



SERIES “HYPOXIA: ERS LUNG SCIENCE CONFERENCE”

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The biology of oxygen

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ABSTRACT: The biology of O₂ is extremely complex and defies a comprehensive yet brief summary that showcases its roles across the entire animal and plant kingdom. This necessarily short introduction to the 2006 Taormina Lung Science Conference (Taormina, Italy) on hypoxia will examine key features of the biology of O₂ only within mammalian cells, even that being a daunting task.

KEYWORDS: Hypoxia, hypoxic gene expression, O₂ toxicity, O₂ transport, tissue perfusion

Traditionally, physiologists consider how O₂ travels from the environment to the mitochondria, *i.e.* the transport processes and the limiting factors. Metabolists consider the role of O₂ in energy generation *via* oxidative phosphorylation, *i.e.* both as substrate and regulator of cell processes, including its own utilisation. Biochemists consider, among other things, O₂ as a source of reactive O₂ species (ROS) and how both O₂ (or lack thereof) and ROS may affect transcription of many genes. Pathologists and clinicians consider effects of both insufficient and excess O₂ on tissue structure and function. Oxygen is at the centre of a remarkable, integrated system that scientists are just beginning to piece together, and the goal of the present introduction to the series is to describe how this multifaceted integration may occur.

O₂ TRANSPORT

The lungs

For many years, respirologists' interests in O₂ focused mostly on understanding how O₂ does (or does not) get from the air to pulmonary capillary blood. Stimulated originally by wartime needs ~60 yrs ago, several generations of researchers [1–6] have developed the current physiological understanding of O₂ transport to quite sophisticated levels with the coming together of new ideas and new techniques. A great deal is currently known about ventilation, blood flow, the distribution of one to the other, and gas exchange [6, 7]. Scientists have been able to define when diffusion limitation of gas exchange occurs in the lungs, in both health and disease [8, 9], and have recognised that pulmonary gas exchange affects gas exchange in

the tissues and *vice versa*, that is, the system is clearly integrated throughout the body.

The tissues

Compared with exchange at the lungs, less attention has been given to understanding how O₂ delivered *via* the arterial blood supply of a tissue or organ does (or does not) get to the mitochondria in that tissue. Interestingly, essentially the same physiological barriers as in the lung potentially impede O₂ transport within tissues: heterogeneity of physiological O₂ supply *versus* biochemical demand for O₂; diffusional transport limitation between tissue vascular red blood cells and mitochondria; reduced delivery *via* low blood flow, haemoglobin or O₂ saturation; and shunting of blood around tissue elements that need it. These have proven much more elusive to characterise and thus understand than their pulmonary counterparts but, slowly, “in-roads” are being made into these processes as well. There is still a long way to go, especially in severe diseases marked by multiorgan failure. One system that has been evaluated is skeletal muscle. It is a useful model to demonstrate the integration that revolves around cellular oxygen tension (P_{O₂}).

Based on magnetic resonance spectrometry measurements of cytoplasmic myoglobin saturation [10], myoglobin is almost fully saturated at rest, implying that cytoplasmic P_{O₂} must be fairly close to that of venous blood. However, intracellular P_{O₂} falls during exercise to remarkably low but stable levels of ~0.4–0.5 kPa (3–4 mmHg), indicated by a myoglobin O₂ saturation of ~50%. This low P_{O₂}, which represents the balance between O₂ supply and O₂ demand, has many remarkable implications and consequences and raises several questions as follows.

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STATEMENT OF INTEREST

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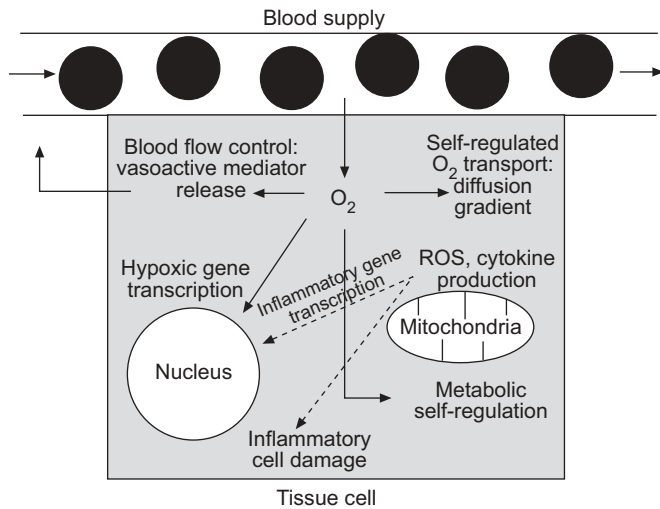


FIGURE 1. Multiple functions of O_2 within tissue cells. While this scheme is very unlikely to describe all ways in which O_2 influences cell structure and function, it serves to illustrate the multiple levels at which oxygen tension plays a role in cellular events such as transport, metabolism, inflammation and gene transcription. ROS: reactive oxygen species.

