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The development of diving in marine endotherms: preparing the skeletal muscles of dolphins, penguins, and seals for activity during submergence

Abstract Myoglobin is an important oxygen store for supporting aerobic diving in endotherms, yet little is known about its role during postnatal development. Therefore, we compared the postnatal development of myoglobin in marine endotherms that develop at sea (cetaceans) to those that develop on land (penguins and pinnipeds). We measured myoglobin concentrations in the major locomotor muscles of mature and immature bottlenose dolphins (*Tursiops truncatus*) and king penguins (*Aptenodytes patagonicus*) and compared the data to previously reported values for northern elephant seals (*Mirounga angustirostris*). Neonatal dolphins, penguins, and seals lack the myoglobin concentrations required for prolonged dive durations, having 10%, 9%, and 31% of adult values, respectively. Myoglobin contents increased significantly during subsequent development. The increases in myoglobin content with age may correspond to increases in activity levels, thermal demands, and time spent in apnea during swimming and diving. Across these phylogenetically diverse taxa (cetaceans, penguins, and pinnipeds), the final stage of postnatal development of myoglobin occurs during the initiation of independent foraging, regardless of whether development takes place at sea or on land.

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Abbreviations *[Mb]* myoglobin concentration · *NST* nonshivering thermogenesis

Introduction

Marine mammals and birds routinely experience prolonged periods of apnea during diving. During these periods, aerobic metabolic processes are supported by the use of on-board oxygen stores. One important store is myoglobin, which supplies oxygen for the locomotor muscles that must provide propulsion during diving and operate in the absence of continuous oxygen delivery by the cardiovascular system. The larger the myoglobin oxygen store, the greater the aerobic dive duration (Kooyman 1989). Consequently, the skeletal muscles of marine mammals and diving birds maintain myoglobin contents that are approximately 10–30-times greater than their terrestrial counterparts (Scholander 1940; Castellini and Somero 1981). This adaptation appears to be especially important for cetaceans and penguins due to their comparatively higher reliance on muscle oxygen stores than pinnipeds, which tend to rely more on blood oxygen stores (Kooyman 1989).

Although it is well known that adult marine mammals and birds have comparatively high myoglobin contents, only a few studies have examined the development of this muscle characteristic in newborn and immature animals. Previous studies concerning changes in myoglobin content during development have primarily focused on pinnipeds (Thorson 1993; Kohin 1998; Burns et al. 2000) and seabirds (Weber et al. 1974; Haggblom et al. 1988; Ponganis et al. 1999). Both groups are born (pinnipeds) or hatched (seabirds) on land; during this time myoglobin content increases before their first trip to sea, primarily in the absence of diving and locomotor demands. In contrast cetaceans are born and develop at sea, and thus immediately encounter the demands of swimming

and diving. Consequently, we asked the question, does the development of muscle myoglobin content in cetaceans differ from that of other swimming and diving endotherms that undergo early development on land?

In this study we compare the postnatal development of the oxygen store in locomotor skeletal muscles of marine endotherms in three categories: marine-born marine mammals (bottlenose dolphin, *Tursiops truncatus*), terrestrial-born marine birds (king penguins, *Aptenodytes patagonicus*), and terrestrial-born marine mammals (northern elephant seals, *Mirounga angustirostris*). Four other cetacean species (Pacific white-sided dolphin, *Lagenorhynchus obliquidens*; common dolphin, *Delphinus capensis*; striped dolphin, *Stenella coeruleoalba*; gray whale, *Eschrichtius robustus*) were sampled as available to determine whether postpartum development of myoglobin content is similar across cetacean species. Comparison of postnatal myoglobin contents across these diverse taxa revealed that neonatal diving marine endotherms have low muscle myoglobin contents, regardless of whether postnatal development occurs at sea or on land. Furthermore, increases in physical activity, thermal demands, and time spent in apnea during swimming and diving are factors that appear to influence myoglobin development. Finally, postnatal myoglobin development appears to be completed after the initiation of independent foraging.

