

Mitochondria and oxygen homeostasis

Mateus P. Mori¹, Rozhin Penjweini², Jay R. Knutson², Ping-yuan Wang¹ and Paul M. Hwang¹ ¹ Cardiovascular Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA² Laboratory of Advanced Microscopy and Biophotonics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

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Correspondence

P. M. Hwang, Cardiovascular Branch, NHLBI, National Institutes of Health, Building 10-CRC, Room 5-5330, 10 Center Drive, Bethesda, MD 20892-1454, USA
Tel: +1 301 435 3068
E-mail: hwangp@mail.nih.gov

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Molecular oxygen possesses a dual nature due to its highly reactive free radical property: it is capable of oxidizing metabolic substrates to generate cellular energy, but can also serve as a substrate for genotoxic reactive oxygen species generation. As a labile substance upon which aerobic life depends, the mechanisms for handling cellular oxygen have been fine-tuned and orchestrated in evolution. Protection from atmospheric oxygen toxicity as originally posited by the Endosymbiotic Theory of the Mitochondrion is likely to be one basic principle underlying oxygen homeostasis. We briefly review the literature on oxygen homeostasis both *in vitro* and *in vivo* with a focus on the role of the mitochondrion where the majority of cellular oxygen is consumed. The insights gleaned from these basic mechanisms are likely to be important for understanding disease pathogenesis and developing strategies for maintaining health.

Introduction

Molecular oxygen (O₂) is one of the most interesting and important molecules that has intrigued the scientific community over the ages. O₂ is an intrinsic part of life that has driven evolution and facilitated broad biological diversification, while reactive oxygen species (ROS) derived from molecular O₂ or the O₂ itself are also suggested to be a major driver of aging [1,2]. Regardless of the perspective, O₂ plays a ubiquitous role in life with the nature of a double-edged sword. The mitochondrion has two demonstrable features with regard to O₂: (1) It utilizes O₂ to form H₂O with such avidity that it can create essential anoxia in a closed environment; and (2) its consumption of O₂ can result in the generation of cellular energy, heat, and ROS. Suggestive of a homeostatic relationship, it is known that higher concentrations of

O₂ in the environment can increase the respiratory capacity of mitochondria while hypoxia may cause its downregulation in tissue culture [3,4]. We review mammalian O₂ homeostasis by revisiting the concentrations of O₂ reported in cells and tissues, summarizing some published work on the role of mitochondria in its regulation, and discussing the relevance of these findings to various disease pathogenesises.

Determinants of physiological O₂ levels (physioxia): association with mitochondrial activity

The diffusion of atmospheric O₂ into tissues is limited by the area of the gas–liquid interface and the low

Abbreviations

DMBA, 7,12-dimethylbenz[a]anthracene; FLIM, fluorescence lifetime microscopy; HIF-1 α , hypoxia-inducible factor 1 alpha; *Mb*, Myoglobin gene; MEF, mouse embryonic fibroblasts; O₂, molecular oxygen; OCR, O₂ consumption rate; PO₂, partial pressure of dissolved O₂; PV, paravascular; ROS, reactive oxygen species; *TP53*, Tumor Protein p53 gene.

solubility of molecular oxygen. These limitations are counteracted by the vast surface area within the lung that permits gas exchange and by the hemoglobin in red blood cells that serves as O₂ reservoir with resultant elevation in the partial pressure of dissolved O₂ (PO₂). The lack of mitochondria and thereby respiration in red blood cells make them ideally suited as O₂ carriers. Before considering the levels of O₂ observed in the different tissues of the body, it is instructive to be reminded of the relevant PO₂ values at the point of gas exchange. The PO₂ of ambient air (20.9% O₂) is ~ 150 mm Hg (25 °C, sea level), but it decreases to ~ 100 mm Hg due to higher temperature and the partial pressures of water vapor and CO₂ in the lower respiratory tract [5]. Upon return to the left atrium after its re-oxygenation at the blood–alveoli interface of the lung (~ 94–98% saturation of hemoglobin), the PO₂ of blood is closer to ~ 95 mm Hg in part due to mixing with venous blood from the bronchial veins.

