

# Hypoxia and hyperbaric oxygen therapy: a review

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**Abstract:** Hypoxia causes a cascade of activity from the level of the individual down to the regulation and function of the cell nucleus. Prolonged periods of low oxygen tension are a core feature of several disease states. Advances in the study of molecular biology have begun to bridge the gap between the cellular response to hypoxia and physiology. Hyperbaric oxygen therapy is a treatment for hypoxic- and inflammatory-driven conditions, in which patients are treated with 100% oxygen at pressures greater than atmospheric pressure. This review discusses hypoxia, the physiologic changes associated with hypoxia, the responses that occur in the cells during hypoxic conditions, and the role that hyperbaric oxygen therapy can play as part of the treatment for many patients suffering from diseases with underlying hypoxia.

**Keywords:** hypoxia, physiology, oxygen signaling, cellular metabolism, hyperbaric oxygen therapy

## Introduction

In the US, diseases such as stroke, cancer, heart disease, and chronic lung disease account for a majority (up to 60%) of the total number of deaths.<sup>1</sup> Hypoxia is a significant component of the pathology of these conditions as the unavailability of oxygen leads to physiological responses that, if not resolved, progress to localized hypoxic responses, cell metabolic inefficiency, organ dysfunction, and finally death. Oxygen is given to most of these patients in the hospital, but the risks of death and mortality remain. A review of how hypoxia develops and the related effects in the body is crucial in understanding the theoretical mechanism of action in hyperbaric oxygen therapy (HBOT) and in determining how to optimally manage these conditions.

## Hypoxia

The term hypoxia is quantitatively related to the organ, tissue, and even cell type. A hypoxic state indicates that an imbalance of oxygen is present and baseline function is compromised as a result of this imbalance. The imbalance of oxygen could result from a lack of oxygen or an excessive demand for oxygen. Baseline function in this context means carrying out normal bodily or cellular functions such as heart muscle beating or a neuron firing an action potential, also known as homeostasis. Hypoxia can be transient, acute, or chronic. Hypoxic conditions occur with a persistent lack of oxygen. Individual tissues have differing oxygen tensions and oxygen demands; on average, tissues at rest utilize 5–6 mL of O<sub>2</sub> per deciliter of blood delivered.<sup>2–4</sup> Hypoxia could be fairly defined as a scenario when tissue fails to receive this amount of oxygen.

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However, hypoxia is better understood as a component of the pathology of many disease states, such as ischemia.

Ischemia is the restriction of blood flow in the body such that oxygen, glucose, and nutrients are not delivered while waste products such as carbon dioxide are not removed. In this state, ischemia is a subtype of hypoxia where additional insults prevent baseline function. Both oxygen and glucose are necessary for aerobic metabolism; thus, hypoxia affects the energy cycle of the cell by creating an oxygen imbalance. This causes an inability of the cells to perform their normal functions and, if the deficit in oxygen delivery is not corrected, anaerobic metabolism takes over. Anaerobic metabolism can only deliver a fraction of the energy needed by the cell. Glucose is used to create pyruvate, resulting in energy production for the cell. Then the pyruvate is turned into lactic acid. If hypoxia cannot be corrected and aerobic metabolism restored, then cell death and tissue scarring occur. This can eventually progress to organ dysfunction and death. Other forms of hypoxia include carbon monoxide (CO) poisoning, asphyxiation, sleep apnea, severe anemia, altitude sickness, and ventilation-perfusion mismatch. Oxygen delivery is impaired in all these conditions just like ischemia, but the mechanisms leading to hypoxia differ while the consequences of hypoxia are shared.

When oxygen is not delivered in sufficient quantities, the body undergoes immediate responses. The fastest response is performed by the glomus cells of the carotid body, which sense oxygen tension in the blood through an incompletely understood mechanism that ends in depolarization within milliseconds.<sup>1,5</sup> This leads to physiologic responses intended to attenuate the hypoxia and prevent progression to dysfunction and death, such as through increasing ventilation and cardiac output. Additionally, at the cellular level, oxygen-sensing enzymes begin to regulate signal cascades to alter the intercellular environment in the same manner. These cascades attenuate hypoxia and prevent progression to apoptosis.<sup>6</sup>

## Cellular metabolism of oxygen

At the level of the cell, 80% of the available oxygen is used by the mitochondria, while only 20% is used by other organelles, indicating the importance of oxygen in metabolism. Mitochondria function as the cell's power station, and ATP is the cell's currency for energy. Oxygen is used as the final electron receptor in the electron transport chain (ETC), and the energy generated by this reaction is used to pump hydrogen ions across an electrochemical gradient outside the mitochondria. The hydrogen then diffuses back into the mitochondria, and the energy generated is used to phosphorylate

ADP to form ATP. Interestingly, although the mitochondrion utilizes most of the oxygen in the cell, the partial pressure is very low, only 1–3 mmHg.

Mitochondria require oxygen to receive the electrons at the end of the ETC at complex IV, cytochrome *c* oxidase, to utilize that energy to make ATP. Observations made of mitochondrial oxygen levels show that only a very small oxygen pressure is required for their function. The concentration of oxygen mainly depends on the rate at which mitochondria utilize oxygen.<sup>7</sup> Pressure at the outer membrane is in excess to the needs of the mitochondria based on myoglobin-facilitated oxygen diffusion. Recent theoretical models have demonstrated that in response to hypoxia, mitochondrial ETC undergoes compensatory changes which reduce their electron carrier levels allowing the mitochondria to maintain homeostasis over a wide range of oxygen levels.<sup>8</sup> While mitochondria have the ability to function under hypoxic conditions, this ability is not indefinite. Once myoglobin-facilitated oxygen diffusion is limited by hypoxia, the mitochondria develop an imbalance that eventually leads to electrons leaking across the mitochondrial membrane before reaching complex IV.

