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Subcellular Energetics and Metabolism: A Cross-Species Framework

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Abstract

While it is generally believed that oxidative phosphorylation and adequate oxygenation are essential for life, human development occurs in a profoundly hypoxic environment and “normal” levels of oxygen during embryogenesis are even harmful. The ability of embryos to not only survive, but thrive in such an environment is made possible by adaptations related to metabolic pathways. Similarly, cancerous cells are able to not only survive, but grow and spread in environments that would typically be fatal for healthy adult cells. Many biological states, both normal and pathological, share underlying similarities related to metabolism, the electron transport chain, and reactive species. The purpose of Part I of this review is to review the similarities between embryogenesis, mammalian adaptations to hypoxia (primarily driven by hypoxia inducible factor-1), ischemia-reperfusion injury (and its relationship with reactive oxygen species), hibernation, diving animals, cancer, and sepsis, with a particular focus on the common characteristics that allow cells and organisms to survive in these states.

Introduction

As organisms that depend mostly on aerobic metabolic pathways for the provision of adenosine triphosphate (ATP), it is not surprising that humans consider oxygen to be an essential component of life. The most ancient forms of life, however, neither consumed nor produced oxygen, utilizing anaerobic anoxygenic photosynthesis (AnAnP) to generate ATP.¹ It was not until the development of oxygenic (oxygen-producing) photosynthesis 2.4–3 billion years ago that the atmosphere began to be “contaminated” with oxygen.^{1,2} The introduction of oxygen to the environment led to the development of primitive mechanisms to protect against oxidative stress, some of which eventually morphed into subcellular pathways capable of consuming this dangerous metabolic byproduct and in doing so producing energy.² The tradeoff between oxidative stress and the production of energy

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(known as the “Respiration Paradox”³), which still exists today, was thus established billions of years ago.

Viruses, Bacteria, and Archaea can survive in completely anoxic environments,⁴⁻⁷ and more recently, Metazoan (kingdom Animalia) species that can tolerate anoxic environments have also been identified.⁸ The questions then arise – if modern, multicellular organisms can survive in completely anoxic environments, are these alternative metabolic pathways utilized by humans, can they be co-opted and used to our advantage, and what can they teach the scientific community about cell death and, potentially, survival in the face of anoxic stress? The purpose of this review is to explore the ability of cells (e.g. cancer) and organisms (e.g. pre-implantation embryos) to survive, and in some cases thrive, in ostensibly hostile environments, and identify common themes that suggest therapeutic applications for humans subjected to hypoxic or oxidative stress.

Human Embryogenesis

Mammalian embryos are a useful starting point for understanding how organisms can adapt to hypoxia because the development and growth of the neurologic system precedes the development of the cardiovascular system. In the adult, hypoxia-induced ischemia appears related to the balance between oxygen delivery and consumption, with the most metabolically active organs (e.g. brain) most susceptible to injury.^{9,10} It is therefore interesting to note that while the adult brain is relatively intolerant of hypoxia, the developing brain *requires* hypoxia to properly form. This counterintuitive phenomenon was demonstrated convincingly by Morriss and New in 1979, who subjected rat embryos to oxygen levels ranging from 5-40% and found that higher oxygen levels prevented neural tube closure [**Figure 1**].¹¹

The dependence of developing organs on subatmospheric oxygen concentrations is unsurprising when one considers that the partial pressure of oxygen (pO₂) ranges from 0.5-30 mm Hg (1-5%) in the intrauterine environment.¹² In contrast, the intracellular oxygen concentrations in most adult cells ranges from 2-9% although exceptions such as the renal medulla safely exist at concentrations as low as 1%.¹³ Studies utilizing the hypoxia marker pimonidazole, which binds protein and DNA at oxygen levels below 2%, demonstrate extensive hypoxia in both the neural tube and developing heart of murine embryos during organogenesis.¹⁴

In fact, during embryogenesis oxygen acts more like a signal and less like an electron acceptor, guiding both stem cell and endothelial progenitor differentiation.^{13,15} Prior to the development of the circulatory system, fetal oxygen levels typically do not exceed 3%, making achievement of “normal” post-gestational levels of oxygen almost impossible.^{16,17} Because oxygen travels by diffusion, an oxygen gradient exists across the developing animal, with higher oxygen levels in areas proximal to maternal spiral arteries, and lower levels dorsally [**Figure 2**].¹³ The fate of individual stem cells is affected by local levels of oxygen. For example, trophoblastic stem cells exposed to high levels of oxygen become giant cells, whereas those exposed to lower levels of oxygen become spongiotrophoblasts.¹⁸ Even in adults, animal data suggests that regional hypoxia helps maintain stem cells in their

pluripotent state.¹⁹ The ontogenic properties of molecular oxygen are not limited to mammals and are shared across species (e.g. in *Drosophila melanogaster*, oxygen levels strongly influence the development of the trachea).²⁰

The link between varying oxygen levels and organogenesis is formed by hypoxia inducible factor 1 α (HIF-1 α), which is expressed almost ubiquitously in the developing embryo.² Both HIF-1 α and vascular endothelial growth factor (VEGF) bind to pimonidazole, suggesting that hypoxia induces adaptive processes such as HIF-1 α upregulation and angiogenesis.^{13,15} Mice deficient in HIF-1 are marked by defective neural tube development and angiogenesis (particularly in neurologic tissue), and do not survive past embryonic day 10.5.^{21,22} It is not clear whether HIFs arose initially to protect against hypoxia (or oxidative stress) and were co-opted to support organogenesis, or vice versa, but it is clear that this class of transcription factors is tightly coupled to oxygen availability and that without it, development and survival are not possible. Because hypoxia is an essential precondition for proper development, and metabolically active organs can develop and grow in low oxygen environments, the question then arises – how is it possible for organ systems (e.g. neurologic system) to thrive in hypoxic environments that would be fatal in the fully developed organism?

Several general strategies have evolved for adapting to hypoxia. Many are shared across organisms, and some are used during development. First, alternative pathways for producing energy may be utilized (e.g. anaerobic glycolysis). Indeed, in the very earliest stages of development (pre-implantation embryo), very little oxygen is consumed by developing cells, and mitochondria demonstrate relatively few foldings, also suggesting minimal aerobic respiration.²³⁻²⁵ Pyruvate is the preferred energy source of the pre-implantation embryo, as it can be used to produce ATP (albeit inefficiently) without the need for oxygen.²⁵⁻²⁷

Second, in low oxygen environments, oxygen delivery can be increased via strategies such as augmenting cardiac output (immediate) or HIF-1 α -mediated angiogenesis (delayed).¹² During embryological development, an increase in cardiac output is not possible until the development of the cardiovascular system is complete (in human embryos, the primitive left ventricle is not formed until approximately ED 30). Angiogenesis is rampant during embryogenesis, primarily driven by hypoxia-responsive factors such as HIF-1 α , HIF-2 α , and VEGF.^{13,15}

Third, when oxygen availability is scarce, energetic needs can be attenuated through a variety of mechanisms, including reductions in adipogenesis, ion-channel activity and protein synthesis (also see **Hibernation**, below).^{13,28} In the developing embryo, which grows at exponential rates, this is a challenge. By the time the blastocyst stage is achieved (post-implantation), oxygen consumption increases markedly and glucose becomes the preferred energy source, with 85% of it being metabolized through aerobic respiration.^{25,27}

Fourth, accumulating data suggest that cellular damage associated with hypoxia may not result only from energetic failure, but also from the production of reactive oxygen species (ROS) that occurs in response to a hypoxic insult followed by re-establishment of normal oxygen levels. This phenomena is also known as ischemia-reperfusion injury (see **Ischemia-**

Reperfusion and Reactive Oxygen Species, below), and may depend on the organism's ability to defend against oxidative stress.²⁹

Lastly, the developing brain may also be resistant to intracellular calcium overload. Calcium accumulation is central to the development of neurologic injury following ischemia. In the classic model of hypoxia-induced neurologic damage, lack of oxygen decreases the activity of ATP-dependent processes such as maintenance of transmembrane ion potentials and neurotransmitter reuptake. The resulting membrane depolarization leads to release of glutamate, dopamine, and other neurotransmitters. These excitotoxins bind to alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors, increasing the concentration of intracellular calcium and ultimately causing cellular damage and death.^{30,31} Limiting calcium accumulation during hypoxia may explain the relative resistance of mammalian neonatal neuronal tissues to hypoxic stress, and inactivation of NMDA receptors has been observed after hypoxia.^{31,32} Interestingly, elevations in intracellular calcium levels may also facilitate HIF-1 expression (see below).³³ [Table 1]

Hypoxia Inducible Factors and Mammalian Adaption to Hypoxia

Evolution has produced mechanisms for responding to hypoxia, all capable of either activating transcription or inhibiting translation to protect oxygen-dependent organisms. These mechanisms include the hypoxia-inducible-factors and mammalian target of rapamycin (mTOR) kinase, the unfolded protein response, and NF- κ B.³⁴⁻³⁶ Of these, the HIFs are the best known and understood. In the presence of oxygen, the α subunits of the HIF proteins (HIF-1 α , HIF-2 α , HIF-3 α) are degraded by HIF prolyl hydroxylases (PHDs).^{3,37,38} When intracellular oxygen drops below a critical value (approximately 10 mm Hg³⁹), PHDs become inactive and HIFs begin to accumulate. This increase in HIFs affects as many as 1,500 target genes through the cis-acting hypoxia response element (HRE), which contains the sequence 5'-RCGTG-3' (where R = A or G).³⁷ With over a thousand target genes, describing the impact of HIFs in the context of a review article is impossible, so we will therefore focus on those most relevant to hypoxia and oxidative stress.

HIF-1 activates several angiogenic and arteriogenic growth factors including VEGF, angiopoietin 2 (ANGPT2), stromal derived factor 1 (SDF1), platelet-derived growth factor B (PDGFB), placental growth factor (PGF), and stem cell factor (SCF). Collectively, these factors increase the delivery of oxygenated hemoglobin to chronically hypoxic tissues.⁴⁰ HIF-2 activates the transcription of genes for erythropoietin (EPO) and proteins needed to absorb and deliver iron, thereby increasing the carrying capacity of blood.^{37,41-43} While these responses can have profound effects over prolonged periods, they do little to ameliorate the effects of hypoxia in the short term.

