A study of the development of diving capabilities in neonate cetaceans. Is myoglobin a limiting factor ?

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Abstract

Myoglobin acts as an oxygen store within mammalian muscle cells, allowing cetaceans to tolerate extended periods of apnea. Our study focuses on neonates and aims to describe how stores of myoglobin build up as the young animals mature.

Samples have been obtained from the local marine mammal stranding network and include a fetus and three neonate odontocetes, along with one mysticete calf, that stranded in Hawaii. Tissue from an adult odontocete was also analyzed for standardization and comparative purposes.

Our early results indicate that levels of myoglobin differ significantly between adult and neonate cetaceans, and are indeed very low in neonates. This may explain their surface persistence during this period.

Introduction

Myoglobin, like hemoglobin, is an oxygen transporter protein. However, whereas hemoglobin is primarily in the blood, myoglobin is found predominantly within muscle cell. This provides cetaceans with an extra supply of oxygen, faciltating prolonged dives and tolerance of extreme apnea.

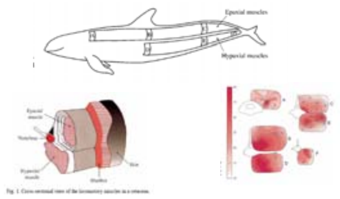
In contrast, neonate cetaceans persist at the surface, and do not make extended dives. Surface drag increases six fold in the top three meters of the water (Hertel 1966) so for calves this surface persistence will increase the energy they expend during swimming, at a time when they are wholly reliant on their mother's milk and therefore her energy reserves. Our study investigates the ontogeny of myoglobin levels in young cetaceans.

In our first investigation we compare adult levels of myoglobin to neonate levels.. Secondly we investigate neonate muscle more closely; in adult odontocetes the muscle myoglobin levels are significantly higher in the inner portions of the muscles (See figure 1). Finally we compare levels of myoglobin in the odontocete neonates to levels in the mysticete neonate, again testing the null hypothesis that again there would be no significant difference between the two groups.

Figure 1.

Heterogeneity of myoglobin in cetacean muscle tissue

From Polasek et al (2004)



Materials and methods

Tissue samples

Samples of locally stranded cetaceans were obtained from the Marine Mammal Stranding Network. We worked with the curator of vertebrates at the Santa Barbara Museum, Michelle Berman, and harvested the muscles during investigative necropsies of the neonates. The humpback whale calf samples were obtained from a calf that stranded on Maui, Hawaii earlier this year.

For the locally stranded animals, the whole carcass was collected, frozen and then thawed for the necropsy. For the humpback whale calf, the calf died on the beach, so the necropsy was performed there and samples were frozen and later flown over to us. All muscles samples were stored at -80°C prior to analysis

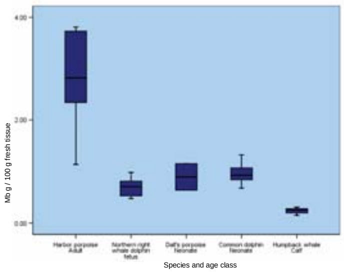
CSUCI students assist with cetacean necropsies

Experimental Procedure

Samples were taken from various different locations (see figure 1), in order to allow comparisons of inner and outer muscle and to compare levels of myoglobin in major swimming vs. non swimming muscles. The myoglobin levels were assessed following established protocols first described by Reynafarje (1963). Each sample was minced, sonicated and homogenized in a phosphate buffer solution, (pH 6.6) using 19.25mL of buffer solution per gram . The samples were then centrifuged for 75 minutes at 19,000g. After centrifugation, the supernatant was collected, and then bubbled with carbon monoxide for 8 minutes. This process ensures that the myoglobin is completely de-oxidized. We used a spectrometer to determine the difference of the absorbance at two key wavelengths – 536 nm and 568nm. This difference between these measurements can be used to estimate levels of myoglobin.

Variation in mean myoglobin levels

between cetacean species and age classes.



