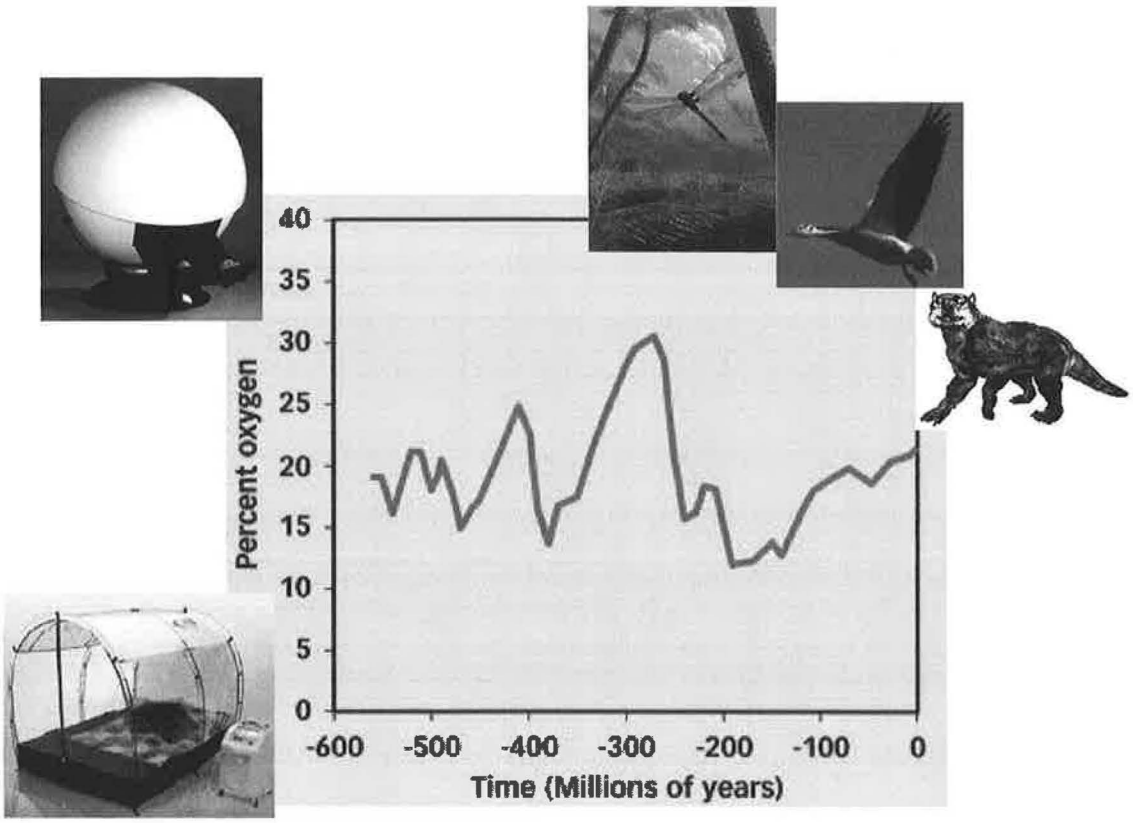
Oxygen Bar or Hypoxic Bed:

What are the optimal requirements?



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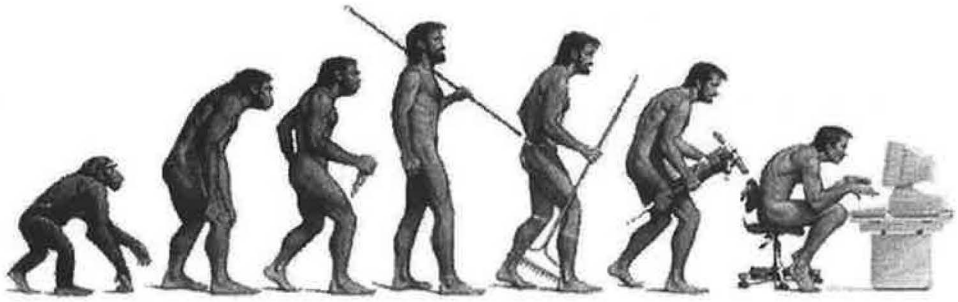

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Professor of Internal Medicine

Research interests:

Pulmonary exercise physiology

Gas exchange

Compensatory lung growth

Introduction

On a recent trip to Irvine, CA I came across an oxygen bar in a shopping mall. For $20 one can inhale 95% pure oxygen for 5 min while drinking fruit punch and watching TV. In fact supplemental oxygen for otherwise healthy people is big business. Oxygen bars are found not only in malls but also in organic cafes, upscale spas, fitness centers and private lounges all over the world; there is an oxygen bar at the Copenhagen Airport. Various forms of personalized oxygen-enriched environment are commercially available, such as the oxygen-enriched car, oxygen-enriched room, and the personal hyperbaric chamber *ala* Michael Jackson. There are books entitled "Flood your body with oxygen". Health food stores sell bottled "super-oxygenated" water as well as portable battery packs to oxygenate drinking water at home. One can also purchase small bottles of oxygenated water for daily supplementation, known as "vitamin O". To counteract the expected increase in oxidative stress, which has been implicated in everything from wrinkles to arthritis to neurodegeneration to cancer, one can simultaneously flood the body with antioxidant supplements, or combine oxygen enrichment with anti-free radical formulas in one product for a counter-balanced effect.