Fewer electrons reaching cytochrome *c* oxidase means that less energy is able to be transferred into creating ATP and that the cell begins building an energy deficit during hypoxic events. As this deficit grows, the body responds, the cellular environment changes, and cell death occurs if the stress is not alleviated by the body's and/or the cell's responses. Furthermore, studies have linked the exposure of hypoxia to increased oxidative stress resulting in generation of reactive oxygen and nitrogen species.<sup>9</sup> Free radical oxygen and nitrogen are extremely toxic to cells and result in damage which induces cellular death, that is, apoptosis. The mechanism of free radical formation is not fully known and the increase of oxygen free radicals during hypoxia seems paradoxical, but has been experimentally observed.<sup>10</sup> ROS are hypothesized to form as a result of direct transfer of electrons from reduced upstream carriers located at complex III in the ETC.<sup>8</sup>

Oxygen's role in providing energy is critical because oxygen deficiency leads to an energy deficit in the cell. Ischemia and hypoxia are interconnected because oxygen is delivered to tissues by plasma; thus, a reduction in blood flow also reduces oxygen tension. While hypoxia is always a component of ischemia, ischemia is not always involved in hypoxia, such as in the case of CO poisoning, during which blood flow is uninterrupted but oxygen delivery is impaired. Thus, oxygen transportation within the body plays a crucial role in the effects of hypoxia.

## Oxygen transport

Oxygen reaches the blood from ambient air during respiration. Air travels through the trachea and conducting zones of the lungs into the respiratory zones, proceeding to terminal bronchioles and eventually alveoli. Here, the pulmonary capillaries bring blood near enough for oxygen to diffuse into the blood, either binding to hemoglobin or dissolving into the plasma, while CO<sub>2</sub> diffuses out of the blood to be exhaled. The now oxygenated blood is then pumped by the heart into the left atria and then left ventricle, from where it is released into the systemic circulation.<sup>2</sup> Oxygenated blood becomes available for delivery to cells once it reaches the corresponding capillary bed.

Oxygen is transferred to cells via plasma, and the amount of oxygen present in the plasma is based on the principles of physics. Oxygen diffuses into the plasma, which can be understood by the application of Dalton's law and Henry's law. Dalton's law states that the absolute pressure of a mixture of non-reactive gases is equivalent to the total partial pressures of the individual gases. The consequence of this is that the partial pressure of oxygen in the air is solely based on the percentage of oxygen in the atmosphere and the barometric pressure, which may vary based on altitude as well as other factors. Given that the Earth's atmosphere contains 20.94% oxygen and the atmospheric pressure at sea level measures 760 mmHg, an oxygen partial pressure of ~160 mmHg at sea level can be calculated. However, air in the trachea will also have a partial pressure similar to humidified water, that is, ~47 mmHg; thus, a more appropriate calculation would use a gas pressure of 713 mmHg and give a partial pressure of oxygen of 150 mmHg (see Table 1).

The partial pressure of oxygen in the trachea that is inspired at sea level is 150 mmHg. Deoxygenated blood entering the lung contains a partial pressure of oxygen of ~40 mmHg. After the diffusion of oxygen occurs in the capillaries of the lung, the ending partial pressure of oxygen in the blood is ~100 mmHg. The partial pressure of the oxygen in the blood is dependent on the gradient between the partial pressure present in the alveoli and in the deoxygenated blood.

A larger difference in these two partial pressures makes more oxygen diffuse from the alveoli into the plasma.

With a given pressure of a gas, it is possible to use Henry's law to determine how much oxygen is dissolved in plasma. Henry's law states, "at a constant temperature, the amount of a given gas that dissolves in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid."<sup>11</sup> This means that the concentration of a given gas into a liquid is in relation to the partial pressure that the gas exerts, as well as its coefficient of solubility. Therefore, at an oxygen partial pressure of 100 mmHg with a solubility coefficient of 0.0024 mL O<sub>2</sub>/(dL blood per mmHg), the amount of oxygen in the plasma at sea level is 0.24 mL O<sub>2</sub>/dL blood (see Table 1).

The maximum concentration of oxygen in plasma at sea level is only 0.24 mL O<sub>2</sub>/dL of blood, but tissues require 5–6 mL O<sub>2</sub>/dL of blood for homeostasis. The delivery of more oxygen than the plasma is capable of carrying is accomplished through hemoglobin, which more than makes up for the low capacity of plasma to carry oxygen. One gram of hemoglobin can carry as much as 1.34 mL O<sub>2</sub> if all four binding sites are occupied on each molecule of hemoglobin. The concentration of hemoglobin can indicate the maximum capacity for carrying oxygen in the blood. Assuming 15 g/dL blood hemoglobin (normal is 11–16 g/dL), a hypothetical total of ~20 mL O<sub>2</sub>/dL blood can be calculated for the carrying ability of hemoglobin. This makes the total oxygen concentration in the blood 20.24 mL O<sub>2</sub>/dL blood at sea level, assuming that the hemoglobin-carrying capacity is maximized (see Table 1).

The capillaries serve as the locations at which oxygen is transferred to tissue from the plasma, and hemoglobin functions as an oxygen reservoir in this context. A maximum of 20.24 mL O<sub>2</sub>/dL of blood enters the capillary bed, and at rest the local tissue consumes 5 mL O<sub>2</sub>/dL of blood, leaving the post-capillary blood with 15.24 mL O<sub>2</sub>/dL blood. During exercise, tissues can consume up to 15 mL O<sub>2</sub>/dL of blood, but may still have higher demands for oxygen.

