

The molecular signals that regulate the ontogeny of aerobic capacity, lipid metabolism and elevated myoglobin concentrations in the skeletal muscles of Weddell Seals

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Project Summary

What are the molecular signals that regulate the changes in skeletal muscle physiology as a young Weddell seals develops into elite divers? We will address this broad question during a three-year study. Results from our previous support (a two year pilot study with a single field season), done in collaboration with the project entitled "Behavioral, Sensory and Physiological Challenges of a Sub-Ice Predator: The Role of Light in the Hunting Behavior and Foraging Efficiency of Weddell Seals" (NSF Award Number: OPP96-14857 and OPP-0125475) characterized the enzymatic, and ultra-structural adaptations for diving that occur in the skeletal muscles of newly weaned, juvenile and adult Weddell seals. In addition we began to define the molecular signals that regulate these ontogenetic changes in the skeletal muscles of the seals. The proposed study builds on these results to investigate the molecular controls that underlie the development of these adaptations. Our first objective is to further characterize the ontogenetic changes in muscle aerobic capacity, lipid metabolism and myoglobin concentration and distribution using enzymatic, immuno-histochemical and myoglobin assays in newly weaned, subadult, and adult seals. Our second objective is to determine the molecular controls that regulate these changes in aerobic capacity, fiber type distribution and myoglobin in skeletal muscles during maturation. Through subtractive hybridization and subsequent analysis, we will determine the differences in mRNA populations in the swimming muscles of the different age classes of Weddell seals. These techniques will allow us to identify the proteins and transcription factors that influence the ontogenetic changes in myoglobin concentration, fiber type distribution and aerobic capacity. These results will increase our understanding of both the ontogeny and molecular mechanisms by which young seals acquire the physiological capabilities to make deep (up to 700 m) and long aerobic dives (ca 20 min). This study will advance our knowledge of the molecular regulation for the adaptations that enable active skeletal muscle to function under hypoxic conditions, which has broader applications for human medicine especially in regards to cardiac and pulmonary disease. This project will support a Hispanic principal investigator, a female Asian-american post-doctoral fellow and two graduate students. This project also has a significant outreach component. In addition to interviews, e-mail exchanges with high school and middle school students, public seminars and presentations at national and international meetings, this project will continue to support our website in collaboration with the Science Teachers Access to Resources at Southwestern (STARS Program) (www.swmed.edu/stars/02antarcticexpedition/) in which we supply weekly

updates about our research efforts during the field season, answer weekly questions submitted by the students and teachers, and continue to supply periodic updates on our results throughout the year.

Summary of Results to date

We have completed the analysis of samples obtained during our pilot study in 2002

After an extremely successful field season in which we attained our sampling goals of biopsies from 5 adult males, 5 newborn and 6 juveniles (3 yearlings and 3 two-year olds), the data are revealing some very interesting results.

Our earlier studies found that the skeletal muscles of marine mammals appear to adapt to the hypoxic conditions of diving in different manners that reflect their diving behavior. Active swimmers such as harbor seals that make short duration dives have enhanced aerobic capacities similar to those of athletic terrestrial mammals to maintain high levels of aerobic

output. Conversely, deep divers such as Weddell seals have an aerobic capacity that is similar to sedentary terrestrial mammals of comparable

