Oxygen in demand: How oxygen has shaped vertebrate physiology

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In response to varying environmental and physiological challenges, vertebrates have evolved complex and often overlapping systems. These systems detect changes in environmental oxygen availability and respond by increasing oxygen supply to the tissues and/or by decreasing oxygen demand at the cellular level. This suite of responses is termed the oxygen transport cascade and is comprised of several components. These components include 1) chemosensory detectors that sense changes in oxygen, carbon dioxide, and pH in the blood, and initiate changes in 2) ventilation and 3) cardiac work, thereby altering the rate of oxygen delivery to, and carbon dioxide clearance from, the tissues. In addition, changes in 4) cellular and systemic metabolism alters tissue-level metabolic demand. Thus the need for oxygen can be managed locally when increasing oxygen supply is not sufficient or possible. Together, these mechanisms provide a spectrum of responses that facilitate the maintenance of systemic oxygen homeostasis in the face of environmental hypoxia or physiological oxygen depletion (i.e. due to exercise or disease). Bill Milsom has dedicated his career to the study of these responses across phylogenies, repeatedly demonstrating the power of applying the comparative approach to physiological questions. The focus of this review is to discuss the anatomy, signalling pathways, and mechanisms of each step of the oxygen transport cascade from the perspective of a Milsomite. That is, by taking into account the developmental, physiological, and evolutionary components of questions related to oxygen transport. We also highlight examples of some of the remarkable species that have captured Bill’s attention through their unique adaptations in multiple components of the oxygen transport cascade, which allow them to achieve astounding physiological feats. Bill’s research examining the oxygen transport cascade has provided important insight and leadership to the study of the diverse suite of adaptations that maintain cellular oxygen content across vertebrate taxa, which underscores the value of the comparative approach to the study of physiological systems.

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Abbreviations: 5-HT, serotonin; ACh, acetylcholine; ACR, air convection requirement; CO, carbon monoxide; CO2, oxygen content; COX, cytochrome C oxidase; E, expiration; EDV, end diastolic volume; ePF, embryonic parafacial respiratory group; ERV, expiratory reserve volume; ESV, end systolic volume; ETC, electron transport chain; fH, heart rate; fV, breathing frequency; H2S, hydrogen sulfide; Hb, hemoglobin; HMR, hypoxic metabolic response; HRV, hypoxic ventilatory response; I, inspiration; LCT, lower critical temperature; MAP, mean arterial pressure; NEC, neuroepithelial cells; NO, nitric oxide; NOS, nitric oxide synthase; Ppa, partial pressure of oxygen at which Hb is 50% saturated; PaO2, arterial oxygen tension; PaCO2, oxygen or carbon dioxide tension; pFRG, pre-Bötzinger Complex; Q, cardiac output; RDS, reactive oxygen species; Ta, ambient temperature; Tb, body temperature; Tbmax, thermoregulatory set point; TNZ, thermoneutral zone; TPR, total peripheral resistance; UCT, upper critical temperature; Vs, stroke volume; Vt, tidal volume.

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1095-6433/© 2015 Elsevier Inc. All rights reserved.
1. Foreword

Bill Milsom has dedicated his career to studying adaptations to varying levels of oxygen. The product of an unparalleled comparative pedigree, Bill has had the great fortune of working alongside many of the other leading comparative physiologists of his day. Bill's work has spanned an astounding breadth of species, developmental stages, physiological systems, and corners of the globe. Although the breadth of Bill's investigations is evident from the studies discussed in this very special issue, his interests are broadly centred on the control of breathing, using species differences and ontogeny to determine the neural basis of respiratory pattern formation and the manner in which this has been shaped by evolution to meet the demands of animals living in extreme environments. Bill's approach to science and life is similar and perhaps best characterized by an unending passion and curiosity that has not been blunted by time. Bill is always up for an expedition, a conference, or an experiment. Following a long day of science in the lab or at a conference he will stay up later than students a third his age, drink most of them under the table, and then be in the lab or lecture hall earlier than they can contemplate, fully recharged and ready to do it all again. Bill has an amazing ability to keenly get to the heart of any scientific problem, which is perhaps best characterized by his knack for sleeping through any presentation only to ask insightful questions at the end. Clearly the “Milsom-nod” has amazing regenerative capacity!

As a researcher, Bill has made many notable contributions to physiology. However, as remarkable as his scientific achievements are, if asked, Bill will always cite the successes of his many excellent trainees as his greatest achievement; his many awards for excellence in mentorship as his most cherished recognitions. As a mentor, Bill achieves a careful balance between encouraging and challenging his students, valuing both hard work and play, and providing help to the mentee in the present while encouraging them to dream about the future.

Indeed, Bill has been an inspiring mentor to each of the authors of this paper and his academic progeny populate the faculties of universities across the globe. Therefore, it is with considerable pleasure that we, the final brood of Milsomites, contribute this review of physiological responses to hypoxia in his honour.

2. Introduction

The primary function of the cardio-respiratory system is to extract oxygen from the atmosphere and deliver it to the mitochondria of cells. At the cellular level, mitochondria utilize oxygen as the terminal electron acceptor to produce ATP through the electron transport chain (ETC) via the biochemical process of oxidative phosphorylation. Together, the series of physiological events that connect environmental oxygen to cellular metabolism is termed the oxygen transport cascade (Fig. 1). This cascade is composed of four primary steps: ventilation, diffusion of oxygen from the air into the blood, circulation, and diffusion of oxygen from the blood into the cells. At sea level, oxygen makes up ~20% of inspired air (~160 Torr), but at each step of the oxygen transport cascade this percentage becomes markedly reduced, such that at the cellular level the oxygen tension ($P_{O2}$) may be as low as ~5 Torr (Weibel, 1984; di Prampero, 1985). Maintaining this gradient is essential to the function of the oxygen transport cascade as the diffusion of oxygen from the atmosphere into the blood, and from the blood into the tissues, is a passive process. Nonetheless, despite the relatively low oxygen saturation at the tissue level in normoxia, there is still abundant oxygen for the mitochondrial synthesis of sufficient ATP to meet cellular energy requirements.

During periods of hypoxia, however, oxygen availability becomes limited, oxidative phosphorylation is impaired, and metabolic throughput along the ETC is greatly decreased. Hypoxemia (i.e. hypoxia of the blood) occurs due to a variety of factors, including reduced environmental oxygen availability, physical exercise, disease, or a combination of these. Indeed, hypoxic environments are common on Earth and many animals inhabit such regions and perform some or all of their daily activities or life cycle functions in varying or low oxygen (Bickler and Buck, 2007). For example, many animals are adapted to life at high-altitude, where ambient oxygen levels are reduced relative to sea level due to lower barometric pressure (see Section 7). These animals must develop, reproduce, and exercise in hypoxia, and this challenge has driven the evolution of a variety of adaptations that enhance oxygen delivery at various stages of the oxygen transport cascade and/or reduce metabolic demand at the cellular level. These adaptations mitigate the impact of reduced environmental oxygen availability by enhancing its delivery through the body, or in situations where enhanced delivery is not sufficient, by reducing the need for oxygen to generate cellular energy at the tissue level (i.e. by decreasing metabolism).

Common adaptations in the oxygen transport cascade include changes in the sensitivity of chemosensory cells and organs to hypoxia, alterations in the anatomy, mechanics, and neural/cellular control of ventilation and cardiac function, biochemical changes to the oxygen carrying capacity of the blood, anatomical adjustments that alter the diffusion distance across which gases must travel between the blood and tissues (and vice versa), and systemic adaptations that minimize energy demand, either through reduced behaviours (i.e. torpor or hibernation), shutting down non-essential tissues to preserve energy for oxygen-
sensitive organs, or by reducing energy demand at the cellular level. Examining differences in the function and composition of the various components of the oxygen transport cascade and their control between species adapted to environments with variable oxygen availability provides important insight into the evolution and function of oxygen-dependent processes in nature. Therefore, the aim of this review is to discuss what is known regarding these adaptations across vertebrate species in both developmental and evolutionary contexts. The anatomy, mechanics, and cellular control of chemotransduction and related physiological responses to changes in oxygen availability will be compared and contrasted to evaluate divergent strategies employed by diverse species in a comparative context, and to highlight gaps in the literature that require further study. We address the components of the oxygen transport cascade in turn, starting with chemotransduction. We then discuss the components of the oxygen transport cascade that are responsible for increasing oxygen delivery to the tissues – ventilation and perfusion. We next turn our attention to systemic mechanisms of metabolic depression that reduce oxygen demand. Lastly, we discuss specific model organisms that are capable of remarkable feats in hypoxia and detail the physiological adaptations at each step of the oxygen transport cascade that permit these animals to function in levels of ambient oxygen that are fatal to most species.

3. Arterial chemoreception of systemic hypoxemia

The ability of an animal to adapt to changes in environmental oxygen and carbon dioxide is predicated on their ability to rapidly detect and respond to changes in these environmental variables via the process of chemoreception. In most vertebrates there are several central and peripheral chemoreceptive sites that detect PO2, oxygen content (CO2), carbon dioxide tension (POT2), and pH in the blood (Gonzalez et al., 1994, 1995; Milsom and Burleson, 2007). When any of these variables deviate from their normal range, chemoreceptors elicit physiological responses that modulate ventilation and perfusion to maintain homeostasis (Lopez-Barneo et al., 1988; Buckler, 2007). For example, in a hypoxic environment, adjustments are made to increase oxygen uptake into the body, maximize oxygen delivery to the tissue, and minimize energy expenditure. This usually results in an increase in breathing frequency (fR) and/or tidal volume (VT) in order to increase the amount of oxygen being moved across the gas exchange surface of the lungs or gills (see Section 4). Cardiovascular responses vary between animal taxa and may consist of an increase or a decrease in heart rate (fH) and/or blood pressure (see Section 5). Peripheral arterial chemoreceptors (i.e. chemoreceptor glands found in arteries outside of the central nervous system – the CNS) are particularly critical for providing sensory input to the cardiovascular and respiratory centres of the brainstem to initiate these changes (see Section 5). Arterial chemoreceptors may function as a part of another organ (i.e. on the gills of fish) or chemoreceptor cells may form a discrete organ or body such as the carotid labyrinth of amphibians and the carotid and aortic bodies of birds and mammals. This section will contrast the evolution and development of chemoreceptive systems across vertebrate taxa, and their role in responding to changes in environmental oxygen. The evolution of central chemoreceptors has been recently reviewed in a phylogenetic context (Milsom, 2010b), and so in the present review we focus on the mechanics and evolution of peripheral chemoreceptive sites.

3.1. Cellular mechanics of chemotransduction

The oxygen-sensing mechanism of chemoreceptors hinges on a unique K+ current, which is mediated by an unidentified oxygen-sensitive membrane spanning K+ channel. This channel is inhibited during hypoxia, thereby decreasing the cellular K+ current when PO2 decreases. The reduction of this K+ current results in a build-up of positive charge within the chemoreceptor cell, leading to membrane potential depolarization, which activates voltage-gated Ca2+ channels and permits Ca2+ influx. The resulting cytosolic Ca2+ accumulation then activates vesicular fusion and the release of neurotransmitters into the synaptic cleft, which excites afferent neurons (Fig. 2) (Nurse, 2005). The specific neurotransmitters released by the chemoreceptor cells vary between vertebrate groups. For example, in fish, serotonin (5-HT) is the primary, and often only, neurotransmitter released upon exposure to hypoxia (Coolidge et al., 2008), whereas in the mammalian carotid body, acetylcholine (ACh) and adenosine are of primary importance (Nurse, 2005; Prabhakar, 2006).
The mechanism via which a reduction in $P_o_2$ closes $K^+$ channels and produces the reduction in $K^+$ current that underlies chemotransduction is poorly understood. Currently there are three competing hypotheses to explain this phenomenon. First, the classic “membrane hypothesis” of chemotransduction posits that hypoxia directly inhibits $K^+$ channels, resulting in membrane potential depolarization (Fig. 2) (Gonzalez et al., 1995). In support of this, $K^+$ channels of the Kv3 and Kv4 families have been reported to be oxygen-sensitive in rabbit and mouse carotid body chemoreceptor cells (Lopez-Lopez and Perez-Garcia, 2007); however, there is no definitive mechanism to explain how hypoxia inhibits these channels. Second, the “metabolic hypothesis” posits that reductions in $P_o_2$ lead to an inhibition of mitochondrial cyclochrome C oxidase (COX) activity, leading to a reduction in ATP levels in the cell (Fig. 2) (Lopez-Barneo et al., 1988; Prabhakar, 2006). This reduction may then cause $K^+$ channel inhibition via a reduction in MgATP or via an activation of AMP kinase. Third, a more recent theory of chemotransduction proposes that the stimulation or inhibition of gasotransmitter levels (namely nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide ($H_2S$)) may be responsible for the inhibition of $K^+$ currents or activation of $Ca^{2+}$ currents in chemoreceptive cells of the mammalian carotid body (Fig. 2) (reviewed in Prabhakar and Peers (2014)). $NO$ is produced in cells by NO synthases (NOS), a family of $O_2$-sensitive enzymes, that normally have an inhibitory effect on $Ca^{2+}$ channels and an excitatory effect on $K^+$ channels. Hypoxia decreases NOS activity, resulting in a decrease in intracellular $NO$, which leads to a reduced inhibition of $Ca^{2+}$ channels and a reduced excitation of $K^+$ channels (Prabhakar et al., 1993). Together these effects can result in cellular depolarization and neurotransmitter release. Similarly, $CO$ is generated when heme oxygenases degrade heme in an $O_2$-dependent reaction. Under hypoxic conditions, CO generation decreases and the activation of maxi$K^+$, or big potassium (BK) channels is decreased, leading to channel closure, membrane potential depolarization, and neurotransmitter release (Prabhakar, 2012). Finally, $H_2S$ generation by cystathionine $\gamma$-lyase increases during hypoxic exposure and $H_2S$ inhibits both twin acid-sensitive $K^+$ (TASK)-like and BK channels. This inhibition results in a reduced $K^+$ current, which may lead to membrane potential depolarization and neurotransmitter release (Peng et al., 2010). It is likely that these gasotransmitters contribute to the hypoxia-sensitivity of chemoreceptors, but the details of these pathways are still being explored.

