Oxygen regulation in biological systems

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OXYGEN IS A FASCINATING MOLECULE. Much of the animal kingdom depends on it for survival, yet it is also a poison. Humans and other animals must inhabit environments with varied availability of oxygen, and either through necessity or choice, can be acutely exposed to hypoxia or hyperoxia. Furthermore, both cellular hypoxia and oxidative stress have been identified as major factors in the development of multiple pathological states. These considerations provided the impetus for a call for papers in this journal, entitled “Oxygen as a Regulator of Biological Systems.” Herein, I discuss some of the recent advances in this field identified in the pages of American Journal of Physiology-Regulatory, Comparative and Integrative Physiology. Rather than provide detailed descriptions of the findings described in these articles, I attempt to highlight the critical new concepts that have arisen in the hope that the interested reader will go to the original sources.

At the outset, we should define what we mean by “normoxia,” “hypoxia,” and “hyperoxia” A normoxic environment could be defined as that encountered by air-breathing terrestrial animals at sea level, or by aquatic animals near the surface of the water at sea level. At greater altitude, or greater depth in a body of water, oxygen tension progressively falls. The partial pressure of oxygen in both terrestrial and aquatic environments can also be reduced by the presence of oxygen-consuming microorganisms (18). Thus hypoxia can be defined in relative terms, as anything less than normoxic. However, more often, we use this term to refer to environments in which the relative lack of availability of oxygen has pathological consequences (e.g., high altitude). Similarly, tissue and cellular hypoxia could encompass any situation in which the balance between oxygen delivery and oxygen consumption changes in such a way that the partial pressure of oxygen falls. But when we use these terms we usually mean situations where lack of oxygen availability drives pathological processes. This definition is complicated by the fact that there is considerable variability in tolerance to hypoxia, both between various species of animal, and between various organs and tissues within the same species. For example, in mammals the renal medulla is normally exposed to a much lower tissue PO2 than the renal cortex (9).

Furthermore, various organs have differing tolerance (41) and cellular responses (39) to reduced oxygen delivery. Thus herein we use the word hypoxia in both the sense of a relative lack of oxygen and in the sense of a pathological deficit in oxygen. Hypoxia can similarly be defined as increased environmental or tissue PO2 in the sense of a critical level of increased oxygen availability that drives pathological or therapeutic processes. It is, of course, not something encountered in the natural world, although there have been times in the Earth’s history when atmospheric levels of oxygen were much greater than they are now (18). Furthermore, humans are exposed to hyperoxia both for therapeutic (e.g., hyperbaric oxygen therapy) and recreational [e.g., scuba diving (43)] purposes.

Chemoreceptor Reflexes

Hypoxia activates peripheral chemoreceptors leading to increased ventilatory drive. Many of the neural pathways within the brainstem that mediate this reflex have been characterized. However, pontine and hypothalamic pathways have not been well characterized. King and colleagues (15) recently showed that selective ablation of catecholaminergic neurons that project to the paraventricular nucleus blunts the ventilatory response to hypoxia in conscious rats. Thus catecholaminergic inputs to the paraventricular nucleus, mostly from the brainstem, appear to play a major mediating role in the ventilatory responses to acute hypoxia. Furthermore, Damasceno and colleagues (5) provided evidence of an important role of the Kölliker-Fuse region of the parabrachial nucleus (PBN), but not the lateral PBN, of the dorsolateral pons in control of respiratory drive under resting conditions and also during hypoxia and hypercapnia. In contrast, the lateral PBN appears to be important in mediating cardiovascular but not ventilatory responses to chemoreceptor activation.

Carotid chemoreceptors appear to contribute to circulatory and ventilatory control during exercise, even when exercise is not accompanied by hypoxia or hypercapnia. Edgell and Stickland (7) recently provided insight into the mechanisms underlying this phenomenon. They found that systemic hyperoxemia reduced muscle sympathetic nerve activity in humans in which blood flow to the arm was occluded immediately following hand-grip exercise. Thus their findings indicate that activation of the muscle metaboreflex can activate the carotid chemoreflex, presumably through altering central integration of carotid chemoreceptor feedback.

