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Review

The diving paradox: new insights into the role of the dive response in air-breathing vertebrates

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Abstract

When aquatic reptiles, birds and mammals submerge, they typically exhibit a dive response in which breathing ceases, heart rate slows, and blood flow to peripheral tissues is reduced. The profound dive response that occurs during forced submergence sequesters blood oxygen for the brain and heart while allowing peripheral tissues to become anaerobic, thus protecting the animal from immediate asphyxiation. However, the decrease in peripheral blood flow is in direct conflict with the exercise response necessary for supporting muscle metabolism during submerged swimming. In free diving animals, a dive response still occurs, but it is less intense than during forced submergence, and whole-body metabolism remains aerobic. If blood oxygen is not sequestered for brain and heart metabolism during normal diving, then what is the purpose of the dive response? Here, we show that its primary role may be to regulate the degree of hypoxia in skeletal muscle so that blood and muscle oxygen stores can be efficiently used. Paradoxically, the muscles of diving vertebrates must become hypoxic to maximize aerobic dive duration. At the same time, morphological and enzymatic adaptations enhance intracellular oxygen diffusion at low partial pressures of oxygen. Optimizing the use of blood and muscle oxygen stores allows aquatic, air-breathing vertebrates to exercise for prolonged periods while holding their breath.

Keywords: Dive response; Air-breathing vertebrates; Aerobic metabolism; Weddell seal; Hypoxia; Mitochondrial volume density; Myoglobin; Model

Contents

1. Introduction

A paradox that has confounded physiologists for the past 60 years is the balance between diving and exercise responses in air-breathing vertebrates. When aquatic reptiles, birds and mammals submerge, they typically exhibit a dive response involving apnea (cessation of breathing), bradycardia (reduction in heart rate), and peripheral vasoconstriction (Andersen, 1966; Blix and Folkow, 1983; Butler and Jones, 1997). Under these conditions, the animals must rely on oxygen stored in the blood, muscles and lungs to support aerobic metabolism or change to anaerobic metabolic pathways. Beginning in the late 19th century, the dive response, historically called a "reflex",

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has been studied by forcibly submerging captive animals (Irving, 1934; Scholander, 1940; Elsner, 1965). Under these artificial conditions, bradycardia decreases resting cardiac output by 90%, which causes a profound reflex peripheral vasoconstriction to maintain central arterial blood pressure (Irving et al., 1942). The resulting decrease in convective oxygen transport conserves blood oxygen for the brain and heart, two organs vital for life (Zapol et al., 1979). Energy metabolism in peripheral organs and tissues either decreases and/or switches to anaerobic glycolysis resulting in the formation of lactic acid. The profound dive response that occurs during forced submergence protects the animal against immediate asphyxiation. However, normal organ function and physiological homeostasis are severely disrupted.

In contrast to forced submergence, most voluntary dives are probably within the animal's aerobic dive limit (ADL) (Kooyman et al., 1980; Butler and Jones, 1997) and are usually associated with submerged swimming (Davis et al., 1999; Williams et al., 2000). A dive response still occurs during voluntary dives, but it is more variable and less intense (Kooyman and Campbell, 1972; Hill et al., 1987; Fedak et al., 1988). Most organs and tissues receive sufficient oxygen from the circulation or endogenous oxy-myoglobin to maintain aerobic energy production (Kooyman et al., 1980). However, the details of blood flow regulation and tissue oxygen consumption during voluntary dives are poorly understood because of the difficulty of making in vivo measurements in free-ranging marine mammals, birds and reptiles (Bevan and Butler, 1992; Kooyman et al., 1999). Measurements of blood chemistry (Castellini et al., 1988), hepatic perfusion and renal glomerular filtration rate (Davis et al., 1983), muscle temperature (Ponganis et al., 1993) and oxy-myoglobin desaturation (Guyton et al., 1995) indicate that some degree of peripheral blood flow is maintained. Organs such as the liver, kidneys and gastrointestinal tract continue to function (Davis et al., 1983), and physiological homeostasis is maintained during aerobic dives. However, little is known about the balance between convective oxygen transport and the oxygen requirements of active skeletal muscle during voluntary dives.

These observations led us to ask the question: If blood oxygen is not sequestered for brain and heart metabolism, then what is the purpose of the dive response during aerobic dives? Why reduce tissue perfusion at all, especially during submerged swimming when muscle metabolism is elevated? To answer these questions, we used a two-fold approach. The first was to model convective oxygen transport and tissue oxygen consumption at different levels of muscular exertion during aerobic dives. The second involved histological and enzymatic studies of muscle and organs. Together, they provide new insights into the role of the dive response in air-breathing vertebrates while exercising submerged.