3.2. Evolution of chemoreceptors

The identification of peripheral chemoreceptive sites in terrestrial vertebrates has been a lifelong interest for Bill and work from his laboratory supports the hypothesis that these have evolved from chemoreceptors associated with the aortic arches of fish, which run between the gill arches (Milsom and Burleson, 2007). The 1st and 2nd aortic arches of fish are lost in all higher vertebrates and the 5th aortic arch is lost in almost all higher vertebrates, persisting only in some amphibians. The 3rd, 4th and 6th aortic arches have persisted in terrestrial vertebrates as the internal carotid artery, the systemic aorta and the pulmonary artery, respectively. Based on the known homology between the aortic arches of fish and the arteries of higher vertebrates, chemoreceptors of the gills are hypothesized to be homologous to the carotid artery, aorta, and pulmonary artery chemoreceptors found in terrestrial vertebrates.

Generally, there has been a phylogenetic trend in carotid artery chemoreceptors across animal taxa, moving from multiple dispersed sites of chemoreception in fish to a single concentrated site of chemoreception in mammals (Gonzalez et al., 1994; Milsom and Burleson, 2007; Coolidge et al., 2008); however, our understanding of this pattern is incomplete. While chemoreception has been studied most thoroughly in mammals, chemoreceptive sites have been identified and described in the arteries of almost all other vertebrate groups except reptiles. The ensuing paragraphs will provide a general review of what is known of chemoreception in all the vertebrate groups and identify areas that require further research. It is interesting to note that the evolutionary divergence of chemoreception in all vertebrates has largely resulted in major anatomical differences, whereas the underlying neurotransmitter pathways and cellular mechanisms of chemotransduction appear to have been largely conserved across highly divergent species. Unfortunately, the majority of studies examining chemoreception in non-mammals have focused on anatomy and mechanics rather than cellular function; most of what is known of cellular chemotransduction has been ascertained from studies of mammals.

Fish take up oxygen and excrete carbon dioxide across their gills. Neuroepithelial cells (NECs), which are found on the gill arches and oro-branchial cavity in fish, are known to be chemoreceptive and in many species are oriented in such a way that they simultaneously detect $P_o_2$ changes in both the blood and the surrounding water (reviewed in Milsom (2012)). The distribution of receptors across the different gill arches is not uniform and this is clearly demonstrated by differences in the bradycardia response to hypoxic water, which can be abolished by ablation of the 1st gill arch in some species (Sundin et al., 1999), but not in others (Micheli-Campbell et al., 2009). This difference is partially due to the fact that in some species cranial nerve X is solely responsible for the innervation of the receptor cells while in other species both cranial nerves X and IX are involved. Bill was involved in many of the early physiological and neurophysiological studies on the $O_2$-sensitivity of gill NECs in a variety of fish species (Milsom and Brill, 1986; Burleson and Milsom, 1993). These seminal works would lay the foundation for modern research into the specifics of fish gill chemoreception (see also the contribution by Jonz et al., in this issue). Recent work with fish NECs has relied on immunohistochemical and patch-clamp techniques to visualize the cells, determine their neurochemical makeup, and elucidate the mechanism of oxygen chemotransduction. These studies have revealed species-specific NEC distribution (Coolidge et al., 2008), demonstrated the importance of 5-HT, ACh, and catecholamines in signal transduction from NECs (Porteus et al., 2013), and also the presence of an oxygen-sensitive outward $K^+$ current similar to those observed in mammalian chemoreceptor cells (Jonz et al., 2004; Burleson et al., 2006).
Similar to fish, many amphibians use gills for respiration during their larval stage of development. Ablation of the 1st gill arch in bullfrog tadpoles results in a loss of response to hypoxic water (Jia and Burggren, 1997), suggesting that similar to fish, the oxygen chemoreceptors responsible for controlling ventilation reside at this site in amphibians. Conversely, in adult amphibians, the chemoreceptive cells of the 1st gill arch converge into dense capillary beds called the carotid labyrinth, which sit on the bifurcation of the left and right carotid arteries. Two distinct cell types have been described in the amphibian carotid labyrinth, one type being chemoreceptive and the other playing a supportive role (Rogers, 1963; Ishii and Kusakabe, 1982). The chemoreceptive ability of the carotid labyrinth was first hypothesized based on histological studies of neurochemical content and denervation studies (Smyth, 1939; Chowdhary, 1951; Rogers, 1963), and was later confirmed through electrophysiological recordings (Ishii et al., 1966). Cells resembling chemoreceptors are also present in the aortic wall (Ishii et al., 1985), and the pulmocutaneous artery of amphibians (Wang et al., 2004), although the importance of these sites in cardiopulmonary control has yet to be determined. More recent studies in amphibians have focused on isolating the effects of reduced Po$_2$ versus reduced Co$_2$ (Andersen et al., 2003), and also on the effects of carotid versus pulmonary artery chemoreceptor stimulation (Wang et al., 2004).

Despite their diversity, Class Reptilia is often ignored in comparative literature, commonly being lumped under the heading “ectotherms” with fish and amphibians. Such a grouping rarely does reptile anatomy and physiology justice, as the differences seen between lizards, snakes, turtles, and crocodilians can be just as drastic as the differences between fish, reptiles, and mammals. This is particularly true when it comes to reptilian cardiovascular anatomy and physiology. Recent postulations regarding where the chemoreceptor cells reside in the vasculature of reptiles are derived from morphological studies of the early- to mid-twentieth century and have been almost entirely restricted to carotid chemoreception. A review of the current knowledge of reptilian cardiovascular anatomy and carotid chemoreceptor function can be separated into the four major groups of reptiles: Chelonia (turtles and tortoises), Lacertilia (lizards), Crocodyilia (crocodiles, caiman and alligators), and Ophidia (snakes).

While there are no physiological studies of chemoreception in cheloniomorphs, morphology and development studies can be used to predict the location of a carotid body homolog. The carotid bifurcation in turtles is believed to be a secondary bifurcation, with the region homologous to the mammalian bifurcation lying at the base of the neck. No physiological or histological studies have been published to date that demonstrate a chemoreceptive function of this area. Unlike chelonians, there is no discreet carotid body organ in Lacertilia. Early morphological studies noted the existence of a small gland at the carotid bifurcation and erroneously named it the carotid body (Adams, 1958), but this has since been identified as the parathyroid III gland. Further visual studies of the carotid bifurcation have revealed clusters of cells that resemble the glomus cells of the mammalian carotid body (Adams, 1953); however, the chemoreceptive abilities of these cells have not yet been demonstrated. As in the Chelonia, the branching of the internal and external carotid artery in the Crocodyilia occurs shortly after the aortic trunk leaves the heart. To date, there has been no carotid body homolog described in any crocodilian species. Finally, in Ophidia the carotid bifurcation is found just distal to the head and is regarded as homologous to the mammalian bifurcation (Ask-Upmark, 1935; Boyd, 1942). To date there has been no record of a discreet body in the region, nor any cells at the bifurcation that resemble those found in the mammalian carotid body.

Neuroepithelial cells of the first aortic arch in birds converge into a mass but there is an absence of the dense capillary beds seen in amphibians. This mass (called the avian carotid body) contains two distinct cell types, similar to the carotid labyrinth (Hodges et al., 1975), but they sit at the beginning of the common carotid artery rather than at the bifurcation. Little work has been done examining chemoreception in the aorta and pulmonary arteries in birds, but innervation and histology studies suggest the presence of chemoreceptor cells in these regions (Bennett, 1971).

In mammals, chemoreceptive cells of the carotid body are called glomus (or Type I) cells and are supported by sustentacular (Type II) cells (Gonzalez et al., 1994; Peers and Kemp, 2001). Type I cells have a clear round nucleus and a granular cytoplasm with dense-core vesicles containing the neurotransmitters involved in initiating the hypoxia response (Fig. 3) (Gonzalez et al., 1994). While the carotid body contributes to both cardiovascular and respiratory changes in response to hypoxia, chemoreceptors of the aorta (collectively called the aortic body) appear to only play a significant role in the cardiovascular response to hypoxia (Biro et al., 1973). The contribution of pulmonary artery chemoreceptors to the cardioventilatory response to hypoxia is unclear, as there have been few studies conducted on these chemoreceptors and those that have been conducted have yielded conflicting data (Duke et al., 1963; Sampson and Hainsworth, 1972).

3.3. Future directions

Overall, the similarities in the location, structure, response to stimuli, and discharge characteristics of the amphibian, avian, and mammalian chemoreceptors suggest homology with fish gill NECs. These persistent similarities have allowed us to track the evolution of chemoreceptors over time and determine with reasonable certainty that these structures are homologous and advantageous to each animal group; however, there are still considerable gaps in our knowledge of non-mammalian chemoreception sites. The most obvious gap in our current understanding of arterial chemoreception lies within Class Reptilia. Virtually no work has examined arterial chemoreception in reptiles since the 1950s and the majority of our current knowledge is based on anatomical homologies between reptiles and other vertebrate groups. Because of the large diversity of cardiovascular anatomy outlined above and the antiquity of the divergence between snakes, turtles, lizards and crocodiles, chemoreceptor characteristics should be investigated separately in each group as there are likely to be large intraclass differences.

Efforts to fill this gap in our knowledge form an area in which Bill has been a pioneer. Recent work in Bill’s laboratory has found evidence of putative chemoreceptor cells in the aorta and pulmonary and carotid arteries of turtles and rattlesnakes (Reyes et al., submitted for publication) and in the carotid arteries of tegu lizards (Reichert et al., 2015). These putative chemoreceptors share many similar characteristics to mammalian glomus cells, but are more dispersed and do not appear to form a discreet organ as in the carotid body. As reptilian chemoreceptor sites are described, we can begin to compile a more complete picture of...
how the transition from water to air ventilation affected chemoreception in vertebrates.

In addition to the characterization of reptilian chemoreception, the interplay between the three peripheral chemoreceptor sites (carotid, aortic, and pulmonary) and central chemoreceptors is also an area in need of further research. Currently, there are conflicting data as to whether the various chemoreceptive sites act in a hypo-additive, hyper-additive, or additive manner when PCO2 and/or PO2 are changed independently at different sites. A better understanding of the neural pathways responsible for communication between the multiple sites of chemoreception may help in the examination of these properties. Also, current studies of additivity have been limited to mammals and it may be of interest to determine how central and peripheral chemoreceptive sites interact in animals with long non-ventilatory periods (i.e. breath holds). Finally, communication between chemoreceptive sites and other control centres of the brain, such as the respiratory centres, is another area of interest.

4. The brains and brawn of breathing: an overview of the respiratory centres and muscles during the breathing cycle

Information from peripheral chemoreceptors (see Section 3), pulmonary mechanoreceptors, and other afferent inputs are directed towards the nucleus tractus solitarius (NTS) within the brainstem. The NTS is part of a highly integrated network, incorporating complex feedback loops, which projects to the central rhythm generators to recruit respiratory muscles and produce a “simple” and appropriate breathing response to maintain homeostasis (Molkov et al., 2013). The following section provides a comparative overview of the neural control of breathing, examines differences in the dominant respiratory rhythm generation sites of vertebrates, outlines the breathing phases of vertebrates, and speculates on the benefits of active expiration in the mammalian system. As we will see, the simple task of breathing is not quite so simple.

4.1. The neural control of breathing

Respiratory muscles operate under neural control and there are a number of areas in the CNS that contribute to the generation and modulation of the breathing response (Fig. 4). In the early 1800s, Jean Legallois discovered that breathing in rabbits ceased after he removed the rostral medulla; suggesting that located near cranial nerve VIII, there exists a critical region required for the production of breathing (Finger, 1994). The hunt for a more defined group of cells that are critical for the generation of breathing in mammals continued into the 1990s, when Smith et al. (1991) progressively sliced the en bloc brainstem preparation in order to isolate and define the region of the rostral medulla that is critical for generating breathing motor output (Smith et al., 1991). These authors also used transverse medullary slices that included the rostral medulla while recording from the hypoglossal nerve. Progressively thinner slices revealed an enclosed region, named the pre-Bötzinger Complex (preBötC), as being sufficient and necessary for the generation of breathing motor output.

With the discovery of the preBötC, research interest was directed to determining the cellular makeup of this region. There are currently two main hypotheses regarding the mechanism of preBötC rhythm generation: the first hypothesis posits that synaptically connected neurons (i.e. the neural network hypothesis) generate this rhythm; while the second hypothesis posits that the respiratory rhythm is generated by a population of pacemaker cells (i.e. the pacemaker hypothesis) with inherent rhythmicity (Feldman et al., 2013). In adult mammals, evidence to date suggests that respiratory rhythm is generated as a result of a network of excitatory (glutamate) and inhibitory (GABA and glycine) neurons, providing evidence for the neural network hypothesis. Conversely, the pacemaker hypothesis emerged in the early 2000s, when a number of studies identified two groups of pacemaker cells within the preBötC (Del Negro et al., 2002; Pena et al., 2004). These cells were found to either be dependent on a persistent Na+ current (INaP), or a Ca2+-activated non-specific cation current (ICan) (Del Negro et al., 2002). INaP pacemaker cells are dependent on persistent Na+ channels, also known as cadmium-insensitive neurons (Pena et al., 2004). ICAN pacemaker cells are dependent on Ca2+-activated non-specific cation channels, and are also known as cadmium-sensitive neurons (Pena and Ramirez, 2002). The prevalence of INaP and ICAN changes with development, such that the majority of pacemaker cells are dependent on INaP channels between postnatal day P0 and P5 (Pena et al., 2004). Interestingly, the majority of pacemaker cells are dependent on ICAN channels after P5 and INaP cells are critical for the production of the gasping response observed in hypoxic conditions (Pena et al., 2004). The importance of both pacemaker cells within neural networks is still unclear; preBötC rhythm generation may be a combination of the two, rather than one or the other exclusively (Feldman and Del Negro, 2006). However, experimental results may be influenced by age, preparation type, and the presence of certain chemicals in the experimental preparation (such as riluzole and flufenamic acid), which confound comparison between studies.