Hypoxia and Developmental Programming of the Heart

During development, chronic hypoxia can have major effects on multiple body systems, including the heart. Studies of developmental programming in mammals are complicated by the difficulties in teasing out direct effects of hypoxia on the fetus from effects mediated secondarily through maternal effects of hypoxia. Such difficulties can be avoided in studies of egg-laying animals such as chickens. Two recent reports described studies of the effects of chronic hypoxic incubation of chicken eggs on cardiac structure and function of embryos on day 19 of the 21-day incubation period. In both studies, absolute heart mass was relatively insensitive to the effects of hypoxic incubation, with a small deficit found in one study...
[15% oxygen (26)] but no significant difference in the other [14% oxygen (12)]. Nevertheless, the balance of evidence from these studies indicates that hypoxia reduces the proliferation of myocytes in chick embryos, but not cardiac myocyte size (26). Importantly, chronic hypoxia led to impaired cardiac systolic and diastolic function in the chick embryo prehatching (12). Furthermore, even relatively brief exposure to hypoxia during cardiogenesis in the chicken embryo can render the embryonic heart susceptible to later hypoxia, perhaps in part due to alterations in adenosinergic signaling pathways (34). These findings demonstrate the utility of the chicken for investigation of developmental programming of cardiac structure and function, particularly in terms of teasing out the direct effects of embryonic hypoxia. Importantly, the findings of these studies in chickens align well with recent observations in mammalian species. For example, in the fetal sheep, chronic hypoxemia induced by placental restriction was associated with the presence of fewer cardiomyocytes (2) and diminished cardiac delivery of oxygen and glucose, even though cardiac blood flow was not significantly affected (30). The pathological effects of hypoxia during fetal development have recently been highlighted by the observation that, in adult male rats that were exposed to hypoxia in utero, aerobic exercise training results in deterioration of cardiac function rather than the improvement seen in control animals (31).

**Hypoxia Signaling Pathways**

Hypoxia-inducible factors (HIFs) are major regulators of hypoxic signaling. The manner in which HIFs are activated is organ dependent. For example, in the brain HIFα-activation appears to be dependent on neuronal nitric oxide (NO) synthase, yet it appears to be relatively independent of neuronal NO synthase in the kidney (8, 39). Such tissue specificity of hypoxia signaling is likely critical, from an evolutionary perspective, in the tailoring of organ responses to hypoxia for optimization of function.

But do HIFs exert beneficial or detrimental effects in disease states associated with hypoxia? The growing consensus is that HIFs are adaptive in conditions associated with transient hypoxia in organs such as the heart and kidney, but induce more detrimental effects when hypoxia is prolonged. But dissection of the seemingly complex interactions of duration and severity of hypoxia with hypoxia signaling mechanisms has proved difficult in mammalian species. Zarndt and colleagues (29, 44) recently reported an approach in the fruit fly *Drosophila* that could cut through some of this complexity. Unlike mammals, *Drosophila* only have a single HIFα homolog (*simia*). This, and the fact that these animals are tractable to genetic analysis, makes the fruit fly a very attractive model for investigation of the physiology and pathophysiology of hypoxic signaling. As appears to be the case for HIFα in mammals, its *Drosophila* equivalent *simia* seems to mediate adaptive responses in the heart tube of *Drosophila* during short-term hypoxia but more maladaptive responses to chronic hypoxia. Thus *Drosophila* may be an excellent model organism for studying hypoxic signaling in the heart.