**Table 1** Oxygen content

	Ambient air	100% Oxygen	Hyperbaric oxygen (3 atm)
Oxygen partial pressure (mmHg)	150	713	2,233
Plasma oxygen content (mL O <sub>2</sub> /dL blood)	0.24	1.71	4.8
Oxygen content of blood (mL O <sub>2</sub> /dL blood)	20.24	21.71	24.8
Net change in plasma oxygen content (%)	Not applicable	+1.47 (7.26%)	+4.56 (22.5%)

## Tissue and organ responses to hypoxia

To meet the increased oxygen demands, the body undergoes physiologic processes that involve the lungs, heart, and vasculature. Cardiac output is increased as needed by increases in stroke volume and heart rate, delivering more blood, and hence, more oxygen to the capillary beds per unit of time. Pulmonary vessels constrict shunting blood from areas of low oxygen tension in the lungs to areas with higher oxygen tension, thereby maximizing the exchange of oxygen in the hemoglobin and plasma. This allows for the maintenance of the reservoir of oxygen stored by hemoglobin in red blood cells. Systemic vessels dilate to perfuse tissues with higher oxygen demand, which also aids in blood delivery, and hence, oxygen delivery.

Local oxygen delivery is based upon the components discussed above and is a direct result of the concentration of oxygen in the blood and the amount of blood delivered. The body has many mechanisms to adjust oxygen delivery and maintain adequate oxygenation, including ventilation rate, cardiac output, stroke volume, hemoglobin concentration, dilation of systemic capillaries with constriction of pulmonary capillaries, and increasing the size of alveoli. A careful balance is maintained by coordinating all of these systems; however, the cellular response plays a major role in how hypoxia is handled at the cellular level, and this is where disease begins.

## Cellular responses to hypoxia

As the level of oxygen drops in the blood, the body undergoes responses such as increasing respiration and blood flow. Simultaneously, individual cells experiencing hypoxia begin reacting to the decreased oxygen tension. The cells sense low oxygen via cellular signaling that starts with the enzyme class prolyl hydroxylase domain (PHD) proteins.<sup>1,6,12</sup> This class of enzymes contains oxygen-sensing hydroxylases that will hydroxylate specific proline residues on the  $\alpha$ -subunit of the transcription factor hypoxia-inducible factor (HIF), which signals for the destruction of HIF. HIF is a heterodimer composed of subunits HIF-1 $\alpha$  or HIF-2 $\alpha$ , which dimerize with a HIF-1 $\beta$  subunit. HIF-1 $\alpha$  is a ubiquitous subunit, meaning that it is produced in all cell types, and the HIF-2 $\alpha$  subunit is found in myeloid cells, liver parenchyma, vascular endothelia, type II pneumocytes, and renal interstitium.<sup>6</sup> PHD forms part of the oxygen-dependent system that regulates the inhibition of HIF. Another molecule, known as factor-inhibiting HIF (FIH), serves as another oxygen-dependent system that regulates HIF.<sup>1</sup>

HIF is found in all nucleated cells of metazoan species (which includes humans). This enzyme is activated under low

oxygen tension and leads to the regulation of certain genes. In the presence of low oxygen levels, PHD is unable to cause the degradation of HIF, and HIF enters the nucleus and affects transcription. HIF directly binds to promoter sequences in the DNA, leading to increased transcription rates of certain hypoxic response genes.<sup>1,6,13-17</sup> This binding results in changes in glucose metabolism from aerobic to anaerobic, increased cytochrome oxidase transcription, inhibition of lipid catabolism, and promotion of lipid storage. Additionally, the transcription rate of some genes is indirectly decreased by the actions of HIF. For these genes, HIF increases the transcription of repressor proteins and the microRNAs that inhibit transcription.

Through this regulation of gene expression, HIF accomplishes the switch from oxidative metabolism to glycolytic metabolism. HIF also plays a role in increasing the expression of erythropoietin and vascular endothelial growth factor.<sup>16</sup> The enzymes of the glycolytic cycle, such as lactate dehydrogenase A and pyruvate dehydrogenase kinase 1, are directly elevated by the actions of HIF-related increases in transcription.<sup>1,17,18</sup> Enzymes that use acetyl-CoA in the tricarboxylic acid cycle to perform oxidative phosphorylation are indirectly shut down by HIF. The upregulated enzymes then function to inhibit the formation of acetyl-CoA from pyruvate, and thereby inhibit the formation of ATP from oxidative phosphorylation. This shunts all of the pyruvate made from glycolysis into forming lactate, causing acidosis. These processes demonstrate that HIF is capable of activating anaerobic respiration in response to hypoxia.

As hypoxia sets in, the function of the ETC in mitochondria declines due to electrons leaking out of the mitochondria before reaching cytochrome *c* oxidase. With the mitochondria not functioning well, the cell stops feeding acetyl-CoA to the citric acid cycle to prevent overwhelming the mitochondria. Instead, the cell concentrates on using glucose for fuel and disposes of the by-products of this process in the form of lactate. HIF serves as the signal for the cell to abandon aerobic metabolism in favor of anaerobic metabolism.