While being enticed with supplemental oxygen during the day, various enclosures are also commercially available for the purpose of sleeping, working or training in a hypoxic environment in order to take advantage of the "Live high, train low" idea for improving exercise performance. Daily intermittent exposure of 8-10 hr to a simulated altitude above 8,000 ft. allows ventilatory acclimatization and stimulation of erythrocyte production, reaching peak effects after 4 to 6 weeks. These personalized enclosures can simulate altitudes up to 15,000 ft. and essentially provide athletes a drug-free method for improving their speed and endurance, largely via increasing blood volume and hematocrit.

The marketplace, therefore, mirrors our schizophrenic relationship with oxygen, a vital gas and a lethal toxin, the classic example of biological trade-off and the double-edged sword. By enabling life as we know it, oxygen also makes death inevitable. Supplemental oxygen is clearly useful for correcting arterial hypoxemia in disease. However, in the absence of disease is there any reason to receive more or less oxygen? Is there an optimal biologic requirement? In this review I will address: **a)** how the earth got its oxygen, **b)** how oxygen drives biological evolution, **c)** how cells sense oxygen, and **d)** how much oxygen is optimal.

Evolution of oxygen on Earth

Although oxygen is one of the most abundant elements in supernova and galaxies including the Milky Way, the primordial Earth contained little free oxygen. The earliest rocks bearing traces of organic life, discovered in Greenland, date to ~3.8 billion years ago (BYA). The earliest methanococcus and cyanobacteria (blue-green algae) fossils, found in Australia, are of a similar age. However, there is no direct evidence that life did not exist before this time. The infant earth experienced many major impact events that could have easily wiped earlier life forms before 3.8 BYA. For example, a meteor the

size of Mars is believed to have gouged out the moon around that time. The microfossil discovery of filamentous bacteria, associated with undersea hydrothermal vents and utilizing hydrogen sulfide of volcanic origin as fuel, suggests at least the possibility that unicellular life in the ocean depths could have been present even before 3.8 BYA.

Because the earliest bacteria appear fully formed in fossil records, even respected scientists have suggested that they might have been seeded from outer space on the tails of comets (1). The cyanobacteria live in shallow water colonies forming large layered sedimentary structures called "stromalite". Living stromalite formations thrive at coastal locales to this day. Contemporary cyanobacteria are morphologically similar to their fossil relatives, suggesting an extremely slow evolutionary process. It is not known exactly when these bacteria acquired chlorophyll and began photosynthesis, because for millions of years the O_2 they produced was scavenged by iron and sulfur in the soil and water forming ferric oxides (e.g., hematite Fe_2O_3) and sulphates. The occurrence of ferric oxides and sulphates indicates an oxidizing atmosphere, and sedimentary rocks have provided key evidence for the evolution of terrestrial fauna and flora.

How atmospheric oxygen drives biologic evolution (2, 3)

From ~2.2 BYA atmospheric O_2 concentration slowly began to rise. The present day level of 21% O_2 was first reached about 600 million years ago (MYA). The initial rise in O_2 caused the first and the worst mass extinction, known as the oxygen holocaust, as most of the primitive anaerobes died out. The survivors either moved deep into the soil or to the ocean floor, or adapted their metabolic machinery to detoxify O_2 and eventually to utilize it. Aerobic respiration essentially utilized the same electron transport chain that already developed in anaerobes, but substituted O_2 instead of nitrite as the final electron acceptor. As aerobic respiration is 16 times as efficient in ATP production as anaerobic respiration, aerobic organisms flourished and diversified in ways that could not be achieved by anaerobes. In the last 600 million years, atmospheric O_2 level has fluctuated periodically owing to the opposing effects of biological mass that increases O_2 production and geological processes such as erosion and volcanic eruption that consume O_2. These fluctuations coincide with major evolutionary milestones:

The aquatic-to-terrestrial transition probably began during a period of O_2 deprivation in the water ~400 MYA. Water is 1,000 times more dense and 50 times more viscous than air; O_2 content is only 3% of that in an equal volume of air and decreases with water depth; hence extracting O_2 from water requires much more metabolic energy than from air. The evolution of limbs can be deduced from the ancient lobe-finned fishes: lungfish, coelacanth, and rhipidistian (now extinct) (4). Gill-to-lung transition is very versatile, exemplified by the tadpole/frog as well as salamanders that can develop gills only, lungs only, gills and lungs, or neither. It was the swim bladder, not the gills, which evolved into lungs. The swim bladder is a gas exchanger surrounded by a dense capillary counter-current exchange system. When blood becomes acidic, capillary hemoglobin releases O_2 into the bladder to increase buoyancy. When blood becomes less acidic O_2 is taken up by hemoglobin. This property, called the *Root effect*, is analogous to the Bohr effect in mammalian hemoglobin only much more sensitive to hypoxia.

In the carboniferous period (~300 MYA), atmospheric O_2 rose steadily to 30-35%) associated with the emergence of giant trees as well as giant insects, millipedes, dragonflies and other arthropods (5). These species obtain O_2 mainly through diffusion across their skin or exoskeleton and so thrive in hyperoxia. The higher air density associated with hyperoxia also provided greater lift for flight and could have facilitated the development of wings, which independently evolved several times during periods of relative O_2 abundance (6, 7).

Towards the late Permian and early Triassic periods (~240-220 MYA), atmospheric O_2 plunged to 13-15%, triggering the Late Permian Extinction. The reason for this decline is not clear. Carbon isotope analysis of rocks from that period shows a shift towards increasing atmospheric levels of CO_2. One theory is volcanic eruption or tectonic plate shift releasing large amounts of methane that turned into CO_2 and lowered atmospheric O_2 level. Climate warming and environmental degradation were also possible factors (8). Fossil records indicate a major reduction in biodiversity. Small ferns replaced large trees, and an estimated 70-95% of marine and terrestrial species became extinct. The surviving species clustered near sea level; most were burrowing animals and amphibians, that is, animals already used to a hypoxic environment.