We usually think of tissue hypoxia as a driver of pathology, but recent findings indicate that it also has important roles in normal physiological functions. For example, tissue hypoxia is a critical component of the process of wound healing. Inflammatory processes, especially infiltration of immune cells into the wound area, augment local oxygen consumption, which in turn renders the tissue relatively hypoxic (21). Local hypoxia, in turn, appears to drive many of the events in the proliferative phase of wound healing. The inflammation and fibrosis observed in chronic diseases of the bowel, lungs, liver, and kidneys appear to represent a situation in which these processes do not resolve (21). Hypoxia signaling may also play an important role in the development of the mammary gland during pregnancy and early lactation (37). Local hypoxia in mammary epithelial cells appears to upregulate glucose transporter 1 (GLUT1), contributing to the cellular delivery of glucose to enable production of milk (37). Another example of the physiological function of hypoxia is its role in the regulation of local blood flow. Multiple mechanisms drive local vasodilatation in tissues under hypoxic conditions. One such mechanism is the release of ATP from erythrocytes (33). This mechanism is impaired in diabetes, which likely contributes to the development of peripheral vascular disease (33). Importantly, treatment with insulin alone appears to be insufficient to restore this mechanism. Richards and colleagues recently showed that release of ATP from hypoxic erythrocytes could be rescued by coinubcation with physiological levels of insulin and peptide C. Peptide C, a sequence within proinsulin, is released from the pancreas with equimolar amounts of insulin (32, 33). These findings may provide a new approach to prevention of peripheral vascular disease in patients with insulin-dependent diabetes.

Hypoxia might even have therapeutic value. Intermittent hypoxia has been linked to multiple pathological conditions, especially in individuals who suffer from sleep apnea. However, there is also evidence that intermittent hypoxia can have beneficial effects, including in patients with sleep apnea, chronic obstructive pulmonary disease (COPD), hypertension, and even myocardial infarction. Navarrete-Opazo and Mitchell (24) reviewed the extensive literature in this field, concluding that beneficial effects are more often seen when the “dose,” defined by the severity, frequency, and duration of the stimulus, is relatively low. For example, a 14-day protocol in healthy humans, comprising 5–10 daily cycles of 5–6 min of inhalation of 10% oxygen interspersed with 4 min inhalation of room air, shifted the oxyhemoglobin equilibrium response to the right (45). This phenomenon would be expected to enhance oxygen delivery to tissue.

**Tissue Oxygenation and Organ Dysfunction in Pathological Conditions**

Oxygen delivery to tissue is compromised in a range of chronic medical disorders, including COPD, intermittent claudication, and chronic kidney disease. A better understanding of the mechanisms underlying impaired tissue oxygen delivery in these conditions should lead to the development of new therapies. For example, it is well established that, in COPD, oxygen delivery to tissue is limited by impaired cardiorespiratory capacity. Medeiros and colleagues (22) provide evidence that peripheral factors might also limit tissue oxygenation and skeletal muscle function in individuals with COPD. The precise nature of these factors remains to be determined. One candidate is tumor necrosis factor-α, which is upregulated in the lung in COPD. It can induce oxidative stress in exercising...
muscle, which might contribute to the development of muscle weakness in COPD (47). Potentially, this could be mediated by multiple downstream actions of reactive oxygen species, including microvascular dysfunction and reduced efficiency of oxygen utilization in skeletal muscle.

Intermittent claudication is a medical condition associated with atherosclerotic peripheral artery disease, characterized by leg pain during ambulatory exercise as a result of relative ischemia. Roseguini and colleagues (35) investigated the effects of sildenafil (a.k.a. Viagra, an inhibitor of cGMP breakdown by phosphodiesterase) on leg muscle oxygenation and exercise tolerance in patients with intermittent claudication. Intriguingly, they found that sildenafil improved muscle oxygenation during exercise in these patients, but did not improve clinical measures of the severity of intermittent claudication (pain-free walking time and maximal walking time). Thus the jury remains out as to whether this treatment could be a useful addition to the therapeutic armory in intermittent claudication.

Polycystic kidney disease (PKD) is the most common genetic form of chronic kidney disease. Renal tissue hypoxia has been observed in multiple forms of chronic kidney disease, providing part of the evidence for the proposition that hypoxia represents a major driver of the progression of kidney disease. Ow and colleagues (27) observed marked renal tissue hypoxia in a rat model of PKD. Deficiency in renal oxygen delivery was identified as a major cause of this hypoxia. However, their finding that renal oxygen consumption in rats with PKD was similar to that of control rats, despite markedly less sodium reabsorption in the polycystic kidney, indicates that inappropriately high renal oxygen consumption also makes a contribution. Thus, although ischemia is a common pathway to tissue hypoxia, oxygen consumption is also an important determinant of tissue oxygenation that merits consideration in studies of the mechanism underlying organ and tissue hypoxia.

