State of the art review: from the seaside to the bedside: insights from comparative diving physiology into respiratory, sleep and critical care

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ABSTRACT

Anatomical and physiological adaptations of animals to extreme environments provide insight into basic physiological principles and potential therapies for human disease. In that regard, the diving physiology of marine mammals and seabirds is especially relevant to pulmonary and cardiovascular function, and to the pathology and potential treatment of patients with hypoxaemia and/or ischaemia. This review highlights past and recent progress in the field of comparative diving physiology with emphasis on its potential relevance to human medicine.

INTRODUCTION

Anatomical and physiological adaptations underlie the dive capacities, foraging behaviours and ecology of marine mammals and seabirds. The dive response (the bradycardia and vasoconstriction associated with a breath hold), enhanced oxygen stores, hypoxaemic tolerance and pressure tolerance of these animals allow for remarkable physiological and behavioural feats. Severe bradycardias (figure 1), arterial pO_2 near 10 mm Hg (1.3 kPa) (figure 2) and maximum dive depths of 500–1500 m occur in multiple species at sea.[1] The deepest and longest dives have been reported for Cuvier's beaked whale at 2992 m and 163 min, respectively.[2 3]

These physiological adaptations for diving are also relevant to basic physiological principles and to potential therapies in cardiovascular and respiratory medicine. For instance, the activation of trigeminal nerve reflexes and the dive response by cold water application to the face is a classic vagal manoeuvre to treat supraventricular tachycardia.[4] In addition, simultaneous activation of both sympathetic and parasympathetic reflexes during cold water immersion of humans has been considered to contribute to autonomic conflict and cardiac arrhythmias.[5]

In this paper, I will review the relevance of comparative physiology and some of the adaptations for diving to the teaching of basic physiological principles and to potential therapies in medicine. These topics include (A) airway anatomy and respiratory flow rates, (B) alveolar collapse at depth, and surfactant function, (C) the roles of the dive response, vena caval sphincter and aortic bulb of seals in regulation of myocardial oxygen supply/demand, (D) haemoglobin (Hb) oxygen affinity in relation to oxygen transport during hypoxaemia, (E) cerebral hypoxaemic tolerance, (F) antioxidants and avoidance of reperfusion injury, and (G) the potential roles of the gasotransmitters—nitric oxide, hydrogen sulfide and carbon monoxide.

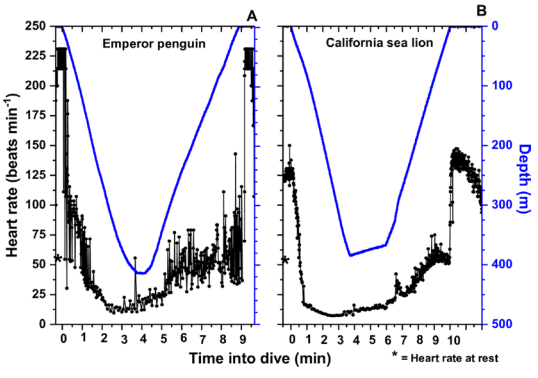

Figure 1 Heart rate profiles in dives to greater than 350 m depth of emperor penguins (A) and California sea lions (B) demonstrate the bradycardia of diving.[105 106] In such deep dives, heart rates routinely approach 10 beats per minute, and at times exhibit considerable variability (adapted from McDonald and Ponganis [105] and Wright *et al* [106]).

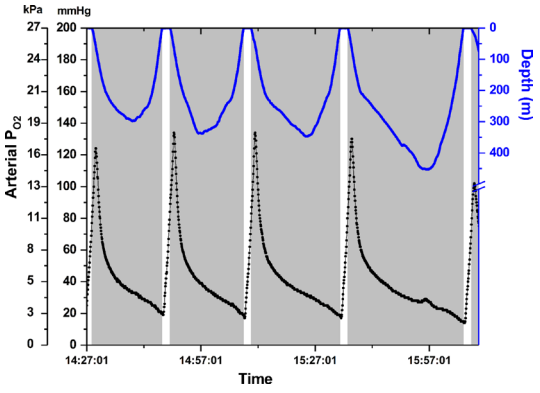

Figure 2 During these routine 15–20 min dives of northern elephant seals,[60] arterial pO_2 profiles demonstrated an initial compression hyperoxia, and then a gradual decline to about 20 mm Hg (2.67 kPa). Data were collected with an intravascular electrode and backpack recorder.Shaded area indicated dive (adapted from Meir *et al* [60]).