## Regulation of HIF

As previously noted, HIF is regulated by two oxygen-dependent mechanisms. PHD represents one branch of the oxygen-dependent regulation of HIF, while FIH represents the other. The enzymes of the families of both PHD and FIH use ascorbate,  $\alpha$ -ketoglutarate, and O<sub>2</sub> as their substrates, while their catalytic center contains Fe<sup>2+</sup>.<sup>19,20</sup> The function of PHD is to bind specific residues of HIF-1 $\alpha$  or HIF-2 $\alpha$  subunits and cause ubiquitination, leading to the proteasomal destruction of HIF, thereby preventing HIF from affecting gene

transcription. The role of FIH is to block the co-activators to HIF and prevent its activation. For both FIH and PHD, oxygen serves as a limiting substrate. Thus, when the levels are normal, PHD and FIH are active. When the levels are low, they are both inactive.

The HIF that becomes hydroxylated by PHD is recognized as von Hippel Lindau (VHL) protein. VHL protein is a part of the E3 ubiquitin ligase complex that ubiquitinates proteins for destruction in the proteasome and is mutated in VHL disease.<sup>6,21</sup> With oxygen present, HIF is destroyed in the proteasome and the cell continues aerobic respiration. FIH is also a hydroxylase, but it targets asparagyl residues on the HIF subunits, thereby reducing the transcriptional activity of HIF.<sup>6,22</sup> HIF requires the binding of the co-activators p300/CBP to be transcriptionally active. P300/CBP requires asparagyl residues to identify and activate HIF. When FIH hydroxylates the asparagyl residues, the co-activators are blocked from interacting with HIF.<sup>16</sup>

Based on the  $K_m$  of their respective hydroxylase activity, FIH is functional at lower oxygen levels compared to PHD.<sup>23,24</sup> Of interest, the  $K_m$  of PHD appears to be higher than tissue oxygen partial pressures, leading to a problem which has not been adequately addressed and, therefore, is an area in need of more research. Because FIH is able to repress the hypoxic response at low oxygen tension, a system is needed to regulate FIH, so that HIF can be active in the event of hypoxic conditions. A class of X11 proteins has been found in macrophages with the ability to suppress FIH. Specifically, the protein Mint3/APBA3 is able to interact with the N-terminus to bind and sequester FIH, thereby enabling HIF to avoid the hydroxylation of asparagyl residues.<sup>20,25</sup> With less enzymatically active FIH, HIF can activate, dimerizing the subunits to form a helix–loop–helix transcription factor. HIF can then associate with co-activators p300/CBP and travel to the nucleus to bind to hypoxia response elements (HREs), which are the promoter regions for the genes activated by HIF.

## Effects of HIF

An understanding of how HIF regulates the cellular response is critical for evaluating the effects of this pathway on a larger scale in tissues. Overall, HIF-1 $\alpha$  and HIF-2 $\alpha$  share HRE-binding sites, but activate different sets of genes.<sup>26,27</sup> HIF-1 $\alpha$  is characterized by activation of glycolytic genes, promotion of angiogenesis, induction of apoptosis, and pH regulation via carbonic anhydrase. In contrast, HIF-2 $\alpha$  targets angiogenesis also, but uniquely promotes cell proliferation by transforming growth factor alpha, de-differentiation, and invasion. Hypoxia has been shown to induce an inflammatory response on

this scale, as observed in mountain sickness where hypoxia leads to excessive amounts of proinflammatory cytokines, cell signals (such as IL-6 and its receptor), and C-reactive protein.<sup>6,28</sup> The levels of these cytokines cause the development of vascular leakage and subsequently result in cerebral or pulmonary edema. These cell signals are a direct consequence of HIF and its alteration of transcriptional activity.

Studies have been conducted on healthy volunteers, who stay at elevation and then have their cytokine levels studied. These studies show increases in inflammatory marker levels.<sup>22</sup> Mice studies show that cytokine levels can increase even within relatively short periods of low oxygen tension.<sup>6</sup> Evidence of how hypoxia can induce inflammation in humans can be observed in graft transplantation. Organ grafts are extremely sensitive to hypoxia via ischemia, and the risk of graft failure or rejection increases once hypoxia begins to induce inflammation.<sup>29</sup> However, not coincidentally, inflammation can lead to decreased oxygen delivery and can increase the risk of hypoxia, thus appearing to induce a cycle in which hypoxia can cause inflammation and inflammation can cause further hypoxia.

In the case of inflammatory bowel disease, the link between inflammation and hypoxia is evident. Inflamed mucosa of the intestine in Crohn's disease has been demonstrated after surgical resection to contain cells experiencing more hypoxia within the area affected compared to the non-inflamed mucosa.<sup>6,30</sup> Additionally, levels of both HIF-1 $\alpha$  and HIF-2 $\alpha$  are elevated within these lesions.<sup>31</sup> This provides a physical basis to demonstrate that inflammation can cause hypoxia and that hypoxia can cause inflammation in a positive feedback loop. The consequence of this is that hypoxia and inflammation go hand in hand in disease pathology.

HIF also regulates a variety of angiogenic factors in response to hypoxia. Both HIF-1 $\alpha$  and HIF-2 $\alpha$  increase the transcription of vascular endothelial growth factor, which promotes endothelial cells to proliferate and form new vessels.<sup>32</sup> Through a variety of proangiogenic genes HIF stimulates local angiogenesis by inducing the production of angiopoietin, platelet-derived growth factor, fibroblastic growth factor, and basic fibroblast growth factor.<sup>33</sup> HIF-2 $\alpha$  uniquely regulates erythropoietin (EPO) synthesis and the metabolism of iron. The EPO HRE is an enhancer region for hepatocyte nuclear factor-4, which coincides with EPO production by the liver and kidneys.<sup>34,35</sup> The full description of factors that work along with HIF-2 $\alpha$  in this process have yet to be described.

