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HIF-1 ACTIVATION AND INFLAMMATORY RESPONSES TO HYPOXIA

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Acute hypoxia is a significant physiological danger during high-altitude flying and military aircraft missions. The human brain requires a constant supply of oxygen to function properly. It is consequently susceptible to settings with low availability of air oxygen. At high altitude, the decreased barometric pressure (hypobaria) decreases the partial pressure of inspired oxygen (PiO2), resulting in hypoxic hypoxia, henceforth referred to as hypoxia . The subsequent hypoxemia induces a metabolic shock that inhibits brain function and, as its severity increases, leads to loss of consciousness and death. For instance, an inboard leak within a pressurised cabin or when ascending in an unpressurized aircraft beyond 10,000 feet, hypoxia may develop gradually (Shaw et al., 2021).

In addition, the emergence of tightly regulated metabolic pathways that exploit the chemical diversity of molecular oxygen has played a crucial role. The majority of eukaryotes have adapted to rely on chemical reduction of molecular oxygen during mitochondrial oxidative phosphorylation as their principal source of energy for fundamental function and survival (Wilson, 2017). The coordinated function of the respiratory, cardiovascular, and red blood cell systems in animals is to systemically regulate oxygen delivery for optimal maintenance of tissue and organ metabolism. However, hypoxia can influence inflammatory signalling, and both central and systemic hypoxia responses can activate HIF pathway genes. Furthermore, HIFs are critical molecules that regulate inflammation and hypoxia, ensuring appropriate cell function and survival (Mesentier-Louro, 2021).

Central and Systemic Hypoxia Responses

There is substantial heterogeneity in oxygen tension across the body due to organ/tissue-specific changes in vascularization, tissue diffusion characteristics, cell-specific metabolism, and oxygen demand. Normoxic tissue pO2 values range from 90 to 1 mmHg, with a typical value of approximately 30 mmHg (Roy & Secomb, 2019). Different organs are hypoxic due to differences in tissue oxygen tensions, which are determined by differences in aerobic metabolism. Thus, a drop in oxygen availability that is insufficient to meet metabolic demand results in hypoxia, the condition in which insufficient oxygen reaches the body's tissues. Hypoxia can be caused by a decrease in partial oxygen pressure (PO2) in the environment, problems with breathing and/or oxygen transport, or the inability of tissues to utilise oxygen. Hypoxia may be caused by the extreme environmental conditions seen at high altitudes or deep sea levels. Extremely hypoxic individuals have the most dramatic systemic and neurological adaptations to persistent hypoxia (Cowburn et al., 2017). Hypoxia-tolerant hibernating animals, such as painted turtles, have developed specialised methods to deal with hypoxia by lowering metabolic rate and inhibiting ion channel activity (Jackson, 2002). These adaptations occur over longer time periods than acute responses of aerobic species that are not accustomed to extreme environments. The reaction of mammals to hypoxia appears to have two general roles. Hypoxia produces signals that accelerate the development of the cardiovascular system during gestation. Adaptation to oxygen gradients in moderately hypoxic conditions is essential for the development of particular

cardiovascular structures, including coronary arteries, outflow tracts, and the placenta (Giussani, 2021). In adults, hypoxia provides mechanisms that respond synchronously to the failures of the oxygen delivery system. Depending on the context of the hypoxic signal, mechanisms that activate cell survival or death signalling pathways are activated under both conditions (Schönenberger & Kovacs, 2015). At the cellular level, hypoxia induces substantial metabolic changes that are not only passive effects of oxygen substrate deficiency. Under these conditions, active cellular responses that offset decreased respiratory efficiency induce non-oxygen-dependent alternative metabolic pathways. Increased expression of glucose transporters and activation of glycolytic enzymes mediate the transition from aerobic metabolism to anaerobic glycolysis (Melkonian & Schury, 2019). In addition to hyperventilation, erythropoiesis, and angiogenesis, further tissue-level and systemic changes occur to enhance oxygen delivery. Hypoxia is frequently associated with a variety of pathological conditions, such as those leading to insufficient perfusion of organs and tissues (cardiac arrest, congestive heart failure, stroke), respiratory dysfunction (obstructive sleep apnea or lung dysfunction), neurodegenerative diseases (e.g., Alzheimer's), and neoplastic disorders (Sforza & Roche, 2016).