1. Should one look at such a low PO_2 as hypoxia (a failure of supply to keep up with demand) or is this a regulated value (*i.e.* normoxia) that serves major biological purposes? Keeping in mind that mitochondria can operate at full speed at such low PO_2 levels [11], it is easy to argue that a PO_2 of 0.4–0.5 kPa (3–4 mmHg) is all that is needed to run the system. Indeed, more would be wasteful and might possibly predispose to excess generation of ROS and therefore, perhaps, to cellular O_2 toxicity. In other words, the system may be regulated to a cell PO_2 of 0.4–0.5 kPa (3–4 mmHg), which is advantageous.

2. Additionally, this low PO_2 means that the PO_2 gradient from red cells to mitochondria is maximised, favouring diffusive transport of O_2 , the only way for O_2 to reach mitochondria. However, a system that needs the gradient maximised suggests that O_2 transport from red cells to mitochondria is diffusion-limited, and there is much evidence to support this notion at maximal oxygen uptake [12–14]. In turn, this implies that maximal O_2 utilisation is limited by its own transport rather than by the capacity of the cells to use O_2 , for which there is also evidence [14–16].

3. The low PO_2 is also important in local blood flow regulation, since local hypoxia dilates local tissue microvessels [17–19], resulting in the augmentation of flow when and where metabolic rate increases to help match O_2 supply to O_2 need. Therefore, it is easy to hypothesise that this low PO_2 is regulated to optimise O_2 transport.

4. As mentioned hereafter in more detail, low PO_2 values lead to gene activation for systems that are upregulated in response to reduced PO_2 in a manner designed to augment O_2 availability. This happens through the hypoxia-inducible factor (HIF) system [20] but may also occur through other pathways. For example, a low PO_2 enhances muscle expression of vascular endothelial growth factor (VEGF) [21], likely to occur (in part at least) *via* HIF-1 α [22]. Over time, this may lead to

angiogenesis, which permits greater blood flow to support a greater metabolic rate. It is easy to speculate for muscle that, without a low intracellular PO_2 during exercise, adaptive gene expression (*i.e.* exercise training) might not occur.

In summary, PO_2 inside the myocyte falls dramatically during exercise due to multiple, quite different but complementary reasons: 1) to maximise diffusive O_2 transport; 2) to regulate ROS generation; 3) to regulate local perfusion to serve local metabolic need; 4) to regulate the speed of metabolic reactions to match O_2 availability; and 5) to stimulate adaptive gene expression to enhance future exercise.

O_2 AND THE TISSUES: NOT GOOD TO HAVE TOO MUCH OR TOO LITTLE

In recent years, the study of O_2 has taken some dramatic new turns. Rather than simply worrying about how O_2 transport occurs, researchers have turned their attention to how O_2 (and especially its reduction or excess stemming from altered transport) may affect cell, tissue and organ function; O_2 is far more than a substrate for energy production (fig. 1).

Researchers have known about excess O_2 causing pulmonary O_2 toxicity for many years. From initial physiological descriptions it is clear that excess O_2 leads to high levels of ROS, inflammation and tissue damage. It has also been well known for many years that reduced O_2 causes serious disturbances, such as tissue death in heart, brain, extremities, gut, *etc.* Hypoxic pulmonary vasoconstriction affects pulmonary circulation, ultimately contributing to potentially fatal illnesses, such as high-altitude pulmonary oedema [23]. Researchers have recognised that acute hypoxia in peripheral tissues causes vasodilatation in a compensatory effort to restore O_2 transport and that persistently reduced O_2 (*e.g.* at altitude) during development and early post-natal life stimulates a number of characteristic changes, including increased lung volumes and blood O_2 transport ability [24–26]. Additionally, post-natally reduced O_2 stimulates typical responses, the most evident of which are hyperventilation *via* chemoreceptor stimulation and erythrocytosis *via* erythropoietin, both apparently targeted to restore O_2 availability.

