https://doi.org/10.1038/s44161-022-00178-7

The Goldilocks Oxygen Principle: not too little and not too much

Will R. Flanigan & Isha H. Jain

Nature has evolved creative ways to maintain oxygen homeostasis, but what happens when these adaptations are insufficient? Here we discuss biochemical failure points across the oxygen spectrum from 'too little' to 'too much' oxygen and their potential contributions to cardiovascular disease.

Joseph Priestly (the scientist) and Robert Southey (the children's author) walk into a bar. The bartender asks, "Good sirs, how much oxygen would you like today?" They reply in unison, "not too little and not too much." And so was born the Goldilocks Oxygen Principle. Priestly discovered oxygen in the late 1700s and quickly realized that it was both vital and toxic for life. He claimed, "I have procured air [oxygen]… between five and six times as good as the best common air that I have ever met," touting the benefits of oxygen for life. He also warned of the toxicities of excess oxygen, saying: "As a candle burns out much faster in dephlogisticated [oxygenated] air, so we might, as may be said, live out too fast…in this pure kind of air." Priestly's observations have largely been substantiated over the last 250 years, confirming that deviations from 'just the right' amount of oxygen can be catastrophic for human health. Here, we set out to contextualize this framework through recent advances in oxygen biology research, together with a broader discussion of its relevance to cardiovascular disease.

The Goldilocks Oxygen Principle holds true in nearly every system tested — in vitro and in vivo. Immortalized cells proliferate more slowly when cultured in extreme hypoxia or hyperoxia. Interestingly, the optimal local oxygen tension, hereafter termed O_2^{opti}, varies across systems. For example, cancer cell lines that have evolved in normoxic laboratory environments will have different O_2^{opti} values than stem cells or primary cells that are directly removed from endogenous microenvironments in the body (where O_2 ranges from ~13% in lung alveoli to <1% in parts of the brain and bone marrow[1]). It will be interesting to investigate how evolution has determined O_2^{opti} for different tissues and cell types, and the underlying genetic and epigenetic modifications that establish this value. Notably, the same concepts hold true at the tissue and whole-body level. Extreme hypoxia or anoxia is toxic to all metazoans studied. As discussed below, the exact cause of damage is still incompletely understood. Nonetheless, some organisms are more anoxia tolerant than others — for example, naked mole rats can survive nearly 30 minutes of anoxia whereas closely related mice can only survive for 1-2 minutes[2]. At the other extreme, 100% O_2 is toxic within days to nearly every organism tested, primarily due to pulmonary or central nervous system toxicity[3]. Of note, survival in both hyperoxia and hypoxia can be extended by prior preconditioning in equivalent oxygen tensions. More extensive comparative physiology will be required to mechanistically understand different O_2^{opti} values at the whole-organism level.

The Goldilocks Oxygen Principle is particularly relevant to the pathologies and care of cardiovascular diseases. During a myocardial infarction (MI) or stroke, the tissue is initially faced with ischemic stress — oxygen and nutrient deprivation (Fig. 1a). Of note, ischemic and hypoxic stress likely result in overlapping and distinct molecular defects that remain to be disentangled at the biochemical level. In these situations, the tissue is exposed to oxygen tensions below O_2^{opti}. However, the same organ is then acutely reperfused as the primary form of clinical management, resulting in an abrupt burst of oxygen and nutrients. If the organ has already adapted to consume less oxygen during the ischemic phase, reperfusion will result in relative hyperoxia (that is, relative to what the organ has adapted to). The resulting ischemia–reperfusion injury is estimated to account for up to 50% of tissue damage following MI[4]. Thus, in the same disease state, both hypoxia and hyperoxia contribute to negative patient outcomes. As another example, states of respiratory dysfunction (chronic obstructive pulmonary disease, acute respiratory distress syndrome, COVID-19, etc.) result in whole-body hypoxia due to impaired pulmonary gas exchange. Chronic hypoxia as a result of lung disease or exposure to high altitude is designated by the World Health Organization as a Group 3 cause of pulmonary hypertension, which, if left untreated, can lead to right heart failure and death. In these settings, supplemental oxygen is the mainstay of treatment. Yet, although supplemental oxygen has shown clinical benefit in patients with pulmonary hypertension and hypoxemia (REVEAL trial[5]), recent prospective studies indicate that excess supplemental oxygen worsens outcomes following MI (AVOID trial[6]). Thus, several cardiovascular disease states and treatments straddle the line between oxygen deprivation and excess. By better considering this balance of oxygen supply and demand, perhaps we can better titrate our therapies to achieve O_2^{opti} for each individual.