In 2003, Onimaru and Homma used Ca2+ imaging to demonstrate that a group of cells near the facial nerve fire before cells in the PreBötC (Onimaru et al., 2003). This region of cells is known as the parafacial respiratory group (pFRG). Less is known about the cellular makeup of the pFRG; however, there is evidence that the rhythm generated by the pFRG is dependent on pacemaker cells (Thoby-Brisson et al., 2009). The current prevailing hypothesis is that the preBötC generates the inspiratory motor output, while the pFRG generates the expiratory motor output, and that these two oscillators are coupled (Fig. 4) (Janczewski and Feldman, 2006). The cellular mechanisms via which these two oscillators communicate remain unclear and several potential pathways have been proposed. First, communication may be mediated via a “handshake model”, wherein the preBötC and pFRG are coupled to form a rhythm-generating network. According to this theory, the pFRG provides excitatory feed-forward inputs to the neurons of the preBötC, while the preBötC provides inhibitory feedback inputs to the neurons of the pFRG. Exciting the preBötC would thus trigger the

![Schematic representation of the neural control of breathing. The pFRG excites the preBötC, which then inhibits the pFRG. These centres within the medulla are influenced by afferent vagal feedback, and descending pontine control. The motor output from the preBötC and pFRG triggers the breathing response via phrenic and abdominal motor neurons.](http://dx.doi.org/10.1016/j.cbpa.2014.10.029)
disinhibition of the pFRG, which would then inhibit the preBötC (Wittmeier et al., 2008; Lal et al., 2011). An alternative model is that a third neuronal group (potentially the Bötzinger Complex) is involved (Kuwana et al., 2006; Lal et al., 2011). In this model, the preBötC excites the third neural group, which then inhibits the pFRG (Lal et al., 2011). Further study is required to determine the mechanism of network coupling in this system.

In addition to regulation by the preBötC and pFRG, afferent feedback from the vagus nerve and descending inputs from the pons are also known to modify the respiratory response by influencing the rate and extent of lung inflation and deflation, and the duration of inspiration, respectively (Molkov et al., 2010). Afferent vagal feedback from stretch receptors in the lungs plays a role in the Hering–Breuer reflex, which ends exhalation when the lungs are deflated, and also ends inhalation to prevent over-inflation of the lungs (Moore, 1927; Heck and Levitsky, 2008). In support of this, vagotomy decreases $f_R$ and increases both inspiratory time (i.e. prolongs inspiration) and $V_i$ in most species (Milsom and Jones, 1980; Fedorko et al., 1988; Reid et al., 2000; Harris and Milsom, 2001).

The pons plays a critical role in transitioning between the inspiratory and expiratory phases. Classic studies by Lumsden (1923a, 1923b) and Stella (1938) showed that when both the rostral pons and afferent vagal feedback are removed, the breathing pattern shifts from eupnea (normal breathing) to apneaus (a prolonged inspiration) (Lumsden, 1923b; Stella, 1938). These results suggest that there is a pneumotaxic centre in the rostral pons, which acts to “turn off” inspiration. Because both the pons and afferent vagal feedback must be removed for the production of apneaus, it is suggested that if either the pons or vagal feedback is removed in isolation, the other acts as a “failsafe mechanism” so that inspiration is still switched off (Morschel and Dutschmann, 2009). The pons plays a role in ending inspiration; based on the cyclical nature of the breathing cycle, the pons also plays a role in activating expiration. Although little work has been done to investigate the role of the pons in the production of active expiration (which will be discussed in greater detail below), there is evidence that descending input from the rostral pons regulates late expiratory activity. For example, Abdala et al. (2009) found that removal of the pons eliminates active expiration in vagotomized juvenile rat preparations, and that it is not recoverable even with increased respiratory drive (i.e. due to hypercapnia) (Abdala et al., 2009).

Taken together, it is clear that a number of brain centres and complex feedback mechanisms are critical to the formation of the “simple” breathing response. Determining when these control centres become active during development is of great interest, as the neural control of breathing is critical to ensure that newborn animals are able to breathe immediately upon birth (Greer et al., 2006). Research examining developmental changes in the control of breathing has revealed that a number of changes take place embryonically to ensure that neural control functions appropriately at birth. The embryonic parafacial respiratory group (epF) is the homolog of the pFRG in developing mammals and epF neurons fire before the preBötC neurons in embryonic mice (Thoby-Brisson et al., 2009). If you consider that the preBötC generates inspiratory motor output and that the pFRG (or epF) generates expiratory motor output, it is clearly critical that inspiratory activity be silenced when expiratory activity is present, and vice versa (i.e. it would seem counterproductive to breathe in while also breathing out). However, when both centres are first present embryonically, both groups of neurons fire in synchrony (Thoby-Brisson et al., 2009). It appears that at this point in development, that the epF couples and entrains the preBötC, establishing the breathing frequency (Thoby-Brisson et al., 2009). This is of course different from the oscillating bursting of the pFRG and preBötC cells at birth. Interestingly, in mice at embryonic day 14.5 (E14.5), when epF cells are active, the intracellular $\text{Cl}^-$ concentration is high due to the expression of the $\text{Na}^+–\text{K}^+–\text{Cl}^-$ co-transporter, which pumps $\text{Cl}^-$ out of the cell (Delpy et al., 2008). High intracellular $\text{Cl}^-$ renders $\text{Cl}^-$ an excitatory ion and thus glycine and GABA are excitatory neurotransmitters at this stage of development. Intracellular $\text{Cl}^-$ is still elevated on E15.5, when the preBötC cells are active. One day following the appearance of preBötC cells (~E16), expression of the K$^+$–$\text{Cl}^-$ (KCC2) co-transporter, which pumps $\text{Cl}^-$ out of the cell, increases, while the expression NKCC1 co-transporter decreases. This lowers the intracellular $\text{Cl}^-$, and shifts the effect of glycineric and GABAergic neurotransmission from excitatory to inhibitory. This shift in the $\text{Cl}^-$ gradient is considered critical to ensure that the two groups oscillate out of phase at birth.

There are also changes in the breathing response observed postnatally in rodent development. Bill’s research has investigated the presence of episodic breathing (and the apnoeas between these episodes) in a number of vertebrate species (Milsom et al., 1997; Fong et al., 2009). The in vitro isolated brainstem spinal cord preparation has been used to demonstrate how changes in early development (occurring between postnatal day 0 (P0) to postnatal day 4 (P4)) influence the breathing pattern. When recording fictive breathing from the inspiratory motor output of spinal nerves (the fourth cervical nerve (CiV)) of younger preparations (P0), episodic breathing (clusters of fictive breaths separated by periods of apnoea) is observed (Fong et al., 2009). This is rarely seen in older preparations (P4) and later that often exhibit continuous fictive breathing. It is important to note that the changes observed with in vitro rat preparations may be species specific, and these changes in breathing pattern may not be consistent in other species or vertebrate groups.

4.2. The neural control centres of vertebrates

While the majority of research has addressed the anatomy and neural control of mammalian breathing patterns, Bill is quick to point out that examining the evolution of these systems across other vertebrate species offers important insights into their development and organization. Breathing strategies vary across the vertebrate groups, with amphibians and lungfish using a buccal pump, and mammals, reptiles, and birds using an aspiration pump. With these different breathing strategies come variations in the neural control centres. From an evolutionary perspective, it has been hypothesized that there are three steps in the transition from the buccal pump to the aspiration pump (Brainerd and Owerkowicz, 2006). First, inspiration in non-amniotic vertebrates is actively powered with a buccal pump, while expiration is passive. For example, lungfish use a buccal pump to force air into the lungs, but rely on the elastic recoil of the lungs to passively exhale. The pause between breaths is a breath hold, resulting from the active closure of the glottis after inspiration (Fig. 5). (It should be noted that air-breathing fish employ one of two different breathing strategies: either a four-phase breathing cycle, or a two-phase breathing cycle, but for the scope of this review, we will focus on the sacropetrgyn (lungfish) two-phase breathing cycle.) Second, in a number of amphibian species an expiration pump evolved that preserves the buccal force pump for active lung inflation, but which actively powers expiration via the abdominal muscles (namely the transversus abdominis). For example, study of salamander breathing has shown that their expiration is active, due to the presence of an expiration pump (Brainerd and Owerkowicz, 2006). This pump is powered by the abdominal muscles, but with greatest contraction of the transversus abdominis (Brainerd, 1998; Simons and Brainerd, 1999). The third step in the evolution of breathing mechanics is the aspiration pump, which replaced the buccal force pump to power active inspiration in mammals, reptiles, and birds (Brainerd and Owerkowicz, 2006). With the aspiration pump, negative pressure is utilized to draw air into the lungs.

With a love for a comparative physiology and the neural control of behaviours, Bill has worked to compile data from his lab and others into an evolutionary story (Milsom et al., 2004; Milsom, 2010a). There are two key observations in the evolution from the buccal pump to the aspiration pump that are pertinent to our understanding of the evolution of the neural control of breathing. The first is that the buccal pump is innervated by the cranial nerves, whereas the aspiration...
pump is innervated by spinal nerves. The second is a mirrored caudal displacement of the rhythm generating sites that control respiratory rhythm used by aspiration pump breathers compared to buccal pump breathers (Milsom, 2010a). For example, in anuran amphibians, the dominant rhythm generating site appears to be near the parafacial motor nuclei and powers lung inflation (Milsom, 2010a). In contrast, the transition to an aspiration pump shifts the dominant sites for respiratory rhythmogenesis caudally. In reptiles, the dominant site is near the parafacial nucleus and powers lung deflation; in mammals, the dominant site is near the paravagal region and powers lung inflation. There is evidence to suggest that while there is a shift in the major respiratory rhythm-generating sites across the vertebrate groups, all the sites are conserved, and that these sites are capable of creating rhythm independently, under appropriate conditions (Milsom, 2010a). With the evidence of a caudal shift in the respiratory rhythmogenic sites across vertebrate species, there is also evidence that these sites are indeed homologous, and conserved across vertebrate groups. For example, mammalian research shows that the cells of the preBotC are opioid-sensitive, while the cells of the pFRG are opioid-insensitive (Takeda et al., 2001; Mellen et al., 2003). This trend is mirrored in the adult anuran, as the lung oscillator is opioid-sensitive, while the buccal oscillator is opioid-insensitive (Vasilakos et al., 2005). The conserved expression of Krox20 during early development provides evidence of homologous parafacial areas between mammals and birds (it still remains unclear if the parafacial oscillators are indeed homologous) (Chatonnet et al., 2006). This indicates that while different breathing patterns and body plans may influence the breathing output, the respiratory rhythm generating sites are conserved in all vertebrates, to ensure that oxygen is appropriately transported from the external environment into the animal.

4.3. The breathing cycles of vertebrates

In all air-breathing vertebrates, the “simple” task of breathing is dependent on the coordination of the respiratory muscles to ensure that air is directed into (inspiration) and out of (expiration) the lungs. The coordination of the respiratory muscles plays a central role in the breathing response, specifically enhancing or repressing \( f_b \) and/or \( V_t \).

Reptiles begin their breathing cycle with an active expiration, followed by an active inspiration. The pause between breaths is a breath hold, due to active glottal closure after inspiration (Fig. 5) (Gans, 1970). While reptiles typically rely on costal pumping for ventilation, there is some variability between species. It was originally thought that lizards rely on their costal muscles for both ventilation and locomotion, which would prevent breathing when the animal moves (Carrier, 1987). However, varanids use gular pumping to supplement breathing while they use their costal muscles to move (Owerkowicz et al., 1999). Crocodilians use a diaphragmatic muscle — which is not homologous to the diaphragm muscle of mammals — to help draw air into the lungs, especially during exercise (Munns et al., 2012). Turtles face an even greater challenge, as their ribs are fused to their shell. They utilize pectoral and inguinal muscles to expand and compress their body cavity to power inspiration and expiration. Birds have a slightly more complex respiratory system, with posterior and anterior air sacs that direct air into and out of the lungs and both expiration and inspiration are active to maintain continuous breathing (Fig. 5) (Kadono et al., 1963; Codd et al., 2005). (Details of the lungfish and amphibian breathing phases are discussed above.)

Within the mammalian pulmonary system, the respiratory muscles are generally considered to be either inspiratory or expiratory. Specifically, the diaphragm is a distinguishing feature of mammals, and acts as the primary inspiratory muscle, although certain intercostal muscles also contribute to inspiration (De Troyer and Boriek, 2011). With the exception of dogs, horses, and opossums, most mammals exhibit passive expiration at rest, such that air is exhaled passively due to the relaxation of the diaphragm (Koterba et al., 1988; Feldman and Del Negro, 2006; Reilly and White, 2009). Conversely, in these exceptions, or when respiratory drive increases in all other mammals, expiration becomes active. Active expiration forces air out of the lungs due to a contraction of the internal intercostals (with some variation along the rostrocostal and dorsoventral gradient), and the abdominal muscles (De Troyer and Boriek, 2011). Furthermore, whereas all of the abdominal muscles can play a critical role in posture, to date it appears that the transversus abdominis contributes most to active expiration (Koterba et al., 1988; Iscoe, 1998; De Troyer et al., 1999; O’Halloran et al., 1999; Reilly and White, 2009).

At rest, and with the notable exception of the aforementioned mammals that employ active expiration, the mammalian breathing cycle is divided into three phases (Fig. 5). The breathing cycle starts with an active inspiration (I), followed by expiration.Expiration is divided into two phases; the first phase is known as expiratory braking (E1). This phase acts to slow expiration, due to the prolonged contraction of the diaphragm (Gautier et al., 1973; Feldman et al., 2013). It is suggested that E1 plays a role in prolonging and enhancing gas exchange (Davis and Bureau, 1987). E1 is followed by the expiratory pause (E2), in which no airflow occurs, leading up to the next inspiration (Richter, 1996; Feldman and McCormin, 2003). The accepted explanation is that if respiratory drive is increased in adult rodents, active expiration occurs at the end of the expiratory cycle (E3), just before the following inspiration (Pagliardini et al., 2011). The addition of active expiration therefore results in a four phase breathing cycle.

When respiratory drive is increased in the mammalian breathing cycle (i.e. during exercise), active expiration typically occurs in the E3 phase. This suggests that active expiration recruits the expiratory reserve volume (ERV) (Sherrey et al., 1988; Abe et al., 1996; De Troyer et al., 1999; O’Halloran et al., 1999; Abdala et al., 2009; Molkov et al., 2010). Recruiting the ERV would expel more air during expiration, and produce a larger \( V_t \) with the following inspiration. To date, there is little evidence to suggest that active expiration occurs in different parts of the breathing cycle (exceptions include newborn rats (Iizuka, 2009) and opossums (Reilly and White, 2009)); however, there may be cases in which active expiration could be beneficial in a different expiratory phase. For example, if there is a need to increase \( f_b \), then it may be critical to recruit active expiration in the E1 phase. At rest, E1

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The occurrence of active expiration may also be connected to developmental stages. To date, there are some discrepancies related to the presence of active expiration in newborn rodents. Using the in vitro brainstem spinal cord preparation, E1 (pre-inspiratory) activity in the pFRG has been observed in P0–P1 preparations but becomes less prevalent in P2–P4 preparations (Oku et al., 2007). It is hypothesized that this is due to a suppression of the preBötC after birth. In contrast, EMG recordings from in vivo newborn rats show that abdominal muscle EMG activity occurs in both the E1 (just after inspiration) and E3 (just before inspiration) phases in newborn rats at rest (Iizuka, 2009). The differences observed may be due to variations in the method of recording active expiration (motor output compared to EMG activity), or perhaps due to variations in preparations (in vitro compared to in vivo). In addition, there is discrepancy between the influences of anaesthetic on the presence of active expiration. Some evidence suggests that abdominal muscle activity is present (although muted) in anesthetized neonatal rats; however, it is not at all present in adult rats when the animal is anesthetized (Iizuka, 2009; Pagliardini et al., 2011). It is not clear why there may be a greater need for active expiration in newborn rats (especially in the E1 phase), but this may be due to the compliance of the chest wall, or higher airway resistances in the younger animal.