LUNG MECHANICS AND AIR FLOW

The importance of tracheobronchial anatomy and compliance in respiratory mechanics is exemplified in the flow volume curves, maximum expiratory flow rates and tidal volumes of cetaceans (whales and dolphins).[6] In contrast to human subjects, and, especially, patients with emphysema (figure 3), flow volume curves of the bottlenose dolphin and of excised harbour porpoise lungs are remarkable for

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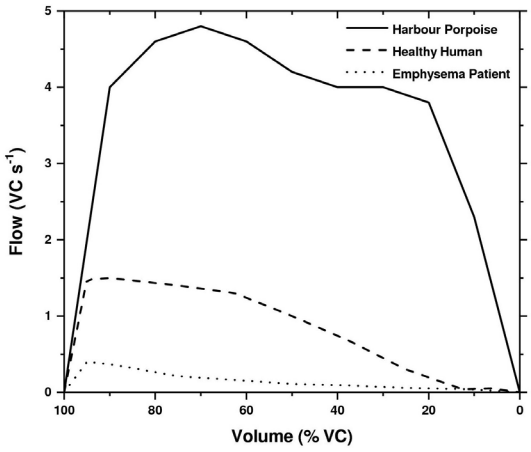

Figure 3 Flow volume curves (expiratory flow rate vs lung volume) demonstrate the magnitude of high flow rates as well as the maintenance of high flow at lower lung volumes in the harbour porpoise.[8] In comparison, flow rates (expressed in % of vital capacity (VC)) of a healthy human and a patient with emphysema are considerably lower and not maintained at lower lung volumes.[107] Maintenance of such high flow rates in the porpoise is attributed to cartilaginous reinforcement throughout the distal airways (adapted from Kooyman and Sinnett [8] and Hyatt *et al* [107]).

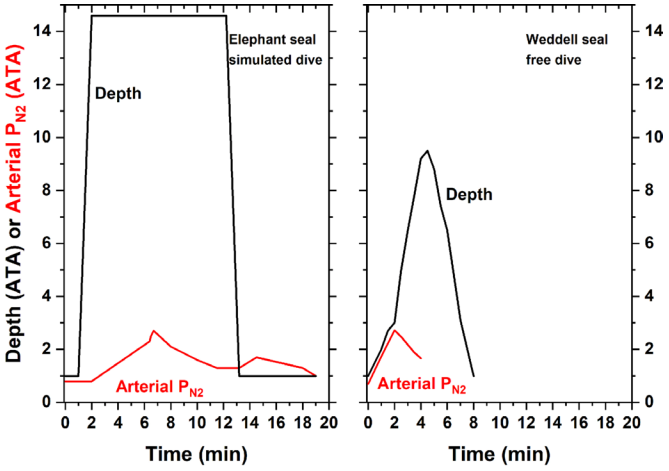

Figure 4 The arterial partial pressure of nitrogen (P_aN_2) remained low during a free dive of a Weddell seal (WS) to about 90 m depth, and during a simulated dive of an elephant seal (ES) in a pressure chamber to 136 m depth.[22 23] Alveolar collapse and lack of gas exchange was estimated to occur at 20–50 m depth from these data. Depth and P_aN_2 are both expressed in atmospheres absolute (ATA). In the absence of a pulmonary shunt or alveolar collapse, arterial P_aN_2 should approach alveolar nitrogen levels, approximately 80% the ambient pressure at depth (adapted from Falke *et al* [22] and Kooyman *et al* [23]) .

the maintenance of high flows at low lung volumes.[7 8] Furthermore, maximum expiratory flow rates in cetaceans and sea lions (expressed in terms of vital capacity (VC)) are in the range of 5–8 VC/s [7–9], much higher than maximum human expiratory flow rates of about 2 VC/s [10]. Such flows allow for a tidal volume as high as 88% of total lung capacity (TLC) in the pilot whale (*Globicephala melena*),[11] and 95% of TLC in bottlenose dolphins (*Tursiops truncatus*).[12] In comparison, the average human tidal volume is less than 10% of TLC.[10]

