The oxygen trail: tissue oxygenation

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Aerobic cellular respiration depends on the efficient supply of oxygen and substrate to the mitochondria. There is an oxygen cascade from the environment to the subcellular environment. Efficient oxygen delivery depends on the coordinated interaction between the respiratory and circulatory systems. The circulation at both macro- and microvascular levels is under the control of humoral and neural factors. There is local autoregulation of flow at the tissue level by metabolic factors which reflect the energy state of the tissues. The response to hypoxia involves the activation of cytokines and genetically controlled factors which maximise capillary perfusion and haemoglobin concentration, and regulate cell metabolism. The formation of reactive oxygen species under such conditions has a detrimental effect on the mitochondria with respiratory chain dysfunction, increased permeability transition, and cell death. This review aims to explore the mechanisms by which the body attempts to maintain tissue oxygen levels at conditions optimal for cell survival.

Evolutionary adaptations have resulted in many changes that have conferred survival advantage. Central to this is the evolution of aerobic cellular metabolism with more than 90% of the body’s oxygen consumption being utilised by a single enzyme, cytochrome oxidase. This is part of the oxidative phosphorylation pathway which generates ATP. Cells derive their energy requirements from high energy phosphate bonds, predominantly ATP. This results in hydrolysis of ATP to ADP, Pᵢ (inorganic phosphate) and H⁺ ions. ATP is then regenerated by the mitochondria from these same metabolites in the presence of molecular oxygen. The most efficient means of producing ATP is by oxidative phosphorylation with glucose as substrate and molecular oxygen as the terminal oxygen acceptor. A total of 38 molecules of ATP are generated per molecule of glucose compared to just 2 by anaerobic metabolism. Anaerobic respiration utilises the glycolytic pathway as well as pathways that utilise the creatine kinase reaction. H⁺ ions are generated as a consequence and can lead to a systemic metabolic acidosis if severe.

The function of the lungs, heart and vasculature is to ensure a continuous and adequate supply of oxygen and substrate to the tissues.
to maintain cellular integrity and function. Under normal physiological conditions the amount of oxygen delivered to the tissues (approximately 1000 ml/min) is considerably in excess of the body’s requirements (approximately 250 ml/min). This ‘spare capacity’ enables the body to cope with a fall in oxygen delivery ($DO_2$) without initially compromising aerobic respiration and oxygen consumption ($VO_2$). This is known as supply-independent oxygen consumption (Fig. 1). However, beyond a certain point, $VO_2$ falls in line with falls in $DO_2$ (supply-dependency). In elegant laboratory studies on dogs, Schlichtig demonstrated this to consistently occur at an oxygen delivery of around $3.03 \pm 1.08$ ml/kg/min. He coined the phrase ‘dysoxia’ to describe tissue status below this point.

Anaerobic metabolism will thus occur either when $DO_2$ falls below a critical point (e.g. with severe haemorrhage, heart failure or hypoxaemia) or oxygen demands increase beyond the ability of oxygen supply and tissue oxygen extraction to meet that need (e.g. beyond $VO_2$ max with severe exercise). A third possibility is that sufficient oxygen may be available to the mitochondria but they are unable to utilise it. This may be due to inhibition either at the level of cytochrome oxidase or at a point upstream in the electron transport chain. This ‘histotoxic hypoxia’ is recognised in cyanide and carbon monoxide poisoning; it may also be implicated in the pathophysiology of sepsis-related organ dysfunction, possibly via nitric oxide and its metabolites, in particular peroxynitrite, which have been shown ex vivo to inhibit Complexes I and IV of the mitochondrial electron transport chain.

**Oxygen transport**

The transport of oxygen from air to mitochondria involves a series of convective and diffusive processes. Atmospheric oxygen is moved by convection down the airways to the alveoli. Thereafter, the oxygen undergoes a process of alveolar mixing, primarily by diffusion though aided by convective forces within the lungs. Oxygen diffuses out of alveolar gas into the pulmonary capillaries. This is a passive process and the degree to
which haemoglobin is loaded with oxygen during capillary transit of pulmonary blood depends on the diffusive conductance for oxygen. Diffusion of oxygen is more complete with higher diffusive/perfusive conductance ratios. The oxygen bound to haemoglobin in red blood cells is then convectively transported to the tissues. Finally, oxygen diffuses out of the microcirculation, across the interstitium and cell membranes, and finally into the mitochondria. The ratio of organ tissue diffusive conductance to organ perfusive conductance determines the degree to which unloading of oxygen is complete.