4.4. Future directions

In the last decade there has been a rediscovery of expiration, much to Bill’s delight. However, most, if not all, studies of active expiration to date have focused on the role of increasing respiratory drive to breathe. Of particular interest is the breathing response of mammals that have low respiratory drive, such as hibernating ground squirrels, and whales. When hibernating, ground squirrels transition from continuous breathing to episodic breathing (Milsom, 1991). This episodic breathing pattern is very similar to what is seen in reptiles at rest, and perhaps, this similarity in breathing pattern brings about a shift to active expiration. In addition, the breathing pattern of diving mammals, such as whales and seals, is similar to the breathing pattern of reptiles. Currently the Milsom lab is investigating how modifications to reduce drive to breathe, in addition to those that increase the drive to breathe, may influence all the breathing phases in general, and the presence of active expiration specifically, in other species.

A number of studies have investigated the neural control of breathing in mammals. However, from an evolutionary perspective, it is critical to extend this focus to all vertebrate groups. It is clear from reviews of the evolution of breathing that differences in breathing mechanics (i.e. between the buccal pump, expiration pump, and aspiration pump) influence the active phases of the breathing cycle (Brainerd and Overkowicz, 2006). These changes are likely paralleled by modifications to the respiratory rhythmicogenic sites across the vertebrate groups (Milsom, 2010a). A truly comparative approach is required to fully delineate how the neural control centres have changed to produce the breathing response in all vertebrates. In addition, a number of questions regarding the neural control of breathing of mammals still remain unanswered, and in particular the role of descending pontine influence and afferent vagal feedback on the presence of active expiration presents a novel opportunity for study.

Although breathing is often defined in very simple terms (inspiration followed by expiration), it is a highly complex, integrated behaviour. The coordination of appropriate cellular and synaptic oscillations within the medulla to simply produce the required muscle contractions for breathing is complicated in isolation. Additional complexity is added by the need to carefully control the relative timing and contribution of the respiratory muscles themselves to produce appropriate breathing responses to maintain homeostasis in varying environments. Thus, the simple act of breathing is clearly not so simple after all! To add further complication, there is significant overlap between the neural circuitry of the ventilatory and cardiac control centres. This overlap links cardio-respiratory responses (Feldman and Ellenberger, 1988), and aids in matching the flow of oxygen from the lungs to the circulatory system, which is critical for maintaining the successful flow of oxygen to the tissues via the oxygen transport cascade.

5. The cardiovascular system: modulation of perfusion during exercise and hypoxia exposure in vertebrates

Along with ventilation, circulation represents a critical mechanism by which the oxygen transport cascade can respond to variations in environmental oxygen availability or systemic oxygen demand. Circulation is a vital component of the oxygen transport cascade because of its role in transporting oxygen acquired during ventilation to the body tissues. During situations of increased oxygen demand (e.g. exercise) or decreased oxygen supply (e.g. environmental hypoxia) there are three main responses by which the cardiovascular system can contribute to the maintenance of adequate oxygen supply to the body tissues: 1) enhanced blood oxygen carrying capacity, 2) increased tissue oxygen extractions, and 3) increased perfusion (Fig. 1).

Blood oxygen carrying capacity and tissue oxygen extraction are influenced by a number of factors that together determine blood-oxygen loading and unloading kinetics. Intrinsic variables influencing blood-oxygen loading include Hb-O2 affinity, mean cellular haemoglobin (Hb) concentration, and haematocrit, such that increases in any of these variables increase blood-oxygen carrying capacity, and vice versa. The intrinsic Hb-O2 affinity is determined by the Hb isomer, which is mutated to favour increased O2-affinity in some animals living in areas of low ambient Po2 (e.g. high altitude animals such as bar-headed geese (Anser indicus; see section 7) and deer mice (Peromyscus maniculatus); (Mairbaurl and Weber, 2012)). Ultimately, increased Hb-O2 affinity enhances arterial oxygen loading, whereas decreased Hb-O2 affinity enhances oxygen delivery to the tissues. Mean cellular Hb concentration determines the capacity of a single red blood cell to bind oxygen, while haematocrit determines the overall capacity of the blood to carry oxygen. Together, these variables determine the intrinsic capacity of the blood to load oxygen. Though haematocrit changes can be triggered by external factors, such as exposure to hypoxia at high altitude, beyond splenic release, the manufacture of red blood cells is slow and haematocrit changes are therefore also relatively slow. In contrast, the effect of allosteric modulators on Hb is rapid. The major allosteric modulators for Hb are organic phosphates, H+, NO, and CO, which can influence Hb-O2 affinity. For example, Hb binding to H+ via the Bohr effect will decrease Hb-O2 affinity, facilitating oxygen unloading at the tissues. Organic phosphates differ between fishes (ATP or GTP), mammals (2,3 diphosphoglycerate), and birds (inositol pentaphosphate); however, when bound to Hb, they all reduce Hb-O2 affinity (Mairbaurl and Weber, 2012). Similarly, temperature also has a rapid effect on Hb-O2 affinity, though inverse in its effect (an increase in temperature decreases Hb-O2 affinity, thereby facilitating O2 unloading at the tissues). While tissue O2 extraction is a function of many morphological factors, such as diffusion distance and capillary tortuosity, it is also inversely related to Hb-O2 affinity, which increases the diffusion gradient for O2 from the red blood cell into the tissues. Thus, Hb-O2 affinity can be
optimized for a variety of different physiological circumstances, and plays a critical role in determining cardiovascular oxygen supply.

When alterations of respiratory function and haematological adjustments are insufficient to adequately load the blood with oxygen, the most rapid means for the circulatory system to maintain adequate oxygen supply to the tissues is to alter perfusion. When perfusion is considered in conjunction with allosteric Hb modulation, circulation is arguably the most critical component in terms of maintaining oxygen supply during periods of increased oxygen demand (e.g. during exercise) because of its rapid ability and wide breadth of capacity to respond. As such, perfusion will be the focus of the remainder of this section. In all vertebrates, oxygen delivery to the tissues is a very dynamic process driven by an active fluid pump: the heart. Driven by this pump, perfusion in its simplest form can be modulated either by frequency (i.e. heart rate; \(f_{H} \)) or volume (i.e. stroke volume; \(V_{S} \)). While vertebrates have the capacity to regulate their cardiovascular system via either of these methods, the ability to increase perfusion upon demand is of foremost importance. The differences in vertebrate evolutionary and ecological life histories have yielded diversity within the heart’s structure, as well as its modulation of perfusion by either \(f_{H} \) or \(V_{S} \).

5.1. The vertebrate cardiovascular system

The cardiovascular system of animals in the Phylum Chordata can be characterized by the following four factors: 1) a single, ventrally-located myogenic heart; 2) blood ejection from the heart directed anteriorly, 3) arterial and venous valves whose movement is dictated by blood flow, and 4) muscular vessels capable of constriction and dilation driven by an active fluid pump: the heart. Driven by this pump, perfusion in its simplest form can be modulated either by frequency (i.e. heart rate; \(f_{H} \)) or volume (i.e. stroke volume; \(V_{S} \)). While vertebrates have the capacity to regulate their cardiovascular system via either of these methods, the ability to increase perfusion upon demand is of foremost importance. The differences in vertebrate evolutionary and ecological life histories have yielded diversity within the heart’s structure, as well as its modulation of perfusion by either \(f_{H} \) or \(V_{S} \).

5.2. Cardiac anatomy

Fishes comprise the majority of extant vertebrates and live in many diverse and extreme environments. Their hearts have evolved accordingly to support a wide range of metabolic demands and ecological challenges. Briefly, the agnathous heart resembles that of aquatic gnathostome fishes, although it is far more primitive. All gnathostome fishes have a heart composed of a sinus venosus, atrium, ventricle, and bulbus arteriosus (also referred to as a conus arteriosus or bulbus cordis in cartilaginous fishes; Burggren et al., 1997; Farrell, 1991b).

Deoxygenated blood flows from the heart to the gills for oxygenation and then through the systemic circuitry before returning to the heart. Thus, unless the heart has a separate coronary artery supply of oxygenated blood, the myocardium is dependent upon the deoxygenated luminal blood supply to support myocardial oxygen consumption. Coronary blood supply is associated with the presence of a compact – rather than solely spongy – myocardium. Air breathing in fishes has evolved independently several times (Graham, 1997), with either intermittent (e.g. Amia calva; Farmer and Jackson, 1998) or primary (e.g. adult Arapaima gigas; Brauner et al., 2004) dependence on breathing air for gas exchange. In many air-breathing fishes deoxygenated systemic blood mixes completely with oxygenated blood from gas exchange structures prior to returning to the heart. Interestingly, lungfish have a highly diverged and complex circulation, which allows for systemic and pulmonary venous blood to return to the heart separately. Accordingly, the lungfish heart has evolved in order to minimize mixing of oxygenated and deoxygenated blood, with structures such as a partially divided atria, highly trabeculate ventricles, and partially divided bulbus cordis (Burggren et al., 1997; Graham, 1997). Thus, within fishes alone there is a wide variety in cardiac form and function.

Most amphibian cardiovascular systems have completely divided right and left atria, allowing for separation of deoxygenated and oxygenated blood returning from the systemic and pulmonary circuits, respectively. Their ventricle is highly trabeculate, allowing for partial separation of deoxygenated and oxygenated blood, even during ventricular systole (Shelton, 1976; Burggren et al., 1997). Depending on the amphibian, oxygenation of blood occurs across the lungs or the gills and also, in a number of genera, percutaneously. The reptilian heart varies significantly between families. Squamates (snakes, tortoises, turtles, and lizards, with the exception of varanid lizards) have two anatomically separate atria and three distinct ventricular cava (cavum arteriosum, cavum venosum, and cavum pulmonale), allowing for highly flexible control of central blood flow (Burggren et al., 1997). Crocodilians have a left aortic arch that arises from their right ventricle, allowing for blood to be diverted away from the lungs along a right-to-left shunt in situations of apnoea or submersion (Grigg, 1992; Hicks and Wang, 1996). With the exception of their left aortic arch, the crocodilian heart is anatomically comparable to that of birds and mammals. Avian and mammalian hearts have anatomically separated atria and ventricles. These chamber divisions accompany the shift to animals capable of substantially higher metabolic rates, allowing for complete separation of deoxygenated and oxygenated blood, and a division of the pressurization of the pulmonary and systemic circuit between the right and left ventricle, respectively. Both ventricles are comprised of a single lumen and compact walls, usually with a coronary circulation. Although bird and mammal hearts are equivalent on a gross anatomical and functional level (Rowlatt, 1980; Smith et al., 2000), it is important to note that this evolution of anatomically separated atria and ventricles has occurred independently at least twice — first giving rise to the arcosaurs (crocodilians and birds), and second giving rise to the synapsids (mammals; Burggren et al., 1997).

5.3. Cardiac output as a function of heart rate and stroke volume

Differences in cardiac anatomy among vertebrates have led to different methods of modulating perfusion. With regards to the cardiac muscle, cardiac output (\(Q \)) — or perfusion — is equal to the product of \(f_{H} \) and \(V_{S} \). Vagal activity can regulate \(f_{H} \) by altering either the depolarization rate of the sinoatrial node (chronotropy), the conduction velocity of cardiac fibres (dromotropy), or cardiac contractility (inotropy; Klubunde, 2012). Stroke volume is equivalent to the difference between end diastolic volume (EDV; maximum cardiac cycle volume) and end systolic volume (ESV; minimum cardiac cycle volume), and thus can be altered by changes in these variables. While externally originating signals from the nervous and endocrine systems play a role in regulating cardiac contractility by changing either the rate (affecting \(f_{H} \)) or strength of contraction (affecting ESV), the EDV of the heart is largely autoregulated through an intrinsic mechanism called the Frank–Starling effect. Simply put, the Frank–Starling effect states that increases in EDV (driven by increases in preload or venous return) increase the force of ventricular contraction. This increase in contraction is due to changes in the degree of actin and myosin overlap up until a maximum level of venous return. In this way the heart can intrinsically compensate for increases in preload (Farrell, 1991a; Berne and Levy, 2001; Klubunde, 2012).

Afterload and preload both greatly affect \(V_{S} \) (Fig. 6). Increases in preload (or venous return) will increase the force of contraction, thereby increasing \(V_{S} \). Increased preload is proportionally related to venous pressure (driven by venous compliance and blood volume) and ventricular compliance, factors that would increase venous return and the ability of the ventricle to expand. It is also proportionally related to outflow resistance and afterload, which impair the ability of the ventricle to empty, and thereby increase preload. Increased preload is inversely

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proportional to $f_H$, as it results in decreased filling time (Farrell, 1991a; Olson and Farrell, 2006; Klabunde, 2012). Afterload is the force that the heart must contract against when ejecting blood. Thus, increases in afterload decrease $V_s$. Afterload is proportionally related to arterial pressure and venous resistance (Klabunde, 2012). This mechanism of cardiac filling, driven by the kinetic and potential energies associated with venous return, is called vis-a-tergo, and is present in the hearts of most mammals, birds, reptiles, amphibians, and some fishes. Certain elasmobranches and active teleosts can use another mechanism called vis-a-fronte filling, which is driven largely by ventricular contractions that distend the atrial wall and decrease the atrial pressure to promote filling (Farrell, 1991b). In these fishes, vis-a-fronte filling sustains lower $V_s$ ranges, whereas vis-a-tergo filling is required to achieve maximum $V_s$ (Farrell, 1991b; Burggren et al., 1997; Olson and Farrell, 2006). While vis-a-tergo filling is generally associated with a flexible pericardium and ambient venous filling pressure, vis-a-fronte filling is often associated with a functionally rigid pericardial chamber and subambient venous filling pressures (Farrell, 1991a, 1991b). Thus, $V_s$ is ultimately determined by cardiac anatomy and varies in some cases as a function of ventricular contraction, but primarily as a function of venous return and system resistance.