The reinforcement of the distal tracheobronchial tree in marine mammals is considered to promote such rapid air exchange in marine mammals. The anatomical basis of such reinforcement varies in different species, and includes the presence of (A) cartilaginous structures in the distal airway walls of cetaceans and sea lions, (B) prominent muscle fibres in the bronchial walls of seals, and (C) large vascular plexuses that have been postulated to become engorged at depth to reinforce the airways in deep-diving whales.[13–16]

Lastly, a recent report of high collateral ventilation flow rates in the unicameral dolphin lung should be of interest in relation to the role of collateral ventilation in emphysema and to its effects on outcomes from endoscopic lung volume reduction procedures.[17] In a novel hypothesis, it has been postulated that high collateral flow and constriction of the dolphin's peribronchiolar sphincter muscles during a dive contribute to optimisation of ventilation-perfusion matching.[18]

ALVEOLAR COLLAPSE AND SURFACTANT

Scholander's observations of cartilaginous distal airways in whales led him to hypothesise that more rigid airways would also allow (1) movement of air into those airways during compression of the lungs at depth, (2) collapse of alveoli, (3) cessation of gas exchange at depth, and (4) the avoidance of excess nitrogen absorption during dives.[16] This concept has been supported by (A) low relaxation volumes of excised lungs of various marine mammals relative to those of terrestrial counterparts,[8 19 20] (B) pulmonary shunts in pressure chamber studies,[21] (C) blood nitrogen levels that were less than expected in the absence of shunting or alveolar collapse at depth[22–24] (figure 4), (D) characteristic arterial pO_2 profiles at threshold depths in sea lions,[25] and (E) modelling studies based on lung volumes and respiratory compliance measurements.[26 27] Although the depth at which gas exchange ceases may vary in marine mammals (above references[22–27]), routine collapse and re-expansion of marine mammal lungs during dives has led to the question as to whether there are adaptations in the pulmonary surfactant of these animals. Elephant seals, for example, spend at least 80% of a 3-month trip to sea underwater at average depths of 400 m with typical dive durations of 20 min and surface intervals of only 2 min.[28] Although alveolar collapse was originally considered to occur at 20–50 m depth in these seals,[22 23] modelling studies now suggest a deeper threshold, near 160 m depth.[29] Regardless of the exact depth of alveolar collapse, most gas exchange is limited to the brief surface intervals during which lung expansion and function are critical.

Analysis of bronchoalveolar lavage fluid from young seals, sea lions and other marine mammals revealed a greater concentration of phospholipid and more fluidic species of phosphatidylcholine than in terrestrial mammals.[30 31] This was considered consistent with more rapid spreading of phospholipids during alveolar re-expansion. Surfactant from elephant seals produced moderately elevated minimum surface tension measurements. Poor surface activity and minimal reduction of surface tension were also later observed in analyses of lavage fluid from excised lungs of other pinniped species.[32] In addition, further analyses of surfactant composition of pinnipeds revealed a relative decrease in anionic phospholipids, an increase in short-chain phospholipids and a decrease in surfactant protein B.[33 34] Based on all these findings, it has been suggested that the primary function of surfactant in deep-diving mammals may be an antiadhesive function rather than only surface tension reduction.[35] This antiadhesive function has been considered to include the reduction of adhesive interactions between respiratory surfaces due to variation in surface tension.[36] In this situation, as two surfaces,

apposed against each other in a collapsed alveolus, begin to pull apart during lung expansion, surfactant lipids are considered to rise to the surface at the air–liquid interface, thereby lowering surface tension and decreasing the work required to expand the lung.