**Implications of the oxyhaemoglobin dissociation curve**

The non-linearity of the O₂ dissociation curve affects O₂ diffusivity (Fig. 2). For any given diffusive conductance the O₂ flux will be a function of the driving PO₂ gradient across that diffusive barrier. This system is optimised for maximum O₂ diffusion in the lungs. The PO₂ is well preserved despite a maximal O₂ flux during the initial steep part of the curve and falls only in the flat part of the curve when unloading is complete. The converse is true of O₂ unloading to the tissues. Hence, in normoxia, O₂ loading in the lungs is often complete whereas tissue unloading is compromised when the system is stressed by exercise or disease⁴-⁷. The rate of O₂ unloading to the tissues is slow as the red blood cell passes through the microcirculation; the diffusion constants are 100,000-fold lower than in alveolar gas. This may reflect an adaptive mechanism which overcomes the microvascular heterogeneity of O₂ supply.

**Factors affecting oxygen delivery (DO₂)**

Tissue O₂ delivery is the product of cardiac output (Q) and arterial O₂ content. Since dissolved oxygen accounts for a negligible fraction of the oxygen transported in blood, under normal atmospheric conditions:

\[
DO₂ = Q \times Hb \times SaO₂ \times 1.39
\]

Eq. 1
where 1.39 (Huffner's constant) is the amount of O\(_2\) (in ml) carried per g of Hb at sea level.

Factors that affect DO\(_2\) are those that affect cardiac output and its regional distribution and those which control the arterial oxygen content.

**Factors affecting oxygen uptake in the lungs**

**Pulmonary function**

Under basal conditions, most of the O\(_2\) transfer across the alveolar-capillary membrane occurs within one-third of the transit time for blood in the pulmonary capillaries. Even in healthy volunteers, the presence of a ventilation perfusion (V/Q) mismatch and O\(_2\) diffusion limitation will reduce SaO\(_2\) below that expected at VO\(_2\) max. In cardiovascularly trained athletes, the diffusive/perfusive conductance ratio is reduced\(^8\); as these individuals have high blood flows, however, there is no improvement in O\(_2\) diffusive conductance with training. Therefore, at VO\(_2\)max, there is a more marked desaturation in this group\(^9\). This effect can be overcome by increasing the inspired O\(_2\) concentration\(^8\). There has been some debate as to whether mechanical ventilatory limitations affect convective transfer of O\(_2\) to the alveoli and, thereby, affect O\(_2\) transport\(^11,12\). The current consensus is that this is not likely to be profound except in trained athletes where the O\(_2\) cost of breathing is very high at VO\(_2\)max.

Lung disease will produce hypoxaemia through a number of mechanisms. V/Q mismatch is often the major cause of inadequate pulmonary gas exchange while diffusion limitation is not a significant factor except in diseases with interstitial fibrosis. There is an attempt at passive compensation for diffusion limitation by increasing alveolar PO\(_2\) through reducing O\(_2\) flux into the pulmonary capillaries, thereby maintaining the diffusion gradient\(^12\). In most acute forms of lung disease, there is an element of right to left shunting of blood through inadequately oxygenated alveoli. In chronic diseases, V/Q mismatch plays a more significant role\(^12-14\). Both intrapulmonary shunts and V/Q mismatch can be worsened by inappropriately high or low cardiac outputs\(^15-17\).

In health, the arterial Hb is virtually fully saturated with O\(_2\) at sea level\(^5\). Further increasing the inspired oxygen to 100% at sea level augments VO\(_2\)max only slightly\(^18\). With altitude, VO\(_2\)max decreases when the steep portion of the O\(_2\) dissociation curve is reached.