5.4. Modulation of cardiac output in vertebrates during exercise and hypoxia

In response to situations of increased oxygen demand, such as exercise, increases in $Q$ can be met by increases in $f_H$ or $V_s$ alone, or in combination. The relative contribution of frequency and volume to modulate increases in $Q$ differs across vertebrate taxa (Shiels and White, 2008). Cyclostomes, elasmobranches, and most teleosts increase $Q$ by increasing $V_s$ by means of the Frank–Starling mechanism (Farrell, 1991b, 1993; Burggren et al., 1997). Though both $f_H$ and $V_s$ increase during aerobic exercise, $V_s$ is the primary contributor to elevated $Q$, increasing by up to twofold. Thus, in most fishes the heart is very sensitive to changes in filling pressure, allowing for rapid changes in $V_s$ (Farrell, 1993); most fish are reliant on volume modulation of the heart to produce increased $Q$ during exercise (Farrell, 1991b; Farrell and Jones, 1992). During hypoxia exposure, hagfish increase both $Q$ and $V_s$ (Forster, 1991); however, many fishes respond with a substantial decrease in $f_H$ (i.e. bradycardia) when exposed to hypoxia. $Q$ is maintained during this severe $f_H$ depression because of large increases in $V_s$ (for review see Farrell (2007)). Thus, despite their incredible diversity, most fishes have maintained the ability to dramatically increase $V_s$ in order to support or increase $Q$ during exercise or hypoxia.

In contrast, the relative contributions of $f_H$ and $V_s$ to $Q$ in amphibians and reptiles are varied. Heart rate has previously been described as the greatest contributor to $Q$ in amphibians and reptiles (Burggren et al., 1997); however, the methods employed for measuring these variables are diverse and limited research is available that is derived from direct measurements. Data suggest that the amphibian heart modulates $Q$ by increasing both $f_H$ and $V_s$ (Shiels and White, 2008), and that some
species have a relatively large capacity to increase $V_S$ (e.g. *Bufo marinus*; McKeen et al., 1997). Studies in turtles, snakes, varanid lizards, and crocodiles indicate that their myocardium is sensitive to the Frank–Starling mechanism (Shiels and White, 2008). Reliable in situ measurements confirm that $V_S$ is the major contributor to $Q$ in turtles (Farrell et al., 1994; Franklin, 1994; Burggren et al., 1997). Similar to fishes, turtles also increase $V_S$ (up to fourfold) to maintain $Q$ while offsetting bradycardia, in this case during diving (Burggren et al., 1997). Increases in systemic perfusion during exercise in the python are mediated by a combination of $f_H$ and $V_S$ (Secor et al., 2000). In exercising iguanids and varanids, increases in $Q$ are driven almost entirely by $f_H$ (Gleeson et al., 1980; Hicks et al., 2000). Increased filling pressures in the in situ crocodile heart causes increases in $Q$ with minimal changes in $f_H$ (Franklin and Axelson, 1994). Thus, the relative contributions of $f_H$ and $V_S$ to $Q$ vary across amphibian and reptile taxa.

It is well understood that the mammalian heart increases $Q$ by a combination of changes to $f_H$ and $V_S$, with $f_H$ as the primary contributor (Shiels and White, 2008). For example, both acute hypoxia exposure and exercise lead to increased $Q$ and $f_H$ in humans (Gamboa et al., 2003; Kontos et al., 1967; Vogel and Harris, 1967; Vogel et al., 1967), with large increases in $f_H$ and any increase in $V_S$ being small (10–30%) or negligible (Burggren et al., 1997).

Modulation of perfusion in the avian heart is often described along-side that of the mammalian heart — increasing perfusion primarily by $f_H$ — as they are similar in form and function (Burggren et al., 1997). However, the avian cardiovascular system is one of the most understudied among the greater vertebrate taxa. Very little is known about the role of the Frank–Starling response in bird hearts at either the cellular or organ level (Shiels and White, 2008), and the evidence that is available is conflicting. While it is generally accepted that birds increase $Q$ during exercise or hypoxia (Faraci, 1986; Shams and Scheid, 1989; Smith et al., 2000), to what degree this increased perfusion is facilitated by $f_H$ or $V_S$ is an area of contention. Birds have a disproportionally large heart for their body size relative to mammals (Grubb, 1983), and thus compared to a mammal of similar body size they exhibit larger heart mass, larger $Q$, larger $V_S$, and lower $f_H$ (Calder, 1968; Grubb, 1983; Smith et al., 2000).

During exercise, changes in $Q$ are reportedly driven by $f_H$ increases in geese (Fedde et al., 1989), ducks (Kiley et al., 1979), and turkeys (Boulanne et al., 1993a, 1993b), while $Q$ is driven by up to 2-fold increases in $V_S$ in emus (Grubb et al., 1983) and chickens (Barnas et al., 1985). Studies concurrently monitoring $f_H$, $V_S$, and $Q$ in birds are few in number (especially during hypoxia) and varied in conclusion. For example, resting ducks breathing hypoxic gas mixtures slightly increase $V_S$ (Shams and Scheid, 1989), whereas during hypoxia due to submersion ducks decrease $V_S$ (Jones and Holeton, 1972). Other studies on bar-headed geese do not report a change in either $f_H$ or $V_S$ during moderate hypoxic exposure at rest (Fedde et al., 1989; Hawkes et al., 2014); however, because bar-headed geese are a high altitude species, it is possible that this hypoxia exposure may not have been sufficiently severe to elicit a response. Thus, modulation of perfusion in the bird heart during hypoxia, and especially during hypoxia in flight, is an area with much research potential.

Hypotheses have been proposed that the transition from ectothermy to endothermy may also be reflected in the apparent change from reliance on a volume-mediated (i.e. $V_S$) increase in $Q$ to a frequency-mediated (i.e. $f_H$) change. The transition to endothermy is associated with many cardiovascular changes; most significantly, the completely divided ventricle allowing for a separation of deoxygenated and oxygenated blood, as well as pulmonary and systemic ventricular blood pressures (Burggren et al., 1997). The production of high systemic blood pressures in the left ventricle could support high endothermic metabolic rates and cardiac outputs, while the comparatively low pulmonary blood pressures allow for very thin blood–gas barriers in the lungs. However, it has also been postulated that the difference in having a preferentially volume- or frequency-modulated $Q$ can be determined by the ventricular makeup. All ectothermic vertebrates (fishes, amphibians, and reptiles) have a very trabecular, sponge-like ventricle. Mammal and bird ventricles, however, are composed primarily of compact myocardium and are much less trabeculate in nature, rendering their ventricular chamber more of a single lumen (Burggren et al., 2014). More trabeculate ventricles could allow for a greater ejection fraction because, according to the Laplace’s Law, these chambers can more efficiently contract when most of the blood resides in the trabeculate cavities (Johansen and Burggren, 1980; Van Mierop and Kutsche, 1985; Burggren et al., 2014). Laplace’s Law describes the relationship between radius, pressure, and wall tension such that larger hearts require a thicker wall to generate the same pressure as a smaller heart. Thus, in trabeculate hearts the trabeculate cavities function as many small pumps, rather than one large pump, optimizing pressure development (Farrell, 1991a). It is also possible that mammalian and bird hearts are primarily frequency-dependent in order to compensate for high metabolic rates. The decreased frequency and filling time allow for faster blood circulation without impeding myocardial oxygen supply, as compact myocardium is usually associated with a coronary circulation, which provides the ventricle with oxygen outside of the lumen (Burggren et al., 2014). While these hypotheses provide good explanations for the correlation between frequency-dependence perfusion and endothermy, as discussed above, $V_S$ is known to increase in some birds during exercise and hypoxia.

Flight is the most energetically costly means of vertebrate locomotion, requiring a 10–20-fold increase in resting metabolic rate (Butler et al., 1977). Increasing $Q$ by a combination of both $V_S$ and $f_H$ during flight would allow for maximum efficiency of oxygen transport, providing blood circulation that is not only high in frequency, but also substantial in volume. This benefit would be especially true during hypoxic flight, where the demand for oxygen is high and the supply of oxygen is low. Examples of where this efficiency could be most beneficial are in the high altitude migrating bar-headed geese and the high altitude resident Andean geese (*Chloephaga melanoptera*). Bar-headed geese biannually perform an impressive high altitude migration over the Himalayan mountains, routinely flying at altitudes of >5500 m (Hawkes et al., 2013) and sustaining high metabolic rates in an environment of lowoxygen supply (see Section 7). In comparison, Andean geese are the only Peruvian waterfowl abundant at altitudes of ~4000 m (McCracken et al., 2009), residing lifelong at very high altitudes. Both species require an efficient means of increasing perfusion during combined stresses of exercise and hypoxia, either due to athletic demand or sustained chronic exposure. However, the relative contributions of $V_S$ and $f_H$ to $Q$ during hypoxic flight in geese are unknown.

### 5.5. Future directions

The study of the relative contributions of $V_S$ and $f_H$ to increased perfusion is abundant in research potential. In general, there is a need for direct (rather than inferred or calculated), simultaneous measurement of cardiovascular variables such as $f_H$, $V_S$, and $Q$. There is also a need for standardization of protocols and techniques, as comparisons between studies are complicated by factors such as differences in duration or intensity of hypoxic exposure or exercise, and differences in the type of hypoxic exposure (e.g. isocapnic versus poikilocapnic). Furthermore, while teleost fishes and mammals have been fairly well characterized with respect to their cardiac responses to hypoxia and exercise, there have been comparatively few studies conducted on amphibians, reptiles, and birds. It is unlikely that broad taxonomic groupings are...
sufficient to determine whether a particular species is dependent on frequency- or volume-mediated perfusion, or a combination of the two. The known variation apparent within vertebrates suggests that cardiac anatomy, activity level, metabolic demand, and environmental stresses could play a large role in determining cardiac strategies for meeting perfusion demand. As such, the diversity of amphibians and reptiles with regards to lifestyle (e.g. aquatic versus terrestrial), metabolic demands (e.g. sedentary versus active), and environmental stresses (e.g. fluctuations in temperature or oxygen and carbon dioxide level) will likely reveal very interesting and novel mechanisms and strategies of perfusion regulation. While most endothermic vertebrates thus far studied have been frequency-dependent, the few studies that report this in birds are largely exercise-related. Concurrently studying fH, VEs, and Q during hypoxia or hypoxic flight would greatly contribute to the field. Theoretically during hypoxic flight there would be benefits of using a combination of frequency- and volume-modulation; however, no studies to date have directly measured all three variables during hypoxic flight. Across the vertebrate taxa there is also great potential for further studies of the adaptive strategies of increasing perfusion in vertebrates on the in vivo, organ, and cellular levels. Animals living and performing in extreme environments represent an excellent opportunity to investigate the wide spectrum of perfusion modulation in vertebrates.

In conclusion, the vertebrate cardiovascular systems and the means via which they are able to increase Q are incredibly diverse. While taxonomic generalities can be made for most fishes (Vb-dependent) and mammals (fH-dependent), active vertebrates within these taxa often pose exceptions to the rule. Amphibians, reptiles, and birds are largely underrepresented in perfusion modulation studies, and the availability of relevant literature varies in result. Understanding the mechanism by which vertebrates increase Q in a variety of different environmental stresses and metabolic demands is key to informing our scientific understanding of vertebrate cardiovascular diversity and evolution.

6. Reducing oxygen demand in hypoxia: ventilatory, metabolic, and thermoregulatory strategies of small neonatal and adult mammals

The preceding sections of this review have focused upon physiological mechanisms within the oxygen transport cascade that serve to increase oxygen delivery to the tissues. We will now discuss the complimentary strategy of decreasing metabolic demand. Many vertebrates, such as hibernating mammals (i.e. heterotherms), reduce oxygen demand and tolerate hypoxia almost entirely through metabolic suppression (Snapp and Heller, 1981; Boyer and Barnes, 1999; Geiser, 2004). By substantially decreasing body temperature (Tb) and metabolic rate, these mammals are able to maintain normal cellular function at oxygen levels too low for most other mammals (Drew et al., 2004). For example, Hiestand et al. (1950) measured the survival time when exposed to a P02 of 33 Torr for multiple species of homeotherms and heterotherms. They found that heterotherms survive longer than homeotherms, with some heterothermic species surviving for more than 3 h (Hiestand et al., 1950). Notably, adult homeotherms can withstand extreme hypoxia for <2 min, however, evidence suggests that as neonates they are just as hypoxia-tolerant as adult heterotherms (Adolph, 1948b, 1969; Glass et al., 1992; Singer, 1999). For this reason, neonates and adult heterotherms are often studied as mammalian models of hypoxia-tolerance; thus, this section will focus solely on them (but see Section 7 for discussion of additional examples of hypoxia-tolerant vertebrates).

What little data there are suggest that all homeothermic and heterothermic neonates respond to a reduction in oxygen availability by reducing energy demands (i.e. metabolic suppression). As heterotherms develop from a neonate into an adult their strategy for tolerating hypoxia transitions from decreasing oxygen demand to increasing oxygen supply (Mortola, 1991; Frappell and Mortola, 1994). Conversely, similar to neonates, adult heterotherms also respond to hypoxia by significantly decreasing oxygen demand (Mortola, 1991; Frappell et al., 1992; Frappell and Mortola, 1994; Barros et al., 2001; Tattersall and Milson, 2003a). Therefore, in heterotherms, the ability to reduce oxygen demand in response to lowered oxygen availability as adults may underlie the increased hypoxia tolerance of this group. However, despite the few seminal studies documenting that neonatal mammals in general, and heterothermic adults in particular are hypoxia-tolerant, our current understanding of how mammalian hypoxic responses change throughout postnatal development is limited. Whereas augmented ventilation and heart rate to increase oxygen supply are the typical responses of most adult homeothermic mammals to hypoxia, neonates and adult heterotherms are capable of extreme reductions in metabolism and Tb in response to hypoxia. It seems probable that differences in hypoxia tolerance among homeotherms and heterotherms may reflect developmental changes in the sensitivity and control of thermoregulatory, metabolic, and ventilatory responses under hypoxic conditions. Therefore, the overarching objective of this section is to contrast the different strategies homeothermic and heterothermic mammals use to match oxygen supply with oxygen demand in hypoxia. Specifically, we will focus our discussion on the importance of metabolic suppression and the reduction in the thermoregulatory set-point in hypoxia in order to understand the basis of enhanced hypoxia tolerance in neonates and heterothermic mammals.