Materials and methods

Experimental design

The postnatal development of myoglobin content of the primary locomotor muscle was examined for three categories of diving endotherms. Postnatal development on land and at sea was compared. The bottlenose dolphin was the primary species used to represent the first category, marine-born marine mammals. This category was supplemented with four other cetacean species (Pacific white-sided dolphin, common dolphin, striped dolphin, and gray whale) to determine whether the developmental pattern for muscle myoglobin content was similar across cetacean species. The king penguin represented the second category, terrestrial-born marine birds. Lastly, the northern elephant seal represented the third category, terrestrial-born marine mammals. Myoglobin contents of the *m. longissimus dorsi* of northern elephant seals were obtained from previous reports (Thorson 1993; Kohin 1998).

Specimens

Cetaceans

The Atlantic bottlenose dolphins used in this study were collected by the Northeast and Southeast Regional Marine Mammal Stranding Networks. Animals were divided into three age classes depending on the presence of neonatal characteristics and/or their length according to the methods of Dearolf et al. 2000. Floppy dorsal fin and floppy tailflukes were used to classify specimens as neonates, as these characteristics are common to dolphin calves that are less than 2-weeks-old (McBride and Kritzler 1951; Tavolga and Essapian 1957; Crockcroft and Ross 1990). All neonates were less than 120 cm. Animals that lacked neonatal characteristics but were less than 200 cm were classified as juveniles. The body lengths of our "juvenile" specimens ranged from 138 cm to 191 cm,

therefore our "juvenile" age class represents animals that are less than 1.5-years-old, according to the mean length-at-age of bottlenose dolphins (Read et al. 1993). Dolphins 200 cm or greater were classified separately as "adults". The body lengths of our "adult" specimens ranged from 204 cm to 260 cm. A 204-cm animal is 1.5–3.4-years-old, therefore our "adult" age class represents animals that are at least 1.5–3.4-years-old or older according to the mean length-at-age of bottlenose dolphins (Read et al. 1993). Three to five individuals were analyzed for each age class depending on the availability of specimens. Additional cetacean species (Pacific white-sided, common, and striped dolphins, and gray whale) were acquired opportunistically from Northeast and Southeast Regional Marine Mammal Stranding Networks, NMFS, Southwest Fisheries Science Center, Los Angeles Natural History Museum, and Long Marine Lab. These specimens were assigned to three age classes (neonate, juvenile, and adult) according to the presence of neonatal characteristics and by body length determined during necropsy (A. Pabst, J. Heyning, D. Casper, personal communication). Muscle samples were taken only from carcasses that were considered in fresh condition (Smithsonian Condition Code 2). Samples were collected from a primary locomotor muscle (m. longissimus dorsi) and stored following protocols outlined previously in Noren and Williams (2000).

Penguins

The king penguin chicks examined in this study were hatched from eggs collected in the Antarctic late in the breeding season. The chicks were reared in a simulated Antarctic environment at Sea World in San Diego, California where temperature, light, and diet were controlled. Chicks were weighed often to ensure that good body conditions were maintained. All chicks used for this study had died of natural causes. A total of 23 chicks were used, ranging in age from 0 days to 136 days. The pectoralis muscle, the major locomotor muscle of this species, was dissected within 1–2 h of death and stored frozen at −80 °C until myoglobin content analysis. Penguins were assigned to age groups that were associated with specific developmental periods. The 0- to 6-day-old group represents chicks immediately following the energy demanding activity of pipping. Two groups, 8- to 29-day-old penguins and 33- to 51-day-old penguins, represent the early and late incubation/ brooding stage (Moore et al. 1999). Two groups, 59–84-day-old penguins and 136-day-old penguins, represent the early and late crèche/fasting stage (Moore et al. 1999). Since the oldest specimen studied was only 136-days-old, we included the myoglobin content of the adult king penguin from Kooyman (1989) for the purposes of our analyses.