Despite its small size (2% of body weight), the mammalian brain receives a disproportionately high proportion of total cardiac output (20%), accounting for a significant portion of total body metabolism. Neurons utilise aerobic and anaerobic metabolism through direct and indirect pathways (through intermediates of astrocytic metabolism) to maintain optimal ATP generation (Yang et al., 2013). By continuously controlling ionic gradients, which are required for membrane excitability, much of this ATP produced supports neural network activity. In particular, about 50% of a neuron's energy is utilised to maintain ionic gradients and fluxes (Didier et al., 2018). Normal brain function is particularly sensitive to changes in oxygen delivery due to the brain's limited energy reserves; hence, prolonged disruptions in blood flow and oxygen supply are uncomfortable. In contrast, high levels of oxygen generate reactive oxygen species, which may cause oxidative stress and cell damage. Neurons are particularly susceptible to the damaging effects of excessive reactive oxygen species due to their low potential for cell division and regeneration, if any. Thus, the mammalian brain contains complex oxygen-sensing mechanisms to carefully regulate oxygen homeostasis and prevent the possibility of metabolic impairment or severe oxidative stress (Garbarino et al., 2015).

In response to systemic hypoxia, compensatory mechanisms are activated to restore the equilibrium of oxygen delivery between tissue and its consumption. Conventionally, the immediate systemic responses to hypoxia have been attributed to oxygen-sensitive peripheral chemoreceptors of the aortic and carotid bodies, which are specialised for converting the stimulus of a lowered blood O2 tension into corrective cardiorespiratory and autonomic reflexes (O'Driscoll et al., 2008). These peripheral chemoreceptors are

considered the first line of defence for detecting changes in arterial blood gases since the circulation time from the lungs to the central nervous system is shorter (Prabhakar et al. 2015). In contrast, medullary central chemoreceptors have primarily been regarded as CO2-sensitive. In response to low arterial oxygen partial pressure, peripheral chemoreceptors transmit afferent signals to the medullary brainstem via branches of the glossopharyngeal and vagus nerves. This sensory input to the brainstem modifies sympathetic efferents to generate systemic cardiorespiratory responses, including increased respiratory rate (hypoxic ventilatory response), increased cardiac output, vasodilation, and increased cerebral blood flow (Guyenet, 2014). The responses to subnormal oxygen levels involve various mechanisms, pathways, and organs, especially the brain, and are not just dependent on the quick oxygen-sensing capabilities of the peripheral chemoreceptors.

In addition to systemic homeostatic mechanisms, mammals have also acquired central cell intrinsic mechanisms to overcome tissue hypoxia (Lee et al., 2020; Yahaya et al., 2019). All cells of the central nervous system, particularly neurons, sense oxygen and modulate their activity in response to hypoxia. For the majority of neurons, the general consequence is a reduction in activity, which reduces metabolic demand and the need for ATP synthesis. However, not all neurons respond in this manner under hypoxic conditions, making generalisations challenging. Research has demonstrated variety in responses to hypoxia among distinct neuronal networks, even within the same nucleus (Bonkowsky & Son, 2018). There are groups of neurons that monitor oxygen levels in the brain and, when engaged, behave similarly to conventional peripheral oxygen chemosensors. These central oxygen chemosensors appear to be critical for both short-term and long-term adaptation to hypoxia (Flor et al., 2018).

The infusion of cyanide into the medulla to imitate localised hypoxia activates sympathoexcitatory neurons, resulting in hyperventilation independent of peripheral chemoreceptors (Dempsey et al., 2014); these are foundational observations of central O2 sensitivity. Briefly, the posterior hypothalamus, the C1 sympathoexcitatory region of the rostral ventrolateral medulla (RVLM), the pre-Botzinger complex, and the nucleus tractus solitarius (NTS) all contribute to the acute hypoxia ventilator response (HVR). Sun and her colleague were among the first to report that neurons in the C1 area of the brainstem are intrinsically sensitive to oxygen deprivation. Neurons in this region are essential for the formation of tonic vasomotor tone and the integration of blood pressure reflex changes (Sun et al., 1992). According to Guyenet et al. (2013), oxygen-sensitive neurons in the posterior hypothalamus boost sympathetic activity directly and also project to the C1 sympathoexcitatory area. The pre-Botzinger complex, the known site of respiratory rhythm production, generates gasping, which is a form of autoresuscitation (Peña-Ortega, 2012). The complex is next to the C1 area, indicating that both nuclei share oxygen-sensing systems. In spite of this, both remain phenotypically and functionally diverse due to variations in receptor expression and sympathetic and respiratory