In the late Triassic Period (~180 MYA), atmospheric O_2 began rising slowly again reaching ~16% by 100-65 MYA, corresponding to the gradual emergence of dinosaurs, flying reptiles, and mammals. Placental birth and bipedal locomotion developed around this time. By ~50 MYA, atmospheric O_2 reached 21% and global temperature increased significantly, associated with the emergence of flying mammals and the precursors of large cattle. By 25 MYA, atmospheric O_2 peaked at 23%, associated with the prevalence of mega-mammals (sloth, giant rhino, etc). These mega beasts gradually became extinct as ambient O_2 level drifted downward to reach the present level of 21%. Overall, fossil evidence suggest that atmospheric O_2 level determines the species diversity and maximum body size attainable by organisms during evolution.

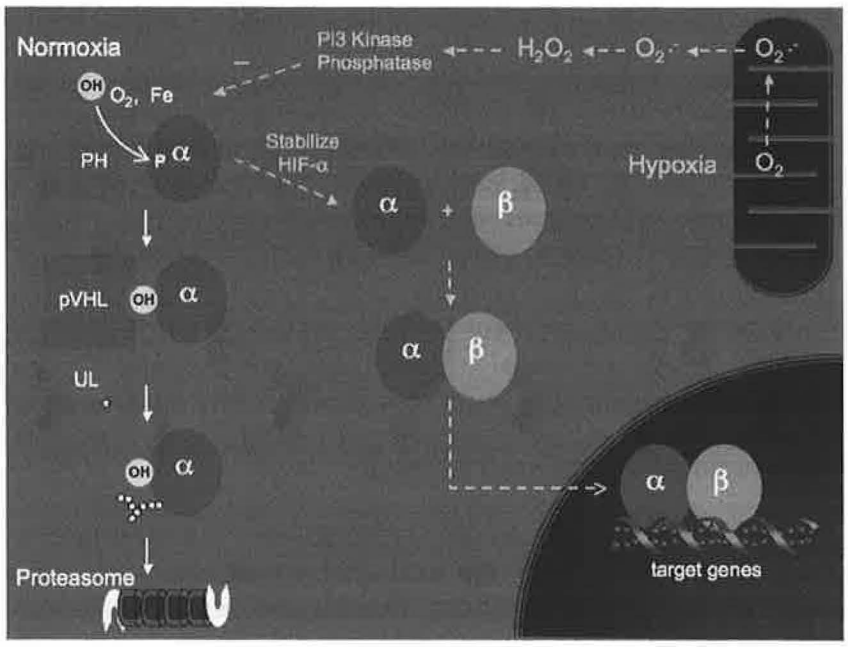
How cells sense oxygen tension

H. Franklin Bunn defines an O_2 sensor as "...the molecule that will pick up changes in the pressure of O_2 in the cell and transduce a signal". The primordial O_2 sensor molecule almost certainly contains one or more transitional metals (such as Fe, Cu, S) and probably forms part of a hemoprotein that generates reactive oxygen species (ROS) (9, 10). The mitochondrial model of O_2 sensing is described below. Other putative O_2 sensors include the flavoheme oxidoreductases, NADPH oxidase in pulmonary neuroepithelial bodies, tyrosine hydroxylase in the glomus cells of the carotid body as well as heat shock proteins and the mammalian target of rapamycin (mTOR) pathway. Oxygen sensing is such a critical primordial function that it should not be surprising to find multiple sensors in a cell.

It makes teleological sense that the site of oxidative respiration should also be the site of oxygen sensing. Electrons are passed down the hemoprotein complexes of the mitochondrial respiratory chain until they are ultimately used to reduce a terminal electron acceptor. About 1-3% of the O_2 entering the electron transport chain is only

partly reduced and discarded from the cytochrome chain as superoxide, mostly from Complexes I and III (11, 12). During normal metabolism, antioxidant enzymes present in the mitochondrial matrix neutralize superoxide anions; Mn-SOD converts superoxide to H_2O_2, which is then inactivated by glutathione catalyzed by glutathione peroxidase or catalase. Hence, mitochondrial ROS normally do not leak into the cytosol.

When mitochondrial respiration is stressed, i.e., by intracellular hypoxia, ischemia-reperfusion, aging or chemical inhibition, superoxide generation increases. With fewer O_2 molecules to accept electrons at Complex IV, unpaired electrons back up along the chain and some gain access to molecular O_2 in unregulated, non-enzymatic reactions creating ROS. Nitric oxide could exacerbate ROS accumulation. The final enzyme, cytochrome c oxidase (Complex IV), is homologous to bacterial nitrate reductase. Primitive bacteria can use either O_2 or NO as the terminal electron acceptor. Eukaryocytes prefer to use O_2, but NO retains its much higher affinity to cytochrome c oxidase than O_2; when O_2 tension declines more NO could competitively bind to this enzyme causing relative inhibition of O_2 binding and back-up of ROS at the proximal steps. Thus, contrary to common expectation, <u>hypoxia actually creates oxidative stress</u>.