More immediately, cells deprived of oxygen can adapt by shifting energy production away from oxidative phosphorylation. Oxidative phosphorylation, in which NADH and FADH₂ donate electrons to the electron transport chain (ETC) to drive ATP synthase using oxygen as the terminal electron acceptor, is highly efficient, producing more ATP per molecule of

substrate than any other known form of energy production. However, the coordinated passage of electrons across the cytochromes and dehydrogenases that make up the electron transport chain is a finely tuned process, and oxygen tensions that deviate in *either* direction from normality lead to the loss of electrons and production of free radicals which not only reduce the amount of ATP generated but can lead to extensive cellular damage.³⁷ To promote the use of alternative metabolic pathways in the setting of hypoxia, HIF-1 upregulates pyruvate dehydrogenase lipoamide kinase isozyme 1 (PDK-1) and consequently pyruvate dehydrogenase (PDH), which prevents the conversion of pyruvate to Acetyl-CoA for use in oxidative phosphorylation.^{37,44}

HIF-1 also suppresses fatty acid β -oxidation (another source of Acetyl CoA) through inhibition of acyl-CoA dehydrogenases.⁴⁵ Both of these activities deprive the TCA cycle of acetyl-CoA, attenuate the ability of NADH and FADH₂ to donate electrons to the ETC, and decrease oxygen dependent ATP production. HIF-1 also inhibits the TCA cycle through indirect inhibition of aconitase⁴⁶ and in doing so modifies oxidative phosphorylation directly. Because the mitochondria serve as a receptacle for a molecule (O₂) necessary to preserve continuous oxidative phosphorylation, the activities of HIF-1 on PDK-1 and PDH help maintain cell function in the face of decreased O₂ supply.⁴⁷

Shutdown of the TCA cycle and oxidative phosphorylation would be fatal unless energy were available from other pathway(s). Towards that end, HIF-1 also activates lactate dehydrogenase-A (LDHA), promoting the conversion of pyruvate to lactate, shunting pyruvate away from the TCA cycle (the “Pasteur effect”), and allowing energy production to continue.³⁷ Because this pathway is less efficient than oxidative phosphorylation, greater supplies of glucose are necessary to maintain stable levels of ATP. Fortunately, HIFs upregulate genes encoding glucose transporters.⁴⁸ HIF-1 also facilitates a subunit switch in cytochrome c oxidase (COX) that increases the efficiency of electron transfer and attenuates the production of ROS (H₂O₂) at lower oxygen levels.^{49,50} This subunit switch is important because hypoxia does not shut down oxidative phosphorylation completely and ROS are harmful. HIF-1 also upregulates the micro-RNA miR-210, which inhibits ETC Complex 1 and reduces ROS production.⁴⁶ Lastly, HIF-1 can induce mitochondrial autophagy, which decreases oxidative phosphorylation by reducing the supply of mitochondria through its interactions with BNIP3 and BNIP3L.⁵¹ Hypoxia inducible factor HIF-2 α plays a similar role in blocking the conversion of pyruvate to acetyl-CoA and thereby depriving the TCA cycle of a crucial intermediary.³ In mice deficient in the conversion enzyme PHD-1, HIF-2 α levels are elevated when compared to wild-type mice.⁵²

Knockdown of PHD-1 leads to an upregulation of peroxisome proliferator-activated receptor alpha (PPAR α), a “master regulator” of metabolism that can reduce glucose oxidation through its effects on pyruvate dehydrogenase lipoamide kinase isozyme 4 (*Pdk4*).⁵² PPAR α -mediated activation of PDK-4 inhibits PDH.⁵³ Skeletal muscles from PHD-1 deficient mice consume less oxygen and tolerate ischemia better than their wild-type counterparts,⁵² likely due to a combination of decreased oxidative phosphorylation, reduced ROS production, and increased glycolysis (also mediated by PPAR α).⁵² Additionally, in the setting of ischemia, PHD-1 deficient mice preserve high energy phosphates better than wild-type mice.⁵² [Figure 4] HIFs also exert their hypoxia-moderating effects through EPO. In

addition to increasing oxygen carrying capacity, which is a long term approach, EPO also activates anti-apoptosis proteins bcl-2 and bcl-XL, inhibits brain derived neurotrophic factor, caspases, and decreases the development of inflammation and excitotoxicity.⁵⁴⁻⁵⁶ These short term effects are particularly relevant in protecting the central nervous system from hypoxia,⁵⁴⁻⁵⁶ although human trials to date have been negative.^{57,58}

Physiologic adjustments to hypoxia are also mediated through non-HIF pathways. Because protein synthesis is so energetically intense, hypoxic stresses such as changes in redox state, glucose availability, glycosylation, and energy loss lead to accumulation of unfolded or misfolded proteins in the endoplasmic reticulum.³⁵ These proteins then activate an unfolded protein response (UPR), a system of signal cascades including activation of PKR-like kinase [PERK], activating transcription factor 6 α [ATF6 α], and inositol requiring 1 α [IRE1 α]. These responses ultimately reduce RNA translation, protein production, and therefore energy expenditure.^{35,59} Similarly, hypoxia downregulates the activity of the a serine/threonine kinase mTOR, a protein that modifies protein synthesis in response to stress, nutrient deprivation, and hypoxia.⁶⁰ Because mTOR drives energy consuming processes such as protein synthesis and cell growth, effects of hypoxia on mTOR lead to decreased cell growth.³⁵ Importantly, the ability of hypoxia to stimulate mTOR is independent of HIF.⁶⁰⁻⁶²

Hypoxia also activates NF- κ B,⁶³⁻⁶⁵ which regulates apoptosis, inflammation, both the innate and adaptive immune responses, the cellular stress response, cell adhesion and proliferation, and tissue remodeling.³⁴ Normal NF- κ B function is essential for survival, perturbations in NF- κ B activity are linked to cancer development, and abnormalities in NF- κ B function have been documented in autoimmune diseases and sepsis.^{66,67} Although NF- κ B activation is a HIF-independent response to hypoxia, both the HIF and the NF- κ B hypoxia responses are mediated by prolyl hydroxylases.⁶³

Ischemia-Reperfusion and Reactive Oxygen Species

In 1960, Jennings et al. first suggested that reperfusion of previously unperfused tissue beds may contribute to injury.⁶⁸ In 1977, Bulkely and Hutchins observed that the majority of perioperative infarctions occurring in patients undergoing coronary artery bypass grafting (CABG) surgery occurred in well-perfused, grafted territories of the myocardium.⁶⁹ A decade later, Parks and Granger demonstrated that histologic injury was worse after three hours of ischemia followed by one hour of reperfusion than after four hours of ischemia with no reperfusion in a feline intestinal ischemia model.⁷⁰ Taken together, these landmark findings raised the possibility that restoring oxygen delivery to previously hypoxic tissues may itself be harmful. At the level of the cell, injury caused by ischemia and reperfusion is multifactorial and incompletely understood, but includes alterations in ion pump function (and resultant ionic disturbances), expression of pro-inflammatory genes, repression of “protective” genes, activation of apoptosis, autophagy, and necrosis, and activation of both the innate and adaptive immune systems (autoimmunity).^{71,72}

A common source of injury following reperfusion is the combination of accumulated reactive oxygen species (ROS) combined with reduced anti-oxidant capacity.⁷³ During normal aerobic respiration, an estimated 1-20% of the electrons destined to flow through the

ETC chain are ejected prematurely, leading to the creation of superoxide ($\bullet\text{O}_2^-$) which subsequently interacts with a variety of molecules to produce both ROS and reactive nitrogenous species (RNS).⁷⁴⁻⁷⁸ While the quantity and sources of these electron “leaks” are not completely understood, both complexes I and III appear to be major contributors.⁷⁹⁻⁸⁴ Supranormal levels of ROS have been demonstrated following ischemia-reperfusion in a variety of animal and human models.⁸⁵⁻⁸⁷ While the source of ROS is not completely understood, ROS begins to accumulate even during the ischemic process when tissue oxygen levels are lower.^{47,82,86,88} This accumulation is then compounded by an additional ROS “burst” that occurs with re-introduction of O_2 to ischemic cells.⁸⁹⁻⁹² ROS and RNS are highly reactive species which, after overwhelming the body's endogenous antioxidant systems, disrupt membrane integrity, and contribute to necrosis and cell death by lipid membrane peroxidation, and alteration of cellular proteins and ribonucleic acids.^{72,93}

The theory that ROS/RNS play a prominent role in injury following ischemia and reperfusion is indirectly supported by several findings. First, free radical scavengers attenuate ischemia-reperfusion injury.⁹⁴⁻⁹⁶ Second, when compared to wild-type cells, HIF-1 α knockouts cultured in a hypoxic environment (1%) die prematurely, despite higher intracellular levels of ATP.⁴⁸ This suggests that hypoxia-induced decreases in oxidative phosphorylation may not be the primary cause of death after hypoxic insult. HIF-1 α knockouts cells subjected to hypoxia also produce lethal levels of ROS.⁸⁸ Current data suggest that mitochondrial ROS lead to HIF-1 stabilization⁹⁷⁻¹⁰⁰ and that ROS scavengers inhibit HIF-1 stabilization.^{47,98,99,101} Third, skeletal muscle of mice deficient in PHD1 consumes less oxygen than muscle from wild-type mice and displays less necrosis and reduced ROS production with limb ischemia.⁵² While ROS and RNS are not the only sources of injury following ischemia and reperfusion (see above), understanding the impact of ROS/RNS formation is important because it is potentially modifiable and may present clinicians with an interventional opportunity.

Hibernation

Several animal species periodically enter controlled, photoperiodically determined states of hypometabolism known as hibernation.¹⁰² A universal component of hibernation is a decrease in core body temperature, which decreases the metabolic rate and the need for oxygen. In small animals, decreased body temperature is accompanied by reductions in cardiac output, ventilation, and in some cases increased peripheral vasoconstriction.¹⁰³⁻¹⁰⁶ Collectively, these macro-physiologic changes decrease VO_2 to approximately 5% of euthermic baseline levels.¹⁰² Complementing this decreased need for oxygen are decreases in immunological and hematologic function, and increased antioxidant activity.³⁰

For reasons that are unclear, many hibernating animals (rodents in particular) undergo brief periods of arousal during the hibernating state, which increases VO_2 and subjects them to the risk of oxygen supply:demand mismatching. During these periods, PaO_2 as low as 10 mm Hg has been recorded in some animals.^{102,104} Despite such arousals, these animals exhibit normal levels of plasma lactate and brain iNOS expression, suggesting they experience only moderate oxidative stress. This ability to tolerate what would be considered severe oxygen supply:demand mismatching is thought to be mediated by increased reliance

on anaerobic and non-carbohydrate metabolic pathways,^{107,108} ion channel arrest,^{109,110} and preconditioning-like phenomena.^{104,111} Interestingly, both preconditioning and hibernation upregulate a similar set of genes involving metabolism, immune responsiveness, and ion channel activity.^{112,113}

Some have suggested that hibernating animals constantly subject themselves to oxidative stress during euthermia to precondition themselves in preparation for hibernation and arousal/rewarming.¹⁰⁴ The arctic ground squirrel, for instance, maximally expresses HIF-1 and iNOS during a euthermic state (when PaO₂ ~ 60 mm Hg), despite decreases in PaO₂ to approximately 10 mm Hg during arousal periods.¹¹⁴ Arctic ground squirrels (AGS) thus experience mild, chronic hypoxia during euthermia, which increases HIF-1 and iNOS levels and provides protection during arousal/rewarming.^{114,115} While ROS may also trigger beneficial effects downstream, they are potentially harmful and mitigated by concomitant antioxidant production (e.g. ascorbate) and redistribution.¹⁰³

Even in their euthermic state, hibernating animals (e.g. ground squirrels, hamsters, and bats) tolerate hypoxia better than their homeothermic counterparts (e.g. rats, guinea pigs).^{104,111,116,117} AGS, for instance, can tolerate 8-10 minutes of asphyxia leading to cardiac arrest with almost no neurologic damage.^{108,116,117} Potential mechanisms for this resiliency include improved hypothermia tolerance (which occurs naturally when oxygen supplies are low and the metabolic rate is regulated downward), higher plasma levels of β -hydroxybutyrate (which can be utilized as an alternative energy source), stronger antioxidant defense systems, and alteration of voltage-sensitive calcium channels (which prevents calcium accumulation and improves cerebral ischemia tolerance).^{104,109,118,119}

Of particular interest is the ability of the AGS to prevent ischemic depolarization (ID) and subsequent intracellular calcium accumulation. During the induction stage of ischemia, electron transport is inhibited, intracellular ATP stores decrease, intracellular acidosis ensues, and the excitatory neurotransmitter glutamate is released, causing intracellular calcium accumulation. Additionally, genes controlling free radical production are unregulated. These changes conspire to activate at least five independently damaging events, called “perpetrators.” Both glutamate excitotoxicity and calcium accumulation are critical components of the transition from reversible cellular stress to irreversible cell death.¹²⁰ As described above (**Human Embryogenesis**), neonatal rat pups are relatively tolerant to hypoxia in part because of the ability of neuronal tissue to prevent hypoxia-induced calcium influx and glutamate accumulation.³²