6.1. Ventilatory and metabolic responses to hypoxia

Under resting conditions in normoxia, heterotherms have lower levels of ventilation and metabolism than homeotherms (Frappell et al., 1992; Barros et al., 2001). Thus, the ventilation to metabolism ratio of heterotherms (i.e. the air convection requirement; ACR) is significantly lower than the ACR of homeotherms of the same size (Mortola, 1991; Frappell et al., 1992; Barros et al., 2001). As a direct result of this reduced ACR, for every breath heterotherms take they are extracting more oxygen than homeotherms. Despite this, their lower level of ventilation results in less oxygen and more carbon dioxide in arterial blood compared to homeothermic mammals. As a consequence of these reductions in metabolism and ACR (i.e. enhanced oxygen extraction), larger reductions in environmental oxygen are required to elicit ventilatory and metabolic responses in heterotherms (i.e. they exhibit blunted hypoxic sensitivity).

When mammals are exposed to low oxygen environments, their immediate compensatory response is to hyperventilate (Powell et al., 1998). Hyperventilation in hypoxia, either through an increase in ventilation (i.e. the hypoxic ventilatory response; HVR), a decrease in metabolic demand (i.e. the hypoxic metabolic response; HMR), or both, compensates for the decrease in oxygen availability (Frappell and Mortola, 1994). Energy balance is disrupted when oxygen supplies fail to meet oxygen demands. This imbalance can ultimately lead to tissue hypoxemia, organ failure, and, consequently, death. All mammals studied to date elicit a well-documented biphasic ventilatory response in hypoxia (Powell et al., 1998). The first phase of the HVR is an immediate increase in ventilation within one breath of a change in Pao2, mediated by the stimulation of the peripheral chemoreceptors (see Sections 2–5) (Eldridge and Millhorn, 1986; Bisgard and Neubauer, 1995). The second phase of the ventilatory response begins with a slow decline in ventilation to a steady lower level, mediated by: 1) central mechanisms, such as the reduction in temperature at which they regulate their Tb (their thermoregulatory set-point; Tbset), metabolism; and as well 2) peripheral mechanisms — due to the time dependent decline in the sensitivity of the carotid bodies to hypoxic stimuli (Bisgard and Neubauer, 1995).

Adult homeotherms respond to hypoxia primarily by increasing ventilation, and typically maintain ventilation at a level that is 30–50% above their normoxic value (Mortola, 1991; Frappell et al., 1992). Traditionally, adult heterotherms are thought to have a reduced HVR relative to adult homeotherms; however, this is not always the case. In some heterotherms, oxygen thresholds and sensitivities are slightly lowered (e.g. hamster; Mortola, 1991), while other studies have found that heterothermic oxygen response thresholds do not differ from typical
mammalian values (Mcarthur and Milsom, 1991; Osborne and Milsom, 1993; Barros et al., 2001). Neonates, on the other hand have a drastically reduced HVR (Mortola et al., 1989). In fact, in neonates, ventilation decreases back to, or below normoxic levels within minutes of hypoxic exposure (Saetta and Mortola, 1987; Martinbody and Johnston, 1988; Mortola and Rezzoconi, 1988; Moss, 2000). This reduced HVR reflects the greater fall in metabolic rate with time in neonates (Hill, 1959; Taylor, 1960; Mortola and Feher, 1998).

Because ventilation is regulated to meet oxygen demands, one would expect that with more extreme levels of hypoxia (i.e. lower levels of $O_2$), ventilation would increase for any given level of metabolism, as each volume of air inspired contains less oxygen than in normoxia. For most mammals this is the case, resulting in an increase in the ACR (Osborne and Milsom, 1993; Saiki and Mortola, 1996; Barros et al., 2001). On the other hand, if metabolism is reduced in hypoxia, then the ACR may remain constant. The limited data available suggest that an increase in ventilation in response to hypoxia becomes progressively more apparent with postnatal development (Bonora et al., 1984; Wangsnes and Koos, 1991). As homeotherms develop there is a shift from reducing oxygen demand as neonates, to increasing oxygen supply as adults in order to try to meet cellular oxygen requirements in hypoxia. Although there is a slight shift in how heterotherms meet oxygen requirements in hypoxia with development, this shift is much less pronounced in heterotherms than homeotherms, and remarkably, adult heterotherms rely more on reducing oxygen demand and hence retain a similar ACR to neonates (Mortola, 1991). Therefore, just as in normoxia, for every breath adult heterotherms and neonates take they extract more oxygen than adult homeotherms. Adult homeotherms are also capable of suppressing metabolism and Tb in hypoxia; however, they do so to a much lesser degree than neonates and adult heterotherms (Challet et al., 1997; Mortola and Seifert, 2000).

Interesterly, the developmental transition from decreasing oxygen demand as a neonate, to increasing oxygen supply as an adult appears to coincide with the loss of the ability of homeotherms to tolerate hypoxia (Singer, 1999).

### 6.2. Ventilatory and metabolic responses to hypoxia in cold

When mammals are exposed to environmental temperatures they increase thermogenesis, metabolism, and ventilation, and a constant Tb is maintained (Lim, 1960; Gautier et al., 1992; Barros et al., 2001). With extreme cold exposure, however, the gradient between Tb and ambient temperature (Ta) becomes larger and thermogenesis does not suffice to maintain Tb; eventually Tb falls with Ta. This fall in Tb has its own inhibitory effect on ventilation and metabolism, due to the $Q_{10}$ effect of temperature, and eventually hyperthermia ensues (Saiki and Mortola, 1996; Tattersall and Milsom, 2003b). Neonates drop their Tb more rapidly than adults in the cold as a result of their poor thermoregulatory capacity, yet their ability to tolerate cold is about equal to that of adult heterotherms, and much greater than that of adult homeotherms (Adolph, 1948a, 1948b, 1951). Interestingly, decreasing the Tb of neonates and adults in hypoxia significantly extends the amount of time they can survive low oxygen availability. For example, neonates exposed to cold have increased survival rates in hypoxia compared to neonates held at Ta's within their thermoneutral zone (TNZ), the range of Ta's over which Tb is held constant, while metabolism is at a minimal and steady value. This is also true of adult heterotherms, who have an increased survival time in a Po$_2$ of 38 Torr when their Tb is reduced from 37 to 35 $^\circ$C, and decreased survival time when Tb is elevated to 40 $^\circ$C (Artru and Michenfelder, 1981). Similarly, adult heterotherms have been found to tolerate anoxia for up to two hours when hibernating, but less than 5 min when euthermic (Biorck et al., 1956). Exposure to hypoxia produces two opposing effects on the drive to breathe, reflecting a balance between 1) an increase in chemosensitive drive, due to the stimulation of the peripheral chemoreceptors; and 2) a reduced metabolic drive, linked to the central depression of Tb$_{set}$ and metabolism. Available evidence suggests that the magnitude of the hypoxia-induced reduction in Tb is greater in the cold than within the TNZ (Barros et al., 2001; Tattersall and Milsom, 2003b, 2009). Therefore, the enhanced ability of animals to survive hypoxia in the cold may be caused by a greater decrease in Tb$_{set}$ at lower Ta's, and the effect of a reduced Tb$_{set}$ on thermogenesis, metabolism, and ventilation (Gautier, 1996). One could therefore predict that the larger reduction in Tb of neonates and adult heterotherms in hypoxia would be beneficial, as it would ultimately reduce oxygen demand and abolish the need to increase oxygen supply.

In all euthermic adults, metabolism is higher at lower levels of Ta, ventilation increases proportionally, and a constant ACR is maintained (Barros et al., 2001). The ACR is also maintained in neonates exposed to cold; however, unlike adults, their metabolism and ventilation are lower at lower levels of Ta (Saiki and Mortola, 1996). These data emphasize that in the cold the regulation of breathing in neonates and adults is precisely adjusted to meet metabolic needs. In hypoxia however, when adult homeotherms and heterotherms are exposed to Ta's below their TNZ, the ACR increases at all Ta's, suggesting that adult homeotherms and heterotherms respond to hypoxia by hyperventilating (Gautier and Bonora, 1992; Barros et al., 2001). Within the TNZ, the HMR of adult homeotherms is minimal, yet the magnitude of the HVR increases substantially. In the cold (10 $^\circ$C) however, adult heterotherms respond to hypoxia by both suppressing metabolism and increasing ventilation. Adult heterotherms on the other hand, slightly increase ventilation and lower metabolism even within their TNZ. In the cold, adult heterotherms respond with a greater reduction in metabolism as well as lower levels of ventilation than at temperatures within their TNZ, and also than homeotherms. In fact, adult heterotherm ventilation in the cold can be lower in hypoxia than in normoxia (Barros et al., 2001). Hence, in both adult homeotherms and heterotherms, exposure to the same degree of hypoxia results in hyperventilation, but with very different strategies for fulfilling their oxygen requirement. Collectively, these data suggest that in both homeotherms and heterotherms the magnitude of the HMR is enhanced and the HVR is reduced at Ta's below their TNZ compared to those at Ta's within their TNZ. Although the evidence is sparse, neonates respond to hypoxia within their TNZ and in the cold by suppressing their metabolism (Mortola and Dotta, 1992); however, simultaneous measurements of ventilation and metabolism in the cold are needed in order to understand how neonates match oxygen demand and oxygen supply with changes in Ta and inspired oxygen. The mechanisms involved in the regulation of metabolism, ventilation, and thermoregulation during hypoxia in the cold, and how they change during postnatal development, are not completely understood.

### 6.3. The effects of hypoxia on thermoregulation and the zone of thermoneutrality

When environmental oxygen is low most mammals reduce their Tb$_{set}$ (Barros et al., 2001; Tattersall and Milsom, 2003a). This hypoxia-induced reduction in Tb$_{set}$ results in an inhibition of thermogenesis and a transient increase in heat loss, which lead to a rapid reduction in Tb. For example, in neonates, exposure to hypoxia causes a depression of behavioural responses to cold, an increase in body surface area, and a reduction in inter-animal huddling in favour of heat dissipation (Mortola and Feher, 1998). In adults, exposure to hypoxia abolishes shivering thermogenesis (Mortola and Feher, 1998; Barros et al., 2001), favours heat dissipation at the periphery (Tattersall and Milsom, 2003b), and blunts the normal circadian oscillation in Tb and metabolism (Bishop et al., 2000; Mortola and Seifert, 2000).

Although it is possible that the inhibition of thermogenesis in hypoxia is due to a lack of oxygen supply to the central thermoregulatory system, over the last few years a large body of evidence has accumulated in support of the hypothesis that the drop in Tb results from a regulated reduction in Tb$_{set}$ (Hill, 1959; Steiner and Branco, 2002; Tattersall and Milsom, 2008). The most compelling evidence for this hypothesis has come from a study done on the Columbian ground squirrel (Urocitellus columbianus).
(Tattersall and Milsom, 2009). In this study, the authors directly heated and cooled the central thermoregulatory control centre (i.e. the hypothalamus) of ground squirrels exposed to normoxia and multiple levels of hypoxia (PO\textsubscript{2} = 91, 68, and 53 Torr). They found that Tbset is reduced from 38 °C in normoxia, to as low as 28 °C in hypoxia. The magnitude of the Tbset reduction is dependent on the level of hypoxia, with a greater reduction in Tbset and lower thermogenic responses with lower oxygen exposure. Furthermore, they found that thermogenesis could still be recruited in hypoxia by cooling the hypothalamus, albeit at reduced levels. Collectively, these data strongly support the hypothesis that the mammalian thermoregulatory system functions properly in hypoxia, and that the threshold temperature for thermogenesis (i.e. the lower critical temperature of the TNZ; LCT) is shifted downward, resulting in a widening of the TNZ.

In adult rats, the zone of thermoneutrality generally ranges from 29 to 34 °C. Below the LCT animals stimulate thermogenesis and metabolism increases. In hypoxia, however, the LCT is reduced to 22 °C and the increase in metabolism below the LCT is drastically reduced. In fact, in some species of rodents, exposure to severe hypoxia (PO\textsubscript{2} = 53 Torr) nearly abolishes the thermogenic response to cold (Barros et al., 2001; Tattersall and Milsom, 2009). As a result, Tb is drastically reduced and in many circumstances, the TNZ is undefined. In contrast to their adult counterparts, neonates have a narrow thermoneutral range of 30–32 °C (Taylor, 1960; Mortola and Dotta, 1992). At Ta’s below their LCT neonates slightly increase their metabolism in an effort to maintain a constant Tb; however, due to their poor thermogenic capacity the gradient between Tb and Ta rapidly becomes too large, and a decline in metabolism and Tb below the LCT is typically observed, a relationship which is linearly dependent on Ta (Taylor, 1960; Mortola and Dotta, 1992). In hypoxia, however, their thermogenic response to cold is completely abolished and results in a reduced Tb and metabolic rate relative to in normoxia (Mortola and Dotta, 1992).

### 6.4. The effects of hypoxia on behavioural and physiological thermoregulatory mechanisms

When exposed to cold, neonates, like adults, can combine behavioural and physiological thermoregulatory mechanisms to maintain a constant Tb. Unlike adults however, most neonates lack a dense pelage, liberal quantities of subcutaneous fat, and the ability to produce heat through shivering thermogenesis (Alexander, 1975; Maxwell and Morton, 1975; Newkirk et al., 1995). In fact, some of the most immature rodents display little or no thermogenic response to cold at birth (e.g. rats and hamsters, respectively) (Newkirk et al., 1995). Neonatal rodents primarily rely on parental care for thermoregulation; however, they are also capable of repositioning within their nest, huddling with siblings, and changing their posture in order to reduce the amount of body surface available for heat loss (Mortola and Feher, 1998). As neonates develop, their external insulation along with their control of internal insulation increase rapidly, and their metabolic response to cold approaches that of an adult within a few weeks (Alexander, 1975).

