Unsteady-state gas exchange and storage in diving marine mammals: the harbor porpoise and gray seal

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Boutilier, R. G., J. Z. Reed, and M. A. Fedak. Unsteady-state gas exchange and storage in diving marine mammals: the harbor porpoise and gray seal. Am J Physiol Regulatory Integrative Comp Physiol 281: R490–R494, 2001.—Breath-by-breath measurements of end-tidal O₂ and CO₂ concentrations in harbor porpoise reveal that the respiratory gas exchange ratio (Rₐ); CO₂ output/O₂ uptake) of the first lung ventilation in a breathing bout after a prolonged breath-hold is always well below the animal’s metabolic respiratory quotient (RQ) of 0.85. Thus the longest apneic pauses are always followed by an initial breath having a very low Rₐ (0.6–0.7), which thereafter increases with each subsequent breath to values in excess of 1.2. Although the O₂ stores of the body are fully readjusted after the first three to four breaths following a prolonged apneic pause, a further three to four ventilations are always needed, not to load more O₂ but to eliminate built-up levels of CO₂. The slower readjustment of CO₂ stores relates to their greater magnitude and to the fact that they must be mobilized from comparatively large and chemically complex HCO₃/CO₂ stores that are built up in the blood and tissues during the breath-hold. These data, and similar measurements on gray seals (12), indicate that it is the readjustment of metabolic RQ and not O₂ stores per se that governs the amount of time an animal must spend ventilating at the surface after a dive.

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CETACEANS ARE EXCLUSIVELY aquatic mammals that range in size from the smallest porpoises (70–100 kg) to the largest animal on the planet, the 150-ton blue whale. They spend the vast majority of their life underwater and surface only periodically to ventilate their lungs with a single breath. The largest whales, with their unusually large blood volumes and high Hb and myoglobin concentrations, can take aboard enough oxygen at the surface to sustain their low mass-specific metabolic rates for dives lasting >1 h (6, 7, 14). Because harbor porpoises represent the lower extreme of cetacean body size, they are of intrinsic interest to respiratory physiologists because they are presumed to have the highest mass-specific metabolic rates and to enjoy the most active lifestyle of all whales.

We had a unique opportunity to study the respiratory physiology of captive harbor porpoises in a strandings rehabilitation facility for marine mammals in Harderwijk, Netherlands. Our experiments on juvenile harbor porpoises confirm that they have the highest oxygen consumption per kilogram body weight and the highest heart rates of all cetaceans studied thus far (13). Whereas their diving O₂ stores in blood, lung, and muscle are slightly greater than one would predict for a terrestrial mammal of comparable size, their high mass-specific metabolic rate dictates a lifestyle that restricts aerobic underwater activities to 3–4 min [their so-called aerobic dive limit (ADL)] (10, 13) before having to surface to breath. This offers them little scope to achieve great diving depths and almost certainly limits their foraging activities to shallower environments than the larger whales. Indeed, recent field studies of free-ranging juvenile harbor porpoises instrumented with time-depth recorders bear out our measurements of a 3- to 4-min ADL (13) as maximum depths and dive durations were on the order of 150 m and 3.5–4.7 min (8, 15). After such dives, harbor porpoises normally visit the surface for up to 6 “rolls” spaced ~10 s apart (15). Each roll coincides with a lung ventilation, or blow, lasting <1 s (13) during which time all gas exchange must occur. Our survey of cetacean ventilatory dynamics (13) indicates that rapid, high-flow velocity breathing is a characteristic feature of all whales, enabling them to exchange large percentages of their total lung gas during the brief periods when they “porpoise” through the air-water interface.

Current concepts of diving in marine mammals focus on oxygen as a “resource” that must be periodically recharged at the expense of loss of time underwater. As such, the time taken to “gather” the resource at the surface is viewed as an inescapable “cost” of the diving habit. We present data showing that the O₂ store of the porpoise is fully readjusted after the first three to four breaths after a prolonged apneic pause. However, a further two to three ventilations are needed, not to load more O₂ but to eliminate built-up levels of CO₂. Although the slower readjustment of CO₂ stores is a predictable feature of unsteady-state gas exchange, its
potential importance as the proximate signal that brings the surface period to an end has been largely ignored.