Similarly, the AGS survive both oxygen and glucose deprivation *despite* significant depletion of ATP, suggesting that the ability of these animals to tolerate ischemia is due not only to reduced oxygen consumption but also the ability to cope with depleted intracellular energy stores.¹¹⁷ Inhibition of the epsilon protein kinase C (ePKC) protein, which inhibits both the Na/K-ATPase and voltage-gated Na channels, severely attenuates the AGS’ tolerance for hypoxia and shortens the time to ischemic depolarization (ID), providing further evidence that AGS and other hibernating animals tolerate loss of high energy substrates by preventing membrane depolarization and the subsequent electrolyte disarray that ultimately leads to cell death.¹¹⁶

Even more surprising, when AGS finally succumb to oxygen supply:demand mismatching and ischemic depolarization does occur, glutamate efflux is less harmful to these animals.^{121,122} This finding is likely due to decreased expression of NMDA receptors and lower levels of glutamate in the AGS.¹²³ AGS also activate mitogen activated protein kinase (MAPK) stress pathways during euthermia and arousal.¹¹⁵ MAPK activation correlates with HIF-1 expression, can be triggered by small increases in intracellular calcium,¹²¹ and has been associated with attenuation of calcium accumulation in an *in vitro* preconditioning model (rat hippocampal slices).¹²⁴

Diving Adaptations

While humans can typically only tolerate several minutes of apnea, some species of diving whales (*Physeter catodon*) and seals (*Mirounga leonina*, *Cystophora cristata*) can remain under water for much longer periods of time – in some cases, over an hour.¹⁰² These species have developed several macro-physiologic adaptations that allow them to store large amounts of oxygen, including higher hemoglobin levels (up to 25 g/dL) and myoglobin (which also behaves as an antioxidant), high tidal volumes when surfacing, increased tissue capillary density, the ability to cool during submergence, and redistribution of blood flow during periods of apnea.¹⁰²

Despite these macro-physiologic adaptations, diving animals eventually deplete most of their oxygen stores. Seals may achieve PaO₂ levels as low as 12 mm Hg.¹²⁵ Remarkably, seals can maintain cerebral integrity (measured using electroencephalography) to PaO₂ levels as low as 7-10 mm Hg,³⁰ which are substantially lower than the “critical” value of 25-40 mm Hg that affects ATP production in non-diving mammals.¹²⁶ Clearly, other adaptations exist to maintain organ function and promote survival. Some diving animals have an increased ability to utilize anaerobic pathways. Because such pathways are inefficient compared to oxidative phosphorylation, increased substrate availability is needed for this mechanism and increased glycogen stores, increased glycolytic capacity, and an enhanced ability to metabolize lactic acid have been documented in these animals.¹²⁷⁻¹²⁹

Additionally, diving mammals may be able to transiently “switch off” the metabolic activity of certain tissue beds. In particular, *in vitro* studies have demonstrated that hepatic and renal tissue can transiently reduce their ATP consumption to 1% of resting of resting rates. These organs may also reduce ion permeability through channel arrest, which is particularly important as maintenance of ionic gradients is highly energy-intensive.¹²⁹⁻¹³¹ Interestingly, myocardial and hepatic tissue in non-diving mammals may also reduce ATP demand and utilization during hypoxia, likely through inhibition of cytochrome c oxidase and mitochondrial function.^{132,133}

Tissue from diving mammals subjected to oxidative stress produces more superoxide than non-diving animals, suggesting that diving mammals have adapted mechanisms to tolerate, rather than prevent, the formation of ROS.¹³⁴ Diving seals, for instance, express large amounts of antioxidant enzymes¹³⁵ and exhibit higher levels of superoxide in several tissue beds than the shorter/shallower diving dolphins, despite experiencing less oxidative stress.¹³⁶ Such behavior may be mediated by HIF-1, as its expression has been identified in

seals and is associated with lower levels of protein oxidation.^{137,138} Neuroglobin, a recently discovered protein located in the central nervous system,¹³⁹ may play a role in attenuating ROS damage in diving animals, although its exact function is controversial.^{134,140}

Cancer

The study of cancerous cells is useful to investigators interested in subcellular energetics for two reasons. First, cancer is the second leading cause of death in the United States¹⁴¹, and understanding how cancerous cells survive, thrive, and spread in austere environments may lead to potentially therapeutic interventions. Second, the survival strategies utilized by cancerous cells may be applicable to other cell types and have implications for non-cancerous disease states (sepsis) and various biological threats (hypoxia).

Non-cancerous cells have narrow tolerances for pH, oxygenation, and nutrient supply. In contrast, cancerous cells survive in unfavorable conditions without consistent access to the bloodstream and at lower pH (typically 6.2-6.8, as opposed to 7.2-7.4 for normal cells).^{35,142,143} Oxygen diffusion occurs at distance no more than 200 μm (up to 20 cell layers) and the partial pressure of oxygen, which varies cyclically, may be as low as 5 mm Hg in some cancerous cells.^{39,144} Thus in order for tumors to grow, neovascularization, hypoxia tolerance, or both are required.³⁵ Hypoxia in cancerous cells leads to HIF-1 mediated angiogenesis via VEGF and angiopoietin 2.³⁹ VEGF-mediated angiogenesis is essential for cancerous cells to grow beyond a few millimeters in size and is thought to force metastatic cells out of dormancy.^{145,146} **[Figure 5]**

Rapidly-dividing cells consume increased amounts of oxygen, worsening local hypoxia.¹⁴⁷ Because cancerous cells often have less access to oxygen (especially prior to VEGF-mediated angiogenesis), alternate metabolic pathways such as anaerobic metabolism are required. Interestingly, cancerous cells often preferentially convert glucose to lactate even under *normoxic* conditions, a behavior known as the Warburg effect.¹⁴⁸ Because anaerobic metabolism is significantly less efficient than oxidative phosphorylation, cancerous cell thus have unusually high glucose requirements (forming the basis for [¹⁸F]-deoxyglucose positron-emission tomography as a tumor identification tool).¹⁴⁸ Thus, in cancerous cells, HIF-1 stabilization also leads to upregulation of GLUT1 and GLUT3 glucose transporters, further facilitating the use of inefficient anaerobic metabolic pathways.¹⁴⁹

In addition to its effects on angiogenesis and inducing a shift from oxidative phosphorylation to glycolytic energy production, HIF-1 also reduces the mitochondrial supply by inhibiting mitochondrial biogenesis through a C-MYC dependent mechanism. HIF-1 thus protects the growing tumor from oxidative stress, and impacts genes that influence metastasis.^{39,148,150} Hypoxia also induces autophagy via BNIP3 and BNIP3L, thus further promoting tumor progression.⁵¹

Both hypoxia and ROS activate HIF-1.^{47,97-101} HIF-1 stability is particularly affected by both hydrogen peroxide (H_2O_2) and nitric oxide (NO).¹⁵¹⁻¹⁵⁷ Many forms of cancer over-express NO synthase (NOS) which increases endogenous levels of NO, stabilizes the HIF family of transcription factors, and initiates the pro-survival metabolic changes described

above even when oxygen levels are normal (see Hypoxia Inducible Factors and Mammalian Adaption to Hypoxia).^{158,159} Coincidentally, hypoxia, which induces a pro-survival response in cancerous cells, also confers resistance to both radiation therapy and chemotherapy through a variety of mechanisms.^{144,160} Oxygen increases DNA damage and higher levels of oxygen thus increase the efficacy of radiation therapy (and some chemotherapeutic agents).¹⁴⁴ While radiation therapy leads to increased local levels of oxygen due to destruction of susceptible cells and reduced oxygen consumption, HIF-1 is up-regulated following radiation therapy, likely due to ROS production.¹⁶¹ Similarly, fewer cells cycle (and thus susceptible to chemotherapy) in low oxygen environments.³⁹ Additionally, the same limited blood supply that delivers oxygen also delivers lower levels of chemotherapy, making drug delivery a challenge.¹⁴⁴

Hypoxia tolerance by cancer cells, while generally pro-survival, may also lead to oncologic treatment opportunities. As compared to hypoxia-sensitive human glioblastoma multiforme cells, hypoxia tolerant human glioblastoma multiforme cells maintain ATP concentrations and stable mitochondrial membrane potentials in the setting of hypoxia. While the mechanism(s) responsible for this preservation have yet to be clarified, the development of hypoxia tolerance is accompanied by increased sensitivity to various mitochondrial inhibitors, a potentially useful characteristic in the development of chemotherapeutic agents.¹⁶² Other treatment strategies based on the hypoxic behavior of cancer cells includes the use of prodrugs which are activated by hypoxia (thus leaving normal cells alone), HIF-1 targeting, hypoxia-selective gene therapy, and even recombinant anaerobic bacteria (for a excellent review on the subject, see Semenza¹⁴⁹ and also Brown and Wilson¹⁴⁴).

Sepsis

Globally, uncontrolled infection (sepsis) is responsible for 721,000 to 2.1 million deaths/year.¹⁶³⁻¹⁶⁶ As in many disease processes, oxygen supply and demand and/or subcellular derangements are thought to play a prominent role in the manifestation of sepsis. Specifically, septic pathology may be thought of in terms of three distinct strata, all of which may be abnormal - the macrovasculature (blood pressure, cardiac output, hemoglobin concentration, arterial oxygen saturation, mixed venous oxygen saturation), the microvasculature (small vessels which bring red blood cells into close proximity to tissues that need oxygen and are deranged during sepsis), and the subcellular apparatus (mitochondria and electron transport chain). **[Figure 6]** Therapies designed to improve macrovascular function have largely been a failure.¹⁶⁷⁻¹⁶⁹ Trials targeting microcirculatory dysfunction have yielded conflicting results.¹⁷⁰

Targeting subcellular processes in sepsis has been challenging. Mitochondrial dysfunction plays a prominent role in sepsis.^{171,172} Total detectable cytochrome levels fall during the initiation of sepsis,¹⁷¹ and cytochrome aa₃ transitions from an oxidized to a reduced state as sepsis progresses in a variety of sepsis models,¹⁷³⁻¹⁷⁶ despite the maintenance of adequate oxygen delivery. Animal studies of experimental sepsis (lipopolysaccharide [LPS] infusion) have demonstrated mitochondrial swelling, respiratory dysfunction, and partial uncoupling of oxidative phosphorylation.¹⁷⁷

Several investigators have attempted to better understand oxygenation at the level of the cell, using tissue oxygen tension measurements. These studies, which have been conducted in septic rat models (LPS and cecal ligation and puncture [CLP]), pigs (LPS), and humans, have not produced consistent results.^{179,182,186,190,196,201-206} Hotchkiss examined the impact of CLP in rodents using the cellular hypoxia marker [18F]fluoromisonidazole, and found no evidence of cellular hypoxia during sepsis.¹⁹⁵ Of note, these studies examined intracellular oxygenation at varying time points, ranging from 0-42 hours after initiation of experimental sepsis.