**Macroversal factors**

**Cardiovascular function**

Maximal VO\(_2\) in health correlates with cardiac output and organ blood flow. Blood flow is probably the most influential factor that determines
tissue oxygenation as it determines the rate of convective transport of \( \text{O}_2 \) to the organs. However, it is not the only critical factor. The ratio of diffusive to perfusive conductance appears to be inversely related to blood flow. As diffusive equilibration of oxygen is an exponential function of the ratio of diffusive/perfusive conductance, both uptake and unloading of \( \text{O}_2 \) may be impeded when blood flow is increased\(^{19}\). Therefore, the beneficial effect of increased blood flow on convective \( \text{O}_2 \) transport is offset by its negative effect on diffusive \( \text{O}_2 \) transport. In disease states, the ability to sustain increases in cardiac output may be limited and there may also be aberrant control of microvascular distribution of organ blood flow. The presence of extra/intracellular oedema may also result in reduced local perfusion and diffusive conductance.

**Haemoglobin concentration (Hb)**

The perfusive \( \text{O}_2 \) conductance is the product of the slope of the oxyhaemoglobin dissociation curve and blood flow\(^{19}\). The Hb concentration determines the slope of the \( \text{O}_2 \) dissociation curve and, hence, the rate of diffusive equilibration in the lung and tissues. Changes in Hb or blood flow affect \( \text{O}_2 \) transport similarly by affecting both convective and diffusive pathways. However, these changes could also affect other parts of the oxygen transport chain, resulting in additional changes. A reduction in Hb results in reduced impedance and an increase in cardiac output and regional flow by the Hagen-Poiseuille equation. It may also have an effect on the distribution of blood flow both between and within organs. A reduction in Hb will cause a fall in diffusive \( \text{O}_2 \) conductance *per se*\(^{20,21}\). The surface area available for effective transfer of \( \text{O}_2 \) between red blood cell and capillary wall is reduced\(^{23}\). A reduced Hb results in a reduction in the number of binding sites for molecular oxygen, and reduces the rate of combination of Hb with \( \text{O}_2 \). Finally, it is also possible that a reduced Hb results in an increased heterogeneity of red blood cells in the microcirculation.

**Microvascular factors**

These include both central and local factors\(^{24}\). Central regulation of redistribution of regional blood flow is mediated by sympathetic vasoconstrictor tone whereas local regulation of perfusion within the organs is mediated by vasodilator tone and the recruitment of capillaries. This is subject to autoregulation\(^{25}\).

Vascular reactivity determines the distribution of \( \text{O}_2 \) delivery between and within organs\(^{26}\). When whole body DO\(_2\) is compromised, flow is redistributed to organs unable to significantly increase oxygen extraction ratios to sustain activity and function. By the same virtue, flow is reduced to those organs that are able to extract oxygen from haemoglobin\(^{27}\). This
redistribution of whole body DO$_2$ has been shown to be a determinant of whole-body critical DO$_2$ in a mathematical model$^{27}$. Redistribution of whole-body DO$_2$ to match metabolic requirements and the oxygen extraction ratios of the tissues enables the animal to maintain whole body VO$_2$ in a supply independent state$^{27}$. The sympathetic system which mediates this redistribution of DO$_2$ also improves the efficiency of O$_2$ extraction in response to hypoxia or ischaemia$^{28-30}$ by maintaining arteriolar tone and preventing ‘vascular steal’ from tissues with a high metabolic rate.

**Microvascular architecture**

The microvasculature can be subdivided into flow controlling vessels (medium-sized arterioles) and distribution vessels (smaller arterioles)$^{31}$. Most of the arteriovenous pressure gradient is dissipated at arteriolar level, though the distribution of flow within the tissues is determined by autoregulation at the level of the precapillary sphincters or precapillary arterioles. It has been suggested that arteriolar control of flow is under the regulation of sympathetic vasoconstrictor tone and regional factors$^{32}$, while distributive vessels are subject to metabolic autoregulation. It is the balance between sympathetic vasoconstrictor tone and metabolically regulated vasodilator tone that matches O$_2$ supply to demand. Interstitial PO$_2$, which is determined by the adequacy of tissue oxygenation, may be the mediator of this vasodilation by its effect on vascular smooth muscle. Cardiovascular reflexes in response to hypovolaemia may further augment the efficiency of local O$_2$ extraction. The microcirculation is also under the control of humoral factors (renin, vasopressin) and neural factors (Fig. 3). These factors assume varying significance based on the specific organ and its sensitivity.
Oxygen sensing structures