The levels of PO₂ observed under physiological conditions (physioxia) can vary spatially within organs (Fig. 1). There are at least three factors that could

interact to result in the variability of tissue PO₂: (a) vascularity, (b) blood flow, and (c) oxygen consumption. Because white blood cells generally contain fewer mitochondria and consume less O₂, vascularity and blood flow would be expected to play a major role in determining the PO₂ of immune tissues. Indeed, the evenly perfused vascular bone marrow has a relatively higher PO₂ (~ 55 mm Hg) compared with the thymus, another immune tissue, which is unusually hypoxic with a PO₂ reported at 10 mm Hg [6–8]. Although the functional significance of the hypoxic environment in the thymus is unclear, the latter observation can be explained by cortical networks of capillaries in the periphery of the thymic lobules forming loops that return blood to the postcapillary venules (vascularity) [9]. Regional differences in PO₂ are also observed in tissues that have high O₂ utilization such as in the human brain where 20% of the total O₂ is consumed by 2% of the body weight [10]. Brain tissue O₂ measurements such as in the cerebral cortex have generally revealed higher PO₂ in the more superficial gray matter compared with the deeper white matter [11]. Although

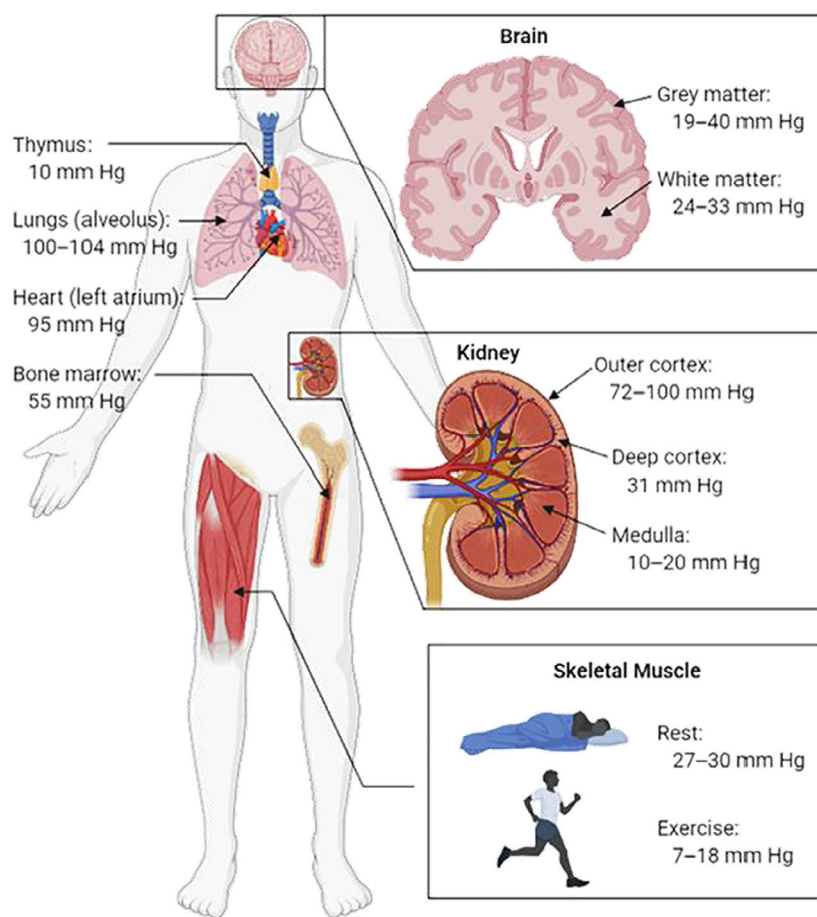


Fig. 1. Variable partial pressure of O₂ (PO₂) in human tissues. Note that the tissue PO₂ under normal physiological conditions is highly variable between different tissue types or organs. There are also regional differences in PO₂ within the same organ such as in brain and kidney. PO₂ can also change depending on functional states such as in exercising skeletal muscle or with cognition in brain. Figure created on BioRender.com website.

this observation could be explained by differences in capillary density and blood flow, O₂ consumption by mitochondria localized to axonal processes in white matter during periods of neural activity is likely to be a contributing factor as well [12].