Rather than focus on tissue oxygen tension, some investigators have attempted to measure high energy phosphate levels. As with the tissue oxygen tension experiments, initial animal work utilizing P₃₁ nuclear magnetic resonance (NMR) to measure high energy phosphates (e.g. ATP) have also produced conflicting results.^{189,193,194,197,198,200} These early studies utilized inconsistent, single time points, and did not discriminate between survivors and non-survivors. A more recent animal study demonstrated nitric oxide overproduction and mitochondrial dysfunction (exhibited by both ATP depletion and complex I inhibition) that generally worsened over time and with increasing sepsis severity.²⁰⁸ Singer and Brealey synthesized data from multiple animal models of varying severity and time points, and found that mitochondrial dysfunction does occur over longer periods of time and with higher severity.²⁰⁹

Early human studies reiterated these longer-term animal findings. Muscle ATP levels are lower in septic patients than in those undergoing elective surgical procedures.^{210,211} More recently, Brealey et al. analyzed vastus lateralis muscle samples in septic humans and found that non-survivors had lower tissue ATP, less complex I (NADH dehydrogenase) activity, and complex IV (aa₃; c oxidase) activity than sepsis survivors and non-septic controls.²⁰⁷ Similarly, Fredriksson et al. found lower ATP levels in the leg muscles of septic patients as compared to healthy controls undergoing elective surgery. Additionally, this group found reductions in complex I and IV activity in intercostal and leg muscles, respectively, as well as evidence of increased mitochondrial oxidative stress.²¹² The growing appreciation that decreased oxygen consumption is due to defects in cellular respiration, rather than abnormal oxygen delivery has led to the development of the term “cytopathic hypoxia.”²¹³ It is not clear whether this mitochondria-driven reduction in oxygen consumption and accompanying “metabolic shutdown” is harmful or an attempt at organ preservation by down-regulating organ function and thus the requirement for high energy phosphates, or some combination of the two. Key elements of this metabolic shutdown include mitochondrial inhibition, reduced mitochondrial biogenesis, and decreased hormonal stimulation.²¹⁴ Sepsis-induced organ quiescence is reversible if the organism survives.^{208,214-216}

Interestingly, the increase in inflammatory mediators that accompanies sepsis (as well as ischemia-reperfusion injury, see **Ischemia-Reperfusion and Reactive Oxygen Species** above) results in overproduction of ROS and RNS, both of which induce mitochondrial dysfunction. HIF-1, which is protective in ischemia-reperfusion injury^{88,217-220} and preconditioning,^{19,20} exerts control over mitochondrial metabolism,⁸⁸ and appears to be upregulated in the setting of sepsis.²²¹ The complex link between HIF-1 and ROS/RNS (ROS stabilize HIF-1,⁹⁷⁻¹⁰⁰ and ROS scavengers have been shown to destabilize

HIF-1,^{47,98,99,101} as described above) may explain why, despite some promising small pre-clinical trials, a phase III trial of NOS inhibition in humans with severe sepsis increased mortality in the treatment group.²²² Further strengthening the theory that hypoxia, HIF-1, and inflammatory disarray (e.g. infection, ischemia) are related is the connection between hypoxia and the immune system - hypoxia increases the expression of Toll-like receptors TLR2 and TLR6, and HIF-1 enhances the bactericidal activity of phagocytes and enhances innate immunity in a variety of cell types.²²³⁻²²⁵

Conclusions

While oxygen is often considered an essential component of life, the natural world provides multiple examples of complex organisms that survive, and in some cases thrive, in hypoxic or anoxic environments. These include, but are not limited to all mammalian embryos, as well as some hibernating and diving mammals. Additionally, the development of hypoxia tolerance improves the survival of ischemic myocardium (through preconditioning) and increases cancer metastasis. HIF-1 is central to hypoxic adaptation, activating over 1500 target genes and leads to angiogenesis, a shift towards anaerobic metabolism, and electron transport chain modifications which increase electron transfer efficiency, enable the activation of iNOS, and control the balance of ROS. ROS are both essential (vasodilation, decreased platelet and neutrophil adhesion) and potentially harmful (oxidative stress), and the ability of organisms to manage ROS is relevant to both preconditioning and the response to uncontrolled infection and inflammation. Greater understanding of the similarities between various pathologies and evolutionary adaptations, especially regarding the electron transport chain, production and attenuation of reactive species, and alternate metabolic pathways, may allow for the development of novel treatment modalities (discussed in the second part of this review).

References

1. Karl DM. Hidden in a sea of microbes. *Nature*. 2002; 415:590–1. [PubMed: 11832923]
2. Dunwoodie SL. The role of hypoxia in development of the Mammalian embryo. *Dev Cell*. 2009; 17:755–73. [PubMed: 20059947]
3. Kelly DP. Hypoxic reprogramming. *Nat Genet*. 2008; 40:132–4. [PubMed: 18227870]
4. Kuypers MM, Sliekers AO, Lavik G, Schmid M, Jorgensen BB, Kuenen JG, Sinninghe Damste JS, Strous M, Jetten MS. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*. 2003; 422:608–11. [PubMed: 12686999]
5. van der Wielen PW, Bolhuis H, Borin S, Daffonchio D, Corselli C, Giuliano L, D'Auria G, de Lange GJ, Huebner A, Varnavas SP, Thomson J, Tamburini C, Marty D, McGenity TJ, Timmis KN, BioDeep Scientific P. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*. 2005; 307:121–3. [PubMed: 15637281]
6. Daffonchio D, Borin S, Brusa T, Brusetti L, van der Wielen PW, Bolhuis H, Yakimov MM, D'Auria G, Giuliano L, Marty D, Tamburini C, McGenity TJ, Hallsworth JE, Sass AM, Timmis KN, Tselepidis A, de Lange GJ, Hubner A, Thomson J, Varnavas SP, Gasparoni F, Gerber HW, Malinverno E, Corselli C, Garcin J, McKew B, Golyshev PN, Lampadariou N, Polymenakou P, Calore D, Cenedese S, Zanon F, Hoog S, Biodeep Scientific P. Stratified prokaryote network in the oxic-anoxic transition of a deep-sea halocline. *Nature*. 2006; 440:203–7. [PubMed: 16525471]
7. Lipp JS, Morono Y, Inagaki F, Hinrichs KU. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature*. 2008; 454:991–4. [PubMed: 18641632]

8. Danovaro R, Dell'Anno A, Pusceddu A, Gambi C, Heiner I, Kristensen RM. The first metazoa living in permanently anoxic conditions. *BMC Biol.* 2010; 8:30. [PubMed: 20370908]
9. Rossen R, Kabat H, Anderson JP. Acute arrest of cerebral circulation in man. *Arch Neurol Psychiatry.* 1943; 50:510–28.
10. Trollmann R, Gassmann M. The role of hypoxia-inducible transcription factors in the hypoxic neonatal brain. *Brain Dev.* 2009; 31:503–9. [PubMed: 19398180]
11. Morriss GM, New DA. Effect of oxygen concentration on morphogenesis of cranial neural folds and neural crest in cultured rat embryos. *J Embryol Exp Morphol.* 1979; 54:17–35. [PubMed: 528863]
12. Okazaki K, Maltepe E. Oxygen, epigenetics and stem cell fate. *Regen Med.* 2006; 1:71–83. [PubMed: 17465821]
13. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol.* 2008; 9:285–96. [PubMed: 18285802]
14. Lee YM, Jeong CH, Koo SY, Son MJ, Song HS, Bae SK, Raleigh JA, Chung HY, Yoo MA, Kim KW. Determination of hypoxic region by hypoxia marker in developing mouse embryos in vivo: a possible signal for vessel development. *Dev Dyn.* 2001; 220:175–86. [PubMed: 11169851]
15. Fraisl P, Mazzone M, Schmidt T, Carmeliet P. Regulation of angiogenesis by oxygen and metabolism. *Dev Cell.* 2009; 16:167–79. [PubMed: 19217420]
16. Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol.* 1992; 80:283–5. [PubMed: 1635745]
17. Mitchell JA, Yochim JM. Intrauterine oxygen tension during the estrous cycle in the rat: its relation to uterine respiration and vascular activity. *Endocrinology.* 1968; 83:701–5. [PubMed: 5693655]
18. Adelman DM, Gertsenstein M, Nagy A, Simon MC, Maltepe E. Placental cell fates are regulated in vivo by HIF-mediated hypoxia responses. *Genes Dev.* 2000; 14:3191–203. [PubMed: 11124810]
19. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci U S A.* 2007; 104:5431–6. [PubMed: 17374716]
20. Jarecki J, Johnson E, Krasnow MA. Oxygen regulation of airway branching in *Drosophila* is mediated by branchless FGF. *Cell.* 1999; 99:211–20. [PubMed: 10535739]
21. Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature.* 1997; 386:403–7. [PubMed: 9121557]
22. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.* 1998; 12:149–62. [PubMed: 9436976]
23. Motta PM, Nottola SA, Makabe S, Heyn R. Mitochondrial morphology in human fetal and adult female germ cells. *Hum Reprod.* 2000; 15(Suppl 2):129–47.
24. Bavister BD, Squirrell JM. Mitochondrial distribution and function in oocytes and early embryos. *Hum Reprod.* 2000; 15(Suppl 2):189–98.
25. Wilding M, Coppola G, Dale B, Di Matteo L. Mitochondria and human preimplantation embryo development. *Reproduction.* 2009; 137:619–24. [PubMed: 19176592]
26. Wilding M, Fiorentino A, De Simone ML, Infante V, De Matteo L, Marino M, Dale B. Energy substrates, mitochondrial membrane potential and human preimplantation embryo division. *Reprod Biomed Online.* 2002; 5:39–42. [PubMed: 12470544]
27. Houghton FD, Thompson JG, Kennedy CJ, Leese HJ. Oxygen consumption and energy metabolism of the early mouse embryo. *Mol Reprod Dev.* 1996; 44:476–85. [PubMed: 8844690]
28. Hochachka PW, Buck LT, Doll CJ, Land SC. Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc Natl Acad Sci U S A.* 1996; 93:9493–8. [PubMed: 8790358]
29. Perrone S, Tataranno LM, Stazzoni G, Ramenghi L, Buonocore G. Brain susceptibility to oxidative stress in the perinatal period. *J Matern Fetal Neonatal Med.* 2015; 28(Suppl 1):2291–5. [PubMed: 23968388]

30. Larson J, Drew KL, Folkow LP, Milton SL, Park TJ. No oxygen? No problem! Intrinsic brain tolerance to hypoxia in vertebrates. *The Journal of experimental biology*. 2014; 217:1024–39. [PubMed: 24671961]
31. Bickler PE, Buck LT. Adaptations of vertebrate neurons to hypoxia and anoxia: maintaining critical Ca²⁺ concentrations. *The Journal of experimental biology*. 1998; 201:1141–52. [PubMed: 9510526]
32. Bickler PE, Hansen BM. Hypoxia-tolerant neonatal CA1 neurons: relationship of survival to evoked glutamate release and glutamate receptor-mediated calcium changes in hippocampal slices. *Brain research Developmental brain research*. 1998; 106:57–69. [PubMed: 9554954]
33. Mottet D, Michel G, Renard P, Ninane N, Raes M, Michiels C. Role of ERK and calcium in the hypoxia-induced activation of HIF-1. *Journal of cellular physiology*. 2003; 194:30–44. [PubMed: 12447987]
34. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol*. 2007; 8:49–62. [PubMed: 17183360]
35. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer*. 2008; 8:851–64. [PubMed: 18846101]
36. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene*. 2010; 29:625–34. [PubMed: 19946328]
37. Prabhakar NR, Semenza GL. Oxygen Sensing and Homeostasis. *Physiology (Bethesda)*. 2015; 30:340–8. [PubMed: 26328879]
38. Singh RP, Franke K, Kalucka J, Mamlouk S, Muschter A, Gembarska A, Grinenko T, Willam C, Naumann R, Anastassiadis K, Stewart AF, Bornstein S, Chavakis T, Breier G, Waskow C, Wielockx B. HIF prolyl hydroxylase 2 (PHD2) is a critical regulator of hematopoietic stem cell maintenance during steady-state and stress. *Blood*. 2013; 121:5158–66. [PubMed: 23667053]
39. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer*. 2008; 8:425–37. [PubMed: 18500244]
40. Rey S, Semenza GL. Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovascular research*. 2010; 86:236–42. [PubMed: 20164116]
41. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. *Cell Metab*. 2009; 9:152–64. [PubMed: 19147412]
42. Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaud C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. *J Clin Invest*. 2009; 119:1159–66. [PubMed: 19352007]
43. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol*. 1992; 12:5447–54. [PubMed: 1448077]
44. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab*. 2006; 3:187–97. [PubMed: 16517406]
45. Huang D, Li T, Li X, Zhang L, Sun L, He X, Zhong X, Jia D, Song L, Semenza GL, Gao P, Zhang H. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. *Cell Rep*. 2014; 8:1930–42. [PubMed: 25242319]
46. Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. *Cell Metab*. 2009; 10:273–84. [PubMed: 19808020]
47. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A*. 1998; 95:11715–20. [PubMed: 9751731]
48. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*. 2006; 3:177–85. [PubMed: 16517405]

49. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell*. 2007; 129:111–22. [PubMed: 17418790]
50. Kwast KE, Burke PV, Poyton RO. Oxygen sensing and the transcriptional regulation of oxygen-responsive genes in yeast. *The Journal of experimental biology*. 1998; 201:1177–95. [PubMed: 9510529]
51. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, Mazure NM. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol*. 2009; 29:2570–81. [PubMed: 19273585]
52. Aragones J, Schneider M, Van Geyte K, Fraisl P, Dresselaers T, Mazzone M, Dirx R, Zacchigna S, Lemieux H, Jeoung NH, Lambrechts D, Bishop T, Lafuste P, Diez-Juan A, Harten SK, Van Noten P, De Bock K, Willam C, Tjwa M, Grosfeld A, Navet R, Moons L, Vandendriessche T, Deroose C, Wijeyekoon B, Nuyts J, Jordan B, Silasi-Mansat R, Lupu F, Dewerchin M, Pugh C, Salmon P, Mortelmans L, Gallez B, Gorus F, Buyse J, Sluse F, Harris RA, Gnaiger E, Hespel P, Van Hecke P, Schuit F, Van Veldhoven P, Ratcliffe P, Baes M, Maxwell P, Carmeliet P. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat Genet*. 2008; 40:170–80. [PubMed: 18176562]
53. Wu P, Peters JM, Harris RA. Adaptive increase in pyruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor alpha. *Biochem Biophys Res Commun*. 2001; 287:391–6. [PubMed: 11554740]
54. Noguchi CT, Asavaritikrai P, Teng R, Jia Y. Role of erythropoietin in the brain. *Crit Rev Oncol Hematol*. 2007; 64:159–71. [PubMed: 17482474]
55. Viviani B, Bartesaghi S, Corsini E, Villa P, Ghezzi P, Garau A, Galli CL, Marinovich M. Erythropoietin protects primary hippocampal neurons increasing the expression of brain-derived neurotrophic factor. *J Neurochem*. 2005; 93:412–21. [PubMed: 15816864]
56. Sun Y, Calvert JW, Zhang JH. Neonatal hypoxia/ischemia is associated with decreased inflammatory mediators after erythropoietin administration. *Stroke; a journal of cerebral circulation*. 2005; 36:1672–8.
57. Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, Schellinger PD, Bohn M, Becker H, Wegrzyn M, Jahnig P, Herrmann M, Knauth M, Bahr M, Heide W, Wagner A, Schwab S, Reichmann H, Schwendemann G, Dengler R, Kastrup A, Bartels C, Group EPOST. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke; a journal of cerebral circulation*. 2009; 40:e647–56.
58. Fauchere JC, Koller BM, Tschopp A, Dame C, Ruegger C, Bucher HU, Swiss Erythropoietin Neuroprotection Trial G. Safety of Early High-Dose Recombinant Erythropoietin for Neuroprotection in Very Preterm Infants. *J Pediatr*. 2015; 167:52–7. e1–3. [PubMed: 25863661]
59. Winnay JN, Kahn CR. PI 3-kinase regulatory subunits as regulators of the unfolded protein response. *Methods Enzymol*. 2011; 490:147–58. [PubMed: 21266249]
60. Arsham AM, Howell JJ, Simon MC. A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. *J Biol Chem*. 2003; 278:29655–60. [PubMed: 12777372]
61. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG Jr. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev*. 2004; 18:2893–904. [PubMed: 15545625]
62. Liu L, Cash TP, Jones RG, Keith B, Thompson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. *Mol Cell*. 2006; 21:521–31. [PubMed: 16483933]
63. Cummins EP, Berra E, Comerford KM, Ginouves A, Fitzgerald KT, Seeballuck F, Godson C, Nielsen JE, Moynagh P, Pouyssegur J, Taylor CT. Prolyl hydroxylase-1 negatively regulates I κ B kinase-beta, giving insight into hypoxia-induced NF κ B activity. *Proc Natl Acad Sci U S A*. 2006; 103:18154–9. [PubMed: 17114296]
64. Culver C, Sundqvist A, Mudie S, Melvin A, Xirodimas D, Rocha S. Mechanism of hypoxia-induced NF-kappaB. *Mol Cell Biol*. 2010; 30:4901–21. [PubMed: 20696840]
65. Taylor CT, Cummins EP. The role of NF-kappaB in hypoxia-induced gene expression. *Ann N Y Acad Sci*. 2009; 1177:178–84. [PubMed: 19845620]

66. O'Sullivan B, Thompson A, Thomas R. NF-kappa B as a therapeutic target in autoimmune disease. *Expert Opin Ther Targets*. 2007; 11:111–22. [PubMed: 17227228]
67. Abraham E. Nuclear factor-kappaB and its role in sepsis-associated organ failure. *J Infect Dis*. 2003; 187(Suppl 2):S364–9. [PubMed: 12792853]
68. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch Pathol*. 1960; 70:68–78. [PubMed: 14407094]
69. Bulkely BH, Hutchins GM. Myocardial consequences of coronary artery bypass graft surgery. The paradox of necrosis in areas of revascularization. *Circulation*. 1977; 56:906–13. [PubMed: 303553]
70. Parks DA, Granger DN. Contributions of ischemia and reperfusion to mucosal lesion formation. *The American journal of physiology*. 1986; 250:G749–53. [PubMed: 3717337]
71. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. *Anesthesiology*. 2001; 94:1133–8. [PubMed: 11465607]
72. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. *Nat Med*. 2011; 17:1391–401. [PubMed: 22064429]
73. Lesnefsky EJ, Dauber IM, Horwitz LD. Myocardial sulfhydryl pool alterations occur during reperfusion after brief and prolonged myocardial ischemia in vivo. *Circ Res*. 1991; 68:605–13. [PubMed: 1991359]
74. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev*. 1997; 77:731–58. [PubMed: 9234964]
75. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J*. 1973; 134:707–16. [PubMed: 4749271]
76. Turrens JF. Superoxide production by the mitochondrial respiratory chain. *Biosci Rep*. 1997; 17:3–8. [PubMed: 9171915]
77. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J*. 1980; 191:421–7. [PubMed: 6263247]
78. Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia--reperfusion, aging, and heart failure. *J Mol Cell Cardiol*. 2001; 33:1065–89. [PubMed: 11444914]
79. Turrens JF, Alexandre A, Lehninger AL. Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch Biochem Biophys*. 1985; 237:408–14. [PubMed: 2983613]
80. Sugioka K, Nakano M, Totsune-Nakano H, Minakami H, Tero-Kubota S, Ikegami Y. Mechanism of O₂-generation in reduction and oxidation cycle of ubiquinones in a model of mitochondrial electron transport systems. *Biochim Biophys Acta*. 1988; 936:377–85. [PubMed: 2848580]
81. Tompkins AJ, Burwell LS, Digerness SB, Zaragoza C, Holman WL, Brookes PS. Mitochondrial dysfunction in cardiac ischemia-reperfusion injury: ROS from complex I, without inhibition. *Biochim Biophys Acta*. 2006; 1762:223–31. [PubMed: 16278076]
82. Becker LB, vanden Hoek TL, Shao ZH, Li CQ, Schumacker PT. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *The American journal of physiology*. 1999; 277:H2240–6. [PubMed: 10600842]
83. Kwong LK, Sohal RS. Substrate and site specificity of hydrogen peroxide generation in mouse mitochondria. *Arch Biochem Biophys*. 1998; 350:118–26. [PubMed: 9466828]
84. Liu SS. Cooperation of a "reactive oxygen cycle" with the Q cycle and the proton cycle in the respiratory chain--superoxide generating and cycling mechanisms in mitochondria. *J Bioenerg Biomembr*. 1999; 31:367–76. [PubMed: 10665526]
85. Kim KB, Chung HH, Kim MS, Rho JR. Changes in the antioxidative defensive system during open heart operations in humans. *Ann Thorac Surg*. 1994; 58:170–5. [PubMed: 8037518]
86. Roberts MJ, Young IS, Trouton TG, Trimble ER, Khan MM, Webb SW, Wilson CM, Patterson GC, Adgey AA. Transient release of lipid peroxides after coronary artery balloon angioplasty. *Lancet*. 1990; 336:143–5. [PubMed: 1973474]
87. Zweier JL. Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. *J Biol Chem*. 1988; 263:1353–7. [PubMed: 2826476]

88. Semenza GL. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim Biophys Acta*. 2011; 1813:1263–8. [PubMed: 20732359]
89. Ambrosio G, Zweier JL, Duilio C, Kuppusamy P, Santoro G, Elia PP, Tritto I, Cirillo P, Condorelli M, Chiariello M, et al. Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. *J Biol Chem*. 1993; 268:18532–41. [PubMed: 8395507]
90. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in “stunned” myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitron. *J Clin Invest*. 1988; 82:476–85. [PubMed: 2841353]
91. Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci U S A*. 1987; 84:1404–7. [PubMed: 3029779]
92. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. *J Mol Cell Cardiol*. 1973; 5:395–407. [PubMed: 4355339]
93. Moens AL, Claeys MJ, Timmermans JP, Vrints CJ. Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. *Int J Cardiol*. 2005; 100:179–90. [PubMed: 15823623]
94. Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, Szeto HH. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J Biol Chem*. 2004; 279:34682–90. [PubMed: 15178689]
95. Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP, Sammut IA. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *FASEB J*. 2005; 19:1088–95. [PubMed: 15985532]
96. Powell SR, Tortolani AJ. Recent advances in the role of reactive oxygen intermediates in ischemic injury. I. Evidence demonstrating presence of reactive oxygen intermediates; II. Role of metals in site-specific formation of radicals. *J Surg Res*. 1992; 53:417–29. [PubMed: 1328767]
97. Mansfield KD, Guzy RD, Pan Y, Young RM, Cash TP, Schumacker PT, Simon MC. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF- α activation. *Cell Metab*. 2005; 1:393–9. [PubMed: 16054088]
98. Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, Simon MC, Hammerling U, Schumacker PT. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab*. 2005; 1:401–8. [PubMed: 16054089]
99. Brunelle JK, Bell EL, Quesada NM, Vercauteren K, Tiranti V, Zeviani M, Scarpulla RC, Chandel NS. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. *Cell Metab*. 2005; 1:409–14. [PubMed: 16054090]
100. Kaelin WG Jr. ROS: really involved in oxygen sensing. *Cell Metab*. 2005; 1:357–8. [PubMed: 16054083]
101. Sanjuan-Pla A, Cervera AM, Apostolova N, Garcia-Bou R, Victor VM, Murphy MP, McCreath KJ. A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1 α . *FEBS Lett*. 2005; 579:2669–74. [PubMed: 15862307]
102. Ramirez JM, Folkow LP, Blix AS. Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. *Annual review of physiology*. 2007; 69:113–43.
103. Toien O, Drew KL, Chao ML, Rice ME. Ascorbate dynamics and oxygen consumption during arousal from hibernation in Arctic ground squirrels. *American journal of physiology Regulatory, integrative and comparative physiology*. 2001; 281:R572–83.
104. Drew KL, Harris MB, LaManna JC, Smith MA, Zhu XW, Ma YL. Hypoxia tolerance in mammalian heterotherms. *The Journal of experimental biology*. 2004; 207:3155–62. [PubMed: 15299037]
105. Milsom WK, Zimmer MB, Harris MB. Regulation of cardiac rhythm in hibernating mammals. *Comparative biochemistry and physiology Part A, Molecular & integrative physiology*. 1999; 124:383–91.
106. Heldmaier G, Ortmann S, Elvert R. Natural hypometabolism during hibernation and daily torpor in mammals. *Respiratory physiology & neurobiology*. 2004; 141:317–29. [PubMed: 15288602]