2. Modeling the aerobic dive response

Unlike most species of diving vertebrates, we now have enough information on Weddell seal (*Leptonychotes weddellii*) morphology, circulation, haematology and exercise metabolism to develop realistic physiological models when in vivo measurements are not possible. We used modelling to ask the following question: Does the dive response play a role in coordinating the use of blood and muscle oxygen stores during aerobic dives, and how does muscular exertion affect this role?

A numerical integration technique was used to model the relationship between regional blood flow and tissue oxygen consumption in a hypothetical Weddell seal during aerobic dives (for details, see Davis and Kanatous, 1999). The numeric process iteratively determined arterial and venous oxygen content for the six largest regional circulations (i.e., cerebral, coronary, skeletal muscle, splanchnic, renal and cutaneous) based on Fick's principle described by the equation:

$$\dot{V}_{O_2} = \dot{Q}(Ca_{O_2} - Cv_{O_2})$$

in which $\dot{V}_{O_2}$ is oxygen consumption (ml O_2/min), $\dot{Q}$ is blood flow rate (l/min), Ca_{O_2} is the arterial blood oxygen concentration (ml O_2/l blood), and Cv_{O_2} is the venous blood oxygen concentration (ml O_2/l blood) (Schmidt-Nielsen, 1975).

The model was run for 7 levels of cardiac output (19%, 37%, 56%, 75%, 94%, 112% and 131% of the resting, predive level) and 16 levels (1–16 times resting) of muscle oxygen consumption. The oxygen consumption of organs and tissues other than skeletal muscle was kept at resting, predive levels. Except for the brain, where circulation was always maintained at a resting level, we assumed that blood flow to the rest of the body decreased proportionately with cardiac output during a dive (Zapol et al., 1979). The aerobic dive limit was reached and the dive terminated when any organ or tissue received insufficient oxygen through convective oxygen transport and oxy-myoglobin (skeletal muscle only) to maintain aerobic metabolism or when the arterial partial pressure of oxygen dropped below 2.93 kPa (22 mm Hg) (Qvist et al., 1986).

Two important conclusions arise from this model. First, a dive response is needed to efficiently use blood and muscle oxygen stores and maximize the aerobic dive limit at low levels (less than ca. 3.5 times resting metabolism) of exertion. Second, the intensity of the dive response should vary inversely with the level of muscle metabolism. The first conclusion has a simple explanation. It results from the presence of two types of molecules that store oxygen in the body that have very different oxygen affinities. Haemoglobin has a P_{50} (i.e., oxygen partial pressure at 50% saturation) of about 27 mm Hg, while myoglobin has a P_{50} of 3 mm Hg. For myoglobin-bound oxygen to become available for aerobic metabolism, the

intracellular partial pressure of oxygen in the muscle must be less than 10 mm Hg; in other words, it must become very hypoxic. We know from studies of Weddell seals making voluntary dives that desaturation of myoglobin begins as soon as the seal submerges (Guyton et al., 1995). At the beginning of a dive when the partial pressure of oxygen in arterial blood is around 100 mm Hg, the best way to make the muscle hypoxic is by ischemia. This is where the dive response plays a major role. Peripheral vasoconstriction reduces convective oxygen transport to the muscle resulting in tissue hypoxia. The partial pressure of oxygen in the muscle becomes low enough for oxygen to dissociate from myoglobin and diffuse into mitochondria. As the dive progresses, hypoxic hypoxia (i.e., a reduction in arterial oxygen partial pressure) also contributes to the level of muscle hypoxia and myoglobin desaturation.

The explanation for the second observation results from the balance between oxygen availability and use within the muscle. If we plot the heart rate (as a percentage of the resting, predive level) that gives the maximum ADL as a function of muscle oxygen consumption and whole body metabolism, the results show that a dive response is necessary to maximize the ADL at low levels of exertion, but that it should be less pronounced as the level of muscular exertion increases (Fig. 1). At routine levels of exertion (whole body oxygen consumption = 4.1 ml O_2 min^{-1} kg^{-1}; (Kooyman et al., 1971; Castellini et al., 1992)) for a Weddell seal, the maximum ADL occurs when heart rate is 45% of the resting, predive level. If the seal were resting while submerged, the maximum ADL would occur with a heart rate of 24% of resting levels. Conversely, if metabolism were greater than 7.5 ml O_2 min^{-1} kg^{-1} (ca. 3.5 times resting metabolism), then the maximum ADL occurs with a heart rate that exceeds resting levels; that is, an exercise response instead of a dive response. Why is this the case?