MATERIALS AND METHODS

Two juvenile harbor porpoises (28.3 and 27.8 kg) were trained in a small tank to breathe freely into a facemask that was positioned over the blowhole each time the animal surfaced to ventilate its lungs. Over a 3-wk training period, the animals were lifted each day from the holding tank (an oval pool 8 × 6-m diameter, 1.5-m deep) to the experimental tank (2 × 0.8 × 0.8-m). During each experimental session, the animal was allowed to breathe freely through a Hall's face-mask (VetDrug, Bury St. Edmunds, UK), and breath-by-breath O2 ([O2]) and CO2 ([CO2]) concentrations were recorded. The facemask was a wide rubber funnel with flexible rims (so that it fit easily over the blowhole without allowing any air to escape), and both the mask and the flow meter had minimal resistance to the porpoise’s respiratory flows, being essentially open tubes. The inspiratory and expiratory flows were measured using an ultrasonic low-resistance flow meter (BRDL, Birmingham Univ.; 20-l/s version; see Ref. 12) mounted on the top of the mask so that the porpoise could breathe freely through the flow meter. Respiratory gases were measured by drawing a continuous subsample of the animal’s expiratory and inspiratory flows at a constant flow rate (600 ml/min) through capillary tubing from the center of the respiratory gas flow, passing through a Ministart drying filter (deadspace 0.1 ml, Sartorius) to Servomex gas analyzers (model 1505 miniature infrared CO2 and 728 zirconia O2; Servomex). O2 and CO2 measures were output as percent end-tidal (minimum) O2 and percent end-tidal (maximum) CO2 and converted to partial pressures (assuming partial pressure of water in air at 37°C is 6.26 kPa). The response time of the oxygen analyzer (installed in the system) was ~500–600 ms, whereas that for CO2 was ~300–400 ms. The maximum possible sample gas flow rates were used to minimize the delay of the system, and the gas analyzers were positioned on a wheeled platform next to the experimental container to minimize the delay between sampling and analysis. The O2 and CO2 gas sensors were calibrated using precision gas mixtures (pure N2, 12% O2 and 4% CO2 in N2; 10% CO2 in N2; supplied and certified to 0.01% by Hoekloos Gases, Amsterdam, Netherlands). Data were sampled and stored by a 386 Dell PC with “ANALYSE” software specifically developed for this work. For further details of the respirometry system, instrument response times, calibration, and system operation, see Reed et al. (12).

RESULTS AND DISCUSSION

Breathing episodes after the longest breath-holds in the present study were normally grouped into distinct bouts of six to eight lung ventilations, with short inter-breath intervals (Fig. 1). Breath-by-breath measurements of end-tidal [O2] and [CO2] in porpoise revealed that the respiratory gas exchange ratio (Rr; the ratio of CO2 output and O2 uptake) of the first lung ventilation in a breathing bout after a prolonged breath-hold was always well below the animal’s time-averaged metabolic respiratory quotient (Rm) of 0.85 (13). Thus the longest apneic pauses were always followed by an initial breath having a very low Rr (0.6–0.7), which thereafter increased with each subsequent breath to values in excess of 1.2 (Fig. 2). It is evident from these data that over the course of the breathing bout, the CO2 stores readjust relatively slowly compared with oxygen.

The effects of differential rates of store adjustment on the Rr can be conveniently illustrated on the O2...
CO2 diagram (5). The gas exchange ratio of the stores is indicated by a fan of lines radiating from the inspired gas composition (Fig. 3). Although diving physiologists have normally assumed the postdive ventilation of marine mammals to be concerned primarily with the recharging of O2 stores, our data for harbor porpoises show that when the O2 stores have attained near-equilibrium values (Hb-O2 saturation, >95%), the CO2 stores are still readjusting (Figs. 1 and 3). Indeed, this is to be expected if one considers that the majority of the CO2 eliminated during breathing must be mobilized from large and chemically complex HCO3/CO2 stores contained primarily within tissues and blood. By comparison, O2 store adjustments proceed more quickly, because they are largely confined to exchanges between the blood and lungs. Although comparatively high myoglobin concentrations can be found in the muscle of diving marine mammals (6), the CO2 capacitance (β-CO2 = Δ[CO2]/ΔPCO2) for body tissues is much higher than the corresponding O2 capacitance (β-O2 = Δ[O2]/ΔPO2), because β-CO2 is determined by both the physical solubility (α-CO2), which is ~25 times higher than for O2, and the chemical binding of CO2 as bicarbonate; i.e., β-CO2 = α-CO2 + (Δ[HCO3]/ΔPCO2), where changes in bicarbonate concentration are stoichiometric with the amount of proton buffering (9). Thus, whereas the major tissue O2 stores are confined to muscle, any tissues with chemical groups that act as H+ acceptors can act as potential CO2 stores. The high capacitance for CO2 compared with O2 means that for a given change in the stores (e.g., if RM = 1), the corresponding change in PCO2 will be much smaller than that for PO2. This is why increases of arterial blood PCO2 of only 10 mmHg are seen during 20-min voluntary dives of Weddell seals, when corresponding oscillations in arterial PO2 reach upwards of 60 mmHg (11), and why lung RR < RM in the initial stages of the postapneic breathing bout (Figs. 1, 2, and 4).