HIF-1 $\alpha$  has a complex relationship with nitric oxide (NO). At low concentrations, NO appears to inhibit the signaling and facilitate the degradation of HIF-1 $\alpha$ .<sup>36,37</sup> High

concentrations of NO appear to stabilize HIF-1 $\alpha$  during normoxic conditions, but in hypoxic conditions, NO antagonizes the effect of HIF-1 $\alpha$  by promoting PHD transcription and activation via increases in intracellular free iron.<sup>38,39</sup> NO mediates vasodilation and the antimicrobial actions of macrophages, which are integrally related to inflammation and immunity. It appears that hypoxia determines the relationship of NO and HIF-1 $\alpha$  in an incompletely understood manner at this time.

## HIF, inflammation, and immunity

One regulator of inflammation, NF- $\kappa$ B, is a fast acting mechanism that responds to elevated levels of stress.<sup>6</sup> This transcription factor controls the gene transcription after the binding of its ligand to the respective receptor, which activates IKK and I $\kappa$ B kinase. Activated IKK phosphorylates the I $\kappa$ B $\alpha$  portion of the NF- $\kappa$ B heterodimer. This leads to the dissociation of I $\kappa$ B $\alpha$  from the transcriptionally active portion, RelA/p50. I $\kappa$ B $\alpha$  is then ubiquitinated and degraded by the proteasome, while RelA/p50 enters the nucleus and affects genetic expression.<sup>40,41</sup> The affected genes include cytokines, immunoreceptors, cell adhesion molecules, and stress response genes, regulators of apoptosis, enzymes, and other transcription factors.<sup>42</sup>

Not surprisingly, NF- $\kappa$ B, which serves as an immune response and a regulator of homeostasis, interacts with PHD.<sup>6</sup> Evidence for this interaction is observed in ischemia in which hypoxia and HIF activation lead to the activation of NF- $\kappa$ B in correlation with increases in inflammatory cytokines such as TNF- $\alpha$ .<sup>43</sup> One of the factors that NF- $\kappa$ B happens to regulate is the transcription level of HIF-1 $\alpha$ .<sup>44,45</sup> Hypoxia serves as a stress to activate NF- $\kappa$ B, and NF- $\kappa$ B also upregulates HIF and the hypoxia response in a positive feedback loop. This provides a cellular basis to assert that hypoxia and inflammation are connected starting at the cellular level, and that this process culminates in the local effects by which both hypoxia and inflammation contribute to disease.

Inflammation is a part of the immune response and is the body's universal response to stress at the level of tissues. NF- $\kappa$ B affects the expression of many aspects of the body's defenses, such as increasing the presence of antimicrobial factors, increasing phagocytosis, and enhancing adaptive immunity via elevating the creation of light  $\kappa$  immunoglobulin chains.<sup>28</sup> Because HIF is connected to inflammation and amplifies NF- $\kappa$ B, HIF can also be inferred to play a role in immunity.<sup>46</sup> This is demonstrated in experiments in which phagocytes that lack HIF-1 $\alpha$  are shown not to be able to eliminate bacteria efficiently and instead form ulcerative

lesions.<sup>47,48</sup> The role of HIF-1 $\alpha$  in the immune system includes increasing ATP in myeloid cells in hypoxic tissue, increasing antibacterial activity, and preventing neutrophil apoptosis, thereby increasing neutrophil life span in hypoxic tissue.<sup>49</sup>

VHL disease serves as a method for determining the effects of excessive HIF in normoxic tissues. In this condition, a defective VHL protein is unable to ubiquitinate and cause the destruction of HIF after hydroxylation by PHD. Neutrophils, under normoxic conditions in VHL disease, demonstrate less apoptosis and more phagocytosis.<sup>50</sup> The influences of HIF on adaptive immunity are observed in mice models in which mice deficient in HIF-1 $\alpha$  develop elevations of anti-double stranded DNA antibodies, rheumatoid factor, proteinuria, and immunoglobulin deposits in the kidney.<sup>51</sup> Additionally, mice models with elevated HIF-1 $\alpha$  show a higher conversion of type 1 helper T-cells into type 2 helper T-cells. The presence of type 2 helper leads to the inhibition of type 1 helper cells through increases in IL-10 and decreases in interferon- $\gamma$ .

Furthermore, HIF leads to the proliferation of another class of T-cells, or regulatory T-cells. These T-cells regulate extracellular adenosine levels, which inhibit the functions of T-cells.<sup>6</sup> During hypoxia, the conversion of ATP to ADP is enhanced, as is the conversion of ADP to adenosine monophosphate and adenosine monophosphate to adenosine.<sup>52</sup> The effects of HIF include elevating adenosine levels and inhibiting the cellular metabolism of adenosine and cellular uptake of adenosine.<sup>6,53</sup> This leads to the attenuation of innate immunity and inflammation due to decreased T-cell activity, but not its complete resolution. Adenosine serves as an anti-inflammatory signal to balance the degree of inflammation, but it cannot completely stop the inflammation process. Conditions of chronic inflammation, such as Crohn's disease, have shown deficiencies in the enzyme that generates adenosine, CD39.<sup>54</sup> Results from the previously mentioned papers and experiments demonstrate how hypoxia, inflammation, and immunity are interrelated in health.

Evidence demonstrating the link between hypoxia and inflammation continues to mount. Inflammation and immunity have, for some time, been shown to be integral portions of the body's defense mechanisms. Research is now beginning to indicate that hypoxia is also connected to immunity. Instead of understanding hypoxia, inflammation, and immunity separately, a better understanding is achieved by realizing that these processes do not induce diseases on their own. The interactions between these three systems are crucial in maintaining homeostasis, and an imbalance in any part affects the entire system. This imbalance disrupts

homeostasis, and it may be helpful to conceptualize the imbalance as a primary or secondary injury in the development of disease that requires correction.