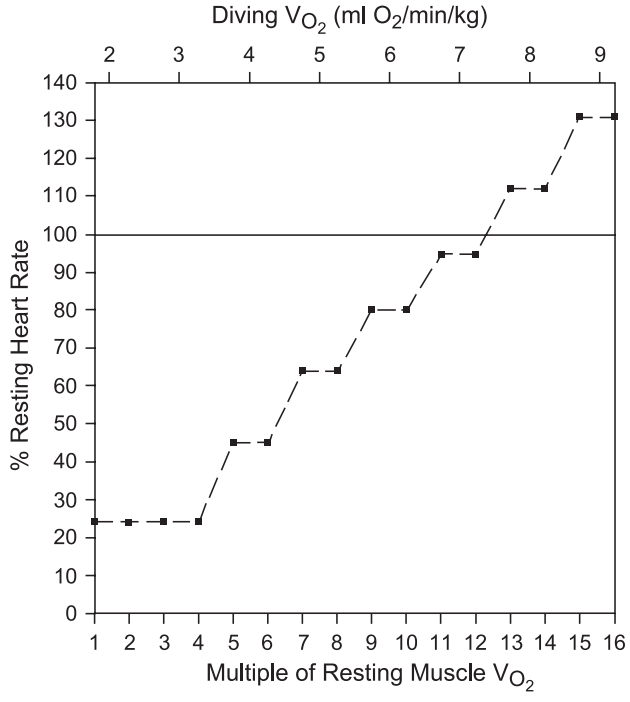

Fig. 1. Optimum heart rate expressed as a percentage of the resting, predive level (52 beats min^{-1}) as a function of skeletal muscle oxygen consumption and whole-body oxygen consumption rate. Optimum refers to the values that give the maximum aerobic dive limit (ADL). The horizontal line is the resting, predive heart rate.

The role of the dive response in maximizing the ADL can be explained by the balance between convective oxygen transport to the muscle ($\dot{Q}_{O_2}$) and muscle oxygen consumption ($\dot{V}_{O_2}$). On average, the optimum muscle $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio at the beginning of a dive is 0.88 for muscle oxygen consumption, less than 10-times resting. At higher levels (10–16 times resting) of muscle metabolism, the optimum $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio is 1.05. In either case, if we assume that the maximum extraction coefficient of blood oxygen is 80% (i.e., maximum extraction coefficient at critical oxygen delivery), then convective oxygen delivery is less than the muscle's aerobic requirement (Lautt and Graham, 1977; Cain, 1978; Cole, 1983; King et al., 1987; Nelson et al., 1988; Samsel and Schumacker, 1994; Torrance and Wittnich, 1994; Noldge-Schomburg et al., 1996). For a $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio that is much less than 0.88 or 1.05, convective oxygen transport to the muscles is too low. As a result, muscle oxy-myoglobin stores are depleted before blood oxygen, and the ADL decreases for all levels of muscle oxygen consumption. For a $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio greater than 0.88 or 1.05, convective oxygen transport to the muscles is too high, and muscle myoglobin is not desaturated because of its higher affinity for oxygen than haemoglobin. In this case, blood oxygen is depleted before muscle oxy-myoglobin stores, and again the ADL decreases. In both cases, an inappropriate $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio results in a decrease in the ADL by as much as 50% because the blood and muscle oxygen stores are not completely used. To maximize the use of body oxygen stores and the ADL for each level of muscle metabolism, the full utilization of available blood (arterial oxygen saturation not less than ca. 38%) (Qvist et al., 1986; Davis and Kanatous, 1999) and muscle oxygen stores must be completed at the same time. This is achieved by adjusting cardiac output according to the level of muscular exertion. As muscle oxygen consumption increases, cardiac output and muscle perfusion must also increase (i.e., the dive response is less pronounced) so that the $\dot{Q}_{O_2}/\dot{V}_{O_2}$ ratio remains at about 0.88 to 1.05 at the beginning of a dive. As the dive progresses, the ratio declines to about 0.3 as arterial oxygen content decreases. However, even toward the end of the dive, the muscle continues to obtain some of its oxygen from the blood. On average, the muscle derives about 70% of its oxygen from the blood and 30% from oxy-myoglobin at the beginning of a dive. By the end of the dive, these values are reversed. Overall, about half of the total oxygen consumed by the muscle