MYOCARDIAL OXYGEN SUPPLY AND DEMAND

The dive response and cardiovascular adaptations of seals provide an excellent demonstration of the regulation and optimisation of myocardial oxygen supply and demand.[1 37] Heart rate reduction during the breath hold is variable, dependent on the nature of a dive and is most severe during forced submersions.[38 39] The bradycardia of diving optimises myocardial oxygen supply and demand by increasing diastolic perfusion time for the heart while at the same time decreasing myocardial workload. In addition, despite increased sympathetic tone and intense vasoconstriction during the severe bradycardia of forced submersion, left ventricular maximum rate of pressure change (dp/dt_{max}) and left ventricular wall segment shortening were decreased in seals.[40 41] Thus, myocardial contractility and its associated oxygen demand are also decreased during the dive response.

Optimisation of cardiac preload is necessary to prevent increased wall stress and overdistention of the heart. In seals, a caval sphincter, composed of striated muscle, and innervated by the right phrenic nerve, surrounds the thoracic portion of posterior vena cava at the level of the diaphragm.[42] The vena caval sphincter, which has been observed to open and contract with each cardiac cycle during forced submersions, represents a unique mechanism to regulate venous return and optimise preload of the heart.[43 44] During the severe bradycardia of forced submersion, decreased left ventricular wall segment dimensions, decreased left ventricular dp/dt_{max}, unchanged left ventricular diastolic pressures, unchanged central venous pressures and decreased stroke volumes were all consistent with decreased ventricular filling, decreased wall tension and decreased contractility.[40 41 45] All these factors would minimise myocardial oxygen demand. The lack of any increase in left ventricular diastolic pressure will also aid in maintenance of myocardial perfusion because the driving pressure for coronary perfusion during diastole is the difference between the arterial pressure and the intraventricular pressure.

In seals, the effects of widespread vasoconstriction and increased systemic vascular resistance on the afterload and oxygen consumption of the left ventricle are minimised by the aortic bulb, an elastic dilatation of the aortic root.[46 47] The distensible walls of the aortic bulb decrease the afterload the ventricle would otherwise face. Thus, the workload of the heart during the dive response is decreased by yet another mechanism. In addition, the elastic wall of the expanded bulb gradually contracts during diastole, maintaining both blood flow and diastolic blood pressure which is necessary for coronary perfusion[48] (figure 5). It is remarkable that the physiological effects of the aortic bulb in seals are exactly analogous to the therapeutic effects (afterload reduction and maintenance of diastolic perfusion pressure) of the intra-aortic balloon pump.[49] Aortic bulbs also occur in other types of marine mammals, and an elastic, highly compliant aortic arch in the large baleen whales is hypothesised to accomplish the same optimisation of myocardial oxygen supply/demand and maintenance of diastolic blood flow.[50 51]

HB'S OXYGEN AFFINITY

Recently, GBT1118, a novel compound that reversibly binds to Hb and increases oxygen affinity, has been demonstrated

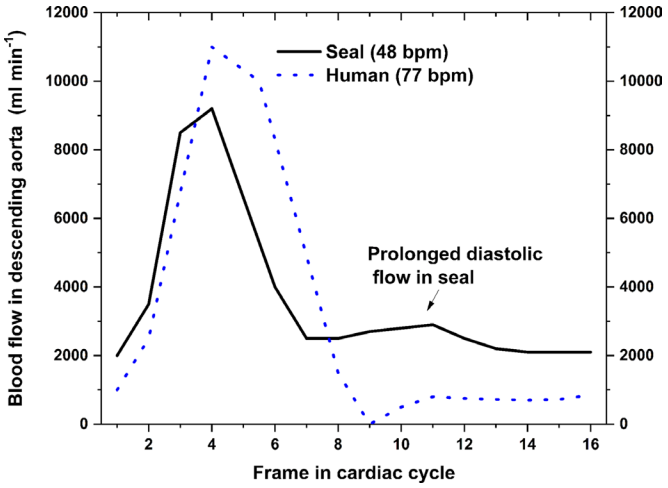

Figure 5 A comparison of blood flow in the descending aorta during a single cardiac cycle in humans and seals illustrated the maintenance of diastolic flow in the seal.[48 108] This higher diastolic flow was attributed to the slow contraction of the distended aortic bulb in the seal. Data were obtained with MRI techniques (adapted from Thornton *et al* [48] and Hochachka [108]) .