Vascular smooth muscle relaxation is induced in hypoxic tissues probably via activation of ATP-sensitive K\(^+\) channels\(^{33}\). These channels are activated by metabolites such as H\(^+\), K\(^+\) ions, adenosine and lactate, which are released by hypoxic or ischaemic parenchymal cells. Ensuing vasodilatation often improves tissue perfusion. Other mechanisms include CO\(_2\) back-diffusion from venous to arterial blood which improves oxygen unloading by the Bohr effect\(^{34}\). There may also be a direct response of arterial vessels to either O\(_2\) content or tension within the vessel.

Endothelial cells are effective O\(_2\) sensors\(^{35}\). They release autocoids which affect vascular tone and platelet function, e.g. prostacyclin (PGI\(_2\)), nitric oxide (NO) and an ‘endothelium-dependent hyperpolarizing factor’ (EDHF). PGI\(_2\) achieves vasodilation by adenylate cyclase stimulation and by increasing intracellular cAMP. NO stimulates cGMP production by guanylate cyclase in vascular smooth muscle while EDHF mediates its vasodilatory effects through the stimulation of ATP-sensitive K\(^+\) channels in vascular endothelium and smooth muscle. These channels are ideally placed to mediate changes in vascular tone in response to changes in the level of intracellular metabolites. Autocoids are released in response to both receptor-dependent mechanisms and physical stimuli. Hypoxia per se is thought to significantly augment NO, PGI\(_2\) and EDHF production and to activate ATP-sensitive K\(^+\) channels\(^{36}\). Endothelial cells have also been shown to be involved in O\(_2\) extraction\(^{37}\), while endothelial sensing of hypoxia in conduit vessels may have a role in the adjustment of global tissue perfusion.

O\(_2\) diffusion from microvasculature to mitochondria

There is probably a finite rate of oxygen diffusion from capillaries to mitochondria which limits mitochondrial O\(_2\) supply except in the case of tissues containing myoglobin. The main impedance to O\(_2\) flux in muscle seems to be over the distance between Hb and sarcolemma and not over the greater distance from sarcolemma to mitochondria\(^{38}\). This has been attributed to myoglobin facilitation of O\(_2\) flux. Capillary surface area and not O\(_2\) diffusion distance seems to determine muscle O\(_2\) diffusional conductance\(^{39}\). Thus the diffusional conductance for O\(_2\) is modified by several factors such as Hb, factors that determine O\(_2\) transfer out of the RBC into plasma and into the cell (e.g. O\(_2\) content, O\(_2\) tension in the capillaries and the interstitium), and myoglobin facilitated diffusion of O\(_2\).
Mitochondria, cell metabolism and oxygen

Over 90% of total body oxygen consumption is used by the mitochondrial respiratory chain enzyme, cytochrome oxidase, as the terminal electron acceptor in oxidative phosphorylation. Oxygen transport is tightly regulated by control of ventilation, circulation and red cell mass. In mammals, hypoxia results in inhibition of conductance through potassium channels in the carotid body. It also causes rapid induction of the gene coding for tyrosine hydroxylase which is the rate-limiting step in the synthesis of the neurotransmitter, dopamine. The consequence is an increase in respiration. Hypoxia also stimulates the production of erythropoietin and results in erythropoiesis. Tissue oxygenation seems to regulate expression of certain genes in endothelial cells, including growth factors such as platelet derived growth factor B, interleukin-1, interleukin-8 and endothelin. Other genes that are regulated include those that code for adhesion molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and endothelial leukocyte adhesion molecule-1. Hypoxia induces the expression of genes which encode a number of cytokines including vascular endothelial growth factor (VEGF) and also acute phase proteins. Cellular oxygen tension regulates intermediary metabolism by affecting the expression of genes encoding enzymes that are responsible for glycolysis, gluconeogenesis, and glucose transport. On the other hand, hyperoxia results in adaptive responses such as the induction of genes that encode enzymes that detoxify reactive oxygen species.