Species	Age class	Inner swimming muscle	Outer swimming muscle	Non swimming muscle
		Units: grams mb / 100 g fresh muscle tissue		
Harbor porpoise *Phocoena phocoena*	Adult	3.77 ± 0.05	2.50 ± 0.21	3.06 ± 0.31
Northern right whale *Lissodelphis borealis*	Fetus	0.55 ± 0.10	0.82 ± 0.07	0.80 ± 0.21
Common Dolphin *Delphinus delphis*	Neonate	1.13 ± 0.20	0.95 ± 0.02	0.72 ± 0.05
Harbor porpoise *Phocoena phocoena*	Neonate	1.27 ± 0.11	1.51 ± 0.05	TBA
Humpback whale *Megaptera novaeangliae*	Calf (<6 months)	0.28 ± 0.02	0.37 ± 0.05	0.27 ± 0.05

Myoglobin measured as grams per 100 grams fresh tissue

Results:

1. Neonate levels of myoglobin are significantly lower than in adults, as reported in other studies.

Differences between age classes were significant (Kruskal Wallis $X^2 = 40.977$; d.f. = 2; p<0.001) , a post hoc Dunnett's T3 test showed that the significant difference lay between the adults vs. the neonates and the fetus; differences between the neonates and the fetus samples were not significant. These results agree with previously published data.

2. Myoglobin levels in our adult samples showed variation between different muscle locations, and between layers, as reported in the literature. In neonates there was no evidence of heterogeneity between different layers or sites.

Variation between layers and sites in the adult was significant (Kruskal Wallis independent means test $X^2 = 14.720$; d.f.=3; p=0.002). Significant differences lay between the inner vs. the outer layers of the main swimming muscle, the *longissimus dorsi*. For the neonates variation was not significant between layers or sites (Kruskal Wallis independent samples test $X^2 = 0.940$; d.f. =2; p=0.625).

3. Levels of myoglobin in the humpback whale calf were significantly lower than in all odontocetes calves; these levels were closer to those recorded for the odontocete fetus.

These measurements are the first reported for humpback whale calves, they were extremely low, and this was clearly significant (two sample test, unequal variances; t= 11.967, d.f.=39, p<0.001). There was also no evidence of heterogeneity in samples taken either from different sample sites or from different layers (ANOVA F=1.569, d.f.=3,12; p=0.248).

Inferences

• Our results show that myoglobin levels in neonate cetaceans are low relative to adult cetaceans. This explains their need to stay at the surface despite the inherent energy costs.

• Myoglobin levels in baleen whale calves are lower than those seen in the odontocete neonates and fetus that we sampled. These low levels of myoglobin may explain why baleen whale mother and calf pairs persist in nursery regions, such as Mexico and Hawaii, despite the lack of nutritional resources. Higher levels of myoglobin, and the consequent increase in their breath holding capacity will aid them on migration. It will reduce the risk of predation and make swimming more efficient, as they will be able to leave the surface waters for longer periods.

• Next we plan to investigate the possible triggers that might control myoglobin production in neonate cetaceans.

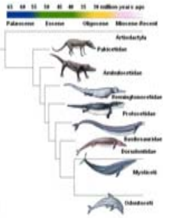
References:
Polasek, L. K and R. W. Davis. 2001. Heterogeneity of myoglobin distribution in the locomotory muscles of five cetacean species. The Journal of Experimental Biology 204, 209–215
Herttel, H. 1966. *Structure, Form and Movement.* New York: Reinhold Publishing Corporation.
Reynafarje, B. Simplified method for the determination of myoglobin. J Lab Clin Med 61: 138-145, 1963.

Fast facts

Evolution of modern cetaceans

Fossils have linked cetaceans to ungulates, which are hooved animals such as hippos, pigs, cattle, and sheep.

Modern cetaceans represent the end result of 60 million years of natural selection allowing them to thrive in the marine environment.



Dolphins vs. Porpoises

Images courtesy of Uko Gorter Natural History Illustrations

The key difference between dolphins and porpoises is the shape of the teeth ; dolphins have conical teeth . Dolphins are generally bigger and many have a pronounced snout or melon

Porpoises are smaller relative to dolphins, they have flattened, spade-shaped teeth and a much smaller snout. There are actually only 6 species of true porpoise

Odontocetes vs. Mysticetes

Odontocetes are essentially small, toothed whales. The largest is the sperm whale, the best known is the bottlenose – seen here. Toothed whales are renowned for their high intelligence and many live in long term, highly organized social groups.

Mysticetes are known as baleen whales, and have baleen plates that hang from the upper jaw and are made of keratin. The largest is the blue whale. They tend to be highly migratory and most associations are short term.

Humpback whale mother and calf pair, with accompanying bottlenose dolphin

Picture provided by: The keiki kohola Project

Taken under NMFS permit# 895-1450