The kidney and skeletal muscle serve as examples of the effects of the interactions among blood flow rate, tissue vascularity, and mitochondrial respiration on tissue PO₂. The PO₂ of the outer cortex of the human kidney is 72–100 mm Hg, while it is only 10–20 mm Hg in the inner medulla in humans (Fig. 1) [13]. Within the cortex, the PO₂ of the deeper layer has been reported to be lower than that of the more superficial layer, suggesting the role of O₂ consumption in determining its tissue levels. In support of this notion, the lower PO₂ observed in the kidney cortex of hypertensive Wistar Kyoto rats compared with that of normotensive rats has been attributed to their increased bioenergetic demand (~15–25% higher O₂ consumption) due to less efficient sodium transport [14].

Skeletal muscle has also been observed to have variable tissue PO₂ depending on activity, but its steady-state PO₂ would be expected to be affected by the increases in oxygenated blood flow associated with exercise. Under rest conditions, the blood flow to skeletal muscle is ~1 L·min⁻¹ (average human cardiac output ~5 L·min⁻¹), but this can increase to 18 L·min⁻¹ during exercise secondary to increased cardiac output (up to 20 L·min⁻¹) and redistribution of blood flow to the muscle vasculature [15]. Despite the potential for such large increases in blood flow to the muscle, the PO₂ can decrease from ~29 mm Hg at rest to as low as ~7 mm Hg (a 75% reduction) with foot-ergometer exercise in the gastrocnemius muscle, and the O₂ saturation of blood returning to the heart via the femoral vein can decrease to 20% with treadmill exercise [16,17]. At the cellular level, the coupled state 3 respiration of mitochondria in permeabilized quadriceps muscle fibers has been shown to exceed that of O₂ delivery during exercise [18]. Such exercise-induced hypoxia demonstrates the extremes of supply-demand mismatch caused by mitochondrial oxygen consumption, which can increase over 100-fold during physical exercise [19].

Given these *in vivo* observations, physioxia cannot be assigned standard values that apply to all tissues under all conditions. Rather, factors such as tissue vascularization, blood flow, and oxygen consumption rate—as well as other determinants such as animal species, functional state, tissue architecture, size of the organ, and even distance from the heart—should be taken into consideration to determine physioxia.

Animal model evidence of O₂ limitation in tissues

Earlier work revealed that the O₂ saturation of myoglobin, an intracellular protein expressed in muscle cells that binds O₂ more tightly than hemoglobin, is very high and not labile under moderate workload changes in an *in vivo* cardiac model using optical spectroscopy, suggesting a lack of O₂ limitation under these conditions [20]. However, subsequent investigations revealed evidence of a significant O₂ gradient in skeletal muscle as monitored by the redox state of mitochondrial NAD(P)H in the perivascular versus intrafibrillar regions using dynamic optical microscopy [21]. As discussed earlier, regional differences in PO₂ within an organ such as the brain or the kidney also suggested the existence of O₂ gradients *in vivo*.

To explore tissue O₂ homeostasis, the gene encoding myoglobin (*Mb*) was disrupted in mice by two independent research groups [22,23]. Myoglobin-deficient mice were initially reported as having no significant cardiac or skeletal muscle dysfunction, questioning the role of myoglobin in O₂ homeostasis and aerobic metabolism. Although there were no overt phenotypes or abnormalities, more detailed characterization did reveal some differences. *Mb*^{-/-} mouse hearts showed increased capillary density (~33%), coronary blood flow, and coronary flow reserve [23]. Notably, a subset of *Mb*^{-/-} embryos at stages E9.5 to E10.5 had defects in cardiac development and associated embryonic lethality, coinciding with the period during which the embryonic heart transitions from glycolytic to aerobic metabolism [24,25]. In addition to the increased capillary density of *Mb*^{-/-} hearts, hypoxia-inducible factor 1 alpha (HIF-1α) and its transcriptional target genes including the blood vessel-promoting gene *VEGF* were upregulated in *Mb*^{-/-} hearts [25]. These observations suggest an adaptive response to decreased O₂ availability and are consistent with the proposed function of myoglobin in facilitating O₂ diffusion to the mitochondria [26].