Excess mitochondrial ROS formed during hypoxia leak into the cytosol via anion channels and are converted to H_2O_2 by superoxide dismutase. H_2O_2 acts as a 2nd messenger to activate various kinases and phosphatases and possibly downregulate the enzyme <u>prolyl hydroxylase</u> (PH), triggering stabilization of <u>hypoxia-inducible factor</u> (HIF)-1-alpha subunit. The various HIF subunits are constitutively expressed in the cytoplasm of nearly all cells. In the presence of O_2 and iron, PH hydroxylates a highly conserved proline residue in HIF-1-alpha, allowing HIF-1-alpha to bind to the von Hippel Lindau protein (pVHL) and forming a complex that activates ubiquitin E3 ligase, resulting in ubiquitination of HIF-1-alpha and its subsequent translocation into proteosomes for degradation. In the presence of cellular hypoxia, PH downregulation slows HIF-1-alpha degradation, allowing unhydroxylated HIF-1-alpha to accumulate in the cytosol forming a stable heterodimer with HIF-1-beta subunit; the heterodimer then translocates to the nucleus where it binds to the hypoxia responsive elements in the promoter region of over 200 target genes.

In support of the mitochondrial model, the action of ROS on HIF-1-alpha can be

mimicked by exogenous H_2O_2, which stabilizes HIF-1-alpha protein in normoxia. Cobalt ion generates H_2O_2 by a non-mitochondrial mechanism and also simulates the effects of hypoxia on HIF-1 stabilization. The iron chelator desferrioxamine acts directly on PI-3 kinases and phosphatases to trigger HIF-1 stabilization in the absence of hypoxia. In murine embryonic cells lacking cytochrome c, mitochondrial ROS activity is absent and HIF-alpha subunits are continually degraded. Exogenous H_2O_2 treatment restores the stabilization of HIF-alpha subunits similar to the effect of hypoxia, indicating that mitochondrial ROS act upstream of PH in this oxygen-sensing pathway. (10, 13, 14)

All of these molecules: hemoproteins, PH and HIF, have ancient homologues traced back to the earliest eukaryocytes and prokaryocytes. Their structure and function have evolved along with atmospheric oxygen. The hemoproteins initially catalyzed nitrogen- and sulfide-based energy production and scavenged reactive nitrogen species in ancient microbes. As ambient O_2 level rose, these proteins were used by anaerobes to detoxify O_2 by using it to convert NO into nitrate, and later were used to channel O_2 into the electron transport chain to drive ATP production.

Function of HIF signaling The very long list of target genes regulated by HIF fall into distinct categories that illustrate a coherent pattern of response to hypoxia (15-18):

Function	**HIF-regulated genes**	**Adaptation**
Anaerobic metabolism	Glycolytic enzymes Glucose transporters	Back-up mechanism to maintain ATP generation
Catecholamine synthesis	Tyrosine hydroxylase	General defense against stress
Suppression of non-vital organ function	Cell cycle regulators	Retard somatic growth
Erythropoiesis, iron and heme metabolism	EPO, Transferrin Heme oxygenase	Increase erythrocyte production, blood volume and hematocrit
Vasomotor regulation	NO synthase Endothelin-1	Optimize blood flow distribution
Angiogenesis	VEGF, NO synthase	Increase capillary density Vascular remodeling
Cytokine growth factors	PDGF, PIGF, TGF-beta...	Cell protection and tissue repair, Growth of gas exchange organs

Many of the HIF-regulated genes subserve multiple interrelated functions. For example, in addition to its endocrine function, EPO is produced in non-hematopoietic tissues and participates in widespread paracrine signaling via EPO-receptors that mediate organ-specific actions (19-22) including: **a)** developmental growth and organogenesis, **b)** injury protection and tissue repair in central nervous, cardiovascular, renal and pulmonary systems, and **c)** compensatory growth of gas exchange tissue.

Another example is NO synthase, which is essential for normal fetal development in addition to vasomotor regulation, and is upregulated during postnatal injury-repair as well as compensatory lung growth. Virtually all of the HIF-responsive cytokine growth factors participate in developmental tissue growth, postnatal injury-repair and compensatory lung growth. This homeostatic system responds to changing ambient

oxygen level by modifying pre-existing molecules and pathways to assume slightly different but related delivery or protective function.

Adaptation of HIF signaling

Phylogenetic adaptation In keeping with the critical function of HIF signaling, we expect to find supply-demand driven adaptation among species. The fruit fly, Drosophila, possesses a gene called *trachealess*, a homologue of the HIF-1-alpha gene that is highly expressed during development. Without this gene the embryonic invagination that eventually forms the trachea does not develop. Aquatic species (16) must tolerate variable and generally low oxygen levels. Tissues from fish show very high basal expression of HIF-1-alpha; imposed hypoxic exposure further increases HIF expression associated with compensatory hypertrophy of the gills. The blind subterranean mole rat (*Spalax*) endemic to the Near East has evolved underground for more than 40 million years. Oxygen concentration inside their burrows averages 7% and fluctuates sharply during seasonal flooding. They have developed coordinated mechanisms to cope with hypoxia (23, 24). In the laboratory the blind mole rat can survive at least 14 h at 3% O_2 whereas ordinary rats (*Rattus*) die after 2 h. Compared to *Rattus*, *Spalax* species exhibit much higher renal HIF-1 and erythropoietin gene expression in normoxia; the levels are further increases during imposed ambient hypoxia. These animals also exhibit higher VEGF activity, erythrocyte count, lung diffusing capacity as well as superior myocardial performance.

Developmental adaptation Actively growing organisms utilize more O_2 per unit of body mass than mature adults. Accordingly, the various components of O_2 sensing pathways show higher expression, and the inhibitor pathways show downregulation, during development. The HIF-alpha subunits and EPO are key factors for normal erythropoiesis and angiogenesis; knockout animals often die *in utero* with various cardio-respiratory malformations.