comes from oxy-myoglobin and the other half from blood oxygen. What happens to the metabolism of the other organs? They have adequate convective oxygen delivery to maintain a predive level of aerobic metabolism and do not become oxygen limited unless cardiac output falls below 19% of the resting level. In fact, the extraction coefficient of oxygen from the blood never rises above 30% for the brain, 50% for the heart, and 60% for the splanchnic organs and kidneys, indicating sufficient convective oxygen delivery to support aerobic metabolism (Davis and Kanatous, 1999).

The need to match an increase in muscle $\dot{V}_{O_2}$ with an increase in $\dot{Q}_{O_2}$ explains why long duration divers such as the Weddell seal and elephant seal (*Mirounga angustirostris*), which maintain a low level of metabolism by using cost-efficient modes of locomotion (Williams et al., 2000; Davis et al., 2001), have a more pronounced dive response than short duration divers such as the Tufted Duck (*Aythya fuligula*), which are very energetic and exhibit an exercise response (i.e., an increase in heart rate and tissue perfusion) during voluntary dives (Butler, 1988). These examples show the influence that muscular exertion has on the optimal level of bradycardia during voluntary dives. Another important factor is the concentration of myoglobin in the muscle. Only those species with a large muscle oxygen store will benefit from a dive response. Those species without an elevated concentration of myoglobin will need to increase muscle perfusion similar to exercising terrestrial animals if their muscles are to remain aerobic. Hence, most terrestrial vertebrates, which have low concentrations of muscle myoglobin, would not be able to maintain aerobic muscle metabolism if blood flow were reduced to active muscles.

3. Tissue adaptations to hypoxia

The model shows that the muscle must be made hypoxic to maximize the ADL. Due to the low P_{50} of myoglobin, the partial pressure gradient for oxygen diffusion within the muscle is very low. As a result, the rate of intracellular oxygen diffusion, as described by Fick's equation for diffusion (Schmidt-Nielsen, 1975), is low. Although the high myoglobin concentration facilitates oxygen storage and diffusion, our histological and enzymatic studies have revealed other possible adaptations. The skeletal muscles (*M. longissimus dorsi* and *M. pectoralis*) of both Weddell seals and harbor seals (*Phoca vitulina*) are composed of roughly equal percentages of Type I slow twitch, oxidative fibers and Type IIa fast twitch, oxidative fibers that are homogeneously distributed throughout the swimming muscles (Watson et al., 2003). It is noteworthy that there are no Type IIb fast twitch, glycolytic fibers. As a result, the muscles of these seals are poised for aerobic metabolism. In addition, capillary density around the muscle fibers is only 60% of that measured in the skeletal muscles of terrestrial mammals with similar volume densities of mitochondria (Fig. 2) (Kanatous et al., 2001, 2002). A muscle poised for aerobic metabolism with reduced capillarity would be difficult to reconcile in a terrestrial mammal or bird (Conley et al., 1987), but it is consistent with the need to reduce convective oxygen transport to seal muscle described pre-