Metabolically, *Mb*^{-/-} perfused hearts showed higher lactate utilization and a shift from predominantly fatty acid oxidation to glycolysis, further indicating the activation of compensatory metabolic processes [25,27]. This shift was also accompanied by decreased expression of β-oxidation pathway genes and an increase in the active form of glucose transporter GLUT4 involved in glucose utilization [27]. Although myoglobin is nonessential for the *survival* of laboratory-bred mice, the relatively small decreases in aerobic metabolism and endurance exercise capacity in the myoglobin-deficient state cannot be disregarded as the magnitude of changes that permit successful adaptation of an

organism in evolution can be very small [28]. Taken together, these data provide *in vivo* support for the notion that molecular O₂ availability can be limiting due to its consumption by mitochondrial respiration under basal and physiological stress conditions.

Mitochondrial respiration-driven hypoxia

Various experimental data obtained from *in vivo* studies and cellular models support the concept that mitochondrial respiration can create an O₂-deficient environment. Morphologically, an innovative study of mouse skeletal muscle revealed a reticular network of paravascular (PV) mitochondria adjacent to capillary vessels that were physically and electrically coupled to mitochondria in the intrafibrillar region [29]. Remarkably, the ratio of mitochondrial respiratory complex IV (cytochrome c oxidase) to complex V (ATPase) in PV mitochondria, presumably with better access to O₂ via the capillaries, was higher compared with that of intrafibrillar mitochondria. One possible interpretation of this observation is that PV mitochondria are specialized to generate the proton-motive force by active respiration due to their proximity to oxygen-rich capillaries, while the intrafibrillar mitochondria produce the ATP at the site of their utilization by the contractile apparatus, supporting the original concept of mitochondria as energy-transmitting cables [30].

Experimentally, increasing mitochondrial capacity in primary human skeletal muscle cells by transducing with the mitochondrial biogenesis promoter PGC-1 α resulted in decreased intracellular O₂ levels, which in turn activate HIF-1 α and its target genes [31]. Consistent with the notion that increased respiration by mitochondria affects O₂ homeostasis, HIF-1 α was observed to be further stabilized upon mitochondrial biogenesis under relatively low oxygen levels compared with ambient air (21% O₂) tissue culture conditions [31,32]. Although these *in vitro* data do not rule out other possible mechanisms of HIF-1 α stabilization, they fit with the well-known observation of exercise-induced hypoxia in skeletal muscle, which associates with acute HIF-1 α stabilization and activation of *VEGF* and *EPO* genes in the human vastus lateralis thigh muscle after exercise [33].

Cellular and mitochondrial O₂ measurements

While there is evidence that alterations in cellular O₂ homeostasis can affect *in vivo* metabolism, there has been substantial controversy regarding O₂ levels and

the existence of O₂ gradients within the cellular microenvironment due to the lack of appropriate intracellular probes [34,35]. The recent development of optical probes sensitive to molecular oxygen has permitted *in vitro* measurements at the cellular level (although these data need to be interpreted with caution given the various limitations of O₂ delivery in the tissue culture geometry setting) [36,37]. Nonetheless, under controlled conditions, these techniques can serve as powerful tools for investigating the effects of mitochondrial respiration on cellular O₂ homeostasis and for developing insights into their *in vivo* significance.