Oxygen sensing

Molecular oxygen has a dual role in that it acts as a biological signal by combination with oxygen sensing structures but, more importantly, has a central role in cell metabolism. Oxygen transport depends on the presence of an adequate haemoglobin mass in circulation that is capable of unloading oxygen to the tissues at relatively high oxygen tensions. To monitor fluctuations in oxygen transport, the sensor needs to be a compound with relatively low oxygen affinity. The biological systems involved appear to be a haem protein, a microsomal mixed function oxidase (involved in peroxide production), NAD(P)H oxidase, and mitochondria. There is preliminary evidence that a multisubunit b-cytochrome, which changes its conformation on binding to oxygen, may also act as a sensor.

The oxygen tension in the mitochondria is very low and is dependent on metabolic fluctuations. The genes responsible for critical mitochondrial functions do not appear to be regulated by an oxygen sensing system. It is well recognised that reactive oxygen intermediates act as major...
intracellular signals for the hypoxic induction of genes such as the erythropoietin (Epo) gene. Even though mitochondria are the main sources of superoxide ion during electron transfer reactions, the levels are dependent on the metabolic activity of the cell. Furthermore, there is an abundance of mitochondrial superoxide dismutase, which makes it unlikely that mitochondrial superoxide is an important signal for gene induction. However, there is some evidence that mitochondria are involved in oxygen sensing in the carotid body.

In summary, it seems that most cells share a common oxygen sensing mechanism in the form of a cytosolic, membrane bound, multisubunit b-like cytochrome. This sensor molecule binds oxygen and reduces it to superoxide, generating reactive oxygen intermediates. These reactive oxygen intermediates act as chemical signals which impact on transcription factors that regulate oxygen-responsive genes.

**Signal transduction**

Signal transduction by hypoxia involves both protein phosphorylation and redox chemistry. A number of genes are induced by reactive oxygen intermediates, and these help protect the cell against oxidant damage. Reactive oxygen intermediates act as chemical signals of changes in intracellular oxygen concentration. They initiate changes in the structure and function of appropriate transcription factors by means of oxidation-reduction (redox) modification of sulphydryl groups in proteins. The common factors studied have been AP-1 transcription factor and NFκB.

NFκB is a trimer composed of two DNA binding proteins and an inhibitory subunit (IκB), which keeps the compound sequestered in an inactive form in the cytoplasm. A variety of signals activate NFκB, by increasing the intracellular levels of reactive oxygen intermediates and depleting the cell of glutathione. NFκB is activated by the dissociation of IκB from the complex, the dimer then binds to the nuclear DNA and causes the induction of genes important in inflammation and immune responses.

Other transcription factors involved include heat shock proteins and basic helix-loop-helix proteins. Hypoxia inducible factor (HIF-1) is a heterodimer composed of two such transcription factors.

**Genetics**

Hypoxia results in the induction of certain physiologically relevant genes, through transcription of HIF-1. The production of Epo is increased up to 1000-fold, due to induction of the Epo gene. In acute hypoxia, the liver contributes up to 33% of total Epo production. VEGF
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is also induced and is responsible for the regulation of angiogenesis as an adaptive mechanism to local hypoxia. Lastly, the genes involved in glucose metabolism are also subject to regulation by hypoxia. Under anaerobic conditions, the cells resort to glycolysis. As the ATP yield during glycolysis is very low, to maintain energy supply, the rate of glucose consumption has to increase significantly. The rate limiting step in this process is the transport of glucose across the plasma membrane. Induction of genes coding for glucose transport is an important adaptive mechanism. Regulation of genes that encode glycolytic enzymes has not been well understood, and the effect of hypoxia varies among cell types. Endothelial cells seem to be better able to tolerate hypoxia than other cell lines, due to a greater level of the high energy phosphates, ATP and GTP. Hypoxic endothelial cells produce a novel set of proteins, such as glyceraldehyde-3-phosphate dehydrogenase, aldolase, triose phosphate isomerase, and lactate dehydrogenase. Hypoxic induction of these genes appears to be isozyme-specific. The balance between glycolysis and gluconeogenesis in the liver is probably determined by the state of tissue oxygenation. In the model of metabolic zonation, the hepatic lobule is divided into zones based on the oxygen tension. In the well oxygenated periportal zone, there is increased expression of phosphoenolpyruvate carboxykinase, resulting in an increase in gluconeogenesis. In the relatively hypoxic perivenous zone, there is an increased expression of hexokinase and pyruvate kinase, thus favouring glycolysis.

