this heme to generate CO; and CO, in turn, modulates mitochondrial respiration and vascular tone. In juxtaposition to the NO• shield proposed for Mb, CO may establish a 'CO shield', acting through COX inhibition and vasodilatation to preserve oxygen flux during intense contractions. This shield could represent an additional layer of regulation in muscle oxygen flux, integrated within the broader context of Mb- and HO-1-mediated control.

From this perspective, Fick's law of diffusion remains fundamental, but it should now be reframed within multiple modulating layers: Mb-mediated NO• metabolism, HO-1-dependent CO production and the organisation of the mitochondrial reticulum. This integrated view helps explain the maintenance of shallow P_{O_2} gradients during exercise: oxygen flux is buffered by complementary biochemical shields, preventing collapse due to non-functional local P_{O_2} levels. By embedding convective and diffusive transport within this gasotransmitter context, we propose a unified framework in which Mb, HO-1 and their products actively shape the muscle fibre's oxygen milieu.

Conclusions and perspectives

The physiological role of Mb is shifting from a traditional view of bulk passive oxygen storage towards a fine dynamic role in the local regulation of oxygen and NO• signalling. Its absence impairs $\dot{V}_{O_2max}$, metabolic flexibility and systemic NO• balance, leaving little doubt that it is indispensable for exercise performance. HO-1 emerges as a convergent partner: by catabolising heme to generate CO, it not only prevents haem toxicity but also introduces an additional gaseous regulator with downstream effects on mitochondrial function and vascular tone.

Although the concept of an NO• shield has been established to describe the buffering of COX by NO•, we propose here an equally compelling extension: that CO, as a product of HO-1 activity, may establish a 'CO shield', juxtaposed to the NO• shield. Acting through COX inhibition and vasodilatation, this shield could represent an additional layer of regulation in muscle oxygen flux, integrated within the broader context of Mb- and HO-1-mediated control.

Thus, the Mb–HO-1 axis may be envisaged as a regulatory module for integrated oxygen and redox signalling in muscle, with the CO shield as a new hypothesis awaiting experimental validation. Future studies using $Mb^{-/-}$ and HO-$1^{-/-}$ models under physiologically relevant P_{O_2} will be essential to test this framework. If confirmed, it would help redefine our understanding of the microatmosphere within the muscle fibre – the finely tuned balance of oxygen, nitric oxide and CO that orchestrates mitochondrial function, vascular adaptation and ultimately exercise capacity.

It may now be timely to expand the oxygen paradigm to embrace not only Mb and the NO• shield, but also HO-1 and a newly proposed CO shield.

Adrian Bayonas-Ruiz[1,2] and Barbara Bonacasa[1,2]

[1]*Department of Physiology, Human Physiology Area, University of Murcia, Spain*
[2]*Research Group of Physical Exercise and Human Performance, University of Murcia, Spain*

Email: bonacasa@um.es

Handling Editors: Paul Greenhaff & Mike Stembridge

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP290460#support-information-section).

References

Adepu, K. K., Anishkin, A., Adams, S. H., & Chintapalli, S. V. (2024). A versatile delivery vehicle for cellular oxygen and fuels or metabolic sensor? A review and perspective on the functions of myoglobin. *Physiological Reviews*, **104**(4), 1611–1642.

Alves de Souza, R. W., Gallo, D., Lee, G. R., Katsuyama, E., Schaufler, A., Weber, J., Csizmadia, E., Tsokos, G. C., Koch, L. G., Britton, S. L., Wisløff, U., Brum, P. C., & Otterbein, L. E. (2021). Skeletal muscle heme oxygenase-1 activity regulates aerobic capacity. *Cell Reports*, **35**(3), 109018.

Clanton, T. L. (2019). Managing the power grid: How myoglobin can regulate PO2 and energy distribution in skeletal muscle. *Journal of Applied Physiology (1985)*, **126**(3), 787–790.

Garry, D. J., Ordway, G. A., Lorenz, J. N., Radford, N. B., Chin, E. R., Grange, R. W., Bassel-Duby, R., & Williams, R. S. (1998). Mice without myoglobin. *Nature*, **395**, 905–908.

Glancy, B., & Balaban, R. S. (2021). Energy metabolism design of the striated muscle cell. *Physiological Reviews*, **101**(4), 1561–1607.