The view of the mitochondrion as an ‘oxygen sink’ has been supported by a recent intracellular O₂ mapping study [35,38]. A Förster resonance energy transfer (FRET)-based O₂ probe was developed by fusing a reporter fluorescent protein mCherry to the O₂-binding protein myoglobin (Myo-mCherry) and imaged using 2-photon fluorescence lifetime microscopy (FLIM) [38]. The cytosolic Myo-mCherry was further subcellularly localized by fusing it to a mitochondrial matrix targeting sequence derived from the human *TFAM* gene. Transient transfection of the mitochondrial probe into the A549 non-small cell lung cancer cells revealed significantly lower concentrations of O₂ in mitochondria compared with the cytosolic probe measurements [38]. Furthermore, the pharmacologic or genetic inhibition of respiration using rotenone or mtDNA depletion (p⁰ cells), respectively, abolished the differences and reversed the lowered intracellular O₂ levels observed in actively respiring cells, confirming the specificity of these measurements and supporting the idea of mitochondria as O₂ sink. The observation of a hypoxic mitochondrial microenvironment can also be interpreted a factor acting against mitochondria being a major source of ROS under physiological conditions as its production is first-order dependent on the concentration of O₂ [39–41].

Increased intracellular O₂ levels in mitochondria-deficient states

If mitochondrial respiration promotes physiologic hypoxia, then impairing it could result in relative hyperoxia, which in turn may be pathologic due to O₂ toxicity. However, the literature linking mitochondrial dysfunction and O₂ homeostasis is scarce, presumably because inhibiting respiration has more immediate and apparent biological consequences. Using fluorescence lifetime imaging microscopy of a metalloporphyrin-based O₂ probe IC60N, we previously demonstrated that the genetic inhibition of cytochrome c oxidase assembly (*SCO2*^{-/-}) in HCT116 human colon cancer

cells resulted in increased levels of intracellular O₂ and high energy reducing equivalents (NAD(P)H), which in combination could contribute to the elevated ROS generation observed in these cells [42]. Furthermore, the nonrespiring *SCO2*^{-/-} cells showed evidence of increased oxidative DNA damage as measured by 8-oxoguanine and phosphorylated histone γ -H2AX markers, which were reduced by decreasing ambient O₂ exposure [42]. These observations were consistent with the prior demonstration that the generation of ROS in mitochondria is O₂ concentration-dependent and that O₂ itself can be mutagenic to prokaryotes [39,40,43].

Translational insights: O₂ as a carcinogen

Higher altitudes that are associated with lower ambient O₂ exposure were reported to be protective against the development of melanoma and cancers of the mouth, esophagus, larynx, and lung with the speculation that pH shifts could affect the proliferation of neoplastic cells [44]. Another explanation was that repair upregulation associated with mild increases in background radiation associated with altitude could confer protection against cancer, but this was subsequently refuted and high altitude *per se* was shown to be inversely correlated with the incidence of certain cancer types, especially of the lung [45]. It was suggested that reduced PO₂ may result in lower damaging ROS generation and protect from cancer. More recently, lung cancer incidence was again reported to be negatively correlated with altitude with a statistical significance ($P < 10^{-16}$) next to smoking, while several other environmental correlates of altitude failed to capture this association [46]. Although it is difficult to delineate the precise mechanism given the many different biological processes involved in lung tissue homeostasis [47], changes in ambient O₂ and oxidative stress associated with altitude could certainly contribute to this phenomenon.

In the laboratory, we have shown that hypoxia can be beneficial by delaying cancer formation. Using tumor-prone *p53*^{-/-} mice, we observed improved cancer-free survival under 10% ambient O₂ exposure in association with increased antioxidant and decreased ROS and 8-oxoguanine levels [48]. Intestinal polyp formation in *Apc*^{Min/-} mice and skin cancer formation using the 7,12-dimethylbenz[a]anthracene (DMBA) chemical carcinogenesis model were also reduced by chronic hypoxia exposure [48]. In another model, mice deficient in the nucleotide excision repair pathway gene *Xpc* are known to develop lung cancer

and their cells have delayed repair of oxidatively modified 8-oxoguanine [49,50]. Compared with wild-type state, *Xpc*^{-/-} mouse embryonic fibroblast (MEF) survival was lower in 20% versus 3% O₂ tissue culture conditions [51]. While DNA repair deficiency states may be more sensitive to oxidative DNA damage, other studies had also shown that chronic exposure to 3% O₂ can delay senescence and mutations even for wild-type MEFs [52,53]. Taken together, it is tempting to speculate that the genotoxicity of O₂ observed in mammalian cell models underlies the effect of altitude on the incidence of specific types of cancer in humans.