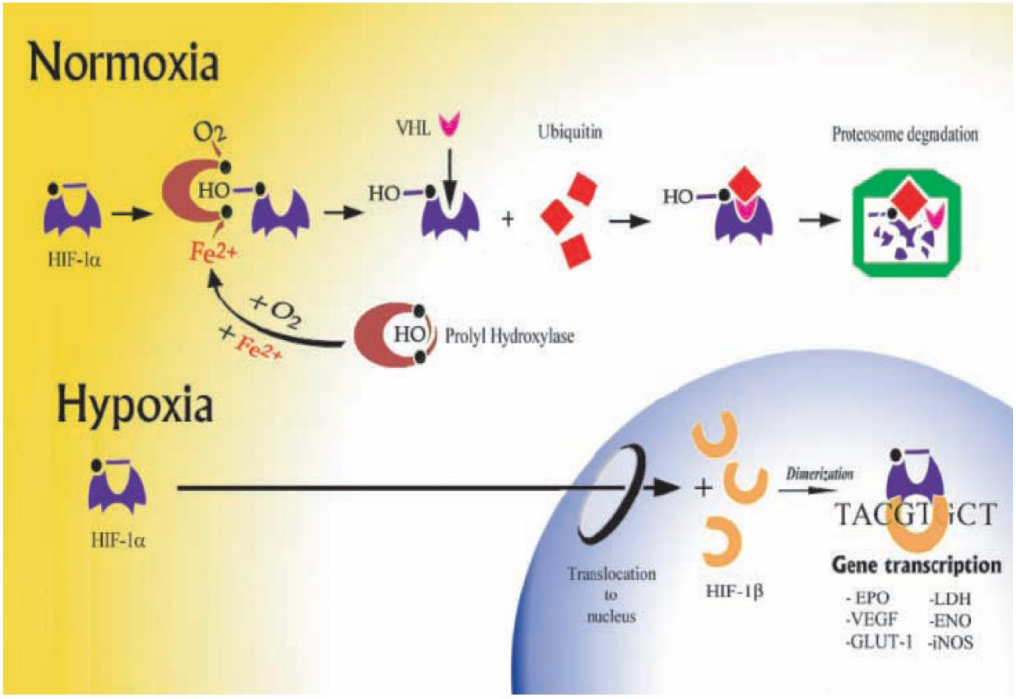
projections (Gourine & Funk, 2017). Additionally, the NTS processes primary afferent signals from a variety of visceral areas and organs, making it an intriguing structure. These afferents consist of chemoreceptors in the carotid and aortic bodies (branches of cranial nerves IX and X, respectively) in addition to arterial baroreceptors from the aorta and carotid arteries. The NTS is the major synapse location of the aorta and carotid bodies, indicating a direct relationship between central and peripheral oxygen-sensitive regions (Kumar & Prabhakar, 2012).

Activation of Hypoxia Inducible Factor (HIF) During Hypoxia

HIF was initially identified as a hypoxia-inducible DNA-binding protein capable of interacting with a hypoxia response element in the 3' region of the Erythropoietin gene (Dengler et al., 2014). Significant progress has been made in our understanding of oxygen physiology as a result of the identification and characterisation of the HIF transcription system (Subramani et al., 2014; Yang et al., 2020). The HIF system is now recognised as a crucial regulator of cellular and systemic hypoxia responses. The HIF transcriptional system is highly conserved among mammalian species and invertebrate organisms like Drosophila melanogaster and Caenorhabditis elegans, further emphasising its importance as a key transcriptional regulator of hypoxia-induced responses throughout evolution (Kierans & Taylor, 2021). Due to more demanding oxygen-sensing requirements, however, larger mammals with more sophisticated respiratory/cardiovascular systems have developed greater degrees of sophistication with regard to constituents and regulation of the HIF pathway (Cerychova & Pavlinkova, 2018). HIF-1 is a heterodimeric transcription factor that regulates the adaptive cellular response to hypoxia (see Figure 1).