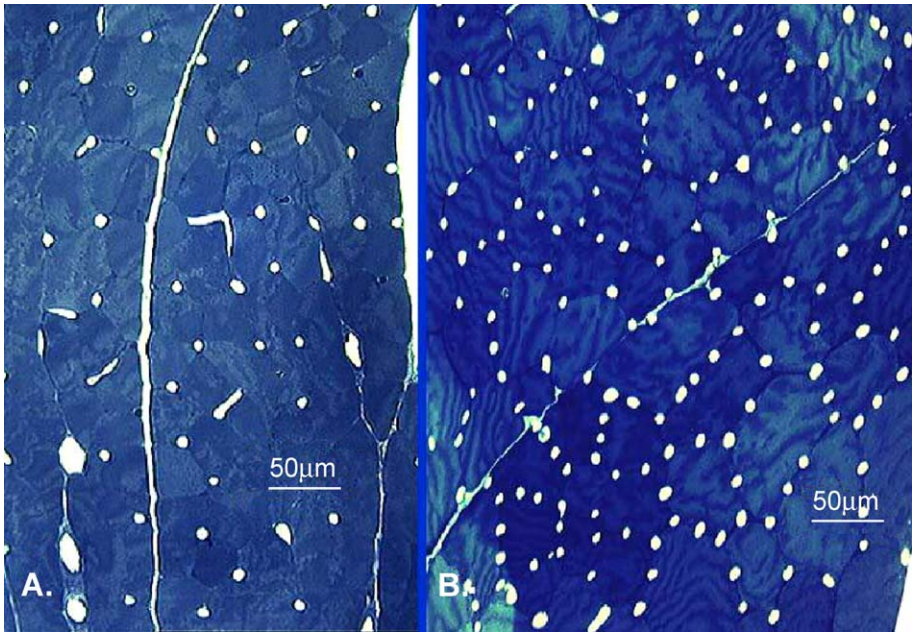

Fig. 2. Light micrographs comparing the capillary density in cross-sections of skeletal muscles with similar volume densities of mitochondria between the (A) harbor seal (*Phoca vitulina*) (*M. longissimus dorsi*) and (B) dog (*Canis familiaris*) (*M. gastrocnemius*). Despite similar volume densities of mitochondria, the seal muscle has 60% fewer capillaries.

viously. It is also consistent with an increased oxygen storage capacity in the blood (due to higher concentration of red blood cells) at the expense of optimizing convective oxygen transport due to a higher blood viscosity (Hedrick and Duffield, 1991; Elsner and Meiselman, 1995).

Mitochondrial volume density and the activity of citrate synthase, an enzyme in the Kreb's cycle, are elevated in harbor seals, especially in swimming muscles, as compared to sedentary terrestrial mammals (Kanatous et al., 1999; Polasek et al., in review). While the total volume density of mitochondria in the skeletal muscles of Weddell seals does not differ from that of sedentary terrestrial mammals, the distribution of the mitochondria throughout the muscles does. As shown previously in harbor seals, the majority of mitochondria are interfibrillar with less than 5% of total mitochondrial volume density being subsarcolemmal. In contrast, subsarcolemmal mitochondria usually account for ca. 10% of the total mitochondrial volume in sedentary terrestrial mammals and in excess of 30% in athletic species (Kayer et al., 1989). In other words, the mitochondria in the skeletal muscles of diving mammals are distributed uniformly throughout the muscle fiber and not concentrated near the sarcolemma and close to capillaries. We hypothesize that the increase in mitochondrial volume density and homogeneous distribution reduces the intracellular distance between mitochondria and oxy-myoglobin, an important source of oxygen which is distributed throughout the cell (Wittenberg and Wittenberg, 2003). This, in combination with myoglobin concentrations that are 20-times greater than in terrestrial mammals (Kanatous et al., 1999), increases the rate of oxygen diffusion into mitochondria and prevents diffusion limitation at low partial pressures, a condition that is required for the dissociation of oxy-myoglobin. Finally, the activity of β-hydroxyacyl CoA dehydrogenase, an enzyme in fatty acid oxidation, in seal muscle is 4–5 times greater than in the dog and rat (Kanatous et al., 1999; Polasek et al., in review), indicating a heavy reliance on lipid for aerobic energy metabolism during most dives (Davis et al., 1991; Davis et al., 1993). In addition to the skeletal muscles, organs such as the liver, kidneys and stomach from the harbor seal show similar increases in mitochondrial volume density and citrate synthase and β-hydroxyacyl CoA dehydrogenase activities as compared to terrestrial mammals (Fuson et al., 2003).

In summary, myoglobin has a much higher affinity for oxygen than haemoglobin, and the muscle must be made hypoxic at the beginning of a dive to access this important store of oxygen and maximize the ADL relative to the level of muscular exertion. This may be the primary role of the dive response during most voluntary dives. We speculate that the evolution of high myoglobin concentrations in the muscles of air-breathing, diving vertebrates as a means of extending the ADL was only possible because the dive response already existed to protect the animal against asphyxia. Now its role may be more subtle in balancing hypoxia and aerobic metabolism in the muscle so that oxy-myoglobin stores can be used to support exercise while the animal is in a breath-hold. Paradoxically, the muscles of diving, air-breathing vertebrates must become hypoxic to maximize the duration of aerobic exercise while submerged. To maintain aerobic metabolism at low partial pressures of oxygen, morphological and enzymatic adaptations have occurred in skeletal muscles and other organs to enhance the intracellular diffusion of oxygen. As a result, aquatically adapted, air-breathing vertebrates are able to exercise for prolonged periods underwater in comparison to their terrestrial counterparts.

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