in murine models to decrease Hb polymerisation in sickle cell disease, attenuate hypoxaemia and pulmonary fibrosis in bleomycin toxicity and improve survival in acute lung injury.[52–54] The rationale for modification of Hb's oxygen affinity to increase blood oxygen content in the treatment of hypoxaemia in human patients is supported by the left shift (increased affinity) of the oxygen-Hb dissociation curve in penguins and high-altitude birds.[55–58] The P_{50} values (pO_2 at 50% Hb saturation) of deep-diving emperor penguins and high-flying bar-headed geese are 10–15 mm Hg (1.3–2.0 kPa) less than those of low-altitude birds (figure 6). However, such leftward shifts in the dissociation curve are not apparent in marine mammals; the P_{50} values of two of the most accomplished pinniped divers, the elephant seal and Weddell seal, were 27–30 mm Hg (3.6–4.0).[59 60] Similarly, although the Hbs of some high-altitude mammals have increased oxygen affinity, the evidence for such an adaptation among all high-altitude mammals was equivocal.[61] Further investigation is necessary.

CEREBRAL HYPOXAEMIC TOLERANCE

In forced submersion studies of cerebral hypoxaemic tolerance, seals tolerated arterial pO_2 as low as 10 mm Hg (1.3 kPa).[62 63] During the routine 20 min dives of elephant seals (figure 7), arterial Hb saturations were consistently between 80% and 20% for most of the dive duration.[60] The lowest saturation recorded was 8%. Significant desaturation also occurred during the frequent spontaneous sleep apnoeas that occur on land (figure 8).[64] The mechanisms underlying such tolerance appear to be multiple, and may provide insights to the design of therapeutic options for cerebral hypoxaemia and ischaemia. In the seal, high Hb concentrations (25 g/dL), hypercarbia (with both its associated rightward shift (decreased affinity) in the oxygen-Hb dissociation curve and its increase in cerebral blood flow) and increased brain capillary density all enhance oxygen delivery under these circumstances.[63] In addition, selective brain cooling can occur via arteriovenous shunting in the foreflippers with brain temperature declines of 3°C–4°C during 15 min forced submersions.[65 66] Such findings lend support to current advanced cardiac life support guidelines in regard to avoidance of hyperventilation during resuscitation

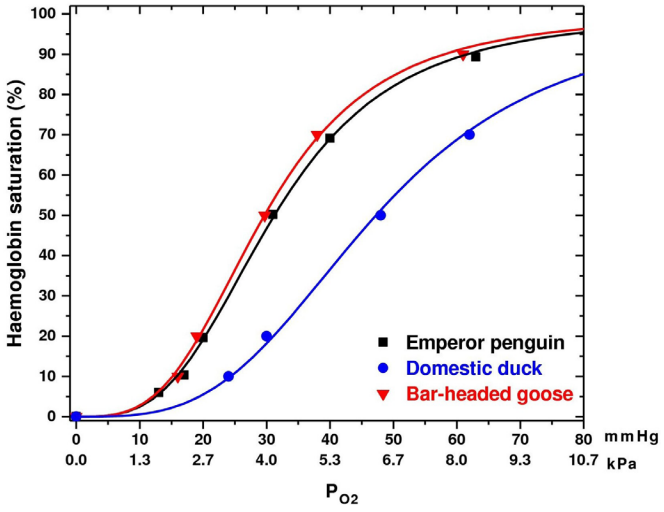

Figure 6 O_2-haemoglobin dissociation curves of the emperor penguin and bar-headed goose demonstrated a left shift relative to that of the domestic duck.[55 56] It was postulated that the increased oxygen affinity of the penguin and high-altitude goose was beneficial in providing higher blood oxygen content at the lower pO_2 experienced by these birds (adapted from Black and Tenney [55] and Meir and Ponganis [56]).

from cardiac arrest and to the potential role of therapeutic hypothermia in neurological recovery from ischaemic insult.