Figure 1

Oxygen-dependent Regulation of Hypoxia-inducible Factor-1α (HIF-1α) Accumulation and HIF-1 activation



Note. HIF-1 is continually expressed, hydroxylated, and destroyed under normoxic circumstances. Prolyl 4-hydroxylases (PHDs) depending on molecular oxygen and reduced iron hydroxylate conserved proline residues on HIF-1, permitting identification by the Von Hippel-Lindau protein (VHL). This recognition is followed by polyubiquitination and proteasomal destruction. When oxygen becomes scarce, the activity of prolyl hydroxylase decreases, allowing HIF-1α to accumulate. The activated transcription factor binds to conserved regions inside the hypoxia response element (HRE) on HIF-regulated genes following translocation and dimerization with the constitutive HIF-1β. This involves the overexpression of several target genes, including erythropoietin (EPO), enolase-1 (ENO), inducible nitric oxide synthase (iNOS), lactate dehydrogenase (LDH), vascular endothelial growth factor (VEGF), and glucose transporter-1 (GLUT-1).

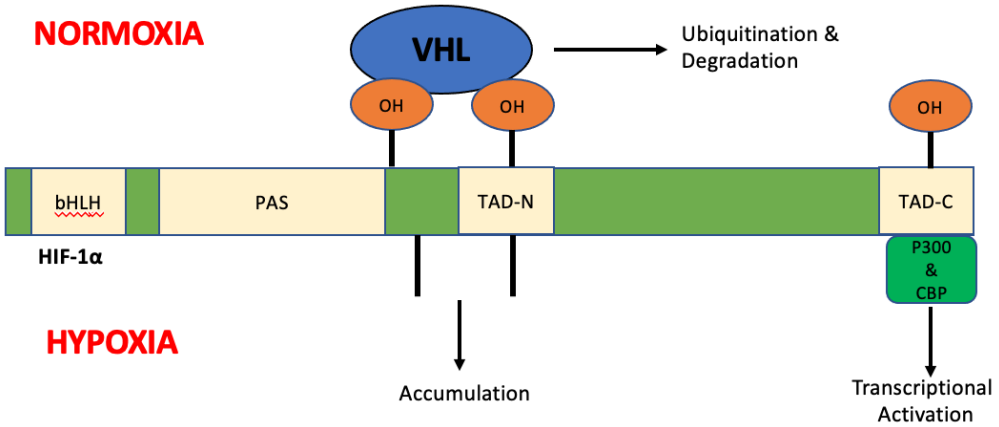
Both α (120 kDa) and β (91-94 kDa) subunits of HIF-1 are required for DNA binding and activation of target genes harbouring hypoxia response elements (HRE). The subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively produced in the nucleus, whereas accumulation of the subunit is often governed by oxygen-dependent post-translational modification. Both subunits are bHLH/PAS transcription factor subunits (Mandl & Depping, 2014). The majority of PAS domain superfamily proteins are signal transduction molecules involved in prokaryotic responses to O_2 concentration, redox status, and other environmental stimuli. Many of these proteins' PAS domains bind to prosthetic groups, such as heme, indicating that O_2 regulates HIF-1 (Ruzila et al., 2010; Wan et al., 2020). Over 100 HIF-1 target genes have been identified, many of which play important roles in physiological

processes, such as vasomotor control, angiogenesis, erythropoiesis, iron metabolism, cell proliferation/death, and energy metabolism.

Throughout the range of physiological and pathological oxygen tensions, HIF activity is tightly regulated by a number of mechanisms that control mRNA expression, protein stability, nuclear translocation, and transactivation activity (Choudhry & Harris, 20018; Ismail et al., 2016). Collectively, they ideally activate and maintain HIF for translocation into the nucleus and transcriptional activity under decreasing oxygen concentrations. As depicted in Figure 1, the predominant mechanism of HIF-α regulation is oxygen-dependent enzymatic hydroxylation followed by proteolysis. During normoxia, the subunit is produced and modified by the EGLN family of HIF prolyl 4-hydroxylase domain-containing proteins (PHDs) (Choudhry & Harris, 20018. These enzymes hydroxylate two conserved proline residues on the subunit and require oxygen (O_2), reduced iron (Fe^{2+}), ascorbate, and 2-oxoglutarate as cofactors (Kuiper et al., 2014). In addition to hydroxylated HIF-α, the byproducts of this enzyme process are carbon dioxide and succinate. The hydroxylated prolines are recognised by the Von Hippel-Lindau tumour suppressor gene (VHL), which functions as the recognition component of a multiprotein ubiquitin complex, thus targeting HIF-α for proteasomal degradation (Figure 2) (Sufan et al., 2004). During hypoxia, oxygen becomes limited and prolyl hydroxylase activity decreases, resulting in an accumulation of nonhydroxylated HIF-α, which permits its translocation to the nucleus and dimerization with HIF-β (D'Angelo et al., 2003). The stabilised and phosphorylated heterodimer binds to DNA at a conserved region, 5'-(A/T)CGTG-3', within the hypoxia response element (HRE) of HIF-regulated genes. Recruitment of the transcriptional adapter/histone acetyltransferase, p300 and CBP, to the promoters of target genes enhances transcriptional activation (Yu et al., 2017).