Fetal lungs normally develop under hypoxic conditions. Levels of HIF-1-alpha and HIF-2-alpha are high during gestation. At term birth HIF-1-alpha declines to negligible levels but HIF-2-alpha remains unchanged; PH level increases after birth consistent with increased HIF-1 degradation. The pro-growth prenatal environment is disrupted by premature birth and neonatal O_2 supplementation, which diminishes alveolar and vascular development and contributes to the development of bronchopulmonary dysplasia (BPD). In vitro hyperoxia downregulates many genes encoding growth factors in the developing lung, especially those associated with angiogenesis, and inhibiting prolyl-4-hydroxylase activity leads to increased HIF-1-alpha and HIF-2-alpha, VEGF and VEGF receptor levels. Postnatal hyperoxic exposure suppresses HIF-2-alpha, VEGF and VEGF-receptors. In fetal mice deficient of HIF-2-alpha, VEGF in alveolar cells is reduced. Mice deficient of the VEGF-164 and VEGF-188 isoforms or of the HIF-binding site in the VEGF promoter die of respiratory distress syndrome at birth, but could be rescued by intra-uterine or postnatal delivery of VEGF, which also stimulates surfactant production (25, 26). In baboons with BPD, downregulation of HIF signaling could be reversed by an inhibitor of PH domain-containing proteins; treatment increases HIF-1alpha but not HIF-2alpha levels and increases platelet-endothelial cell adhesion

molecule 1 (PECAM-1) and VEGF levels relative to untreated controls (27, 28). Thus, modulation of HIF signaling has the potential for enhancing angiogenesis and alveolar development in the prevention and or treatment of BPD.

Induced adaptation In the lungs of growing dogs compared to adult lungs, the already elevated gene and protein expression of HIF-1-alpha and EPO-receptor is further increased during compensatory lung growth following pneumonectomy as well as following increased mechanical strain imposed on the remaining lung. Upregulation correlates with increases in lung cell proliferation index and morphological changes that eventually restores alveolar tissue volume, gas exchange surface areas and lung diffusing capacity to normal (21, 22). It has been suggested that compensatory lung growth reactivates developmental pathways, with the additional requirement that existing structure be remodeled or pruned in order to accommodate the newly generated tissue without causing structural distortion.

Is more oxygen beneficial?

Oxidative stress Oxidative stress derives from 2 sources: **a)** higher O_2 flux above that necessary for respiration, and **b)** free radicals produced by cellular respiration. As molecular O_2 is paramagnetic (having 2 unpaired electrons with parallel spin), it can only absorb another electron with an anti-parallel spin or when energy is transferred to O_2 to overcome the spin restriction. The one-electron reduction of O_2 produces superoxide ($O_2^{\cdot -}$) and a further single electron reduction produces hydrogen peroxide H_2O_2, which readily diffuses across cell membranes. Superoxide and H_2O_2 react to form the hydroxyl radical ($^{\cdot}OH$) catalyzed by transitional metal ions (Fe^{2+} or Cu^{+}).

$$H_2O_2 + O_2^{\cdot -} \xrightarrow{\text{metal catalyst}} {}^{\cdot}OH + OH^- + O_2$$

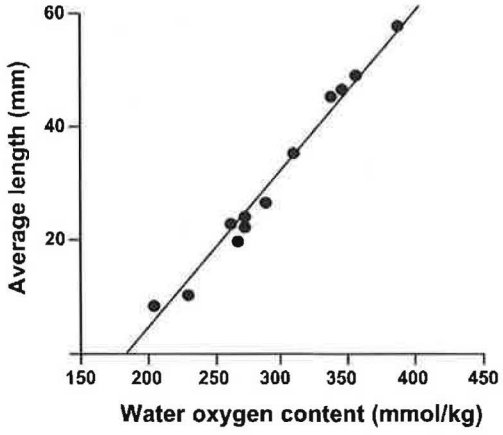
The hydroxyl radical reacts with DNA, protein and lipid and alters their structure leading to cell damage and death. Antioxidant defenses evolved in parallel with O_2 sensing and utilization, and consist of either prevention of ROS generation or elimination of ROS.

Antioxidant strategy	Action	Examples
Sequestration	Trap or exclude ROS	Cell membrane, chloroplast, peroxisome
Binding metal ions to protein	Slows ROS formation	Albumin, transferring, ferritin, ceruloplasmin
Enzymatic diversion and neutralization	Convert hydroxyl radical to H_2O_2 and H_2O_2 to water	Superoxide dismutase, catalase, glutathione peroxidase
Scavenging	Mops up residual hydroxyl or lipid radicals in tissue	Ascorbic acid, tocopherols, uric acid, bilirubin, glutathione
Quenching	Absorbs electrons and energy to prevent ROS initiation	Alpha tocopherol, beta carotene

An example of species-specific antioxidant mechanism is ascorbic acid, produced endogenously by all invertebrates and lower organisms and by most mammals at a rate directly related to physiological stress. Humans, apes, guinea pigs, bats and some

species of fish and bird have lost their synthetic ability due to inactivation of the gene for L-gluconolactone oxidase (GLO) 50-60 MYA (late Cretaceous period). This time point roughly follows the interval when dinosaurs and many other organisms became extinct. One theory is that following a period of great physiological stress and after enough plants have recovered to supply ascorbic acid, the GLO gene mutation could have conferred survival advantage upon the primates by streamlining their metabolism to conserve energy. Ascorbic acid synthesis actually generates H_2O_2, so there may have been selection pressure for its downregulation in favor of more cost efficient antioxidants, perhaps uric acid and superoxide dismutase (SOD). Antioxidant defenses fundamentally play catch up with O_2 utilization and are constrained by the fact that ROS are normally used in cell signaling and regulating gene transcription. It is an irony of evolution that antioxidants cannot be overly efficient; a controlled pro-oxidant environment must remain in order for normal physiologic function to proceed.