Remarkably, synaptic transmission is maintained in seal brain tissue even under conditions of extreme (<1% O_2) hypoxia.[67] In comparison to terrestrial controls, biochemical adaptations and molecular mechanisms that support the seal brain under such conditions include: (A) a threefold elevation in brain glycogen, (B) increased tolerance to both elevated lactate and decreased glucose concentrations, but no increase in pH buffering capacity, (C) normal lactate dehydrogenase (LDH) activity with >70%

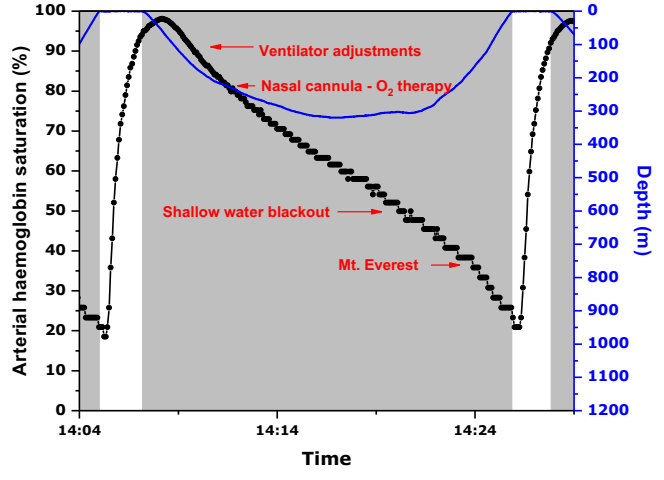

Figure 7 During routine 20 min dives at sea, elephant seals consistently experienced arterial haemoglobin saturations between 80% and 20%.[60] The seal's arterial saturation was at or below the common clinical criterion (90%) for ventilation and inspired oxygen concentration adjustments in ventilated patients, below the supplemental nasal cannula oxygen treatment threshold for patients with severe lung disease, below the human shallow water blackout level and, by the end of the dive, even less than the arterial saturations of humans breathing ambient air on Mt Everest (adapted from Grocott *et al* [109], Lindholm and Lundgren [110], Mason *et al* [111] and Nunn [112]).

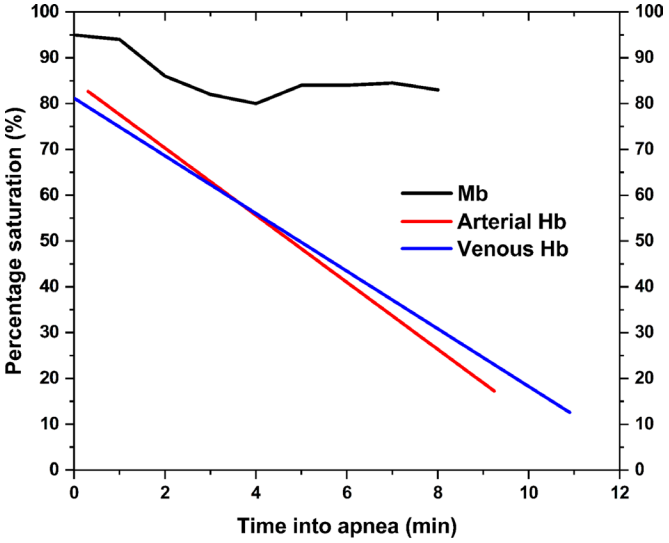

Figure 8 Blood and muscle oxygen store depletion patterns during spontaneous sleep apnoeas of young northern elephant seals revealed near-complete depletion of the blood oxygen store but 80%–90% preservation of the muscle oxygen store.[64 113] Arterial and venous haemoglobin (Hb) data were obtained with intermittent blood sampling during multiple breath holds of different animals; myoglobin (Mb) saturation was determined with ^{1}H NMR spectroscopy (adapted from Stockard *et al* [64] and Ponganis *et al* [113]).

LDH1 and 2 isoenzymes (lactate oxidation), primarily located in glial cells, (D) increased gene expression of S100B (a stress protein with calcium binding activity), clusterin (an extracellular chaperone molecule) and most glycolytic enzymes, but decreased expression of pyruvate dehydrogenase, and (E) normal neuroglobin and cytochrome oxidase gene expression, but, in contrast to terrestrial mammals, located in glial cells not neurons.[68–74] Thus, in the seal in contrast to terrestrial mammals, most oxidative activity and consumption of lactate occurs in the astrocytes, not the neurons. Neither oxidative nor glycolytic activity appears enhanced. And, again in contrast to terrestrial mammals, there appears to be a reverse astrocyte-neuron lactate shuttle (astrocytes consume lactate produced by neurons).