Figure 2

HIF-1 Protein Domain Architectures, Including Basic Helix-loop-helix (bHLH), Per/Arnt/Sim (PAS), N-terminal Transactivation Domain (TAD-N), and C-terminal Transactivation Domain (TAD-C)



Note. Approximately modified amino acid residues are displayed within the domains. Under normoxic circumstances, proline residues are hydroxylated, which results in VHL-dependent polyubiquitination and proteasomal degradation. In addition, factor inhibiting HIF-1 (FIH-1) hydroxylates a conserved asparagine residue, which lowers the transcriptional activity of HIF-1 by blocking its interaction with p300 and CBP. Reduced hydroxylase activity under hypoxic settings permits HIF-1α accumulation and transcriptional activity.

Conclusions

Since it has been proven that the PHD-VHL-HIF axis is critical for intracellular and systems-level regulation of human life, hypoxia is a situation in which insufficient oxygen reaches cells. In reality, the emerging role of HIF in systemic physiology, described here in terms of the response to high altitude, applies to any clinical scenario in which hypoxia is present. Based on the theory that HIF-1 functions as a sensor for hypoxia and activates compensatory and adaptive mechanisms.

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References

Bonkowsky, J. L, & Son, J. H. (2018). Hypoxia and connectivity in the developing vertebrate nervous system. *DMM Disease Models and Mechanisms, 11*(12). doi: 10.1242/dmm.037127

Cerychova, R., & Pavlinkova, G. (2018, August 15). HIF-1, metabolism, and diabetes in the embryonic and adult heart. *Front Endocrinol (Lausanne)*, 9. doi:10.3389/fendo.2018.00460

Choudhry, H., & Harris, A. L. (2018). Advances in hypoxia-inducible factor biology. *Cell Metab, 27*(2), 281-298. doi:10.1016/j.cmet.2017.10.005

Cowburn, A. S., Macias, D., Summers, C., Chilvers, E. R., & Johnson, R. S. (2017). Cardiovascular adaptation to hypoxia and the role of peripheral resistance. *Elife, 6*, e28755. doi:10.7554/eLife.28755

D'Angelo, G., Duplan, E., Boyer, N., Vigne, P., & Frelin, C. Hypoxia up-regulates prolyl hydroxylase activity. (2003). *Journal of Biological Chemistry, 278*(40), 38183-38187. https://doi.org/10.1074/jbc.M302244200

Dempsey, J. A., Powell, F. L., Bisgard, G. E., Blain, G. M., Poulin, M. J., & Smith, C. A. (2014). Role of chemoreception in cardiorespiratory acclimatization to, and deacclimatization from, hypoxia. *J Appl Physiol, 116*(7), 858-866. doi:10.1152/japplphysiol.01126.2013

Dengler, V. L., Galbraith, M. D., & Espinosa, J. M. (2014). Transcriptional regulation by hypoxia inducible factors. *Crit Rev Biochem Mol Biol, 49*(1), 1-15. doi:10.3109/10409238.2013.838205

Didier, M. E. P., Tarun, O. B., Jourdain, P., Magistretti, P., & Roke, S. (2018). Membrane water for probing neuronal membrane potentials and ionic fluxes at the single cell level. *Nature Communication, 9*, Article number: 5287. https://doi.org/10.1038/s41467-018-07713-w