## Hyperbaric oxygen therapy

When hypoxia leads to disease, the first thought that comes to mind is to provide more oxygen to the patient to alleviate the oxygen deficit. However, it is important to consider how much oxygen reaches the hypoxic tissues. Using Dalton's and Henry's laws, we can see that the physiologic effect of HBOT alters the concentration of oxygen in the plasma and assists hemoglobin to achieve full oxygen-carrying capacity.

Because the partial pressure of oxygen does not affect hemoglobin's carrying capacity for oxygen, the maximum amount that hemoglobin can carry is unaltered and remains at 20 mL O<sub>2</sub>/dL of blood. The only change is in the amount of oxygen dissolved in the plasma. Oxygen partial pressure is 713 mmHg in the trachea when 100% oxygen is given to a patient (see Table 1). This partial pressure of oxygen is dissolved with a coefficient of 0.0024 mL O<sub>2</sub>/(dL of blood per mmHg), yielding 1.71 mL O<sub>2</sub>/dL of blood dissolved in plasma. A total of 21.71 mL O<sub>2</sub>/dL of blood is the oxygen content with the application of 100% oxygen. This is a net increase of 1.47 mL O<sub>2</sub>/dL of blood, or only a 7.26% increase. There are limitations to this application, as oxygen can only be delivered to areas receiving adequate blood supply. As in cases of ischemia, the oxygen does not reach the hypoxic tissues, and the administration of 100% oxygen to the patient is insufficient to correct the underlying pathology.

HBOT uses 100% oxygen in a setting of local artificially elevated pressures generally in the range of 1–3 atm.<sup>3,55</sup> This has practical implications for treating conditions in which tissues are damaged due to oxygen deprivation. HBOT is the use of oxygen under pressure "as a drug to treat basic disease processes and their diseases".<sup>56</sup> The effects of hyperbaric oxygen lead to changes in the transcription of DNA, alterations in the organelles of the cell, improved structure of tissue, and more efficient function of the organ. These changes can become permanent within 25–35 treatments.<sup>42</sup>

In the setting of HBOT at 3 atm of pressure, the amount of oxygen dissolved in the plasma is increased due to the increased partial pressure of oxygen. One atmosphere is equivalent to 760 mmHg; therefore, at 3 atm, the pressure is equivalent to 2,280 mmHg. At a pressure of 2,280 mmHg with 100% oxygen, the partial pressure of oxygen in the trachea is 2,233 mmHg, accounting for the partial pressure of water vapor (see Table 1). Taking into account the solubility factor for oxygen, 0.0024 mL O<sub>2</sub>/(dL of blood per mmHg),

as well as a possible arterial partial pressure of oxygen of greater than 2,000 mmHg, gives a total of at least 4.8 mL O<sub>2</sub>/dL of blood dissolved in plasma and a total oxygen content of 24.8 mL O<sub>2</sub>/dL of blood. This is a net increase of 4.56 mL O<sub>2</sub>/dL of blood, or 22.5%. Of note, this is very close to the tissue oxygen demand at rest. Hemoglobin serves as a reservoir of oxygen, and the dissolved concentration in the plasma represents the river delivering the oxygen from the reservoir. HBOT offers a method to significantly enhance the size and flow of the oxygen river, thereby improving its delivery to stressed tissues. HBOT administration can thus be theoretically advantageous in regard to the calculated values of oxygen delivery.

## Mechanism of action

The manner in which HBOT affects the body as a consequence of primary and secondary effects should be understood. The primary, or direct effects, include correcting the hypoxic condition by increasing oxygen delivery and tension, antimicrobial activity, and the attenuation of the HIF-mediated effects. The secondary effects include indirect consequences of HBOT, such as reducing the formation of ROS, increasing the body's ability to heal, vasoconstriction, and angiogenesis, as well as subduing inflammation.<sup>57</sup> The therapeutic pressures used in HBOT are described in terms of atmospheres of pressure ranging from 1.5 to 3.0 atm. In general, lower partial pressures are favored to avoid barotrauma to the lungs, ear drums, sinuses, and teeth. Oxygen toxicity seizures are rare in clinical use of HBOT, but have been reported.<sup>58</sup> Pressures and duration of HBOT differ between treatment centers, and standardized protocols require further investigation.

The direct effects of HBOT can be observed in its use to treat CO poisoning. The pathology of CO poisoning is based upon the 200 times greater affinity of CO to irreversibly bind hemoglobin instead of oxygen. Once CO reaches the alveoli, it outcompetes oxygen for the binding sites on hemoglobin. Carboxyhemoglobin is the result of this interaction, which alters the ability of hemoglobin to release bound oxygen, leading to generalized hypoxia. Without any intervention, the half-life of carboxyhemoglobin is ~5 hours.<sup>41</sup> The effects of low oxygen are also complicated by CO's ability to bind to cytochrome *c* oxidase of the ETC, as demonstrated *in vitro*.<sup>59,60</sup> This can compound the energy deficit due to hypoxia.

The administration of HBOT in the setting of CO poisoning produces robust results. Utilizing 100% oxygen at a pressure of 2.5 atm, the half-life of carboxyhemoglobin can

be reduced to only 20 minutes.<sup>61,62</sup> The higher partial pressure of oxygen involved in HBOT allows oxygen to more rapidly titrate the CO from the hemoglobin, therefore alleviating the oxygen and energy deficits that the body experiences. A faster reversal agent for CO poisoning correlates with less sequelae. This is an example of the direct effect of HBOT on disease.