Another remarkable aspect of neural function in deep-diving marine mammals is the apparent absence of high-pressure nervous syndrome. Recent molecular studies in cetaceans have revealed that substitutions in the N-methyl-D-aspartate receptor change its tertiary structure and may alter activation of the receptor, thus preventing the neuronal hyperexcitability that occurs at high pressure in other animals, including humans.[75]

AVOIDANCE OF REPERFUSION INJURY

Absence of reperfusion injury in marine mammals was well documented more than 40 years ago during complete, warm ischaemia of seal kidneys.[76] The mechanisms responsible for such tolerance are still incompletely understood, but again are potentially relevant to ischaemic preconditioning, organ preservation for transplantation and avoidance of reperfusion injury during revascularisation procedures. In the seal heart, although a 10-fold elevation in glycogen content may provide a large glycolytic energy store and prevent intracellular calcium accumulation during ischaemia/hypoxaemia,[77] an even more impressive 25-fold elevation in glutathione content should enhance the potential for scavenging of reactive oxygen species (ROS) during reperfusion.[78] Significant elevations in glutathione content were

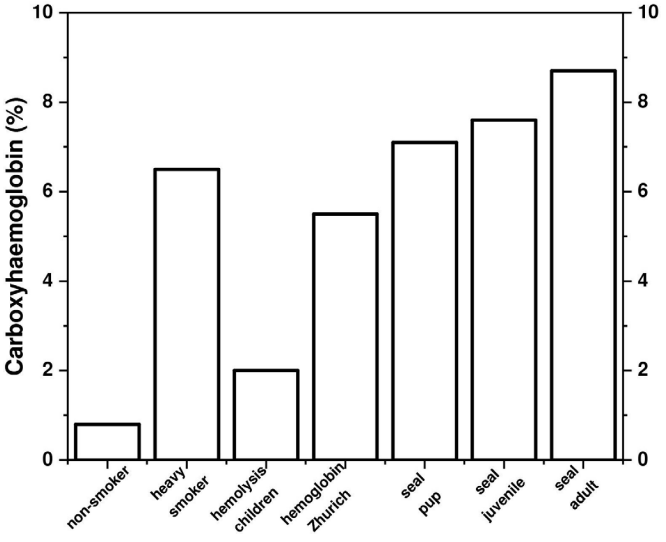

Figure 9 Carboxyhaemoglobin levels in non-smokers, heavy smokers (40 cigarettes per day), children with haemolytic diseases, patients with haemoglobin Zurich and northern elephant seals.[100 114–116] Data are mean values from the studies.

also found in seal kidney, lung and muscle. In all tissues, enzymes associated with the recycling of glutathione were elevated. In addition, multiple other ROS-scavenging enzyme activities are elevated in many tissues of both seals and penguins.[79–81] Lastly, in marine mammal muscle, the elevated myoglobin concentrations may also affect both ROS scavenging and production.[82 83] The peroxidase activity of myoglobin can break down hydrogen peroxide. The nitrite reductase activity of myoglobin may increase nitric oxide production, which, in turn, can inhibit cytochrome c oxidase and decrease ROS formation.

It has also been found that the whole blood inflammatory response of seals on exposure to a potent endotoxin (lipopolysaccharide, LPS) is significantly blunted.[84] Interleukin-6 cytokine production in blood was 50–100 times lower in elephant seals and Weddell seals than in humans. In cell cultures of mononuclear cells from Weddell seal blood, addition of seal serum to the culture media decreased the LPS-induced production of interleukin-6 and other cytokines. It thus appears that seal blood contains an anti-inflammatory compound that may also play a role in protection against ischaemic and hypoxaemic insult. The mechanism(s) of this anti-inflammatory response in seals may represent another route to explore to minimise inflammatory responses in various clinical situations, including acute lung injury and organ transplantation (as emphasised by those authors), and also in others (ie, during cardiopulmonary bypass).[85] Changes in granulocyte and monocyte function induced by pressure exposure have also been reported in the beluga, a deep-diving whale.[86] It was postulated that such alterations in immune function might decrease susceptibility to decompression sickness.