107. Buck MJ, Squire TL, Andrews MT. Coordinate expression of the PDK4 gene: a means of regulating fuel selection in a hibernating mammal. *Physiological genomics*. 2002; 8:5–13. [PubMed: 11842126]
108. Andrews MT. Genes controlling the metabolic switch in hibernating mammals. *Biochemical Society transactions*. 2004; 32:1021–4. [PubMed: 15506953]
109. Gentile NT, Spatz M, Brenner M, McCarron RM, Hallenbeck JM. Decreased calcium accumulation in isolated nerve endings during hibernation in ground squirrels. *Neurochemical research*. 1996; 21:947–54. [PubMed: 8895849]
110. Wang SQ, Lakatta EG, Cheng H, Zhou ZQ. Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. *The Journal of experimental biology*. 2002; 205:2957–62. [PubMed: 12200399]
111. Dave KR, Prado R, Raval AP, Drew KL, Perez-Pinzon MA. The arctic ground squirrel brain is resistant to injury from cardiac arrest during euthermia. *Stroke; a journal of cerebral circulation*. 2006; 37:1261–5.
112. Stenzel-Poore MP, Stevens SL, Xiong Z, Lessov NS, Harrington CA, Mori M, Meller R, Rosenzweig HL, Tobar E, Shaw TE, Chu X, Simon RP. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet*. 2003; 362:1028–37. [PubMed: 14522533]
113. Drew KL, Rice ME, Kuhn TB, Smith MA. Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. *Free radical biology & medicine*. 2001; 31:563–73. [PubMed: 11522441]
114. Ma YL, Zhu X, Rivera PM, Toien O, Barnes BM, LaManna JC, Smith MA, Drew KL. Absence of cellular stress in brain after hypoxia induced by arousal from hibernation in Arctic ground squirrels. *American journal of physiology Regulatory, integrative and comparative physiology*. 2005; 289:R1297–306.
115. Zhu X, Smith MA, Perry G, Wang Y, Ross AP, Zhao HW, Lamanna JC, Drew KL. MAPKs are differentially modulated in arctic ground squirrels during hibernation. *J Neurosci Res*. 2005; 80:862–8. [PubMed: 15884016]
116. Dave KR, Anthony Defazio R, Raval AP, Dashkin O, Saul I, Iceman KE, Perez-Pinzon MA, Drew KL. Protein kinase C epsilon activation delays neuronal depolarization during cardiac arrest in the euthermic arctic ground squirrel. *J Neurochem*. 2009; 110:1170–9. [PubMed: 19493168]
117. Christian SL, Ross AP, Zhao HW, Kristenson HJ, Zhan X, Rasley BT, Bickler PE, Drew KL. Arctic ground squirrel (*Spermophilus parryi*) hippocampal neurons tolerate prolonged oxygen-glucose deprivation and maintain baseline ERK1/2 and JNK activation despite drastic ATP loss. *J Cereb Blood Flow Metab*. 2008; 28:1307–19. [PubMed: 18398417]
118. Buzadzic B, Blagojevic D, Korac B, Saicic ZS, Spasic MB, Petrovic VM. Seasonal variation in the antioxidant defense system of the brain of the ground squirrel (*Citellus citellus*) and response to low temperature compared with rat. *Comparative biochemistry and physiology Part C, Pharmacology, toxicology & endocrinology*. 1997; 117:141–9.
119. D'Alecy LG, Lundy EF, Kluger MJ, Harker CT, LeMay DR, Schlafer M. Beta-hydroxybutyrate and response to hypoxia in the ground squirrel, *Spermophilus tridecemlineatus*. *Comparative biochemistry and physiology B, Comparative biochemistry*. 1990; 96:189–93. [PubMed: 2364670]
120. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev*. 1999; 79:1431–568. [PubMed: 10508238]
121. Ross AP, Christian SL, Zhao HW, Drew KL. Persistent tolerance to oxygen and nutrient deprivation and N-methyl-D-aspartate in cultured hippocampal slices from hibernating Arctic ground squirrel. *J Cereb Blood Flow Metab*. 2006; 26:1148–56. [PubMed: 16395285]
122. Gidday, J., Pérez-Pinzón, M., Drew, KL., Zuckerman, JA., Shenk, PE., Bogren, LK., Jinka, TR., Moore, JT. Hibernation: a natural model of tolerance to cerebral ischemia/reperfusion.. In: Gidday, J., Pérez-Pinzón, M., editors. *nate Tolerance in the CNS: Translational Neuroprotection* 37 by Pre- and Post-Conditioning. Springer; New York: 2012. p. 37-50.

123. Zhao HW, Ross AP, Christian SL, Buchholz JN, Drew KL. Decreased NR1 phosphorylation and decreased NMDAR function in hibernating Arctic ground squirrels. *J Neurosci Res.* 2006; 84:291–8. [PubMed: 16676330]
124. Bickler PE, Fahlman CS. Moderate increases in intracellular calcium activate neuroprotective signals in hippocampal neurons. *Neuroscience.* 2004; 127:673–83. [PubMed: 15283966]
125. Meir JU, Champagne CD, Costa DP, Williams CL, Ponganis PJ. Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *American journal of physiology Regulatory, integrative and comparative physiology.* 2009; 297:R927–39.
126. Erecinska M, Silver IA. Tissue oxygen tension and brain sensitivity to hypoxia. *Respir Physiol.* 2001; 128:263–76. [PubMed: 11718758]
127. Kerem D, Hammond DD, Elsner R. Tissue glycogen levels in the Weddell seal, *Leptonychotes weddelli*: a possible adaptation to asphyxial hypoxia. *Comparative biochemistry and physiology A, Comparative physiology.* 1973; 45:731–6. [PubMed: 4147925]
128. Blix AS, From SH. Lactate dehydrogenase in diving animals--a comparative study with special reference to the eider (*Somateria mollissima*). *Comparative biochemistry and physiology B, Comparative biochemistry.* 1971; 40:579–84. [PubMed: 513336]
129. Murphy B, Zapol WM, Hochachka PW. Metabolic activities of heart, lung, and brain during diving and recovery in the Weddell seal. *Journal of applied physiology: respiratory, environmental and exercise physiology.* 1980; 48:596–605.
130. Hochachka PW, Castellini JM, Hill RD, Schneider RC, Bengtson JL, Hill SE, Liggins GC, Zapol WM. Protective metabolic mechanisms during liver ischemia: transferable lessons from long-diving animals. *Mol Cell Biochem.* 1988; 84:77–85. [PubMed: 3231217]
131. Hong SK, Ashwell-Erickson S, Gigliotti P, Elsner R. Effects of Anoxia and Low pH on Organic Ion Transport and Electrolyte Distribution in Harbor Seal (*Phoca vitulina*) Kidney Slices. *Journal of Comparative Physiology B.* 1982; 149:19–24.
132. Budinger GR, Chandel N, Shao ZH, Li CQ, Melmed A, Becker LB, Schumacker PT. Cellular energy utilization and supply during hypoxia in embryonic cardiac myocytes. *The American journal of physiology.* 1996; 270:L44–53. [PubMed: 8772526]
133. Schumacker PT, Chandel N, Agusti AG. Oxygen conformance of cellular respiration in hepatocytes. *The American journal of physiology.* 1993; 265:L395–402. [PubMed: 8238374]
134. Zenteno-Savin T, Clayton-Hernandez E, Elsner R. Diving seals: are they a model for coping with oxidative stress? *Comparative biochemistry and physiology Toxicology & pharmacology : CBP.* 2002; 133:527–36. [PubMed: 12458181]
135. Vazquez-Medina JP, Zenteno-Savin T, Elsner R. Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. *Comparative biochemistry and physiology Toxicology & pharmacology : CBP.* 2006; 142:198–204. [PubMed: 16269268]
136. Cantu-Medellin N, Byrd B, Hohn A, Vazquez-Medina JP, Zenteno-Savin T. Differential antioxidant protection in tissues from marine mammals with distinct diving capacities. Shallow/short vs. deep/long divers. *Comparative biochemistry and physiology Part A, Molecular & integrative physiology.* 2011; 158:438–43.
137. Johnson P, Elsner R, Zenteno-Savin T. Hypoxia-inducible factor 1 proteomics and diving adaptations in ringed seal. *Free radical biology & medicine.* 2005; 39:205–12. [PubMed: 15964512]
138. Johnson P, Elsner R, Zenteno-Savin T. Hypoxia-Inducible Factor in ringed seal (*Phoca hispida*) tissues. *Free radical research.* 2004; 38:847–54. [PubMed: 15493458]
139. Burmester T, Weich B, Reinhardt S, Hankeln T. A vertebrate globin expressed in the brain. *Nature.* 2000; 407:520–3. [PubMed: 11029004]
140. Burmester T, Hankeln T. What is the function of neuroglobin? *The Journal of experimental biology.* 2009; 212:1423–8. [PubMed: 19411534]
141. Control CfD. *Leading Causes of Death.* 2015
142. Gerweck LE, Vijayappa S, Kozin S. Tumor pH controls the in vivo efficacy of weak acid and base chemotherapeutics. *Mol Cancer Ther.* 2006; 5:1275–9. [PubMed: 16731760]
143. Cardone RA, Casavola V, Reshkin SJ. The role of disturbed pH dynamics and the Na⁺/H⁺ exchanger in metastasis. *Nat Rev Cancer.* 2005; 5:786–95. [PubMed: 16175178]

144. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer*. 2004; 4:437–47. [PubMed: 15170446]
145. Bos R, Zhong H, Hanrahan CF, Mommers EC, Semenza GL, Pinedo HM, Abeloff MD, Simons JW, van Diest PJ, van der Wall E. Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis. *J Natl Cancer Inst*. 2001; 93:309–14. [PubMed: 11181778]
146. Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, Flynn E, Watnick RS, Straume O, Akslen LA, Folkman J, Almog N. A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. *J Natl Cancer Inst*. 2006; 98:316–25. [PubMed: 16507828]
147. Freyer JP. Rates of oxygen consumption for proliferating and quiescent cells isolated from multicellular tumor spheroids. *Adv Exp Med Biol*. 1994; 345:335–42. [PubMed: 8079727]
148. Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, Zeller KI, Dang CV, Semenza GL. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell*. 2007; 11:407–20. [PubMed: 17482131]
149. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. 2003; 3:721–32. [PubMed: 13130303]
150. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell*. 2003; 3:347–61. [PubMed: 12726861]
151. Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJ. Free radical biology and medicine: it's a gas, man! *American journal of physiology Regulatory, integrative and comparative physiology*. 2006; 291:R491–511.
152. Ignarro LJ. Nitric oxide as a unique signaling molecule in the vascular system: a historical overview. *J Physiol Pharmacol*. 2002; 53:503–14. [PubMed: 12512688]
153. Bell EL, Klimova TA, Eisenbart J, Moraes CT, Murphy MP, Budinger GR, Chandel NS. The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. *J Cell Biol*. 2007; 177:1029–36. [PubMed: 17562787]
154. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. *J Biol Chem*. 2000; 275:25130–8. [PubMed: 10833514]
155. BelAiba RS, Djordjevic T, Bonello S, Flugel D, Hess J, Kietzmann T, Grolach A. Redox-sensitive regulation of the HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells. *Biol Chem*. 2004; 385:249–57. [PubMed: 15134338]
156. Sumbayev VV, Budde A, Zhou J, Brune B. HIF-1 alpha protein as a target for S-nitrosation. *FEBS Lett*. 2003; 535:106–12. [PubMed: 12560087]
157. Mateo J, Garcia-Lecea M, Cadenas S, Hernandez C, Moncada S. Regulation of hypoxia inducible factor-1alpha by nitric oxide through mitochondria-dependent and - independent pathways. *Biochem J*. 2003; 376:537–44. [PubMed: 14531732]
158. Tozer GM, Everett SA. Nitric oxide in tumour biology and cancer therapy. Part 1: Physiological aspects. *Clin Oncol (R Coll Radiol)*. 1997; 9:282–93. [PubMed: 9368723]
159. Tozer GM, Everett SA. Nitric oxide in tumor biology and cancer therapy. Part 2: Therapeutic implications. *Clin Oncol (R Coll Radiol)*. 1997; 9:357–64. [PubMed: 9448964]
160. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol*. 1953; 26:638–48. [PubMed: 13106296]
161. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. *Cancer Cell*. 2004; 5:429–41. [PubMed: 15144951]
162. Turcotte ML, Parliament M, Franko A, Allalunis-Turner J. Variation in mitochondrial function in hypoxia-sensitive and hypoxia-tolerant human glioma cells. *British journal of cancer*. 2002; 86:619–24. [PubMed: 11870546]
163. Jawad I, Luksic I, Rafnsson SB. Assessing available information on the burden of sepsis: global estimates of incidence, prevalence and mortality. *J Glob Health*. 2012; 2:10404.

164. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med.* 2008; 36:296–327. [PubMed: 18158437]
165. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA.* 2014; 311:1308–16. [PubMed: 24638143]
166. A Randomized Trial of Protocol-Based Care for Early Septic Shock. *N Engl J Med.* 2014
167. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF, Coats TJ, Singer M, Young JD, Rowan KM, Pro MTI. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med.* 2015; 372:1301–11. [PubMed: 25776532]
168. Pro CI, Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC. A randomized trial of protocol-based care for early septic shock. *N Engl J Med.* 2014; 370:1683–93. [PubMed: 24635773]
169. Investigators A, Group ACT, Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, Higgins AM, Holdgate A, Howe BD, Webb SA, Williams P. Goal-directed resuscitation for patients with early septic shock. *N Engl J Med.* 2014; 371:1496–506. [PubMed: 25272316]
170. De Backer D, Donadello K, Taccone FS, Ospina-Tascon G, Salgado D, Vincent JL. Microcirculatory alterations: potential mechanisms and implications for therapy. *Annals of intensive care.* 2011; 1:27. [PubMed: 21906380]
171. Simonson SG, Welty-Wolf K, Huang YT, Griebel JA, Caplan MS, Fracica PJ, Piantadosi CA. Altered mitochondrial redox responses in gram negative septic shock in primates. *Circulatory shock.* 1994; 43:34–43. [PubMed: 7982271]
172. Levy RJ, Deutschman CS. Cytochrome c oxidase dysfunction in sepsis. *Crit Care Med.* 2007; 35:S468–75. [PubMed: 17713395]
173. Schaefer CF, Biber B, Lerner MR, Jobsis-VanderVliet FF, Fagraeus L. Rapid reduction of intestinal cytochrome a,a3 during lethal endotoxemia. *J Surg Res.* 1991; 51:382–91. [PubMed: 1661795]
174. Schaefer CF, Biber B. Effects of endotoxemia on the redox level of brain cytochrome a,a3 in rats. *Circulatory shock.* 1993; 40:1–8. [PubMed: 8391939]
175. Schaefer CF, Lerner MR, Biber B. Dose-related reduction of intestinal cytochrome a,a3 induced by endotoxin in rats. *Circulatory shock.* 1991; 33:17–25. [PubMed: 1849055]
176. Forget AP, Mangalaboyi J, Mordon S, Guery B, Vallet B, Fourrier F, Chopin C. *Escherichia coli* endotoxin reduces cytochrome aa3 redox status in pig skeletal muscle. *Crit Care Med.* 2000; 28:3491–7. [PubMed: 11057806]
177. Crouser ED, Julian MW, Blaho DV, Pfeiffer DR. Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity. *Crit Care Med.* 2002; 30:276–84. [PubMed: 11889292]
178. Moss GS, Erve PP, Schumer W. Effect of endotoxin on mitochondrial respiration. *Surg Forum.* 1969; 20:24–5. [PubMed: 4910586]
179. Schumer W, Erve P, Kapica SK, Moss GS. Endotoxin effect on respiration of rat liver mitochondria. *J Surg Res.* 1970; 10:609–12. [PubMed: 4924213]
180. Mela L, Bacalzo LV Jr, Miller LD. Defective oxidative metabolism of rat liver mitochondria in hemorrhagic and endotoxin shock. *The American journal of physiology.* 1971; 220:571–7. [PubMed: 4250607]
181. Kilpatrick-Smith L, Erecinska M, Silver IA. Early cellular responses in vitro to endotoxin administration. *Circulatory shock.* 1981; 8:585–600. [PubMed: 6269776]
182. Clemens M, Chaudry IH, Baue AE. Oxidative capability of hepatic tissue in late sepsis. *Adv Shock Res.* 1981; 6:55–64. [PubMed: 7349586]
183. Tanaka J, Kono Y, Shimahara Y, Sato T, Jones RT, Cowley RA, Trump BF. A study of oxidative phosphorylative activity and calcium-induced respiration of rat liver mitochondria following living *Escherichia coli* injection. *Adv Shock Res.* 1982; 7:77–90. [PubMed: 6753540]

184. Tavakoli H, Mela L. Alterations of mitochondrial metabolism and protein concentrations in subacute septicemia. *Infect Immun*. 1982; 38:536–41. [PubMed: 6128308]
185. Garrison RN, Ratcliffe DJ, Fry DE. The effects of peritonitis on murine renal mitochondria. *Adv Shock Res*. 1982; 7:71–6. [PubMed: 7136934]
186. Mela L, Miller LD. Efficacy of glucocorticoids in preventing mitochondrial metabolic failure in endotoxemia. *Circulatory shock*. 1983; 10:371–81. [PubMed: 6352076]
187. Asher EF, Garrison RN, Ratcliffe DJ, Fry DE. Endotoxin, cellular function, and nutrient blood flow. *Arch Surg*. 1983; 118:441–5. [PubMed: 6338864]
188. Geller ER, Jankauskas S, Kirkpatrick J. Mitochondrial death in sepsis: a failed concept. *J Surg Res*. 1986; 40:514–7. [PubMed: 3525991]
189. Jepson MM, Cox M, Bates PC, Rothwell NJ, Stock MJ, Cady EB, Millward DJ. Regional blood flow and skeletal muscle energy status in endotoxemic rats. *The American journal of physiology*. 1987; 252:E581–7. [PubMed: 3555111]
190. Astiz M, Rackow EC, Weil MH, Schumacher W. Early impairment of oxidative metabolism and energy production in severe sepsis. *Circulatory shock*. 1988; 26:311–20. [PubMed: 3208424]
191. Jacobs DO, Maris J, Fried R, Settle RG, Rolandelli RR, Koruda MJ, Chance B, Rombeau JL. In vivo phosphorus 31 magnetic resonance spectroscopy of rat hind limb skeletal muscle during sepsis. *Arch Surg*. 1988; 123:1425–8. [PubMed: 3178491]
192. Dawson KL, Geller ER, Kirkpatrick JR. Enhancement of mitochondrial function in sepsis. *Arch Surg*. 1988; 123:241–4. [PubMed: 3277586]
193. Raymond RM, Gordey J. The effect of hypodynamic endotoxin shock on myocardial high energy phosphates in the rat. *Cardiovascular research*. 1989; 23:200–4. [PubMed: 2686834]
194. Jacobs DO, Kobayashi T, Imagire J, Grant C, Kesselly B, Wilmore DW. Sepsis alters skeletal muscle energetics and membrane function. *Surgery*. 1991; 110:318–25. 25–6. [PubMed: 1650038]
195. Hotchkiss RS, Rust RS, Dence CS, Wasserman TH, Song SK, Hwang DR, Karl IE, Welch MJ. Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [18F]fluoromisonidazole. *The American journal of physiology*. 1991; 261:R965–72. [PubMed: 1928443]
196. Boekstegers P, Weidenhofer S, Pilz G, Werdan K. Peripheral oxygen availability within skeletal muscle in sepsis and septic shock: comparison to limited infection and cardiogenic shock. *Infection*. 1991; 19:317–23. [PubMed: 1800370]
197. Hotchkiss RS, Song SK, Neil JJ, Chen RD, Manchester JK, Karl IE, Lowry OH, Ackerman JJ. Sepsis does not impair tricarboxylic acid cycle in the heart. *The American journal of physiology*. 1991; 260:C50–7. [PubMed: 1987781]
198. Solomon MA, Correa R, Alexander HR, Koev LA, Cobb JP, Kim DK, Roberts WC, Quezada ZM, Scholz TD, Cunnion RE, et al. Myocardial energy metabolism and morphology in a canine model of sepsis. *The American journal of physiology*. 1994; 266:H757–68. [PubMed: 8141377]
199. Taylor DE, Ghio AJ, Piantadosi CA. Reactive oxygen species produced by liver mitochondria of rats in sepsis. *Arch Biochem Biophys*. 1995; 316:70–6. [PubMed: 7840680]
200. Mizobata Y, Prechek D, Rounds JD, Robinson V, Wilmore DW, Jacobs DO. The duration of infection modifies mitochondrial oxidative capacity in rat skeletal muscle. *J Surg Res*. 1995; 59:165–73. [PubMed: 7630122]
201. Rosser DM, Stidwill RP, Jacobson D, Singer M. Oxygen tension in the bladder epithelium rises in both high and low cardiac output endotoxemic sepsis. *Journal of applied physiology*. 1995; 79:1878–82. [PubMed: 8847247]
202. VanderMeer TJ, Wang H, Fink MP. Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. *Crit Care Med*. 1995; 23:1217–26. [PubMed: 7600830]
203. Sair M, Etherington PJ, Curzen NP, Winlove CP, Evans TW. Tissue oxygenation and perfusion in endotoxemia. *The American journal of physiology*. 1996; 271:H1620–5. [PubMed: 8897959]
204. Hasibeder W, Germann R, Wolf HJ, Haisjackl M, Hausdorfer H, Riedmann B, Bonatij J, Gruber E, Schwarz B, Waldenberger P, Friesenecker B, Furtner B. Effects of short-term endotoxemia and