A lack of oxygen predisposes to an increased risk of infection due to weakening of the neutrophils' ability to form oxygen radicals for bactericidal activity.<sup>63,64</sup> HBOT can restore this imbalance of the innate immune system by regenerating the neutrophils' innate bactericidal activity, as well as through its own antimicrobial effect.<sup>41</sup> The bactericidal activity of HBOT acts against some anaerobic bacteria, such as *Clostridium perfringens*. HBOT confers an additional benefit of suppressing alpha toxin activity.<sup>65</sup> Nicotinamide adenine dinucleotide phosphate oxygenase requires oxygen to catalyze the formation of ROS in phagocytes to kill the bacteria. Increased demand for oxygen by the neutrophils can be mitigated by HBOT, thereby allowing the immune system to function properly.<sup>66</sup> Bacteriostatic effects, such as the suppression of bacterial cell growth and division, have been demonstrated against several species of *Escherichia* and *Pseudomonas* and were shown to result from the direct action of HBOT. This direct effect exemplifies oxygen's linkage with the immune response.

As discussed previously, HIF requires low oxygen levels to dimerize and bind to HRE. HBOT creates a hyperoxic condition, in which the activity of both PHD and FIH to block HIF activation has recently come into question. A study involving hypoxia, normoxia, and hyperoxia at 30% oxygen showed that xenografts in hyperoxia expressed higher levels of HIF-1 $\alpha$ .<sup>67</sup> This observation requires verification, and future studies need to assess for the activity level of HIF in hyperoxic conditions. Without further data to indicate HIF activity in hyperoxia, it is hypothesized that the actions of PHD and FIH would break the positive feedback loop mechanism whereby hypoxia induces inflammation and inflammation induces hypoxia, preventing the damage done by this cycle. Additionally, inactivation of HIF helps the cells maintain their oxidative metabolism, thereby making more ATP available. Cells with more ATP are better suited to withstand the insults of hypoxia, and are therefore more likely to avoid apoptosis and dysfunction.

Indirectly, HBOT leads to improved healing, less inflammation, reduced radical oxygen species, vasoconstriction, and angiogenesis. The healing effects of HBOT are due to the secondary effects of hyperoxia on the tissue. Hyperoxic tissue does not dilate (other than in the lung) and instead constricts,

which can cause situational vasoconstriction-induced peripheral ischemia in extreme cases.<sup>43</sup> This is extremely helpful in reducing edema and controlling secondary injury after the initial insult. High oxygen concentrations inhibit the formation of superoxides by the neutrophils, aiding the healing process by preventing further inflammation and tissue damage.<sup>68</sup> Oxygen is also required by the fibroblasts to form collagen and perform angiogenesis. Hyperoxic tissue shows faster and more complete healing due to the readily available oxygen required for collagen formation. With an increased ability to make new vessels, nutrients are delivered more rapidly to the injured area by newly formed capillary beds, allowing for better recovery. The largest factor that HBOT indirectly assists with is the avoidance of reperfusion injury.

Once the insult is resolved, re-establishing oxygen balance leads to reperfusion injury. Hypoxic or ischemic vessels become targets for neutrophils. Neutrophils adhere to hypoxic vessels and begin to scar the local tissue by releasing proteases and free radicals, causing extreme vasoconstriction.<sup>55,69</sup> These effects decrease the blood flow to the local area and end in tissue destruction. The behavior of neutrophils is akin to remodeling a house and tearing down everything to build it back up. However, this process does not always repair the tissue back to complete functional status and, in many cases, can lead to greater dysfunction. HBOT has been shown in rats to inhibit neutrophil adherence to damaged vessels and ease postischemic vasoconstriction.<sup>55,70</sup> More studies are needed to fully appreciate the degree to which HBOT can attenuate reperfusion injury.

The secondary benefits of HBOT include reducing inflammation, attenuating reperfusion injury, promoting wound healing, and improving circulation. The primary benefits include increased oxygen tension, antimicrobial activity, and blocking HIF activity. Overall, HBOT is a safe and well-tolerated therapy when used under the direction of experienced and licensed treatment facilities. Side effects of HBOT are self-limiting and rare due to screening.<sup>71</sup> HBOT has so many potential benefits and modalities for its use. It seems strange that HBOT has failed to gain widespread support. This is likely due to the history of HBOT and public opinion.

## History of HBOT

The history of hyperbaric therapy is linked to diving and diving medicine. The oldest records show that in 4,500 BC, humans were diving at depths of no more than 30 m unaided.<sup>2</sup> Aristotle described cauldrons of air that were used to let divers breathe while going into the water for short periods of time during the fourth century BC. It was not until the



invention of the modern diving bell that humans were able to dive lower for longer lengths of time. The understanding of air pressure discovered in the use of the diving bell led to the creation of first hyperbaric chambers in the 17th century.

In 1662, a British doctor named Henshaw built an airtight chamber known as the “domicilium” and performed the first hyperbaric treatments. Hyperbaric chambers predated the discovery of oxygen and utilized air bellows along with valves to increase or decrease the pressure inside. Oxygen was discovered in 1775 by the Englishman Joseph Priestly, and its toxic effects were later described by Lavoisier and Seguin in 1789.<sup>2</sup> The toxicity of oxygen led to hesitation for the use of HBOT for almost 100 years. During the 1800s, several attempts to use hyperbaric therapy were undertaken by various individuals in Europe to treat pulmonary afflictions, flu, kidney failure, and even nervous disorders.