Flor, K. C., Silva, E. F., Menezes, M. F., Pedrino, G. R., Colombari, E., & Zoccal, D. B. (2018). Short-term sustained hypoxia elevates basal and hypoxia-induced ventilation but not the carotid body chemoreceptor activity in rats. *Front Physiol, 9*, 134. doi:10.3389/fphys.2018.00134

Garbarino, V. R., Orr, M. E., Rodriguez, K. A., & Buffenstein, R. (2015). Mechanisms of oxidative stress resistance in the brain: Lessons learned from hypoxia tolerant extremophilic vertebrates. *Arch Biochem Biophys, 576*, 8-16. doi:10.1016/j.abb.2015.01.029

Giussani, D. A. (2021). Breath of life: Heart disease link to developmental hypoxia. *Circulation, 144*(17), 1429-1443. doi:10.1161/CIRCULATIONAHA.121.054689

Gourine, A. V., & Funk, G. D. (2017). (2017). On the existence of a central respiratory oxygen sensor. *J Appl Physiol, 123*(5), 1344-1349. doi:10.1152/japplphysiol.00194.2017

Guyenet, P. G. (2014). Regulation of breathing and autonomic outflows by chemoreceptors. *Compr Physiol, 4*(4), 1511–1562. doi:10.1002/cphy.c140004

Guyenet, P. G., Stornetta, R. L., Bochorishvili, G., DePuy, S. D., Burke, P. G. R, & Abbott, S. B. G. (2013). C1 neurons: The body's EMTs. American *Journal of Physiology, 305*(3), https://doi.org/10.1152/ajpregu.00054.2013

Ismail, R., Allaudin, Z. N., Abdullah, R., Lila, M. A., Abd, N., Rahman N. M. A., & Rahman, S. A. (2016). Combination of VP3 and CD147-knockdown enhance apoptosis and tumor growth delay index in colorectal tumor allograft. *BMC Cancer, 16*, Article 461. https://doi.org/10.1186/s12885-016-2530-8

Jackson, D. C. (2002). Hibernating without oxygen: Physiological adaptations of the painted turtle. *Journal of Physiology, 543*(3), 731-737. doi:10.1113/jphysiol.2002.024729

Kierans, S. J., & Taylor, C.T. (2021). Regulation of glycolysis by the hypoxia-inducible factor (HIF): Implications for cellular physiology. *Journal of Physiology, 599*(1). https://doi.org/10.1113/JP280572

Kuiper, C., Dachs, G. U., Munn, D., Currie, M. J., Robinson, B. A., Pearson, J. F., & Vissers, M. C. M. (2014). Increased tumor ascorbate is associated with extended disease-free survival and decreased hypoxia-inducible factor-1 activation in human colorectal cancer. *Front Oncol, 4*, 10. doi:10.3389/fonc.2014.00010

Kumar, P., & Prabhakar, N. R. (2012). Peripheral chemoreceptors: Function and plasticity of the carotid body. *Compr Physiol, 2*(1), 141–219. doi:10.1002/cphy.c100069

Lee, P., Chandel, N. S., & Simon, M. C. (2020). Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nature Reviews Molecular Cell Biology, 21*, 268–283. https://doi.org/10.1038/s41580-020-0227-y

Mandl, M., & Depping, R. (2014). Hypoxia-inducible aryl hydrocarbon receptor nuclear translocator (ARNT) (HIF-1β): Is it a rare exception? *Molecular Medicine, 20*(1), 215-20. doi:10.2119/molmed.2014.00032.

Melkonian, E.A., & Schury, M.P. (2019). Biochemistry, anaerobic glycolysis. *StatPearls* [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK546695/

Mesentier-Louro, L. A., Rangel, B., Stell, L., Shariati, M. A., Dalal, R., Nathan, A., Yuan, K., Perez, V., & Liao, Y. (2021). Hypoxia-induced inflammation: Profiling the first 24-hour posthypoxic plasma and central nervous system changes. *PLoS One, 16*(3). https://doi.org/10.1371/journal.pone.0246681

Muthuraju, S., & Pati, S. (2014). Effect of hypobaric hypoxia on cognitive functions and potential therapeutic agents. *Malaysian Journal of Medical Sciences, 21*, 41-45.