Growth, differentiation and longevity The major developmental effect of ambient hyperoxia is increased body size. In aquatic species and at high altitude maximum body size is positively correlated with environmental O_2 tension. For example, the body length of over 1,800 species of marine crustaceans found in 12 ocean sites all over the world is strongly correlated with water O_2 content. (Figure from ref. (29)). The effect can also be experimentally observed. Fruit flies and mealworms raised in hyperoxic chambers grow larger than those raised in normoxia while those raised in hypoxic conditions are smaller (30). Alligator eggs incubated in hypoxic air hatch later than eggs incubated in normoxic air. Eggs incubated in hyperoxic air hatch earlier but embryo mortality is also higher, reflecting a greater oxidation stress.



Normal intracellular O_2 tension averages only ~3-5% (range 0.5 to 10%); considerable intracellular gradient exists across even a few microns. Cellular O_2 tension gradients drive embryonic development. For example, in chick embryos regions of low O_2 tension promote cartilage development, while regions of high O_2 tension promote development of muscle cells (31). Mitotic and growth rates of placental cytotrophoblasts are highest during the 1st trimester of pregnancy when maternal blood flow to the placenta is minimal. As cytotrophoblasts invade the uterine blood vessels, a steep O_2 tension gradient causes the cells to exit gradually from the cell cycle (32). Cells cultured in 21% O_2 are in fact exposed to a supra-physiologic O_2 tension. Human fibrobasts grow faster when cultured at sub-atmospheric O_2 tensions (33). Cytotrophoblasts cultured in 2% O_2 proliferate actively and differentiate poorly. When cultured in 20% O_2, the cells stop proliferating and differentiate normally (34). Myocardial cells cultured at 21% O_2 attain a larger size but grow slower compared to cells cultured at 3% O_2 that are smaller but remain proliferative and mobile (35). Across cell lines, proliferative life span is inversely related to ambient O_2 tension, with hyperoxia inducing downregulation of growth factors

and metalloproteinases, upregulation of pro-inflammatory markers as well as large increases in various antioxidants (36, 37).

Transgenic *Drosophila* strains that overexpress glutathione reductase show lower O_2 consumption in normoxia and increased longevity compared to wild type controls when challenged with 100% O_2 (38). In rodents, acclimatization to sublethal hyperoxia (40-65%) blunts post-pneumonectomy compensatory lung growth (39) but increases survival during subsequent exposure to 100% O_2. Mitochondrial DNA is more susceptible to hyperoxic damage than nuclear DNA. Mitochondrial ROS release appears to determine the rate of apoptosis. Species that release ROS slowly from mitochondria, such as pigeons, live longer than those that release mitochondrial ROS in larger amounts such as rats, even though basal metabolic rates are similar. Transgenic mice overexpressing mitochondrial catalase live 5 mo longer than wild type controls, presumably due to more efficient ROS removal; cardiac pathology, cataract development, H_2O_2 production, oxidative damage and mitochondrial deletions were delayed or reduced (40). Transgenic mice overexpressing human extracellular SOD show a lower inflammatory profile and preservation of alveolar surface and volume density of alveoli during exposure to hyperoxia (41).

Exercise performance Acute hyperoxic exposure during exercise increases endurance and maximal power output, particularly in athletes who develop exercise-induced arterial hypoxemia; lactate production and acidosis are minimized and brain oxygenation improved (42, 43). Maximal O_2 uptake either remains unchanged or increases slightly. Maximal heart rate is reduced but maximal cardiac output is unchanged. Blood flow to the exercising limb is unchanged or reduced (44). Chronic intermittent exercise training during hyperoxia leads to a higher work intensity during training and improved endurance but the effects are modest.

In the presence of arterial hypoxemia (athletes at peak exercise, patients with COPD or heart failure) exercise performance is clearly improved by acute exposure to hyperoxic air (30-40% O_2) compared to normoxic air. Currently there is ongoing debate regarding whether supplemental oxygen should be routinely given as part of cardiopulmonary rehabilitation and whether allowing patients to achieve a higher work intensity or endurance during exercise sessions improves long-term outcome (45).

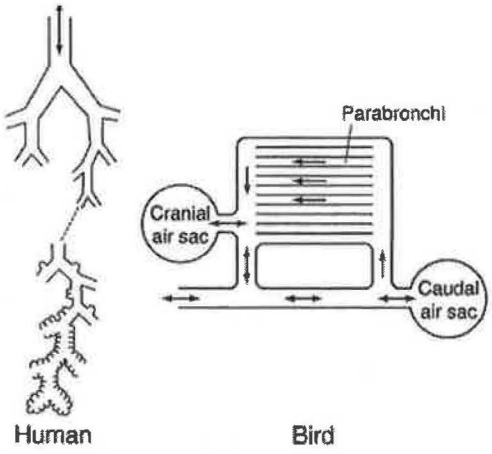
In summary, in subjects with normal arterial blood oxygenation, hyperoxic conditioning increases body size, downregulates hypoxia-responsive genes, and elicits antioxidant defenses to protect against tissue damage during subsequent hyperoxic exposure, but there is no evidence for significant functional benefit in normoxia.

Is less oxygen beneficial?

There are only 3 possible ways to deal with a restricted budget imposed by ambient hypoxia: **a)** maximize O_2 uptake, **b)** improve efficiency of O_2 utilization and **c)** conserve energy expenditure. Both evolutionary history and experimental intervention illustrate these common response patterns.