Keeley, T. P., & Mann, G. E. (2019). Defining physiological normoxia for improved translation of cell physiology to animal models and humans. *Physiological Reviews*, **99**(1), 161–234.

Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., & Shore, V. C. (1960). Structure of myoglobin: A three-dimensional fourier synthesis at 2 Å resolution. *Nature*, **185**, 422–427.

Maines, M. D. The heme oxygenase system: A regulator of second messenger gases (1997). *Annual Review of Pharmacology and Toxicology*, **37**, 517–54.

Millikan, G. A. (1939). Muscle hemoglobin. *Physiological Reviews*, **19**(4), 503–523.

Muthusamy, V. R., Kannan, S., Sadhaasivam, K., Gounder, S. S., Davidson, C. J., Boeheme, C., Hoidal, J. R., Wang, L., & Rajasekaran, N. S. (2012). Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium. *Free Radical Biology and Medicine*, **52**(2), 366–76.

Nath, K. A., Balla, G., Vercellotti, G. M., Balla, J., Jacob, H. S., Levitt, M. D., & Rosenberg, M. E. (1992). Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *Journal of Clinical Investigation*, **90**(1), 267–70.

Peters, T., Jürgens, K. D., Günther-Jürgens, G., & Gros, G. (1994). Determination of myoglobin-diffusivity in intact skeletal muscle fibers: An improved microscope-photometrical approach. *Advances in Experimental Medicine and Biology*, **345**, 677–683.

Poole, D. C., Musch, T. I., & Colburn, T. D. (2022). Oxygen flux from capillary to mitochondria: Integration of contemporary discoveries. *European Journal of Applied Physiology*, **122**(1), 7–28.

Pecorella, S. R., Potter, J. V., Cherry, A. D., Peacher, D. F., Welty-Wolf, K. E., Moon, R. E., Piantadosi, C. A., & Suliman, H. B. (2015). The HO-1/CO system regulates mitochondrial-capillary density relationships in human skeletal muscle. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **309**(8), L857–L871.

Ryter, S. W., & Tyrrell, R. M. (2000). The heme synthesis and degradation pathways: Role in oxidant sensitivity. *Free Radical Biology and Medicine*, **28**, 289–309.

Ryter, S. W., Alam, J., & Choi, A. M. (2006). Heme oxygenase-1/carbon monoxide: From basic science to therapeutic applications. *Physiological Reviews*, **86**(2), 583–650.

van Beek-Harmsen B, J., Bekedam, M. A., Feenstra, H. M., Visser, F. C., & van der Laarse W, J. (2004). Determination of myoglobin concentration and oxidative capacity in cryostat sections of human and rat skeletal muscle fibres and rat cardiomyocytes. *Histochemistry and Cell Biology*, **121**, 335–342.

Vesely, M. J., Sanders, R., Green, C. J., & Motterlini, R. (1999). Fibre type specificity of haem oxygenase-1 induction in rat skeletal muscle. *Federation of European Biochemical Societies Letters*, **458**(2), 257–260.

Wittenberg, J. B., & Wittenberg, B. A. (1961). The secretion of oxygen into the swim-bladder offish. II. The simultaneous transport of carbon monoxide and oxygen. *Journal of General Physiology*, **44**(3), 527–42.

Zoladz, J. A., Grandys, M., Smeda, M., Kij, A., Kurpinska, A., Kwiatkowski, G., Karasinski, J., Hendgen-Cotta, U., Chlopicki, S., & Majerczak, J. (2024). Myoglobin deficiency impairs maximal oxygen uptake and exercise performance: A lesson from mb-/- mice. *The Journal of Physiology*, **602**(5), 855–873.

Additional information

Competing interests

The authors declare no competing interests.

Author contributions

B. B. and A. B.-R. conceptualized and wrote the article. Dr. Bonacasa was the recipient of a Moving Minds 2025 Fellowship awarded by the University of Murcia to conduct a research visit to Prof. Giovanni E. Mann's Physiological Oxygen Laboratory at King's College London. The authors gratefully acknowledge Prof. Mann for the many thoughtful and extended conversations during this stay, which helped shape the conceptual foundations of the CO-shield hypothesis, as well as for his generous time in revising the final version of the manuscript. Adrián Bayonas is supported by a pre-doctoral fellowship at the University of Murcia from the Spanish Ministry of Universities (FPU22/04352).

Funding

None.

Keywords

dioxygenase, heme oxygenase-1, myoglobin

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History