Despite these restorative responses, the hypoxia of altitude produces progressively more extreme and deleterious effects on the body. While some individuals can make the 8,848 m ascent of Mount Everest without breathing supplemental O_2 [27], in spite of the fact that inspired PO_2 on the summit is only 5.3–6.0 kPa (40–45 mmHg) [28] and arterial PO_2 is only 3.3–4.0 kPa (25–30 mmHg) [29], man cannot live and reproduce at altitudes $\sim >5,000$ m for more than short periods of time [30]. Basically, every organ is affected by such degrees of hypoxia, with loss of lean body mass, and cerebral, gastrointestinal and reproductive disturbances that progress to intolerance for that altitude.

Even at sea level and in the absence of parenchymal disease *per se*, sleep-disordered breathing produces levels of hypoxia that lead to many responses considered to be disadvantageous to the organism. Cycles of hypoxia and re-oxygenation are reported to cause systemic inflammation, likely *via* ROS [31], clinically important dysregulation of the autonomic nervous system resulting in systemic hypertension [32, 33] and, it seems, predisposition to the metabolic syndrome [34].

O₂-RELATED PATHOPHYSIOLOGY: MECHANISMS

As these phenotypic responses have been progressively catalogued over the years, research has begun to focus on how changes in O₂ lead to changes in function, that is, the mechanisms. It is known that in many tissues, both high and low levels of O₂ promote greater generation of ROS that may themselves lead to inflammation, signalling and gene activation. It is known that altered levels of O₂ in many tissues affect gene expression directly. The overarching role of HIF in regulation of many genes associated with restorative responses to hypoxia has emerged as one of the major stories of recent years [20]. The stabilisation of HIF by hypoxia [35], which provides enough of this factor to cause transcriptional activation of a whole series of genes whose proteins act to counterbalance the reduction in available O₂, is now quite well understood. Prominent among HIF-regulated genes are those encoding glycolytic pathway enzymes, glucose transporters, erythropoietin and VEGF to name just four categories. Undoubtedly, the mechanisms will prove to be complex, far more so than this trivial description.

O₂: HOW IT IS SENSED

How levels of O₂ may be sensed to initiate the many consequences aforementioned remains a "hot topic" with evidence for various sensors [4, 36] at the mitochondrial level, within the HIF system, during the generation of ROS and possibly others. Interestingly, as the many O₂-dependent pathways are better understood, it seems that the associated responses may not be fully explained by hypoxia alone. For example, it may be that O₂-independent signalling pathways in muscle might be involved in activation of VEGF in concert with hypoxia.

O₂ AND THE GENOME: CAUSE AND EFFECT

Understanding the details of these many, varied and complex signalling pathways remains a major challenge. But even when these are elucidated, there will still exist the need to determine their importance. Some have been established beyond doubt, but appropriate gene manipulation strategies will need developing to be certain about the importance of others. As an example, it is possible to discuss skeletal muscle. It has been found that VEGF is indeed the key angiogenic growth factor in muscle. *In vivo* deletion of this gene in the adult mouse by conditional knockout using Cre-LoxP techniques has demonstrated that two thirds of the muscle capillaries disappear in regions where VEGF is substantially absent [37]. Over ≥ 8 weeks there is no recovery of capillarity and considerable apoptosis occurs. Similar results are seen with lifelong whole muscle VEGF deletion using a cross-breeding strategy to delete VEGF throughout from embryonic stages, but only in muscle [38]. Remarkably, these findings suggest that no adequate parallel or compensatory pathway exists for this gene and tissue. Deletion of VEGF in the lung by similar methods also produces major effects [39], especially apoptosis and an emphysema-like phenotype, but whether this plays into clinical emphysema in humans remains to be determined.

SUMMARY

The objective of the present far-ranging introduction to the 2006 Taormina Lung Science Conference was to briefly cover some of the remarkable developments in the study of the

biology of O₂ over the past century: from physiological interests in its transport to how it acts as a signalling molecule; how it affects tissue structure and function when in high or low concentrations; and how such changes in availability affect genomic responses presumably designed to restore function under such circumstances. These many faces of O₂ coexist of course as a remarkable, integrated system of transport, utilisation, signalling and pathogenesis.

This series will continue the discussion of the many faces of O₂ and will lead the participants down new roads in the unending quest to understand its very complex biology.

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