As it is well established that moderate decreases in Tb are protective to animals in hypoxia (Wood, 1991), activation of behavioural and physiological thermoregulatory mechanisms to favour heat dissipation may represent a defensive response to accelerate the reduction in Tb and metabolism. In hypoxia, adult mammals placed within a thermoneutral temperature will adopt a stretched and extended posture to favour heat dissipation (Mortola and Feher, 1998). In addition to these behavioural thermoregulatory mechanisms, mammals can promote heat loss in hypoxia physiologically by altering their vasomotor tone. In hypoxia, adult ground squirrels vasodilate their peripheral vasculature, and in doing so shift heat away from their core and dump it at their periphery (Tattersall and Milsom, 2003b). Presumably, it isn’t until their Tb reaches their new Tbset that ground squirrels constrict their peripheral vasculature and shift blood to vital tissues (i.e. heart and brain) (Tattersall and Milsom, 2003b). Although the alteration of vasomotor tone in hypoxia has yet to be examined in neonatal rodents, one would predict that if vasomotor mechanisms are functional at birth, then the large, insulation free surface area of the neonate would be advantageous for dumping heat in hypoxia. In the neonate, vasomotor mechanisms used for thermoregulation have been characterized as immature and inferior to that of the adult (Spiers and Adair, 1986). For example, Poczopko (1961) measured skin temperature as a function of Ta and concluded that neonatal rats have no detectable vasomotor mechanisms for effectively altering heat loss until 12 days of age (Poczopko, 1961). Other studies have found that although the peripheral vasculature of infants is morphologically immature at birth, thermoregulation through the alteration of peripheral blood flow is considered to be well developed and functional (Perera et al., 1970).

### 6.5. Future directions

From the evidence presented above it is apparent that hypoxia inhibits thermogenesis and lowers the Tbset in all neonate and adult mammals studied to date. The lowering of Tbset in hypoxia is beneficial because it both allows the lower Tb to be sustained over a wider range of Ta, widening the animal’s TNZ, and reduces the energetic demand that accompanies the recruitment of thermoregulatory mechanisms outside of the TNZ. Although in hypoxia increasing ventilation and cardiac output are effective responses to increase oxygen supply, they are energetically demanding. Therefore, attenuating these responses at lower Tb’s (when metabolic demand is decreased due to the Q\textsubscript{10} effect) is advantageous. Furthermore, the reduction of Tb in hypoxia is protective because a drop in Tb increases the affinity of Hb for oxygen, which results in improved extraction of oxygen at the lungs, a desirable mechanism in conditions of low alveolar oxygen pressure. In adults, it appears that the reduction of Tbset in hypoxia is dependent on both the Ta at which hypoxic exposure occurs, and the level of hypoxic exposure (Barros et al., 2001). The limited data that do exist suggest that in neonates the hypoxia-induced reduction in thermogenesis and metabolism occurs even at thermoneutrality. Studies on neonates within the TNZ indicate that even mild levels of hypoxia (PO\textsubscript{2} = 137 Torr) reduce Tb, whereas PO\textsubscript{2} of 76 to 114 Torr are required in adults (Gautier and Bonora, 1992; Mortola and Dotta, 1992). In neonates, however, the extent to which the relationship between metabolism and Ta is modified by hypoxia has only been addressed in rats and kittens at one level of hypoxia (PO\textsubscript{2} = 76 Torr) (Mortola and Dotta, 1992).

In summary, it appears that the basis of enhanced hypoxia tolerance in neonates and adult heterotherms lies in their inherent ability to suppress their metabolism in a way that balances oxygen supply and demand. Overall, this response to hypoxia is not unique to heterotherms, but rather found in a variety of hypoxia tolerant mammals with diverse lifestyles, suggesting that an enhanced HMR is advantageous and conserved response. This strategy not only matches oxygen supply with demand, but also favours oxygen diffusion from the blood to the tissues at low oxygen tensions. A reduction in Tbset also appears to be an important strategy, and is a strategy shared with many other hypoxia-tolerant animals, which are discussed in Section 7. There is substantial experimental evidence in support of the hypothesis that thermoregulatory centres of mammals are adjusted to set Tb at a lower level, and the Tb threshold for thermogenesis is reduced in hypoxia (Barros et al., 2001; Tattersall and Milsom, 2003b, 2009). Interestingly, the developmental changes in behavioural and physiological thermoregulatory mechanisms responsible for accelerating the reduction in Tb and metabolism in hypoxia are still largely unknown. It appears that hypoxia alters behavioural and physiological thermoregulatory mechanisms in favour of accelerating heat loss, and that this changes in different ways in heterotherms and homeotherms throughout development. These generalities regarding how adult homeotherms and heterotherms respond to hypoxia are however based on a few studies comparing the
HVR and HMR for only a handful of species briefly exposed to one arbitrary level of hypoxia.

7. Environmentally driven adaptations to the oxygen transport system

As discussed in section 2, when faced with an environmental challenge, animals must either escape to a less challenging environment or, if their physiology permits it, cope and eventually adapt to their surroundings. Use of oxygen as the terminal electron acceptor is plesiomorphic for all animals, and as such, it presents a universal challenge when oxygen is limiting. Oxygen-limited environments are common on earth and many organisms experience periods of intermittent or prolonged hypoxia in their daily and/or annual life cycles (Bickler and Buck, 2007). Examples of common hypoxic environments include benthic regions, warm or particularly salty waters, stagnant or eutrophic lakes, tide pools, crowded or sealed underground burrows, alpine lakes, and high altitude mountains or plateaus. Many animals living in such environments cope by reducing oxygen demand through metabolic suppression (Boutilier, 2001; Hochachka, 1988; see also Section 6).

For some, however, this is not possible (Hochachka, 1985). Animals who spend their whole lives at altitude or hunt at depth must not only survive in environmental hypoxia, but also perform some of the most energetically costly behaviours of their lives with less than optimal oxygen availability. Investigating and comparing how animals manage the balance between metabolic demands and oxygen-limited energy production in hypoxia sheds light on mechanisms and trade-offs utilized at every level of the oxygen transport cascade. In this section we draw upon knowledge from earlier sections of this review to discuss environmentally driven adaptations to the oxygen transport system in two of Bill’s favourite species: transient, high altitude migrating bar-headed geese, and air breathing mammals that dive to depth. Both encounter oxygen challenges as well as pressure challenges, and cope with them behaviourally as well as physiologically.

7.1. High flyers

Bar-headed geese are a well-studied example of a migratory species whose highest metabolic demand occurs when they experience the lowest oxygen tensions of their lives. These geese breed and moult during the summer in China and southern Mongolia at moderate altitude, migrate across the Himalayas in the late fall to their wintering grounds in India at sea level, and then return across the mountains in spring to their breeding grounds (Hawkes et al., 2011). This is an impressive physiological feat, although there has been discussion regarding exactly how high and fast they fly, and the magnitude of the metabolic demand (i.e. whether they flap aerobically during the flight or glide on the updrafts). Until recently, the most frequently cited estimate of the maximum altitude reached by any one goose was 7290 m during the southbound migration and 6540 m during the northbound migration (Hawkes et al., 2013). The geese, however, generally fly between 4000 and 6000 m, usually about 350 m above the ground, indicating that the birds make use of the passes to cross the Himalayas. They confirmed that during the northbound migration the geese complete the mountain crossing in a single day, usually in about 8 h. This corresponds to an average climb rate of just over 1 km·h⁻¹, with a maximum climb rate of 2.15 km over 1 h, suggesting that the geese complete the climb without any stopovers (Hawkes et al., 2011). This study also investigated the possibility that the geese utilize updrafts to aid in climbing and perhaps take advantage of gliding to lower aerobic demand. They found no support for this hypothesis. Indeed, it seemed the birds avoided updrafts, and instead chose to fly starting at night or very early in the morning, when they likely experience light headwinds (Hawkes et al., 2011). Perhaps they make use of the calmer conditions as well as the cooler, denser air at night. Whatever the ultimate reason, this finding allows us to better estimate the cost of the bar-headed goose migration.

Flapping flight is generally considered the most metabolically costly form of vertebrate exercise (Butler et al., 1977). Birds in general may increase their oxygen consumption up to 30-fold from rest to flight, and bar-headed geese increase their metabolic rate more than 16-fold (Tenney, 1990; York et al., 2014). This work is increased at high altitude due to hypobaria, which reduces the lift and thrust generated by each wingbeat (Altschuler and Dudley, 2006). However, the physiological effects of hypobaria are as yet unexplored in this species. Overall, very little research has specifically investigated the physiology of bar-headed geese in flight, especially in environmental hypoxia. Nonetheless, research conducted thus far on resting or running geese has revealed much about how they are adapted to perform at high altitude and how the oxygen transport cascade can be adapted to maximize oxygen supply under high demand.

Generally, birds are much more tolerant of hypoxia than mammals; a classic experiment by Vance Tucker in 1968 showed sparrows could fly in levels of hypoxia that render mice comatose (Tucker, 1968). This is in part due to the more efficient respiratory system of birds, consisting of stiff lungs ventilated by air sacs. The unidirectional flow of air through the parabronchioles allows for gas exchange to occur in a countercurrent manner with the blood, increasing efficiency (Faraci, 1991). The relatively rigid lung allows birds to decrease barrier thickness, directly increasing diffusion rate (Faraci et al., 1984). Birds also always use active expiration, even in eupnea (Fedde et al., 1985).

At high altitude, birds have an additional advantage due to their insensitivity to hypocapnia. In mammals, hyperventilation occurs at altitude to increase blood P\textsubscript{CO\textsubscript{2}}, but this also causes a drop in blood P\textsubscript{CO\textsubscript{2}} (i.e. an alkalois). The body responds to hypoxia with vasodilation to increase perfusion, but also responds to hypocapnia with vasoconstriction. Thus the net effect is a tendency to restrict cerebral blood flow, which sometimes causes cerebral edema. However, this vasoconstriction does not occur in birds; in fact, cerebral blood flow increases in response to hypocapnic hypoxia in birds (Faraci, 1986). Similarly, mammals respond to hypoxia in the lung with reflexive vasoconstriction to prevent unnecessary lung perfusion in areas of the lung that are damaged or infected; unfortunately, when the whole lung is hypoxic, vasoconstriction can cause pulmonary hypertension or edema and impair gas exchange. Birds do not have a hypoxic pulmonary vasoconstriction response, and their stiff lungs also help protect against edema (Scott et al., 2011a).

Thus, bar-headed geese started with an evolutionary advantage in facing hypoxia common to most birds when the species appeared in the late Pliocene era, likely migrating over the Himalayas, which were lower at that time. The Himalayas thrust upwards over several thousand years during the late Pleistocene, and with them, the migration of the bar-headed goose reached higher and higher altitudes (Swan, 1961; Black and Tenney, 1980). During this time, this species has developed many physiological and morphological adaptations to cope with this...
challenge. Indeed, treadmill-trained bar-headed geese have been found to maintain their maximal running speed (determined at 21% O₂) during sustained running in 7% O₂, while low-altitude migrators cannot sustain exercise in 7% O₂ at all (Hawkes et al., 2014).

Within the respiratory system, bar-headed goose lungs are ~25% larger relative to their body size than other birds, although nothing else is currently known regarding their lung morphology or barrier distance (Scott et al., 2011b). Their hypoxic ventilatory threshold is low (PO₂ = 30 Torr), but when stimulated they are able to increase ventilation up to 7-fold, while other birds increase ventilation by a maximum of 4-fold (Black and Tenney, 1980; Scott and Milsom, 2007). Interestingly, this increase in ventilation is caused primarily by an increase in VT and not, as in some other lowland birds, fR. This response increases respiratory efficiency by minimizing effective dead space, but may be more costly than increasing fR (Milsom, 1991; Scott and Milsom, 2007). This begs the question as to how compliance of the bar-headed goose respiratory system compares to other birds. Further studies are required to resolve this question. Sally Ward and colleagues (2002) find a strong relationship between fH and oxygen consumption in normoxia for exercising geese, although this relationship changes between running and flying birds (Bishop et al., 2002; Ward et al., 2002). Preliminary findings from later wind-tunnel work in Bill’s laboratory suggest that perhaps this relationship could be logarithmic in nature; that is, oxygen consumption increases exponentially in relationship to heart rate from resting to walking to running to flying (York et al., 2014).

At the circulatory level, it is well established that bar-headed geese have a higher Hb-O₂ affinity than other birds studied (Black and Tenney, 1980; Faraci et al., 1984; Faraci, 1991; Scott et al., 2011a, 2011b). This is due to a single amino acid substitution that renders the deoxygenated form of Hb less stable (Jessen et al., 1991; Zhang et al., 1996). Thus, for any given partial pressure of oxygen, bar-head blood has higher oxygen content than other birds. The bar-head P₅₀ (oxygen tension at which haemoglobin is half-saturated) is 27 Torr, whereas in other birds it is around 40 Torr, thus bar-head blood can carry 2.5-fold more oxygen at a given partial pressure compared to blood of other birds (Faraci, 1991).

Oddly, although birds in general have larger hearts than mammals of similar size and therefore have high cardiac outputs, bar-headed geese have, if anything, smaller hearts for their size than other birds, although how V₅ and fH change in flight is still unknown (Black and Tenney, 1980; Scott and Milsom, 2007). They do, however, have about 30% higher capillary density in the heart, especially in the left ventricle cardiac myocytes (Scott et al., 2011b). Bar-headed geese, like other high-altitude adapted species, do not have elevated haematocrit. Even when unacclimated birds are taken to high altitude their haematocrit does not increase, as it does in low-altitude migrating species (Black and Tenney, 1980; Scott and Milsom, 2007). Increasing haematocrit at altitude may not be adaptive due to an increase in blood viscosity, which decreases cardiac output. Bar-head goose blood also has what has been described as an unremarkable Bohr effect (Meir and Milsom, 2013).

Fig. 7. (a) Migratory routes of bar-headed geese tagged in the wild and (b) altitude profile of the migratory route over the Himalayas. At sea level the inspired PO₂ is approximately 159 Torr, at 5000 m above the Tibetan Plateau the inspired PO₂ is approximately 90 Torr, and at 2500 m in Mongolia the inspired PO₂ is approximately 123 Torr. Adapted from Hawkes et al. (2011).
For the bar-headed goose, diffusion and delivery at the muscle tissue is the most rate-limiting step in the oxygen transport cascade (Faraci, 1991; Scott and Milsom, 2007). As in their cardiac tissue, bar-heads have a higher and more uniform capillary density in muscles, as well as mitochondria situated closer to the capillaries in the muscle fibre to minimize diffusion distance (Snyder et al., 1984; Scott et al., 2009). Thus for a given metabolic demand, capillary supply is elevated by 40% compared to other birds, although it must be matched by increased cardiac output to actually increase perfusion (Sillau et al., 1980; Snyder et al., 1984; Scott et al., 2009). These enhancements are on top of the higher mitochondrial density, elevated myoglobin levels, and smaller muscle fibre diameter shared by all avian muscle in comparison to mammalian muscle (Mathieu-Costello, 1990; Faraci, 1991).