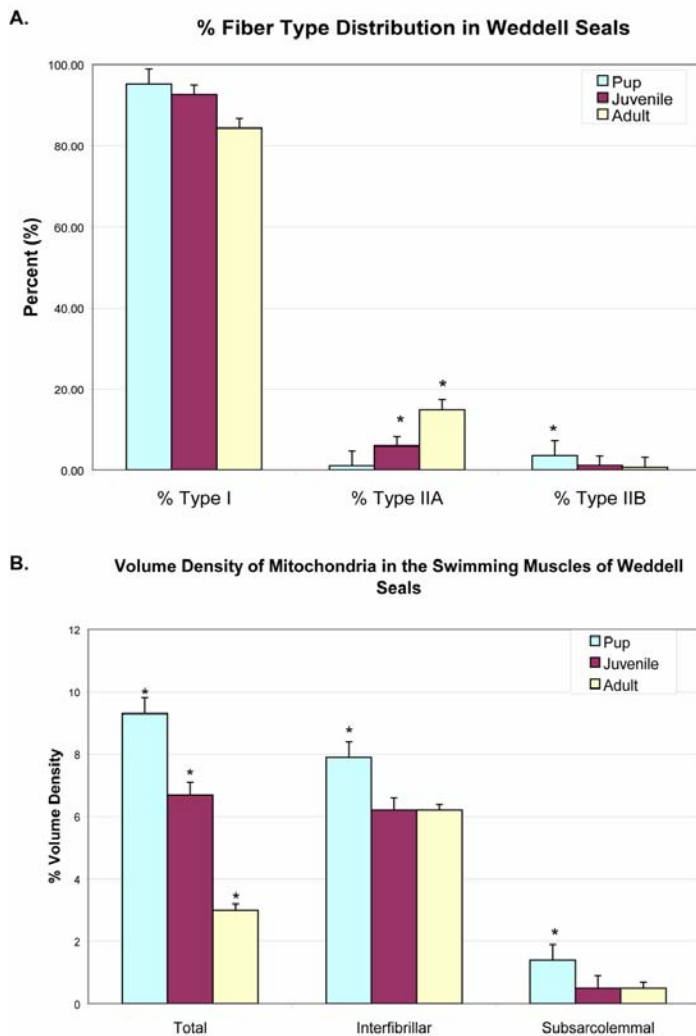


Figure 1. A. Histogram showing the change in fiber type composition, (% of total fiber number) in the swimming (longissimus dorsi) muscles of Weddell seals as they mature. B. Histogram showing the decrease in the volume density of mitochondria in the swimming muscle of Weddell seals as they mature. * denotes significantly different (p -value < 0.05).

size and reflects their low levels of aerobic metabolism. In addition, deep divers employ a number of energy conserving modes of locomotion to reduce the overall energetic costs of diving (Kanatous et al., 1999, Kanatous et al., 2002 and Williams et al. 2001).

Our previously funded study (NSF/OPP 0125475) built on these results to investigate the ontogeny of these adaptations and the genetic control of their development. Our first objective was to characterize the ontogenetic changes in aerobic capacity, lipid metabolism, fiber type, and myoglobin concentration in newborn, subadult, and adult seals. Our second objective was to determine the molecular controls for changes in the concentration and distribution of myoglobin in skeletal muscles during maturation.

The results for the adults corroborate those we have previously reported (Kanatous et al., 2002). The swimming muscles of adult Weddell seals are composed of a mixed fiber type population consisting primarily of slow oxidative fibers (Type I) with fast oxidative fibers (Type IIA) and a near complete absence of fast glycolytic fibers (Type IIB) (Figure 1A). The total volume density of mitochondria was also similar to previously reported values and comparable to