Evolutionary adaptation to hypoxia One way to maximize O_2 uptake is to grow efficient gas exchange organs, a universal response across phylogeny. Fish living in stagnant water develop hypertrophy of the gills. Frogs and toads respire through the skin; species that live at high altitudes develop redundant skin folds that increase the surface area for gas exchange. Some lungfish and salamanders switch between lung and gill breathing as needed. Mammals native to high altitude show enlarged placenta compared to lowland members of the same species.

The bird lung represents an evolutionary triumph in overcoming the double challenge of ambient hypoxia at high altitudes and the high O_2 demands of migratory flight (46, 47). Their lightweight hollow bones contain numerous air-filled membranes connected to large air sacs and rigid tubes (parabronchi, 3-10μm diameter). Airflow is unidirectional and continuous through the parabronchi without inspiratory-expiratory phases or mixing (no dead space) or any change in lung volume (little tissue stress). The parabronchi are surrounded by a dense honeycomb-like capillary network in which blood and air flow in opposite directions, i.e., cross-current exchange. Oxygen tension in the parabronchi is always slightly higher than in its adjacent capillary, so that even at low tensions O_2 diffuses continuously from air to the blood. The densely packed parabronchi and capillaries mechanically support each other to avoid collapse and little connective tissue is needed for strength. Fossil evidence indicates that many theropod dinosaurs had a flow-through system like that in birds. The dinosaur-bird link remains controversial. While skeletal morphology shows many similarities, dinosaurs almost certainly had a diaphragm while birds don't (48). The growth pattern of Triassic prosauropods was variable like reptiles, while that of later dinosaur species more resembles birds and mammals (49).



The diving animals evolved special respiratory features for withstanding the compression pressure at depths (>1,000m) and the mechanical stress associated with repeated diving and surfacing (50, 51). As alveoli collapse at ~50m depth, alveolar septa in whales and dolphins have a thick connective tissue core and a bi-layer capillary bed. Cartilage extends down the entire airway tree to increase the effective dead space for accommodating the air forced from the collapsing alveoli. The terminal bronchioles and pulmonary venous plexus contain myosphincters to prevent retrograde air and blood flow. Snorkeling animals like the elephant also develop thick connective tissue septa in the lung and the pleural space to withstand an increased gravitational and frictional stress during respiration.

Metabolic suppression is a common adaptation to periodic severe O_2 deprivation. For example, the freshwater turtle reduces its metabolic rate to an absolute minimum and becomes comatose for an entire winter season, tolerating a blood lactate up to 200 mmol/liter; the H^+ release is buffered by the carbonate from its shells (52). The crucian

carp not only survives several months in completely deoxygenated water but remains active the whole time (53). Their hemoglobin has an extremely high O_2 affinity (P_{50}= 2.6mmHg). The gills undergo extensive apoptosis and then hypertrophy in hypoxia water. The liver is huge and full of glycogen. Protein synthesis is downregulated; energy is obtained by converting glucose to ethanol; lactic acidosis is avoided. Brain adenosine receptors are activated, which maintains brain ATP levels and depresses metabolism. The Weddell seals also drastically reduce their metabolic rate during a dive and can stay submerged for up to 90min but they preferentially maintain blood flow to the brain so as to remain alert to passing prey.

The Antarctic icefish exhibits a puzzling response to hypoxia; they completely lost the hemoglobin gene 12-5 MYA; some species have also inactivated the myoglobin gene in independent mutations. They directly extract dissolved O_2 from frigid water (-1.8 to +1.5°C). Their blood is transparent and muscle pale. The lack of an O_2 carrier is compensated by gross cardiac hypertrophy, wide blood vessels, high tissue capillarity and high mitochondrial density associated with exceptionally elevated NO synthase (54, 55). Whether these mutations are adaptive or not depends on comparison with other species and in reference to a specific environment. Clearly hemoglobin is not needed to survive for eons in their restricted habitat, so the loss must be "good enough", but the streamlining does leave them fewer options when faced with changing conditions, e.g., global warming, rising water temperature and increased population of predators.

Induced adaptation to hypoxia Chronic hypoxia elicits a biphasic dose-dependent metabolic response. While severe hypoxia (equivalent to >4,500m altitude) globally blunts metabolic processes, reduces body mass, retards growth and increases mortality, moderate hypoxia (<4,000m) activates HIF signaling pathways and stimulates compensatory responses in all steps of the oxygen transport chain:

Step	**Effect**	**Result**
Neural control	Hyperventilation	Increase alveolar PO_2
Lung	Alveolar growth and remodeling Increase alveolar volume and surface area Thinning of blood-gas barrier	Increase lung diffusing capacity
Circulatory	Pulmonary vasoconstriction Right ventricular hypertrophy Peripheral vasodilation	Improve blood flow distribution
Hematologic	Increase erythropoiesis Increase blood volume	Increase O_2 carrying capacity
Skeletal muscle	Lower muscle mass and fiber diameter Higher capillary and mitochondrial density Higher myoglobin concentration Upregulate glycolytic enzymes	Improve efficiency of aerobic and anaerobic metabolism

Growing new alveolar tissue on a low budget is no easy task; hypoxia-induced alveolar growth differs from developmental growth in the requirement for extensive remodeling of a well-differentiated scaffold. While new alveolar septal tissue is added slowly, alveolar ducts become smaller as alveolar surfaces unfold. The cells and matrix within alveolar

walls are redistributed. Alveolar capillaries distend as erythrocyte mass and blood volume increase. The net effect is progressive decrease in blood-gas barrier resistance to O_2 diffusion and a corresponding increase in lung diffusing capacity (56-58).