Oxygen delivery to multiple tissues, including skeletal muscle, is impaired in diabetes, which in turn renders diabetic individuals exercise intolerant (14). This effect is likely multifactorial, including deficits in cardiac, vascular, and muscle function. Yamakoshi and colleagues (42) recently investigated the interplay between these mechanisms by investigating the effects of progressive hyperbaric hyperoxia on the dynamics of microvascular oxygenation and cardiovascular function. Somewhat counterintuitively, they found that muscle microvascular oxygenation increased faster in diabetic rats than controls at the onset of hyperoxia and was maintained at a higher level for longer when hyperoxia was withdrawn. They proposed that the apparent enhanced effect of hyperoxia in the diabetic animals could be explained, at least partly, by a more sluggish cardiovascular response to increase oxygen availability. Regardless of the underlying mechanisms, their findings are potentially important as they indicate that the effectiveness of hyperbaric oxygen therapy should not be diminished by the presence of diabetes.

Adaptations in Oxygen Utilization Induced by Endurance Training

Endurance training increases the ability of skeletal muscle to utilize oxygen and also the ability of the cardiovascular and pulmonary systems to extract oxygen from the environment and deliver it to exercising tissues (46). Indeed, not only is maximal oxygen utilization increased at steady state, but the time constants for changes in oxygen utilization are reduced (46). The mechanisms underlying such changes in dynamic regulation of oxygen utilization have been difficult to study in intact humans. Zoladz and colleagues (46) recently took a novel approach to this problem by combining observations of pulmonary kinetics and muscle biochemistry with simulations from a mathematical model of cellular bioenergetics in skeletal muscle. They found that a 4-wk program of endurance training of a moderate workload reduced the time constant for increased pulmonary oxygen uptake at the onset of exercise. The dominant mechanism underlying this effect appears to be an increase in the intensity of so-called “each-step activation” of oxidative phosphorylation, whereby increased ATP use by skeletal muscle during exercise simultaneously activates multiple complexes in the process of oxidative phosphorylation. Interestingly, it appears that the adaptations associated with endurance training are at least partly mediated by inhibition of HIF signaling and consequent downregulation of pyruvate dehydrogenase kinase-1 (PDK-1). This sequence of events appears to be initiated by upregulation of negative regulators of HIF such as prolyl hydroxylases, factor inhibiting HIF, and the histone deacetylase sirtuin-6 (19).

Life at the Extremes: Adaptations in Diving Mammals and to Hypoxic Environments

Diving. The adaptations that allow diving mammals and birds to remain submerged for long periods, sometimes at great depths, have fascinated physicists for generations. In a personal reflection on the field, Gerald Kooyman (17) reviewed the evidence that structural adaptations in the lungs of these animals have been critical to their success. Specifically, it appears that structural reinforcement of the terminal airways of diving mammals allows them to generate spectacular expiratory flow rates over a wide range of vital capacity. Clearly such adaptations are important for animals that must both expire and inspire during sometimes brief periods at the surface.

High altitude and other hypoxic environments. A major adaptive response to high altitude is accelerated erythropoiesis, driven by release of erythropoietin (Epo) from the kidney. This requires the coordination of multiple systems, including inhibition of the liver-derived hormone hepcidin, which in turn leads to activation of iron efflux from the liver, providing the additional iron required for erythropoiesis (11). It had previously been thought that Epo acts on the liver to directly suppress hepcidin expression. However, Gammella and colleagues (11) recently provided strong evidence that this is an indirect effect mediated through the ability of Epo to increase production of erythroferrone, a newly discovered regulator of iron metabolism.

The physiological adaptations that allow humans and animals that habitually live at altitude to perform better in hypoxic environments than their “lowlander” counterparts has remained a central question in the field of oxygen biology. Lui and colleagues (20) took a novel approach to answering this problem, by contrasting the physiological and phenotypic responses to acclimatization to hypoxia in deer mice derived from “highland” and “lowland” populations (20). Thus their approach allowed at least partial dissection of the mechanisms underlying genomic adaptation to hypoxia as opposed to phenotypic
plasticity (acclimation). Remarkably, they found that the greater performance of highlanders under hypoxic conditions was associated with distinct differences (compared with lowlanders) in muscle phenotype and biochemistry (e.g., capillary density, fiber type, and enzyme activity). In contrast, acclimation to altitude was mainly associated with increased blood hemoglobin content and lung mass, and these changes were greater in lowlanders than highlanders. Thus, while lowlander mammals can acclimatize to a high-altitude environment, they will likely always be at a disadvantage relative to their highlander relatives.