Relationship between the Volume Density of Mitochondria and Body Mass

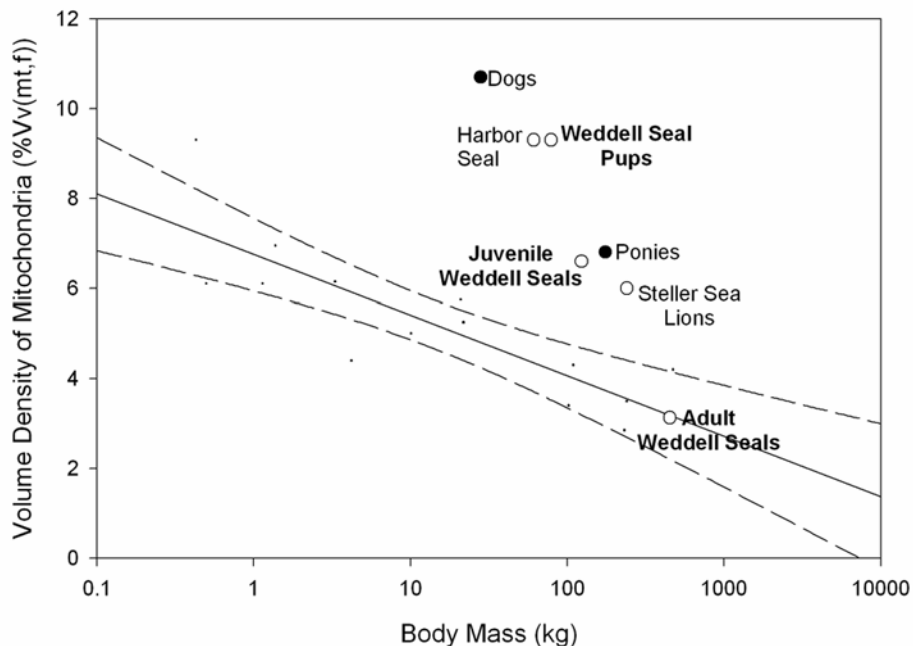


Figure 2. Plot of muscle mitochondrial volume density against body mass in athletic (solid circles) and sedentary terrestrial mammals (small dots) and pinnipeds (open symbols). The linear relationship ($y = 6.75 - 1.34 [\log x]$, $r^2 = 0.70$) was generated from the volume densities of the *vastus medialis*, a primary locomotory muscle, from various terrestrial mammals ranging in size from the dwarf mongoose to the steer (Kanatous et al., 1999; and terrestrial data from Hoppeler et al., 1987). In contrast to the adult Weddell seals; whose volume densities of mitochondria are similar to that predicted for a sedentary terrestrial mammal of comparable size, juveniles and pups have volume densities of mitochondria similar to athletic terrestrial mammals and shorter duration divers of similar size.

sedentary terrestrial mammals of equal body mass (Figure 2).

The fiber type results for all the age classes show a trend towards a decrease in type I fibers with a significant increase in type IIA fibers as the animals mature (Figure 1A). These results are further corroborated by the data

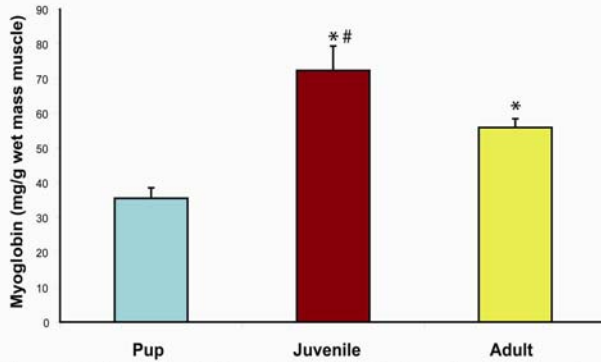


Figure 3. The concentration of myoglobin in the swimming muscles of Weddell seals. The concentration of myoglobin was significantly greater in the swimming muscles of the adult and juvenile seals as compared to the pups. In addition myoglobin was also significantly higher in the swimming muscles of the juvenile as compared to the adult. * denotes significantly greater than the pup. # denotes significantly greater than the adult. ($p < 0.05$)

increase in fast-twitch oxidative fibers (type IIA) with a significant decrease in mitochondrial density as the animals mature. In contrast the locomotor muscles of precocial terrestrial mammals are similar to adults in both mass, as a percent of total body mass, and fiber type composition (Cobb et al., 1994, Dearolf et al., 2000 and Grand 1992). Another unexpected result was found in the concentration of myoglobin (Figure 3). Similar to previous reports, the adults had a significantly greater concentration in their swimming muscles as compared to the pups. The unexpected results which were also verified using quantitative RT PCR and immunoblot western analysis showed that the juveniles had a significantly greater concentration of myoglobin in their swimming muscles as compared to both the adults and pups.

The difference in Weddell seal skeletal muscle physiology may arise from the three very distinct stages of their life history. During the first few weeks of life, seal pups are a non-diving terrestrial mammal that must rely on lanugo (natal fur) for thermoregulation in the extremely harsh environmental conditions of Antarctica. Their increased aerobic capacity may act to provide additional thermogenesis while the pups are on the ice during weaning. As juveniles, they lack sufficient whole body oxygen stores and diving capacity so their skeletal muscle physiology is similar to that of short duration, shallower divers than adults (Burns et al., 1997). As they continue to mature into an elite deep

from the volume density of mitochondria analysis which shows a significant decrease in the volume density of mitochondria as the seals mature (Figure 1B) and indicates that the aerobic capacities of pups and juveniles are significantly greater than in the adults and similar to athletic terrestrial mammals and short duration divers of comparable size (Figure 2). To our knowledge, Weddell seals are the first reported instance where there is a shift away from aerobic type I fibers towards an