At the air-tissue interface the alveolar cells sense a much higher O_2 tension (150mmHg) than most cells in the body; they also detect hypoxia first and mount the first defense. When O_2 tension declines, mitochondrial ROS production increases in alveolar endothelial and vascular smooth muscle cells, triggering the release of intracellular Ca^{2+} stores, recruitment of Ca^{2+} channels in the plasma membrane and activation of vascular contraction (59). This is an attempt to selectively reduce blood flow to hypoxic regions in order to match regional ventilation to perfusion, which optimizes arterial O_2 tension and protects the gradient for O_2 diffusion in the periphery.

The consequence of pulmonary vasoconstriction is right ventricular hypertrophy and increased myocardial O_2 demand. Perhaps to protect against myocardial ischemia, in chronic hypoxia maximal stroke volume is reduced via downregulation of beta-adrenergic receptors (60). It is debatable whether the downregulating signal comes from a central regulator or working skeletal muscle or both. The peripheral vasculature responds to arterial hypoxemia at a much lower O_2 tension, via autonomic and NO-mediated modulation of regional vascular tone to match perfusion to tissue O_2 demand, and via hematologic modulation (increased P_{50} and erythropoiesis). Erythrocyte production increases both convective (O_2 carrying capacity) and diffusive O_2 transport; lung and peripheral diffusing capacities increase owing to better matching between erythrocyte and capillary membranes (61, 62). The skeletal muscle responds to chronic hypoxia by downregulating oxidative capacity, upregulating glycolytic capacity and downsizing muscle mass and fiber diameter, leading to an apparent increase in capillary and mitochondrial densities without an absolute increase in their volumes. Fiber downsizing reduces the resistance for O_2 extraction from capillary to mitochondria. (63)

Intermittent hypoxia has been promoted as a way of **a)** enhancing performance in well-trained athletes, and **b)** inducing general protection against hypoxic/ischemic injury. In rodents, intermittent hypoxia +/- exercise training upregulates muscle glucose transport and glycogen storage, improves glucose tolerance, reverses beta-adrenergic downregulation and prolongs survival under anoxia. In lowland subjects, intermittent exposure to simulated altitude induces ventilatory acclimatization and reduces the incidence of acute mountain sickness; it is a strategy often used by mountain climbers (64). In endurance athletes, hypoxic exposure during exercise training (*Live low, train high*) broadly upregulates genes associated with HIF signaling, mitochondrial enzymes, antioxidants and myoglobin concentration in skeletal muscle (65). Heat shock proteins, prostaglandins and NO synthesis also increase. Mitochondrial respiration becomes more efficient without a significant increase in muscle oxidative capacity (66). Exercise endurance improves with minimal or no change in maximal O_2 uptake or O_2 carrying capacity of blood (67). Thus, intermittent hypoxia training elicits minimal enhancement of aerobic or anaerobic capacity (68, 69).

The opposite strategy, intermittent hypoxic exposure +/- normoxic exercise training (*Live*

high, train low), has been shown to improve athletic performance as well as aerobic and anaerobic metabolic profiles at sea level, predominantly via ventilatory acclimatization and increased erythrocyte volume and O_2 carry capacity of blood (70, 71). While this approach sounds promising for augmenting both athletic performance and physiologic reserves against hypoxic insults, there are a few caveats. *First*, obstructive sleep apnea (OSA) is an extreme manifestation of intermittent hypoxia. Cyclic deoxygenation-reoxygenation in OSA is associated with heightened mitochondrial oxidative stress that correlates with cardiovascular complications (72). Supra-physiologic erythrocytosis further amplifies oxidative stress because the manufacture and maintenance of extra blood incurs a high metabolic cost; long-term consequences of higher than required levels of hematocrit, blood volume and viscosity are undesirable. Athletic animal species (horses, dogs) uniformly possess a large spleen to sequester extra erythrocytes at basal state and releases erythrocytes only upon sympathetic stimulation during exercise or in hypoxia; these animals maintain normal basal hematocrit, blood volume and viscosity despite a large total body blood volume (61, 62). This protective splenic reservoir is absent in humans. *Second*, even short intermittent hypoxia causes systemic and pulmonary hypertension, with resultant left- and right ventricular hypertrophy (73). *Third*, the brain is not so forgiving of periodic hypoxia. In mice exposed to alternating hypoxia-normoxia cycles, CNS angiogenesis is reversibly enhanced but dose-dependent demyelination is irreversible (74); various aspects of cognition are also impaired (75, 76). Patients with OSA show abnormal brain creatine bioenergetics and impaired cognitive function similar to that observed following ischemic preconditioning (77). Combined with the observation that no great intellect ever evolved from a hypoxic environment, it seems that some degree of caution is warranted in the use of intermittent hypoxia for the purpose of improving upon evolution.

Summary

Life on Earth has adapted with amazing variety and success to a wide range of ambient oxygen levels from zero to 35%. The optimal oxygen level is determined by developmental and metabolic need. Excess O_2 acutely improve exercise performance but chronic exposure promotes wasteful oxygen flux, resulting in gigantism, excess ROS production, upregulated antioxidant defenses, and possible tissue injury. Hypoxia promotes conservation of resources, elicits coordinated downregulation of metabolic capacities, improves the efficiency of energy production and utilization, and channels the meager savings into compensatory growth of gas exchange organs. Externally imposed hyperoxia and hypoxia both increase oxidative stress and evoke responses aimed at minimizing damage and enhancing survival, but these responses invariably incur costs and disadvantage in other ways. Understanding the cost-benefit equation of oxygen enables us to make rational decisions about the manipulation of this "deadly elixir of life" for medical or non-medical purposes in order to enhance some aspects of our definition of quality of life and to delay our ultimate demise by oxygen poisoning. As Anna Quindlen puts it, life ought to be looked upon as a terminal illness; only the journey, not the destination, is in doubt (78).

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