- dopamine on mucosal oxygenation in porcine jejunum. *The American journal of physiology*. 1996; 270:G667–75. [PubMed: 8928797]
205. Noldge-Schomburg GF, Priebe HJ, Armbruster K, Pannen B, Haberstroh J, Geiger K. Different effects of early endotoxaemia on hepatic and small intestinal oxygenation in pigs. *Intensive Care Med*. 1996; 22:795–804. [PubMed: 8880249]
 206. Kantrow SP, Taylor DE, Carraway MS, Piantadosi CA. Oxidative metabolism in rat hepatocytes and mitochondria during sepsis. *Arch Biochem Biophys*. 1997; 345:278–88. [PubMed: 9308900]
 207. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet*. 2002; 360:219–23. [PubMed: 12133657]
 208. Brealey D, Karyampudi S, Jacques TS, Novelli M, Stidwill R, Taylor V, Smolenski RT, Singer M. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. *American journal of physiology Regulatory, integrative and comparative physiology*. 2004; 286:R491–7.
 209. Singer M, Brealey D. Mitochondrial dysfunction in sepsis. *Biochem Soc Symp*. 1999; 66:149–66. [PubMed: 10989665]
 210. Liaw KY, Askanazi J, Michelson CB, Kantrowitz LR, Furst P, Kinney JM. Effect of injury and sepsis on high-energy phosphates in muscle and red cells. *J Trauma*. 1980; 20:755–9. [PubMed: 6447800]
 211. Tresadern JC, Threlfall CJ, Wilford K, Irving MH. Muscle adenosine 5'-triphosphate and creatine phosphate concentrations in relation to nutritional status and sepsis in man. *Clin Sci (Lond)*. 1988; 75:233–42. [PubMed: 3416559]
 212. Fredriksson K, Hammarqvist F, Strigard K, Hultenby K, Ljungqvist O, Wernerman J, Rooyackers O. Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure. *Am J Physiol Endocrinol Metab*. 2006; 291:E1044–50. [PubMed: 16803854]
 213. Fink MP. Bench-to-bedside review: Cytopathic hypoxia. *Critical care*. 2002; 6:491–9. [PubMed: 12493070]
 214. Mongardon N, Dyson A, Singer M. Is MOF an outcome parameter or a transient, adaptive state in critical illness? *Current opinion in critical care*. 2009; 15:431–6. [PubMed: 19617821]
 215. Protti A, Singer M. Bench-to-bedside review: potential strategies to protect or reverse mitochondrial dysfunction in sepsis-induced organ failure. *Critical care*. 2006; 10:228. [PubMed: 16953900]
 216. Abraham E, Singer M. Mechanisms of sepsis-induced organ dysfunction. *Crit Care Med*. 2007; 35:2408–16. [PubMed: 17948334]
 217. Ong SG, Lee WH, Theodorou L, Kodo K, Lim SY, Shukla DH, Briston T, Kiriakidis S, Ashcroft M, Davidson SM, Maxwell PH, Yellon DM, Hausenloy DJ. HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. *Cardiovascular research*. 2014; 104:24–36. [PubMed: 25063991]
 218. Feinman R, Deitch EA, Watkins AC, Abungu B, Colorado I, Kannan KB, Sheth SU, Caputo FJ, Lu Q, Ramanathan M, Attan S, Badami CD, Doucet D, Barros D, Bosch-Marce M, Semenza GL, Xu DZ. HIF-1 mediates pathogenic inflammatory responses to intestinal ischemia-reperfusion injury. *Am J Physiol Gastrointest Liver Physiol*. 2010; 299:G833–43. [PubMed: 20689059]
 219. Bao W, Qin P, Needle S, Erickson-Miller CL, Duffy KJ, Ariazi JL, Zhao S, Olzinski AR, Behm DJ, Pipes GC, Jucker BM, Hu E, Lepore JJ, Willette RN. Chronic inhibition of hypoxia-inducible factor prolyl 4-hydroxylase improves ventricular performance, remodeling, and vascularity after myocardial infarction in the rat. *J Cardiovasc Pharmacol*. 2010; 56:147–55. [PubMed: 20714241]
 220. Jones NM, Bergeron M. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. *J Cereb Blood Flow Metab*. 2001; 21:1105–14. [PubMed: 11524615]
 221. Olguner CG, Koca U, Altekin E, Ergur BU, Duru S, Girgin P, Tasdogan A, Gunduz K, Guzeldag S, Akkus M, Micili SC. Ischemic preconditioning attenuates lipid peroxidation and apoptosis in the cecal ligation and puncture model of sepsis. *Exp Ther Med*. 2013; 5:1581–8. [PubMed: 23837035]

222. Hauser B, Bracht H, Matejovic M, Radermacher P, Venkatesh B. Nitric oxide synthase inhibition in sepsis? Lessons learned from large-animal studies. *Anesthesia and analgesia*. 2005; 101:488–98. [PubMed: 16037166]
223. Kuhlicke J, Frick JS, Morote-Garcia JC, Rosenberger P, Eltzschig HK. Hypoxia inducible factor (HIF)-1 coordinates induction of Toll-like receptors TLR2 and TLR6 during hypoxia. *PLoS One*. 2007; 2:e1364. [PubMed: 18159247]
224. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol*. 2009; 9:609–17. [PubMed: 19704417]
225. Mishra KP, Ganju L, Singh SB. Hypoxia modulates innate immune factors: A review. *Int Immunopharmacol*. 2015; 28:425–8. [PubMed: 26184693]

Figure 1.
Thirteen-somite embryo cultured at 40% O₂, demonstrating broad opening of the forebrain (abnormal cranial fold) as compared to embryos cultured at 5% O₂.

a Developing human placenta **b Developing mouse placenta** **Figure 2.**

During development, cells are exposed to varying levels of O_2 , which influences their differentiation.

Figure 3.

Interaction of electron transport chain (ETC) with intermediates (FADH₂, NADH). Note that electrons may be ejected from the ETC prematurely, combining with oxygen to produce reactive oxygen species (ROS, e.g. superoxide) and reactive nitrogenous species (RNS, e.g. peroxynitrate), which can subsequently inhibit various components of the ETC. Also note that conversion of pyruvate to lactate, as opposed to Acetyl-CoA, deprives the TCA cycle of substrate, preventing the formation of FADH₂ and NADH and thus the transfer of electrons to the ETC.

Figure 4.

HIF-1 and HIF-2 activate thousands of genes, many of which impact metabolism and are protective in the setting of hypoxia.

Figure 5. cancerous cells often live in a hypoxic environment. Their ability to survive, and in some cases *thrive*, is due to upregulation of a variety of HIF-1 mediated genes that modify metabolism, inhibit apoptosis, induce angiogenesis, and promote invasion and/or metastasis.

Sepsis Strata

Figure 6.

Sepsis may be best conceptualized in terms of three distinct strata - the macrovasculature (blood pressure, cardiac output, hemoglobin concentration, arterial oxygen saturation, mixed venous oxygen saturation), the microvasculature, and the subcellular apparatus (mitochondria and electron transport chain, reactive species).

Table 1

Strategies for Adapting to Hypoxia

Strategies for Adapting to Hypoxia
Alternative energetic pathways
Increased oxygen delivery
Decreased energy needs
Defense against oxidative stress
Attenuation of calcium accumulation

Table 2

Studies Describing The Effect of Sepsis on Various Components of Subcellular Energetics

Oxygen Utilization and Subcellular Energetics in Sepsis					
Model	Measurement	Time Point	Finding	Author	Year
Rat isolated hepatic mitochondria (LPS)	Polarographic measurement of oxygen uptake	n/a	LPS inhibited citrate, pyruvate, and alpha-ketoglutarate-induced mitochondrial respiration	Moss ¹⁷⁸	1969
Rat isolated hepatic mitochondria (LPS)	Oxygen uptake (citrate oxidation)	n/a	LPS inhibited citrate-induced mitochondrial respiration	Schumer ¹⁷⁹	1970
Rat sepsis (LPS)	ATPase activity	6 hours	Significant reduction in ATPase activity	Mela ¹⁸⁰	1971
Mouse neuroblastoma cells (LPS)	ATP/ADP Ratio	n/a	Decrease in cellular ATP/ADP ratio	Kilpatrick-Smith ¹⁸¹	1981
Rat sepsis (CLP)	O ₂ consumption	16-19 hours	No differences in oxygen consumption between control and septic tissue	Clemens ¹⁸²	1981
Rat isolated hepatic mitochondria (E.coli)	Polarographic (Clark-type electrode) measurement of mitochondrial respiration	3-6 hours	Increased oxidative phosphorylative activity	Tanaka ¹⁸³	1982
Rat sepsis (CLP)	ATP synthesis	6 days	Significant reduction in ATP synthesis	Tavakoli ¹⁸⁴	1982
Rat sepsis (CLP)		2-6 hours	No differences in any measure of mitochondrial function	Garrison ¹⁸⁵	1982
Rat sepsis (LPS)	Mitochondrial O ₂ utilization and ATP synthesis	24 hours	Significant reduction in O ₂ utilization and ATP synthesis	Mela ¹⁸⁶	1983
Rat sepsis (LPS)	Polarimetric measurement of respiratory control index	6 hours	No difference in respiratory control index	Asher ¹⁸⁷	1983
Rat sepsis (LPS, fecal IP injection)	Polarimetric measurements of mitochondrial function	18-24 hours	No differences in any measure of mitochondrial function	Geller ¹⁸⁸	1986
Rat <i>E.coli</i> injection	P ₃₁ NMR	3-5h	No differences in high energy phosphates despite decreased blood flow	Jepson ¹⁸⁹	1987
Rat sepsis (CLP)	Tissue pO ₂	6 hours	No difference in cellular oxygen	Astiz ¹⁹⁰	1988
Rat cecal ligation	P ₃₁ NMR	6, 24, 48 hours	Decrease in both PCr/Pi and ATP/Pi ratios	Jacobs ¹⁹¹	1988
Rat isolated hepatic, muscle, cardiac mitochondria (E.coli)	Polarographic (Clark-type electrode) measurement of mitochondrial respiration	4 hours	Higher respiratory control ratio in mitochondria of infected animals	Dawson ¹⁹²	1988
Rat <i>E.coli</i> injection	P ₃₁ NMR	1-16h	Decreased myocardial ATP and creatinine phosphate at 1 hour	Raymond ¹⁹³	1989
Rat sepsis (LPS)	Cy tochrome aa ₃ oxidation state (NIRS)	0-120 minutes	Significant reduction in cytochrome aa ₃ oxidation state despite stable VO ₂	Schaefer ¹⁷³	1991
Rat sepsis (CLP)	P ₃₁ NMR	24 hours	Increased ATPase activity, decreased PC/ATP ratio, ATP levels maintained	Jacobs ¹⁹⁴	1991
Rat sepsis (CLP)	[¹⁸ F] fluoromisonidazole	36-42 hours	No difference in cellular oxygen	Hotchkiss ¹⁹⁵	1991

Oxygen Utilization and Subcellular Energetics in Sepsis					
Model	Measurement	Time Point	Finding	Author	Year
Human sepsis	Tissue pO ₂		Tissue (muscle) pO ₂ increased significantly	Boekstegers ¹⁹⁶	1991
Rat cecal ligation	P ₃₁ NMR	36-48h	No differences in high energy phosphates	Hotchkiss ¹⁹⁷	1991
Canine <i>E.coli</i> injection	P ₃₁ NMR	~48h	No differences in high energy phosphates	Solomon ¹⁹⁸	1994
Rat sepsis (CLP)	Polarographic (Clark-type electrode) measurement of mitochondrial respiration	16 hours	No difference in mitochondrial oxygen consumption	Taylor ¹⁹⁹	1995
Rat sepsis (CLP)	P ₃₁ NMR	24-48 hours	Normal mitochondrial function despite decrease in high energy phosphate stores in early sepsis	Mizobata ²⁰⁰	1995
Rat sepsis (LPS)	Tissue pO ₂	0-180 mins	Mucosal pO ₂ increased significantly	Rosser ²⁰¹	1995
Pig sepsis (LPS)	Tissue pO ₂	0-180 mins	Mucosal pO ₂ increased significantly	VanderMeer ²⁰²	1995
Rat sepsis (LPS)	Tissue pO ₂	4 hours	Lower muscle pO ₂ in septic animals	Sair ²⁰³	1996
Pig sepsis (LPS)	Tissue pO ₂	210-340 mins	Lower mucosal pO ₂ in septic animals	Hasibeder ²⁰⁴	1996
Pig sepsis (LPS)	Tissue pO ₂	0-6 hours	Lower mucosal pO ₂ and pH in septic animals	Nöldge-Schomburg ²⁰⁵	1996
Rat sepsis (CLP)	Clark electrode (isolated mitochondria)	16 hours	Mitochondrial oxygen consumption increased 35%	Kantrow ²⁰⁶	1997
Human sepsis	P ₃₁ NMR	24 hours	Lower tissue ATP in non-survivors	Brealey ²⁰⁷	2002
Rodent sepsis	P ₃₁ NMR	0-72 hours	ATP depletion, NO overproduction in non-survivors	Brealey ²⁰⁸	2004