O'Driscoll, B. R., Howard, L. S., & Davison, A. G. (2008). *BTS guideline for emergency oxygen use in adult patients*. (SUPPL. 6). http://dx.doi.org/10.1136/thx.2008.102947

Peña-Ortega, F. (2012). Tonic neuromodulation of the inspiratory rhythm generator. *Front Physiol, 3*, 253. doi:10.3389/fphys.2012.00253

Prabhakar, N. R., Peng, Y. J., Kumar, G. K., & Nanduri, J. (2015). Peripheral chemoreception and arterial pressure responses to intermittent hypoxia. *Compr Physiol, 5*(2), 561-77. doi:10.1002/cphy.c140039

Roy, T. K., & Secomb, T. W. (2019). Effects of pulmonary flow heterogeneity on oxygen transport parameters in exercise. *Respiratory Physiology and Neurobiology, 261*, 75-79. https://doi.org/10.1016/j.resp.2018.10.004

Ruzila, I., Zeenathul, N. A., Nik-Mohd-Afizan, N. A. R., Sheikh-Omar, A. R., Norhidayah, M., & Mohd-Azmi, M.L. (2010). Tissue distribution of intramuscularly and intratumouraly administered DNA plasmid harbouring apoptotic gene in mice. *Afr J Pharm Pharmacol, 4*(11):775–82.

Schönenberger, M. J., & Kovacs, W. J. (2015). Hypoxia signaling pathways: Modulators of oxygen-related organelles. *Front Cell Dev Biol, 3*. https://doi.org/10.3389/fcell.2015.00042

Sforza, E., & Roche, F. (2016). Chronic intermittent hypoxia and obstructive sleep apnea: An experimental and clinical approach. *Hypoxia*, 99–108. doi:10.2147/HP.S103091

Shaw, D. M., Cabre, G., & Gant, N. (2021). Hypoxic hypoxia and brain function in military aviation: Basic physiology and applied perspectives. *Front Physiol*, 12. https://doi.org/10.3389/fphys.2021.665821

Subramani, T., Yeap, S. K., Ho, W. Y., Ho, C. L., Omar, A. R., Aziz, S. A., Rahman, N. M. A., A., & Alitheen, N. B. (2014). Vitamin C suppresses cell death in MCF-7 human breast cancer cells induced by tamoxifen. *J Cell Mol Med., 18*(2), 305-13. doi:10.1111/jcmm.12188

Sufan, R. I., Jewett, M. A. S., & Ohh, M. The role of von Hippel-Lindau tumor suppressor protein and hypoxia in renal clear cell carcinoma. *Am J Physiol Renal Physiol, 287*(1), F1-6. doi:10.1152/ajprenal.00424.2003

Sun, M. K., Jeske, I. T., & Reis, D. J. (1992). Cyanide excites medullary sympathoexcitatory neurons in rats. *Am J Physiol, 262*(2 Pt 2), R182-9. doi:10.1152/ajpregu.1992.262.2.R182

Wan, Q., Song, D., Li, H., & He, M-l. (2020). Stress proteins: the biological functions in virus infection, present and challenges for target-based antiviral drug development. *Signal Transduction and Targeted Therapy, 5*, Article number 125. https://doi.org/10.1038/s41392-020-00233-4

Wilson, D.F. (2017). Oxidative phosphorylation: regulation and role in cellular and tissue metabolism. *Journal of Physiology, 595*(23). https://doi.org/10.1113/JP273839

Yahaya, M. A. F., Lila, M. A. M., Ismail, S., Zainol, M., & Afizan, N. A. R. N. M. (2019). Tumour-associated macrophages (TAMs) in colon

cancer and how to reeducate them. *J Immunol Res, 2019*, 2368249. doi:10.1155/2019/2368249

Yang, G., Shi, R., & Zhang, Q. (2020). Hypoxia and oxygen-sensing signaling in gene regulation and cancer progression. *Int J Mol Sci, 21*(21), 8162. doi:10.3390/ijms21218162.

Yang, H., Wu, J., Guo, R., Peng, Y., Zheng, W., Liu, D., Song, Z. (2013). Glycolysis in energy metabolism during seizures. *Neural Regen Res, 8*(14), 1316-26. doi:10.3969/j.issn.1673-5374.2013.14.008

Yu, T., Tang, B., & Sun, X. (2017). Development of inhibitors targeting hypoxia-inducible factor 1 and 2 for cancer therapy. *Yonsei Med J., 58*(3), 489-496. doi:10.3349/ymj.2017.58.3.489