Benefits of limiting O₂ exposure on survival in mitochondrial dysfunction

Given the preponderance of evidence that mitochondrial respiration *in vivo* affects O₂ homeostasis, the question arises as to whether increases in tissue O₂ levels due to its decreased consumption in mitochondrial diseases, for example, can cause toxicity. This query is a corollary of the symbiotic theory of the mitochondrion, which postulated that engulfment of precursors of the modern-day mitochondrion by primordial anaerobic eukaryotes afforded protection from O₂ toxicity as its concentration rose in the ancient atmosphere of the earth [54].

More recently, Mootha and colleagues have elegantly addressed this question in a series of innovative studies using the *Ndufs4*^{-/-} mouse, a model of the complex I mitochondrial disease Leigh syndrome [55–57]. Paralleling the severity of the disease observed in patients who develop neurodegeneration and die within the first few years of life, the *Ndufs4*^{-/-} mice also developed neurologic deficits, failed to thrive, and showed shortening of lifespan (58 d median survival) [56]. As predicted, the age-dependent decreases in whole body O₂ consumption in *Ndufs4*^{-/-} mice were associated with hyperoxia in brain (Fig. 2) [56,57]. Remarkably, chronic exposure of *Ndufs4*^{-/-} mice to 11% O₂, which normalized brain PO₂ levels, prevented the severe neurologic phenotype and increased their lifespan by over fourfold to ~270 days [56]. Although initially thought to be related to the benefits of cellular response to hypoxia, this possibility was ruled out by the lack of significant improvement of the disease upon the activation of the master hypoxia response regulator HIF-1 α . On the other hand, simply decreasing O₂ delivery by low doses of carbon monoxide or by anemia improved the severe phenotype of the *Ndufs4*^{-/-} mice, further supporting a role for direct toxic effects of O₂ [57].

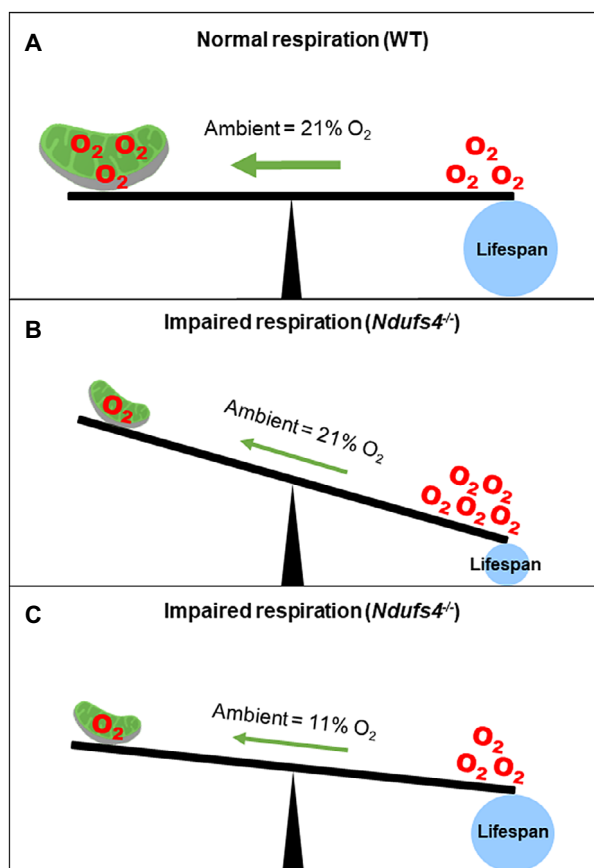


Fig. 2. Oxygen homeostasis associated with mitochondrial respiration may affect aging and lifespan. (Based on the following references: Ferrari *et al.* [56]; Jain *et al.* [57].) (A) Schematic representation of the proposed homeostatic relationship between tissue oxygen under normal ambient room air (21%) and its consumption in the mitochondria of wild-type mice resulting in a normal lifespan. (B) *Ndufs4*^{-/-} mice with impaired mitochondrial respiration have decreased oxygen consumption and thereby elevated tissue PO₂ which can promote oxygen toxicity and decrease lifespan. (C) Lowering ambient oxygen exposure of *Ndufs4*^{-/-} mice by placing in a hypoxia chamber (11% O₂) partially prevents the increase in tissue PO₂ and improves survival time.