The development of ways to improve human performance at altitude has been an enduring goal in high-altitude physiology and medicine. Increasing oxygen delivery to tissue by enhancing NO-mediated vasodilation could be a valid strategy, particularly if this could be achieved by dietary modification rather than pharmacotherapy. Kelly and colleagues (13) recently showed that dietary supplementation with NO3-rich beetroot juice can reduce the oxygen cost of submaximal cycle exercise and improve the kinetics of whole body oxygen consumption, in humans under hypoxic conditions (13). They proposed that this effect could be mediated through the ability of NO3 to be metabolized to NO, which could both improve oxygen delivery to tissue and increase the efficiency of oxygen utilization within mitochondria. Potentially, such effects of NO3 supplementation could help improve performance in individuals exercising under conditions of relative hypoxia, such as high altitude.

One of the risks for lowlanders who ascend to altitude is pulmonary hypertension. Patel and colleagues (28) identified a mechanism that may protect against development of pulmonary hypertension. They found that exposure of mice to 21 days of hypoxia led to inhibition of pulmonary vasoconstriction, in response to acute hypoxia, caused by increased extracellular levels of hydrogen peroxide.

The risk of stroke is thought to be augmented at high altitude (1). Moses and colleagues (23) recently showed that acute hypoxia can lead to recruitment of a patent foramen ovale, presumably due to hypoxic pulmonary vasoconstriction. This observation provides a potential mechanistic basis for increased risk of stroke at altitude, since a patent foramen ovale provides a direct pathway for thrombi that form in the venous circulation to access the cerebral circulation.

One of the ways animals have adapted to hypoxic environments is through the properties of hemoglobin. For example, fish adapted to hypoxic waters tend to have hemoglobin with very high affinity for oxygen. Air breathing in fish is also thought to have evolved as an adaptation to hypoxic aquatic environments (6). But as the efficiency of air breathing improved, selection pressure switched toward hemoglobin with lower oxygen affinity (6). Thus air-breathing fish provide an excellent opportunity to study related species whose hemoglobin have a wide range of affinities for oxygen. Damsgaard and colleagues (6) examined the properties of hemoglobin in the air-breathing Southeast Asian striped catfish (Pangasianodon hypophthalmus) and compared their findings with those of previous studies of air-breathing fishes. Their findings provide evidence that a common adaptation leading to high affinity of hemoglobin for oxygen in such species of fish is relative insensitivity to the chloride ion, which normally acts to reduce the affinity of hemoglobin for oxygen. Snakes also must face a range of terrestrial and aquatic environments. Within specific species, they must also experience large changes in oxygen delivery and demand. For example, some aquatic snakes dive for prolonged periods (e.g., yellow-bellied sea snake), whereas some terrestrial snakes have large postprandial increases in metabolic rate (e.g., pythons and rattlesnakes). Storz and colleagues (38) provide evidence that snake hemoglobins are characterized by a large capacity for regulation of their affinity for oxygen, which may be central to their ability to regulate oxygen delivery to tissue under such diverse conditions.

Some fish, such as the crucian carp (Carassius carassius), are able to survive virtual anoxia for extended periods of time (25). Nilsson and colleagues (25) made the interesting observation that many of the protein kinase signaling pathways activated in cardiac and brain pre- and postconditioning are activated by anoxia in the crucian carp. Seven days of anoxia induced marked increases in the phosphorylation of the mitogen-activated protein kinases (MAPKs) extracellular regulated protein kinase (pERK), c-JUN NH2 terminal kinase (pJNK), p-p38-MAPK in the heart, and p38-MAPK in the brain. Importantly, there was little or no change in the absolute levels of these proteins. The crucian carp may thus be an interesting model for investigation of the molecular pathways driven by oxygen deprivation, particularly those that might drive adaptation to cellular hypoxia.

