Acid-Buffering Capacity and Myoglobin Content in Hooded and Harp Seals

To maintain adenosine triphosphate (ATP) production, muscles working under anaerobic conditions produce lactic acid ($C_3H_6O_3$), which has been linked to muscle fatigue and acidosis. Marine mammals often dive for extended periods of time under hypoxic conditions and have adapted to cope with the build up of lactic acid produced during occasional, very long dives. Hooded (*Cystophora christata*) and harp (*Pagophilus groenlandicus*) seals offer a unique comparison of muscle physiology because hooded seals prefer to forage using long, deep dives (avg. 450 m, 16 min) while harp seals prefer to forage using short, shallow dives (avg. 95 m, 5 min). Our aim was to compare the ability of hooded and harp seals to operate under hypoxic conditions through the comparison of acid buffering capabilities (β) and myoglobin (Mb) content in cardiac and skeletal muscle. For acid buffering, muscle tissue was homogenized in a saline solution and then titrated with NaOH while measuring changes in pH levels. Myoglobin content was determined spectrophotometrically from reduced muscle supernatant. In both species, there was a significant increase in the Mb content of skeletal and cardiac muscle between adults and neonates and an age-related increase in β in skeletal muscle. However, there was no significant difference in β in the cardiac tissue between adults and neonates in either species. This implies that while neonatal cardiac muscle may be able to withstand lactic acid build-up at birth, skeletal muscle buffering capacity and oxygen store capacity needs to develop the as animals age and may dictate juvenile diving and feeding behaviors.

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Introduction

Under aerobic conditions, muscles produce adenosine triphosphate (ATP) through the Kreb's cycle. However, under hypoxic and anaerobic conditions, muscles create ATP through glycolysis which creates 2-hydroxypropanoic acid, more commonly known as lactic acid.[1] Lactic acid causes muscle fatigue and acidosis,[2] which affects the length and level at which muscles may perform under conditions when oxygen supply is limited. Marine mammals often perform long dives during which muscle oxygen supply is reduced. However, their muscles have adapted to cope with these hypoxic conditions. Most dives taken by these animals are generally aerobic because the myoglobin, the main carrier of oxygen begins to run out, has a large capacity for oxygen.[1] However, when oxygen begins to run out, glycolysis occurs and lactic acid production increases. Therefore, during prolonged dives, the animals must have a way to compensate for high amounts of lactic acid that build up in the working skeletal muscles; if they are unable to, their muscles will undergo acidosis and cease to function properly. Therefore, animals that routinely produce lactic acid in their skeletal muscles typically have high buffering ability. In contrast, the cardiac muscle (heart) rarely goes without oxygen and is thus unaccustomed to coping with much lactic acid building up. However, it must be able to cope or else the animal will perish.

Hooded (*Cystophora cristata*) and harp (*Pagophilus groenlandicus*) seals offer a unique comparison of muscle physiology as hooded seals make longer, deeper dives than harp seals (avg 450 m, 16 min vs. 95 m, 5 min).[3,4] In addition, hooded and harp seals differ in their weaning process with hooded seal neonates being nursed for only four days while harp seal neonates nurse for twelve days.[5] This difference suggests that hooded seals may be born further developed and more ready to dive, compared to the harp seal neonates. Our aim was to compare the ability of neonate and adult hooded and harp seals to operate under hypoxic conditions through the comparison of acid-buffering capabilities and myoglobin content in cardiac and skeletal muscle. A significant difference in these parameters would imply that young animals need to develop the physiology necessary to withstand hypoxic environments which may dictate their diving and feeding behaviors.