Potential therapeutic translations

The aforementioned concepts discussed in this review could provide insights into strategies undertaken in the clinics, ranging from cardiovascular diseases to cancer. The observation that the most commonly mutated tumor suppressor gene *TP53* in human cancers promotes mitochondrial respiration under normal conditions could be interpreted as another one of its various antioxidant activities, potentially to protect against oxidative DNA damage and maintain genomic stability [58,59]. With the recent interest in gene versus

environment interactions, it is tempting to investigate whether decreasing exposure to ambient O₂ can have preventive effects in cancer susceptible conditions such as Li-Fraumeni syndrome caused by germline mutations of *TP53* [60].

Even in cardiopulmonary medicine, where lack of O₂ would be considered pathologic, supplemental O₂ therapy beyond what is clinically indicated in acute or chronic pulmonary insufficiency, stroke, or myocardial infarction did not result in improved patient outcomes [61–64]. In fact, two independent meta-analyses of available clinical studies on myocardial infarction were suggestive of harm if O₂ was routinely used in patients without clinical evidence of hypoxia [65,66]. Furthermore, a study of 638 patients admitted for acute myocardial infarction randomized to either O₂ or no supplemental O₂ therapy actually showed evidence of increased tissue injury by blood biomarker and increased size of heart damage in association with O₂ treatment [67]. These negative findings associated with oxygen treatment should give pause to current advocates of intracoronary supersaturated O₂ therapy for acute myocardial infarction given the well-known phenomenon of ischemia-reperfusion injury [68].

The role of the mitochondria in O₂ homeostasis provides lessons not only for common diseases such as cancer and myocardial infarctions but also for rare diseases such as retinitis pigmentosa, a heterogeneous set of genetic conditions resulting in vision loss through photoreceptor degeneration. Although its pathogenesis had been suggested to be due to retinal hypoxia [69], the intraretinal O₂ concentration was actually found to increase in the degenerating photoreceptor layer of a rat model [70]. This observation indicated that retinal hypoxia was unlikely to be driving pathogenesis. In a pilot study of retinitis pigmentosa patients, systemic treatment with the antioxidant N-acetylcysteine resulted in improved visual acuity over a 24 weeks period, suggesting a role for oxidative stress in driving pathogenesis as might be expected in the setting of increased retinal O₂ levels [71]. Although this promising observation needs confirmation through a randomized, placebo-controlled trial, it emphasizes the need for basic mechanistic understanding of disease processes prior to executing therapeutic clinical studies.

Conclusion

There is broad acceptance that mitochondria are the major source of cellular ROS, but the evidence provided in this review suggests that mitochondrial deficiency states may result in even greater O₂ toxicities.

While there is evidence that ROS generated at low levels have physiological functions [72,73], the mitochondria being the main source of damaging ROS is more debatable [41,74–76]. A critical factor in this controversy is the availability of O₂ as a substrate for ROS generation at the level of the mitochondrial enzymes *in vivo*, which the aforementioned study of Penjweini *et al.* finds is lower than that in the cytosol—adding support to prior proposals of the mitochondrion as an oxygen sink [35,38]. Thus, mitochondrial respiration, linked to more complex eukaryotic organisms for bioenergetic reasons, also plays an inextricable role in O₂ homeostasis for protection against its toxicity, in concert with the ancient role hypothesized in the symbiotic theory of the mitochondrion. An optimal level of PO₂ may be dependent on not only the respiratory demands of a given tissue, but also the local vulnerability to oxygen.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

MPM, RP, JRK, PW, and PMH wrote and/or edited the review.

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