For comparative purposes, mean lung R values during postapneic breathing bouts were computed from breath-by-breath measurements of end-tidal PO2 and PCO2 in the porpoise (see Fig. 1) and the gray seal (12). When seals surface to breathe after a dive, they normally remain at the surface with their nares exposed to air for a series of lung ventilations. Our analysis shows that the RR values in the early stages of a postdive breathing bout are much lower than in the later stages of the bout, when RR values increase in both animals to levels far in excess of the metabolic RQ (Figs. 1 and 4). The only significant difference between the postapneic development of an increasing RR in harbor porpoises and gray seals is that the gas composition of the first few breaths after a dive in the seal is thought to reflect that of a largely underperfused lung during the breathhold (12). As the circulation to the periphery of the seal is reestablished, the peak end-tidal PCO2 values increase far less than the corresponding fall in PO2, leading to an overall decrease in RR and to the curvilinear relationship for RR shown in Fig. 4. The fact that we do not see such curvilinear relationships in the

Fig. 3. Moment-to-moment changes in alveolar partial pressures of O2 (Pao2) and CO2 (Paco2) during several breathing bouts after prolonged apneic pauses of 22–44 s in 2 harbor porpoises. RR is shown by the fan of lines that radiates from the point of interception of the PO2 and PCO2 of inspired gases (see Ref. 5).

Fig. 4. RR of 2 gray seals (A: 160-kg female; B: 185-kg male) during postdive breathing bouts. Each breath is expressed as a fractional proportion of the total time spent at the surface after a dive. Data for 5 postdive breathing bouts in each seal were computed from end-tidal PO2 and PCO2 measures found in Ref. 12.
harbor porpoise (Fig. 1) is further support of our contention that the lung is probably used as an O_2 store throughout the breath-hold and that peripheral vasoconstriction is only slow to develop (13).

One may well ask whether the time a marine mammal spends at the surface after a dive always exceeds the time taken to recharge the O_2 stores. For example, in Weddell seals diving voluntarily for up to 57 min, the mean time on surfacing to reach an expired PO_2 of 60 mmHg (90% Hb-O_2 saturation) was 0.56 min, whereas mean surface time was upwards of 3 min (10). In the case of the harbor porpoise, which surfaces for only one breath at a time, this means that after long dives, they may be constrained to a surface for only one breath at a time, this means that after long dives, they may be constrained to a period of porpoising behavior near the surface if they are to readjust fully their body O_2 and CO_2 stores. Given that it takes more time to liberate CO_2 than to uptake O_2, one questions whether such animals would ever forego full readjustment of their CO_2 stores. By rapidly charging themselves with O_2, they could cut the surface period short, still dive aerobically, “put up with” the added CO_2/pH burden, and then eventually offload the built-up CO_2 at some later date by spending even longer times at the surface. There could well be times when loading O_2 quickly might be advantageous if, for example, being at the surface posed a threat or getting underwater quickly (e.g., for feeding) temporarily outweighed the benefits of full readjustment of body gas stores.

The overriding emphasis in current models of marine mammal diving focuses on the effectiveness of O_2 store management during the dive and on the time taken to recharge the O_2 store at the surface. On the other hand, factors effecting the rate-dependent steps in CO_2 storage, transport, and removal have been largely ignored. Taken together, the data we present suggest that we may need to refocus our interpretation of postdive breathing behavior in marine mammals to acknowledge the possibility that it is the readjustment of the body CO_2 store, not the O_2 store per se, that governs the amount of time an animal must spend ventilating at the surface.

**Perspectives**

The extended breath-hold capacities of diving marine mammals raise the question of whether these animals possess any special mechanisms to accelerate CO_2 removal to facilitate greater matching between O_2 and CO_2 exchange rates. Some years ago, Boutilier et al. (1) showed that the nonbicarbonate buffering capacity (β-NB) of the separated plasma of the killer whale and gray seal was two- to fourfold higher than in terrestrial mammals. This suggested that the increased β-NB of separated plasma could facilitate enhanced CO_2 removal directly from the plasma (i.e., thereby avoiding rate-limiting red blood cell anion exchange) as long as there were sufficient amounts of the enzyme carbonic anhydrase in contact with the extracellular compartment. Although the presence of carbonic anhydrase in the pulmonary vasculature of the rat is known to enhance CO_2 excretion (3, 4), it is not considered nearly as important as the erythrocytic enzyme, owing to the relatively low β-NB of separated plasma. All else being the same, the greater β-NB of separated plasma in the diving forms (1) would not only enable greater carriage of blood total CO_2 from the site of production to the lung but, when there, could also facilitate enhanced extracellular formation of CO_2 by providing the protons needed to drive extracellular bicarbonate dehydration. Other buffering characteristics, such as the comparatively high buffering power seen in the muscle of marine mammals (2), indicate to us that prolonged voluntary dives (with large acid-base disturbances and large amounts of tissue CO_2 storage) will be important to focus on in future studies of unsteady-state gas exchange in these animals.

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**REFERENCES**