Methods

Tissue samples (*longissimus dorsi* and cardiac muscle) from 3 adult and 3 neonatal harp and hooded seals were collected in the Gulf of St. Lawrence, Canada in March 2005 and stored at −80°C until analysis. Acid buffering capacity (β) was determined based on Castellini and Somero.[6] Briefly, tissue samples (~0.05–0.3 g) were homogenized (Fisher Scientific Sonic Dismembrator model 500) in 0.9M saline. The homogenate was heated to 37°C in a water bath and titrated from pH 6.0 to 7.0 with 5 or 25 µl aliquots of 0.2N NaOH. Buffering capacity was determined as the moles of NaOH added per gram tissue. Myoglobin content (Mb) was determined following Reynafarje.[7] Briefly, tissue samples (~20–

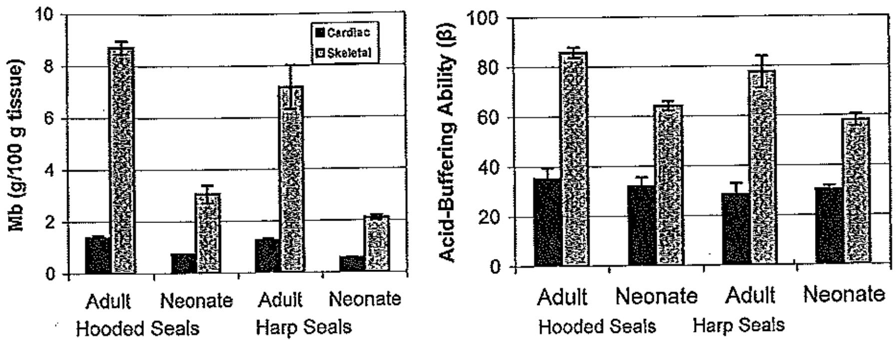

Fig 1. Myoglobin content and acid buffering capacity with standard deviations

30 mg) were homogenized in 2.5 mL of 0.4M phosphate buffer (pH=6.6), and the homogenate centrifuged (Fisher Scientific Marathon 21000R) at 21,150 g for 50 min at 4°C. The supernatant was bubbled with CO for 8 min, an excess of sodium dithionite was added and bubbled with CO for another 2 min. Myoglobin concentration was calculated from $\Delta A_{568-535}$ in a Beckman DU ® 530 spectrophotometer. All samples were run in triplicate and each run included a tissue control from harbor seal, and the Mb assays also included lyophilized horse Mb (Sigma-Aldrich) as a control. Statistical differences for age, muscle and species were determined using Mann-Whitney U tests. Significance was assumed at $P<.05$.

RESULTS

Myoglobin concentrations were significantly higher in both the cardiac and skeletal muscles of adults, as compared to neonates in both species (Figure 1). However, Mb was not different between species in either age class. Acid-buffering capacity was higher in adult skeletal muscle compared to neonates in both species, but was not significantly different in cardiac muscle due to age or species.

DISCUSSION

Because marine mammals rely on oxygen in their muscles to sustain aerobic metabolism while diving, development of muscle oxygen stores (ie, myoglobin) is critical for pups to be able to forage and dive in the same manner as adults. The significantly lower Mb in both harp and hooded seal pups compared to adults may limit the pups' ability to forage efficiently until they mature. In addition, when this oxygen store is depleted, skeletal muscle must buffer the lactic acid produced from anaerobic metabolism. The significantly lower β found in pups of both species compared to adults is further evidence that skeletal muscle is not fully developed at birth and must mature before pups can forage in the same way as the adults. Our finding of no significant difference between the two species in either age class was contrary to our expectation that hooded seals would have a much higher Mb and β than harp seals due to differing diving patterns. Perhaps diving behaviors are not very different in the Gulf of St. Lawrence.

Development of cardiac muscle function is also crucial, as the heart must work whether the animal is in a hypoxic environment or not, and it can not function well in the absence of oxygen. We were surprised to find that cardiac Mb followed the same trends as in skeletal muscle, as pups were born with a significantly lower Mb than adults. This implies that the cardiac tissue in pups must mature before it can carry the same amount of oxygen and sustain long dives like the adults. However, the lack of any statistical difference in β between harp and hooded seal neonates and adults implies that the heart's acid buffering capabilities are almost fully developed at birth. This is likely due to the need for the heart to function even if lactic acid builds up due to low oxygen availability. Like the skeletal muscle, there was no significant difference in Mb or β between hooded and harp seals in either age class. This is once again contrary to our expected results, but may reflect actual diving patterns. Results from this project would be complemented with a larger sample size and dive records of the animals tested.

ACKNOWLEDGMENTS

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