There also appear to be responses to hypoxia that are unique to mammalian species. For example, in some tissues in rodents and primates, hypoxia induces a switch from transcription of paralog 1 of subunit 4 of cytochrome c oxidase (COX) (COX4-1) to paralog 2 (COX4-2) (16). It has been proposed that this switch reduces production of reactive oxygen species by mitochondria under hypoxic conditions. Interestingly, Kocha and colleagues (16) were unable to detect a similar transcriptional switch in a range of fish and reptiles.

Recent Technological Advances and New Techniques

Experimental techniques. Arterial oxygen saturation can be measured indirectly by pulse oximetry, but noninvasive measurement of the partial pressure of oxygen and other gases in the arterial blood of humans has remained problematic. This has limited advances in our understanding of the control of breathing. For example, “dynamic end-tidal forcing” is a method used to experimentally regulate blood gas levels by altering inspired gas concentrations on the basis of their concentrations in end-tidal expired air (PET) (40). But errors in the estimation of arterial PO2 and PCO2, from the concentrations of these gases in expired air, will confound this experimental approach. To address this problem, Tymko and colleagues (40) directly compared the partial pressure of oxygen and carbon dioxide in arterial blood and end-tidal expired air, in response to changes in the inspired concentrations of oxygen and carbon dioxide, both at sea level and high altitude. Their findings confirmed the existence of systematic differences between PET and direct measurement of arterial PO2 and PCO2. However, they also showed using linear regression analysis that equations could be developed to allow both arterial PO2 and PCO2 to be accurately predicted from the concentrations of these gases in end-tidal air. If these equations can be validated in an independent sample, they can be used to accurately estimate...
arterial blood gases in experimental situations in which it is not feasible to use invasive techniques. Studies of the oxygenation of the kidney have been limited by the fact that available methods for measuring absolute renal tissue PO2 have required the use of anesthesia. Calzavacca and colleagues (4) described a method that can be used in unrestrained sheep to provide continuous and simultaneous measurements, in both the renal cortex and medulla, of tissue PO2 (by fluorescence lifetime oximetry) and local perfusion (by laser Doppler flowmetry). In a large animal such as the sheep, this approach can be coupled with techniques for measurement of systemic and renal hemodynamics and oxygenation, as well as neural and hormonal function. Notably, it appears that global measures of renal blood flow and renal oxygen delivery and consumption are very poor predictors of the impact of vasoactive agents on regional kidney perfusion (3). Thus this new approach opens the way for studies of the physiological control of renal oxygenation and the role of renal tissue hypoxia in the development of acute kidney injury and chronic kidney disease.

**Clinically translatable methods.** Prevention of acute kidney injury remains one of the major challenges in management of patients in critical care settings. It has recently been argued that measurement of the oxygen tension in the urinary bladder, perhaps via a fiber-optic probe introduced into a bladder catheter, might provide a means to monitor patients at risk of development of acute kidney injury (10). There has also recently been considerable interest in the potential use of near infrared spectroscopy (NIRS) for assessing tissue oxygen saturation in a variety of clinical and experimental settings. For example, tissue oxygen saturation determined by NIRS is reduced by lower body negative pressure before detectable reductions in arterial pressure. Thus NIRS could be a useful method for triage of patients subjected to blood loss in a military setting. But multiple physiological factors could potentially confound the prognostic value of this approach. With this in mind, Schlader and colleagues (36) investigated the effects of hyperthermia on the ability of NIRS to predict presyncope in response to progressively increased lower body negative pressure. They found that forearm oxygen saturation fell similarly during lower body negative pressure in hyperthermic and normothermic subjects. Yet the hyperthermic subjects reached presyncope at a much lesser lower body negative pressure than the normothermic subjects. Thus, while NIRS is likely an excellent measure of tissue oxygen saturation, its utility as a prognostic marker for triage of patients at risk of cardiovascular collapse after hemorrhage may be limited by the impact of confounding factors such as body temperature.

In conclusion, oxygen is a simple molecule but its biology is complex. A number of recent advances in this field have been described in the pages of this journal. Let us hope there is much more to come in the near future.

**REFERENCES**