NITRIC OXIDE, HYDROGEN SULFIDE AND CARBON MONOXIDE

The diving physiology of marine mammals provides an opportunity to examine the functional roles and therapeutic potential of the gasotransmitters, nitric oxide, hydrogen sulfide and carbon monoxide.[87–89] These intracellular messengers have multiple physiological effects including vasodilatation. In that regard, it is notable that seals, which have common, spontaneous (non-obstructive) sleep apnoeas, do not develop pulmonary hypertension despite the routine hypoxia and hypercarbia that occur during these 6–12 min breath holds.[90]

Increased nitric oxide production in the blood in marine mammals has been postulated because of elevated blood nitrite levels in porpoises and the high Hb concentrations found in many marine mammals.[91] Nitrite is a potential substrate for nitric oxide formation by deoxyhaemoglobin due to deoxyhaemoglobin's nitrite reductase activity.[92–94] The presence of exhaled nitric oxide in porpoises supported this hypothesis.[95] However, nitric oxide was absent in exhaled air of Weddell seals.[96] Falke *et al* suggested that the absence of exhaled nitric oxide may be secondary to reduced nitric oxide production in the seal, although another possible cause was the lack of paranasal sinuses in seals as paranasal sinuses are considered to contribute significantly to nitric oxide levels in exhaled air. Further examination of the role of nitric oxide and nitrite in these animals is needed, and is particularly relevant to current investigations of nitrite therapy in hypoxaemic and ischaemic disease.[97]

Hydrogen sulfide may also play a role in the prevention of pulmonary hypertension in marine mammals. During in vitro exposure to hypoxia, the pulmonary arteries of sea lions exhibit hypoxic pulmonary vasodilation, not hypoxic pulmonary vasoconstriction.[98] Vasodilatation also occurs in response to addition of hydrogen sulfide to the tissue bath. In contrast, bovine pulmonary arteries constrict on exposure to either hypoxia or hydrogen sulfide. It has been postulated that hydrogen sulfide accumulates within smooth muscle cells on exposure to hypoxia in both species, but that the contractile response to this intracellular messenger has changed in the sea lion to that of vasodilatation rather than vasoconstriction as in terrestrial mammals.

Carboxyhaemoglobin levels as high as 10% in elephant seals and elevated blood carbon monoxide levels in Weddell seals raise the possibility of a role for carbon monoxide in vascular regulation and prevention of reperfusion injury in these deep-diving seals.[99 100] Carboxyhaemoglobin levels were as high as in smokers (figure 9). These high carbon monoxide levels have been attributed to generation of carbon monoxide in the metabolism of haem to bilirubin, and to the high Hb concentrations (25 g/dL), blood volumes (200–220 mL/kg body mass) and myoglobin concentrations (5.4–7.8 g 100/g muscle) in these seals.[101] Although paradoxical in that total blood oxygen stores would be decreased by the binding of carbon monoxide to Hb, the left shift (increased O_2 affinity) of the Hb dissociation curve induced by carbon monoxide may be beneficial and actually increase blood oxygen content at low arterial pO_2.

In this era of the design of carbon monoxide releasing molecules and the potential utilisation of carbon monoxide as a therapeutic agent in vascular regulation and prevention of apoptosis/inflammatory responses during reperfusion,[88 102–104] these seals are potentially excellent models for investigation of carbon monoxide's function. The high concentrations of carbon monoxide in seals may play a significant role in the diving physiology of the seal, especially in vascular regulation and hypoxaemic/ischaemic protection.

CONCLUSIONS

In conclusion, the exceptional breath-hold capacities and dive behaviours of many marine mammals and seabirds are dependent on anatomy and physiology. Investigation of these adaptations is relevant to the understanding of their ecology and their responses to environmental challenges. Such research could benefit from collaboration of field biologists, comparative physiologists and medical investigators. Furthermore, the hypoxaemic

and pressure tolerance of these animals and the avoidance of reperfusion injury provide the opportunity to illustrate basic physiological principles and explore potential therapeutic applications.

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