Bar-head flight muscle (the pectoralis and supracoracoideus) is composed of both fast oxidative and fast glycolytic fibres, but with a higher proportion of fast oxidative fibres than flight muscle in related species—giving bar-headed goose flight muscle an increased aerobic capacity (Scott et al., 2009). Myoglobin transports oxygen through the muscle fibre to the mitochondria where it is accepted by cytochrome C oxidase (COX) in the ETC. Bar-head COX has a unique substitution in subunit 3 that is correlated to high oxygen diffusion capacity in cardiac myocytes and may help to reduce reactive oxygen species (ROS) production (Scott et al., 2011a). It should be noted, however, that all of the oxygen flux enhancements thus far discussed were studied in resting or running birds, most of which had never been to high altitude. Very little is known about the plastic phenotypic effects of geese that acclimate to hypoxia, warrant further investigation.

The effects of temperature on the efficiency of oxygen flux in the bar-headed goose present the possibility of interesting new findings with further investigation. Decreased blood temperature increases oxygen loading, and the $P_{50}$ of bar-headed goose decreases by nearly 2 Torr per degree C temperature drop (Meir and Milsom, 2013). The hypothesis being that if blood temperature is cooler at the lung, loading will be enhanced, and when the blood is warmed in the exercising muscle, off-loading will be favoured. Because birds are effectively insulated, heat loss occurs only in the so-called thermal windows of the feet, bill, and eyes (Scott et al., 2008). At the eye, birds have a collection of extracranial arteries and veins called the rete ophthalmicum, where it is thought that heat and gas exchange occurs to maintain brain temperature and oxygen levels (Bernstein, 1990; Faraci, 1991). Bar-headed geese may use this area and/or the respiratory passages to cool the blood before it returns to the heart and then the lungs. Bar-heads and related species of waterfowl decrease Tb in response to hypoxia, but bar-heads do not begin to lower body temperature until a lower inspired oxygen level than other birds, and therefore show a blunt thermoregulatory response to hypoxia. Bar-heads dump heat at the bill by $-0.5\,\text{C}$ for every 1 mmol-L$^{-1}$ decrease in $P_{O_2}$, which may decrease temperature-dependent metabolic demand (Scott et al., 2008). These temperature effects and their relationship to metabolism, especially in hypoxia, warrant further investigation.

Bar-headed geese provide an excellent example of an animal whose metabolic demand is maintained when faced with a low oxygen challenge; indeed, they must increase their aerobic performance as oxygen availability decreases. Work completed in the last 25 years has revealed much about how these birds are adapted to high altitude at every level of the oxygen transport cascade. More work is required in particular at the anatomical and molecular level, in addition to investigations of plastic and temperature effects to have a complete understanding of these high performance birds.

### 7.2. Deep divers

Somewhat akin to high altitude adapted animals, animals that breathe air but hunt, migrate, or live in water encounter two physiological problems when diving: lack of available oxygen and changes in pressure. In this section, we focus on penguins as an example of another bird that regularly encounters hypoxic challenges, but we also include discussions of diving mammals that often exhibit more extreme dives than penguins. While diving, the pressure the animals encounter increases by 1 atm for every 10 m descent into water, creating challenges due to direct effects of high pressure and the potential for decompression sickness during ascent from depth (Butler and Jones, 1997). The severity of the hypoxic challenge is directly related to the amount of time and metabolic energy the animal spends under the surface. Small penguins normally circumvent this challenge by keeping their dives short and shallow: usually less than 2 min dives followed by 1–3 s recoveries on the surface (Millard et al., 1973). The short recovery times indicate that these dives are entirely aerobic, whereas animals that dive longer and deeper sometimes require extended periods at the surface, indicating that they may require this time to flush lactic acid from the system. The cost of relatively short dives decreases as the dive progresses and oxygen becomes more limiting—because the most metabolically expensive part of the dive is the descent against the birds buoyancy, followed by benthic foraging, and then passive ascent (Borg et al., 2004). In both birds and mammals, dive duration and depth are negatively correlated with food availability and success of foraging (Butler and Jones, 1997).

Larger penguins dive down to 500 m, with each dive usually lasting under 12 min, and are able to keep their dives aerobic by relying on respiratory oxygen stores (Kooyman and Kooyman, 1995). Millard et al. (1973) estimated that, while diving, penguins potentially increase their oxygen use by about 10 to 15-fold over resting values. California sea lions (Zalophus californianus) are estimated to have an 8 to 10-fold diving metabolic scope (Ponganis et al., 1991). Weddell seals (Leptonychotes weddellii) probably only increase their metabolic rate 1.5-fold from sleeping to diving, and they are able to stay submerged over an hour, at a depth of typically between 200 and 400 m (Castellini et al., 1992; Schreer and Testa, 1996). Elephant seals (Mirounga leonina) can dive for a maximum of 2 h and usually stay between 200 and 800 m deep (Hindell et al., 1991). Amazingly, during the eight months they spend at sea, elephant seals spend about 90% of their time underwater and only surface for very short periods (maximum surfacing time measured was 5 min).

To cope with extended periods without breathing, deep diving animals generally lower their metabolic rate and redistribute blood flow (Irving, 1938; Pickwell, 1968). Metabolic rate is reduced primarily by bradycardia leading to a reduction of cardiac output and oxygen delivery, and therefore availability and consumption at the tissue and cellular level (Kooyman and Ponganis, 1998). Thus the rate of oxygen consumption is largely controlled via supply by $f_S$ and perfusion (Ponganis, 2011). It is also possible that metabolic demand is reduced by a cooling of Tb in some areas of the body (Butler and Jones, 1997; Ponganis, 2011); however, these measurements and models were originally determined in part from studies employing forced dives, and it is generally accepted that the physiological response to forced dives is an extreme version of what occurs under free-living conditions; some species capable of bradycardia and a controlled drop in metabolism during diving do not actually employ it often in the wild (Borg et al., 2004; Ponganis, 2011). Indeed Meir and Ponganis (2009) did not measure any regular reductions in body temperature in elephant seals during voluntary dives in the wild (Meir and Ponganis, 2009).

Deep diving animals such as elephant seals also employ bradycardia to lower resting levels while diving, and this is matched by a vasoconstriction that reduces blood flow to the skeletal muscle, skin, kidneys, and digestive system such that $V_e$ and blood pressure remain unchanged (Millard et al., 1973; Butler and Jones, 1997). This is part of the “dive reflex” originally described by Scholander in 1940, which is believed to preserve oxygenated blood flow in favour of the nervous system and the heart.

Oxygen stores are maintained in the respiratory system, the blood, and the muscle, and are thus dependent on lung volume, blood volume,
muscle mass, and Hb and myoglobin concentrations (Kooyman and Ponganis, 1998; Ponganis et al., 2011). The amount of oxygen stored by an animal is directly dependent on body mass (Ponganis and Kooyman, 2000). In general, diving mammals have an oxygen storage capacity that is 2 to 5-fold greater than humans (Ponganis, 2011). Deep divers rely on oxygen stores in the blood and the muscle (80–90% of oxygen storage), while shallow divers rely more on respiratory stores (Kooyman and Ponganis, 1998). Indeed, emperor penguins are able to maintain their P\textsubscript{O\textsubscript{2}} near maximum levels for up to 12 min of diving because they maintain 5 to 6-fold more gas storage per body mass than other diving animals (Meir and Ponganis, 2009). Furthermore, deep divers are able to arterialized their venous blood via shunting to optimize oxygen storage, and they generally have higher Hb concentrations than terrestrial mammals. A common trait of all diving animals is a 10–30-fold increase in myoglobin concentrations over terrestrial mammals (Meir and Ponganis, 2009; Ponganis, 2011). Higher myoglobin levels not only increase oxygen storage capacity, but also may help to bind ROS (Ponganis, 2011). Recently, maximum concentrations of myoglobin in the muscle of mammals has been found to be exponentially correlated with myoglobin net surface charge, both of which increase with the animals dive capacity: the most elite divers having the greatest myoglobin concentration and net surface charge, perhaps for increased protein solubility (Mirceta et al., 2013).

During ascent, an anticipatory tachycardia and vasodilation help to further decrease blood P\textsubscript{O\textsubscript{2}} and thus maximize diffusion rates during recovery and minimize time spent at the surface (Millard et al., 1973). At the surface the animal hyperventilates, mostly due to an increase in f\textsubscript{b} but not VT, and restoration of oxygen stores and carbon dioxide wash-out is thought to occur within one circulation of the blood—about 15 s in a diving pup (Borg et al., 2004). Milsom et al. (1983) found that diving Antarctic birds (i.e. penguins) had higher Hb–O\textsubscript{2} affinities and greater Bohr effects than non-diving Antarctic birds (Millard et al., 1973). They postulated that divers had left-shifted Hb–O\textsubscript{2} equilibrium curves to enhance the oxygen loading during surfacing, while the increased Bohr effect allowed for delivery at the tissues.

In addition to these adaptations, deep divers are very tolerant to hypoxemia. For example, elephant seals can drop their P\textsubscript{O\textsubscript{2}} to 12–23 Torr (Meir et al., 2009). This utilization of oxygen stores prevents seals from becoming anaerobic during dives, and therefore avoid becoming truly hypometabolic (Meir and Ponganis, 2009; Ponganis, 2011). In Weddell seals, P\textsubscript{O\textsubscript{2}} drops to 18 Torr (Qvist et al., 1986), while emperor penguins can tolerate P\textsubscript{O\textsubscript{2}} as low as 26 Torr (Ponganis et al., 2007). This hypoxemia tolerance allows these divers to fully utilize their oxygen stores to maximize dive duration.

The chemoreceptor cells of diving mammals are not more or less sensitive to hypercapnia and hypoxia than terrestrial mammals (Skinner and Milsom, 2004). Control of the response to hypercapnia is mediated primarily by the central chemoreceptors, as in other mammals, while the hypoxic response is controlled by the peripheral (carotid body) chemoreceptors (Milsom et al., 1981). In diving animals, hypercapnia primarily results in increases in VT, while hypoxia increases f\textsubscript{b}, so it seems that the hyperventilation during surfacing is brought on primarily by a lack of oxygen, and not a drive to wash out carbon dioxide (Milsom et al., 1981). If the dive is long and therefore at least partially anaerobic, the recovery time must also serve to reduce lactic acid levels within the system. Indeed, after hour-long dives Weddell seals require several hours of recovery and sleep (Kooyman et al., 1980).

High pressure, the other physiological challenge imposed by diving, can lead to nitrogen narcosis due to increased pressure in the lung that leads to passive absorption of nitrogen; if ascent occurs too quickly, gas bubbles can form in the tissues (Kooyman and Ponganis, 1998). To avoid these problems, deep diving mammals are able to collapse their lungs during diving, which minimizes the available nitrogen that can be absorbed as well as facilitating a rapid recovery at the surface, as the first breath inhaled is completely fresh air. Lungs and air passages of marine mammals are reinforced with cartilage or muscle fibres to allow for this collapse (Kooyman, 1973; Ponganis, 2011). The diaphragm and the chest wall assist in collapsing the lungs, and blood is shunted from the pulmonary artery (Kooyman, 1973; Kooyman and Sinnett, 1982; Kooyman and Ponganis, 1998). Surfactants in the lungs not only have a surface tension-reducing function as in other mammals, but also an anti-adhesion function that benefits alveolar re-expansion during recovery (Spragg et al., 2004). Cartilage is also thought to allow high flow rates during recovery, to minimize surfacing time (Ponganis, 2011). Tissues, especially those with air-filled cavities, can become deformed at high pressure, and diving animals are thought to have either eliminated these cavities (such as brain sinuses) or to fill them with blood while diving (Cozzi et al., 2005; Ponganis, 2011).

Overall, animals that dive and animals that fly at high altitude each face oxygen and pressure challenges. Animals that dive are able to partially reduce their metabolic rate if needed, while high altitude migrants must increase metabolic rate due to the requirements of flight. The study of both groups of animals has provided key insights into how the oxygen transport system works and how it can be modified to be more efficient under extreme conditions when oxygen is limited. Comparisons of the various adaptations to low oxygen stress in various hypoxia-tolerant models are particularly informative as it is only through such a comprehensive window that we can see the true diversity of the interplay between adaptations throughout the oxygen transport cascade. It is notable that although it is possible to model elegant solutions to the problem of environmental hypoxia, those potential solutions can be found in the organism in unexpected combinations or sometimes not at all. As George A. Bartholomew reminds us, “Since natural selection demands only adequacy, elegance of design is not relevant... it is to be expected that a given environmental challenge will be met in a variety of ways by different animals. The delineation of the patterns of the accommodations of diverse types of organisms to the environment contributes much of the fascination of ecologically relevant physiology” (Bartholomew, 1964). The discussion of adaptations found in bar-headed geese and in diving birds and mammals serves as a reminder of what combination of changes are adequate for these animals to achieve their unique physiological feats in hypoxia, and highlight the flexibility of the oxygen transport cascade.

8. Concluding remarks

Responding appropriately to changes in environmental and systemic oxygen availability is critical to maintaining cellular and systemic homeostasis in all animals. Sensitive detection of both environmental oxygen availability and changes in physiological oxygen levels, effective integration of these signals with the control of ventilatory and cardiac function, and careful matching of metabolic demand to oxygen supply are key to the survival of all species that rely on oxygen as a terminal electron acceptor in the production of cellular energy. Vertebrates have evolved a remarkably diverse suite of adaptations throughout the oxygen transport cascade that fine-tune these systemic responses to match changes in environmental oxygen availability and/or physiological oxygen demand. These adaptations are unique and specific to each species and serve to extend the environmental range of organisms and to enhance their fitness in the face of daunting external challenges. Due to an overwhelming medical bias, research into these mechanisms has focused largely on mammalian systems, and more so on those of humans and common laboratory model organisms. As a result, despite decades of research, our understanding of the oxygen transport cascade outside of mammals is largely limited to anatomical and mechanical studies. Considerable work remains to elucidate in particular the cellular signalling mechanisms that underlie communication between chemotransduction and the regulation of physiological responses, and also cell-level adaptations to changes in environmental and physiological oxygen availability in non-mammals, throughout development, and also during periods of physical activity. As we follow in Bill's footsteps
using the comparative approach to carefully study these systems in more diverse models across the animal kingdom, and especially in key species with remarkable abilities to maximize oxygen delivery and energy usage, we will gain important insight into the development and evolution of the oxygen transport cascade.

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