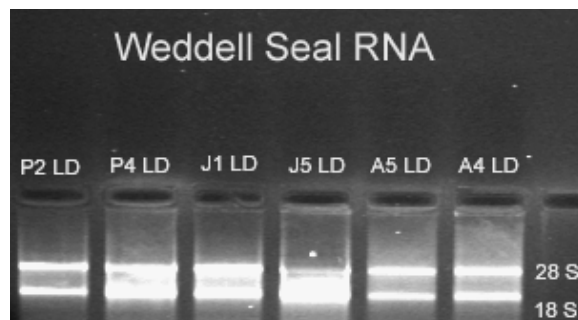


Figure 4. Representative agarose gel verifying the integrity of the RNA isolated from our samples.

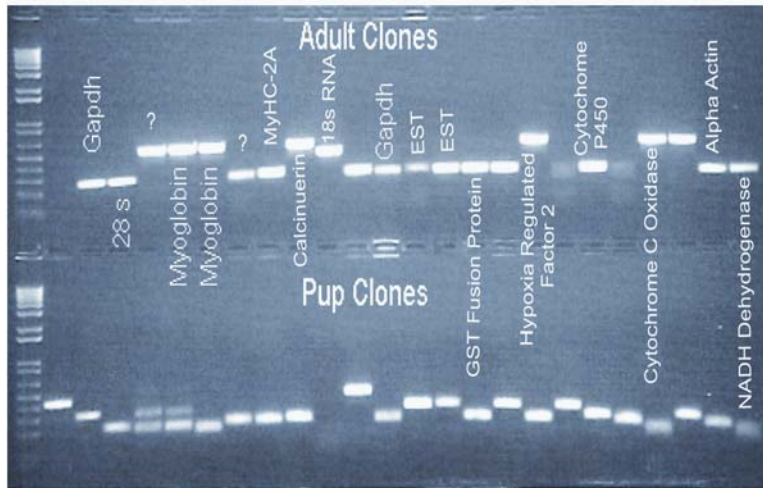


Figure 5. The results from our subtractive analysis between the pup and the adult has clearly defined a genetic profile that unique to the adult as compared to the pup. In addition the results of the subtraction correlate with our physiological results.

diver, their skeletal muscles transform to a more sedentary state in order to maintain low levels of aerobic metabolism under the hypoxic conditions associated with long duration diving. These results are completely contrary to our original hypothesis which expected a shift towards

more aerobic fibers as Weddell seals matured and adapted to the hypoxic conditions associated with prolonged diving. These unexpected results raise interesting questions about the molecular regulation of their skeletal muscle aerobic capacity, fiber type and myoglobin concentration which we will address in our current proposal.

Presently we are completing the aerobic (citrate synthase and β -hydroxyacyl Co A dehydrogenase), and anaerobic (lactate dehydrogenase) enzyme assays. In addition, we have successfully isolated RNA from all of the samples and transformed it into cDNA for our subtractive hybridization analysis (Figure 4). Our initial subtractions between the adult and pups have yielded over twenty transcripts that define a genetic profile that is unique to the adult as compared to the pup (Figure 5). In addition the transcripts identified from the subtraction correlate with our physiological results. As we found in our physiological analysis the adult's had a significantly greater percentage of fast-oxidative fibers as well as myoglobin concentration which was also found in our subtractive analysis.

In summary as newborn pups, Weddell seals have an extremely high aerobic capacity similar to that found in terrestrial animal athletes and short duration divers. However this enhanced aerobic capacity is not an adaptation towards diving but is due to their high fat diet and to offset thermoregulatory costs associated with using their lanugo for insulation in the extremely harsh environment of Antarctica. As they begin to dive and mature into juveniles, their skeletal muscles begin to transform. As juveniles, they initiate the development of fast-oxidative fibers and significantly increase their intramuscular stores of oxygen in the form of oxy-myoglobin. As they continue to mature and increase their diving capacity, Weddell seals increase their anaerobic capacity by significantly increasing their percentage of type IIA fast-oxidative fibers in their skeletal muscles. In addition, their skeletal muscles transform to a more sedentary state in order to maintain low levels of aerobic metabolism under the hypoxic conditions associated with long duration diving. Similar to what has been found in terrestrial mammals; the results of our subtractive analysis indicate these changes in skeletal muscle metabolic potential are regulated in part by calcium signaling and its downstream mediator calcineurin (Figure 6).