The first chamber in the US was built in 1861 in New York. Paul Bert, a French engineer, physician, and scientist, wrote a book entitled *La Pression Barometrique* in 1872. This text was one of the first references which described the physiological effects of air under increased and decreased pressure.<sup>2</sup> His research led him to discover oxygen toxicity causing seizures in 1878. Oxygen continued to be viewed as toxic in response to these findings by Bert, and hyperbaric therapy continued to avoid the use of oxygen.

Despite the information from Bert, hyperbaric chambers continued to be built across the world. The first positive correlation using HBOT came from Valenzuela who published the effects of HBOT on rabbits in 1887.<sup>72</sup> He noted that rabbits given HBOT experienced fewer febrile events and higher survival after injection with “putrid contents” coming from a dead rabbit. By 1891, doctors in the US began examining the use of HBOT for neurological illnesses. Even though hyperbaric chambers became more common, it took until the end of World War I for clinical indications to be developed for HBOT.

In the 1920s, Dr Orval J Cunningham utilized the largest hyperbaric chamber in the world to treat many conditions in Kansas City, KS. Fire from open gas burners caused an explosion in his hyperbaric chamber one day during 1923. Patients were safely evacuated from the hyperbaric chamber without any fatalities.<sup>2</sup> Dr Cunningham continued to treat patients with hyperbaric therapy despite this incident. Then, tragedy struck one night when a mechanical failure led to the loss of pressure of his chamber, killing all the patients inside. After this latest incident, Dr Cunningham sought the support of industrial tycoon HH Timken. Together, Timken and Dr Cunningham built the largest hyperbaric chamber

in the world in Cleveland, OH. This chamber was 64 ft in diameter, five stories tall, and opened in 1928. The floors of this facility housed 12 bedrooms where patients would live for weeks. Dr Cunningham was given opportunities to report on the evidence in support of hyperbaric therapy, but he never added to the literature. He was censured by the American Medical Association in 1928, and by 1937, his five-storied chamber was dismantled and sold for scrap.

Hyperbaric oxygen was first used in 1937 by Behnke and Shaw to treat decompression sickness.<sup>2,73</sup> After the success of Behnke and Shaw, multiple uses for HBOT were explored. A year later in 1938, Ozorio de Almeida and Costa treated patients suffering from leprosy with HBOT. By 1960, Sharp and Smith treated cases of CO poisoning using HBOT. In 1961, Boerema and Brummelkamp used HBOT for gas gangrene. Perrins demonstrated the use of HBOT for osteomyelitis in 1965. Then in 1983, the American College of Hyperbaric Medicine was founded by Dr Neubauer. During this time, the safety features and designs of hyperbaric chambers improved as the indications for therapy expanded. HBOT is currently administered in chambers of varying sizes ranging from personal units to surgical operating rooms every day.

HBOT is currently studied by the use of animal models and in patients with an emphasis on neurological conditions. Studies are examining HBOT in treating subarachnoid hemorrhage, ischemic stroke, intracranial hemorrhage in combination with thrombolysis, stents, or medication, asphyxiation, and traumatic brain injury.<sup>74,75</sup> Results show great promise in the future use of HBOT to alleviate damage to neurons, but further work is needed.<sup>76–80</sup> Other recent studies and reviews have shown mixed results in assessing the therapeutic benefits of hyperbaric oxygen in a variety of conditions such as diabetic ulcers and autism.<sup>81–83</sup> However, these papers recommend that further investigation be performed as there are problems with the current data, including poor documentation, poor methodologies, a paucity of studies, and a lack of randomized clinical trials. As the information accumulates, the uses of HBOT may expand to include treatments for brain injury, osteonecrosis, cerebral palsy, autism, graft or flap transplants, multiple sclerosis, dementia, stroke, drowning, near hanging, cardiac arrest, acute coronary syndrome, neurotoxin exposure, radiation tissue injury, and even cancer.<sup>56,84–91</sup>

## Current indications of HBOT

Current indications for the use of HBOT are shown in Boxes 1–3.<sup>57,92–94</sup>

**Box 1** Current indications of hyperbaric oxygen therapy

Current indications of HBOT		
Carbon monoxide poisoning	Air or gas embolism	Clostridial myositis; myonecrosis
Acute thermal burns	Compromised grafts and flaps	Decompression sickness
Arterial insufficiencies	Necrotizing soft tissue infections	Severe anemia
Delayed radiation injuries	Refractory osteomyelitis	Intracranial abscesses
Osteoradionecrosis	Osteomyelitis of the jaw	Aggressive periodontitis
Compartment syndrome, crush injuries, or other acute traumatic ischemia		
Adjunctive therapy for the placement of implants in irradiated jaws		

**Box 2** Relative contraindications

Relative contraindications		
Seizure disorder	Pregnancy	Claustrophobia
Uncontrolled hyperthermia	History of thoracic or ear surgery	Upper respiratory infection
Emphysema with carbon dioxide retention	Asymptomatic pulmonary lesions seen on X-ray	

**Box 3** Absolute contraindication

Absolute contraindication
Pneumothorax

**Summary**

HBOT has many current indications for treatment due to its ability to counter oxygen deficits, promote healing and angiogenesis, fight infection, and control inflammation. Inflammation is the body's response to all insults and, in some instances, the body loses control over the system. This can lead to chronic disease or play a role in the development of disease. Evidence supporting the utility of HBOT as an anti-inflammatory treatment is growing. HBOT offers the possibility of a new drug class and will require more research to determine its dosing and indications for treating disease. The use of HBOT to treat the secondary injury process that causes damage in acute conditions could prove to be very valuable.<sup>56</sup> Technological advances that make HBOT more available promote its potential to fight death and disability.

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The author reports no conflicts of interest in this work.

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