Reactive oxygen intermediates/ischaemia reperfusion injury

Cell injury is a consequence of hypoxaemia or ischaemia and is often exacerbated by subsequent re-oxygenation or reperfusion. This is mediated by reactive oxygen intermediates (ROI). One of the consistent sources of ROI is the mitochondrial respiratory chain. Approximately 2% of mitochondrial oxygen consumption results in generation of superoxide anion ($O_2^-$). This is due to mono-electron reduction of the oxygen molecule at complexes I, II, and III. Other more reactive species such as the hydroxyl radical (‘OH) and hydrogen peroxide ($H_2O_2$) are formed from superoxide. However, mitochondria have a very efficient anti-oxidant defence system due to the presence of manganese superoxide dismutase, glutathione peroxidase, glutathione reductase, NAD(P) transhydrogenase and compounds such as glutathione, NADPH, vitamins C and E.

Depletion of antioxidants and/or excess production of ROI play a major role in the pathogenesis of cell injury and disease. Mitochondrial redox components (NAD+, ubiquinone, and the cytochromes) are normally in a oxidised form under aerobic conditions. During severe hypoxaemia and ischaemia, they may be almost completely reduced.
This condition is termed ‘reductive stress’ when reactions occur between molecular oxygen and other electron donors in the respiratory chain resulting in the generation of ROI. This escalates further on re-oxygenation or reperfusion. There is also an abnormal oxidation of antioxidants such as NAD(P)H and glutathione with oxidative stress which are then unable to detoxify the ROI. In complete anoxia, electron transfer components are reduced but ROI formation is not possible due to the lack of molecular oxygen. However, on re-oxygenation, generation of ROI is rapidly terminated by re-oxidation of the electron carriers of the respiratory chain. On the other hand, conditions of low flow or intermittent ischaemia/hypoxaemia and reperfusion/re-oxygenation may result in increased formation of ROI, causing further cell injury.

In conditions where the level of anti-oxidants is reduced or the levels of ROI are increased, a state of ‘oxidative stress’ is created. Oxidative alteration of mitochondrial membrane components occur, such as lipid peroxidation and protein thiol oxidation. This results in mitochondrial permeabilisation and dysfunction with mitochondrial swelling, membrane depolarisation and uncoupling of oxidative phosphorylation. Mitochondrial damage caused by Ca\textsuperscript{2+} or other inducers is related to an increase in mitochondrial ROI production and is characterised by oxidation of membrane protein thiols. Intracellular Ca\textsuperscript{2+} enhances the formation of hydroxyl radicals by stimulating superoxide production. This mobilises the intramitochondrial Fe\textsuperscript{2+} necessary to drive the Fenton reaction. When membrane protein oxidation and aggregation becomes extensive, membrane permeabilization becomes irreversible. Ca\textsuperscript{2+} plays a vital role in this process by stimulating the production of ROI in the electron transport chain, stimulating the mobilisation of matrix Fe\textsuperscript{2+} to drive the Fenton reaction, unmasking membrane protein thiol groups and having a direct effect on permeability transition pores. These pores play a vital role in maintaining the integrity of the cell membrane. In conditions of oxidative stress such as ischaemia/reperfusion, hypoxaemia/re-oxygenation, or sepsis with microvascular alterations in DO\textsubscript{2}, mitochondrial damage and apoptosis can thus occur.

**Conclusion**

This review has attempted to demonstrate the complex relationship between oxygen and the body, with the obvious benefits and harm that imbalance in the supply-demand ratio can bring. The body responds to changes in this ratio by respiratory, macrovascular, microvascular, hormonal and cellular mechanisms which inter-relate in numerous ways. Increased understanding of these mechanisms will lead to more appropriate monitoring and interventions.
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