Oxygen-sensitive reactions fail at different oxygen tensions

To understand the toxicities associated with variations in oxygen levels, it is useful to consider the wide range of oxygen-dependent reactions (Fig. 1b). Oxygen is involved in 200+ enzymatic reactions, making it the most widely used enzyme substrate. Perhaps the best-studied oxygen-dependent reaction is the electron transport chain, which uses oxygen as the terminal electron acceptor to maintain the mitochondrial membrane potential and power aerobic ATP production. This reaction is especially relevant to cardiovascular disease because the heart is a highly oxidative organ, deriving 60–90% of its energy from fatty acid oxidation[7]. Essential cellular processes such as fatty acid desaturation, heme[8] and ceramide[9] synthesis, and disulfide bond formation[10] all also require oxygen and have been shown to be sensitive to physiological hypoxia. Additionally, there are over 60 α-ketoglutarate

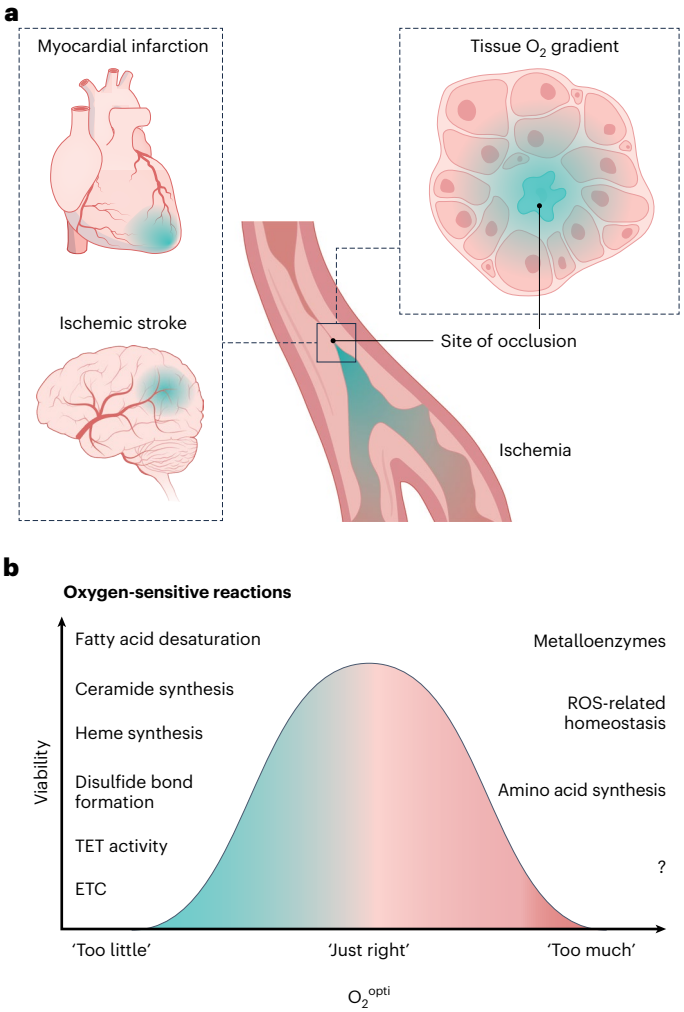

Fig. 1 | Oxygen-sensitive reactions fail as a function of local tissue PO_2. **a**, Myocardial infarction and ischemic stroke result in a gradient of oxygen and nutrient deprivation across the tissue. **b**, Key cellular reactions fail at different oxygen tensions across the tissue, contributing to organ injury. Reactions on the left fail in hypoxia, whereas reactions on the right fail in hyperoxia. Many O_2-sensitive reactions remain to be discovered. ETC, electron transport chain; ROS, reactive oxygen species; TET, demethylation enzyme ('ten-eleven translocation').

(αKG)-dependent dioxygenase enzymes that use oxygen, αKG and iron as substrates. These enzymes have diverse functions, including collagen maturation, histone/nucleotide demethylation (achieved by TET enzymes[11]), ribosome hydroxylation, tRNA modifications and carnitine synthesis.