This project also had a significant outreach component. In addition to interviews, e-mail exchanges with high school and middle school students, public seminars and presentations at national and international meetings, this project continues to support our website in collaboration with the Science Teachers Access to Resources at Southwestern (STARS Program) (www.swmed.edu/stars/02antarcticexpedition/) in which we supply weekly updates about our research efforts during the field season, answer weekly questions submitted by the students and teachers, and continue to supply periodic updates on our results throughout the year.

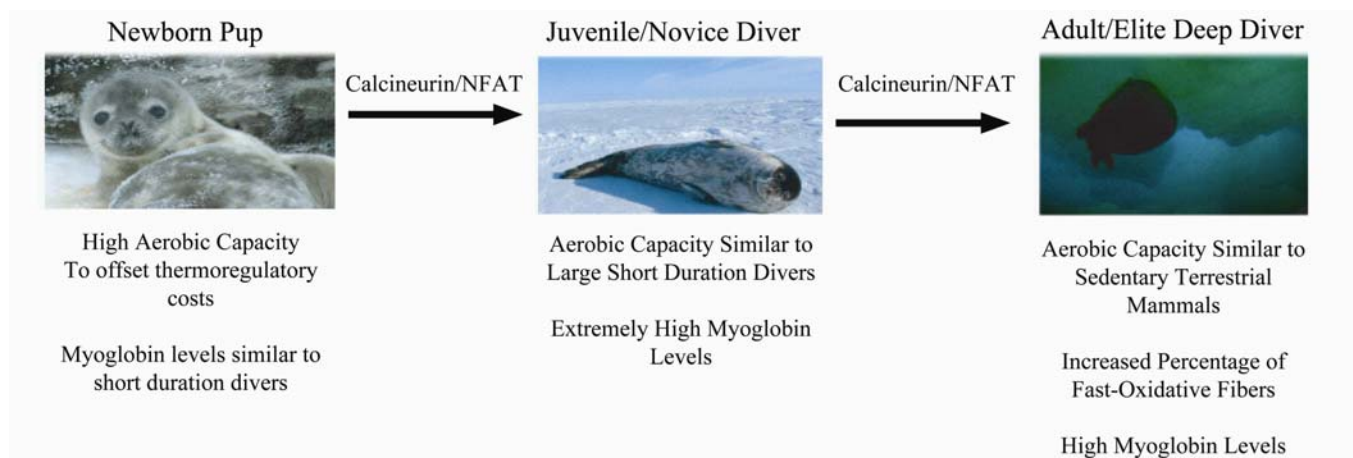


Figure 6. Summary results from our pilot study on the ontogeny of skeletal muscle adaptations the enable long deep dives in Weddell seals . As newborn pups, Weddell seals have an extremely high aerobic capacity similar to that found in terrestrial animal athletes and short duration divers. However this enhanced aerobic capacity is not an adaptation towards diving but is due to their high fat diet and to offset thermoregulatory costs associated with using their lanugo for insulation in the extremely harsh environment of Antarctica. As they begin to dive and mature into juveniles, their skeletal muscles begin to transform. As juveniles, they initiate the development of fast-oxidative fibers and significantly increase their intramuscular stores of oxygen in the form of oxy-myoglobin. As they continue to mature and increase their diving capacity, Weddell seals increase their anaerobic capacity by significantly increasing their percentage of type IIA fast-oxidative fibers in their skeletal muscles. In addition, their skeletal muscles transform to a more sedentary state in order to maintain low levels of aerobic metabolism under the hypoxic conditions associated with long duration diving. Similar to what has been found in terrestrial mammals; the results of our subtractive analysis indicate these changes in skeletal muscle metabolic potential are regulated by calcium signaling and its downstream mediator calcineurin.