To better treat states of ischemia and hypoxia, we must first understand: what goes wrong when there is too little oxygen? Much of the research in the field has focused on the ATP crisis and subsequent ion-channel disturbances that occur during ischemia. Though important, this is likely to be only part of the story. In fact, the K_m for oxygen for cytochrome oxidase (of the electron transport chain) is extremely low, estimated at 0.1% O_2 (ref. [12]). Thus, oxygen is only limiting for these functions in near anoxia. However, ischemic pathologies are characterized by a broad range of oxygen tensions. At the primary site of a blood vessel occlusion, there may be complete anoxia. However, radiating outward from the anoxic core is a gradient of oxygen and nutrients. This region is termed the 'stroke penumbra' or 'border zone' in the context of a stroke or MI, respectively. A similar gradient exists in solid tumors that lack oxygen at the necrotic core but have better-oxygenated regions at the periphery. It is likely that different oxygen-dependent reactions fail as a function of distance from the anoxic core. If we can better understand the precise oxygen sensitivity (that is, the K_m for oxygen) of these pathways, we can better design therapies to bypass the most sensitive reactions, thereby salvaging most of the infarcted organ.

To understand states of oxygen toxicity, we must first understand: what goes wrong when there is excess oxygen? In this realm, the field has primarily focused on the damaging effects of reactive oxygen species. It is well known that superoxide production increases with local oxygen tensions. However, it is still unclear which reactions fail as a function of increasing oxygen levels. Moreover, it is likely that different reactive oxygen species, as well as molecular oxygen itself, have different targets. Interestingly, many oxygen-dependent enzymes require metal cofactors such as iron and copper that themselves are susceptible to oxidation. Thus, perhaps these enzymes and downstream metabolic pathways also have an O_2^{opti}: in states of oxygen deprivation, these reactions are halted due to substrate deprivation; in states of oxygen excess, the same reactions may be halted due to oxidation of necessary cofactors (heme, iron, etc.). Additionally, the synthesis of several amino acids is inhibited in hyperoxia[13]. It will be important to dissect exactly which metabolic pathways fail as a function of excess oxygen to create adjuvant therapies for oxygen supplementation and ischemia–reperfusion injury. Moreover, we recently showed that states of mitochondrial dysfunction cause a mismatch between oxygen supply and demand, resulting in pathological tissue hyperoxia[14]. Thus, such lines of investigation will be relevant for a wide range of clinical conditions.

Although the oxygen biology field has predominantly focused on hypoxia and the primary hypoxia-sensing pathway (the HIF pathway) in recent times, much remains to be understood through the full spectrum of oxygen doses (anoxia to hyperoxia) and durations (acute versus chronic). By approaching cardiovascular disease states with the Goldilocks Oxygen Principle in mind, we can gradually uncover the toxicities of too little and too much oxygen.

Will R. Flanigan [1,2] **& Isha H. Jain** [1,3,4] ✉
[1]Gladstone Institutes, San Francisco, CA, USA. [2]UCSF-UC Berkeley Joint PhD Program in Bioengineering, Berkeley, CA, USA. [3]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, USA. [4] Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, USA.
✉e-mail: Isha.Jain@gladstone.ucsf.edu

Published online: 12 December 2022

References

1. Ast, T. & Mootha, V. K. *Nat. Metab.* **1**, 858–860 (2019).
2. Park, T. J. et al. *Science* **356**, 307–311 (2017).
3. Clark, J. M. & Lambertsen, C. J. *Pharmacol. Rev.* **23**, 37–133 (1971).
4. Frank, A. et al. *Semin. Cardiothorac. Vasc. Anesth.* **16**, 123–132 (2012).
5. Farber, H. W. et al. *J. Heart Lung Transplant* **37**, 948–955 (2018).
6. Stub, D. et al. & AVOID Investigators. *Circulation* **131**, 2143–2150 (2015).
7. Stanley, W. C., Recchia, F. A. & Lopaschuk, G. D. *Physiol. Rev.* **85**, 1093–1129 (2005).
8. Otsuka, S., Matsumoto, K., Nakajima, M., Tanaka, T. & Ogura, S. *PLoS ONE* **10**, e0146026 (2015).

9. Devlin, C. M. et al. *J. Biol. Chem.* **286**, 38069–38078 (2011).
10. Koritzinsky, M. et al. *J. Cell Biol.* **203**, 615–627 (2013).
11. Thienpont, B. et al. *Nature* **537**, 63–68 (2016).
12. Bienfait, H. F., Jacobs, J. M. C. & Slater, E. C. *Biochim. Biophys. Acta* **376**, 446–457 (1975).
13. Boehme, D. E., Vincent, K. & Brown, O. R. *Nature* **262**, 418–420 (1976).
14. Jain, I. H. et al. *Science* **352**, 54–61 (2016).

Acknowledgements
I.H.J. was supported by US National Institutes of Health (NIH) DP5 DP5OD026398. W.R.F. was supported by NIH R01AG065428.

Competing interests
I.H.J. is a consultant for Maze Therapeutics. W.R.F. declares no competing interests.