Analyses

Myoglobin content

Myoglobin content ([Mb]), reported in g Mb (100 g wet muscle)$^{-1}$, was determined using the procedure of Reynafarje (1963). Slightly thawed muscle samples (approximately 0.5 g) were minced in a low ionic strength buffer (40 mM phosphate, pH = 6.6), and sonicated (Sonifier Cell Disrupter Model W185D, Heat systems – Ultrasonics) for 2–3 min on ice. Buffer to tissue ratio was 19.25 ml buffer g^{-1} wet tissue. The samples were centrifuged at −4 °C and 28,000g for 50 min (Sorvall RC – 5B refrigerated superspeed centrifuge, DuPont Instruments). The clear supernatant was extracted and then bubbled at room temperature with pure CO for approximately 8 min. We added 0.02 g sodium dithionite to ensure a complete reduction. The absorbance of each sample was read at room temperature at 538 nm and 568 nm on a spectrophotometer (Shimadzu UV – visible spectrophotometer Bio spec – 1601). All samples were run in triplicate.

Validation for the assay

As controls for the assay, myoglobin contents were determined for the *m. longissimus dorsi* of a 1-week-old northern elephant seal pup and New Zealand white rabbit, and compared to previously pub-

lished values. The [Mb] of the seal pup, 2.4 ± 0.2 g (100 g wet muscle)$^{-1}$, and rabbit muscle, 0.08 ± 0.06 g (100 g wet muscle)$^{-1}$, in the present study were similar to values reported by Thorson (1993) and Castellini and Somero (1981) [2.9 and 0.04 g (100 g wet muscle)$^{-1}$, respectively].

Statistics

Statistical analyses were computed using Sigma Stat Software (Jandel Scientific 1995). Myoglobin contents for dolphin and penguin age classes are reported as means ± 1 SEM. A one-way analysis of variance with a Tukey all pairwise multiple comparison test was used to assess inter-age variability of muscle myoglobin content for the bottlenose dolphin, king penguin, and elephant seal. Inter-age variability of muscle myoglobin content for the common dolphin was determined by a t-test. Linear regressions were determined by least squares method; significance of the regressions was determined using F-tests. Ninety-five percent confidence intervals are shown for significant regressions. A Basic program outlined by Yeager and Ultsch (1989) was used to determine the statistical breakpoints in the data for length or age versus myoglobin content. Results were considered significant at $P \leq 0.05$.

Results

Development of skeletal muscle in cetaceans (marine-born marine mammals)

For the bottlenose dolphin, myoglobin content increased with age and the differences between all age classes (neonate, juvenile, and adult) were significant ($F=28.45$, $P<0.001$, $df=11$, $n=12$; Table 1). Furthermore, muscle myoglobin contents for bottlenose dolphins were described by two relationships depending on length, and hence age. Myoglobin increased linearly with total body length up to 191 cm. This was followed by a breakpoint between 191–204 cm, suggesting that adult myoglobin values are obtained within this range of body lengths for bottlenose dolphins. For the animals analyzed in this study, myoglobin content reached a plateau when body length was equal to or exceeded 204 cm (representing the adult age class). Mean myoglobin content at the plateau was 2.76 ± 0.15 g (100 g wet muscle)$^{-1}$ for the adult bottlenose dolphins (Fig. 1A).

The adult muscle of the common dolphin had significantly greater myoglobin contents than that in the neonate muscle ($t=5.51$, $P=0.012$, $df=3$, $n=4$; Table 1). Small sample sizes for the Pacific white-sided dolphin, striped dolphin and gray whale precluded statistical analyses. However, there was an overall trend showing an increase in myoglobin content with older age classes in all cetacean species examined (Table 1).

Development of skeletal muscle in penguins (terrestrial-born marine birds)

As found for the cetaceans, myoglobin contents for the skeletal muscles of king penguins differed significantly between age classes (Table 2; Fig. 2B, $F=4.27$, $P=0.019$, $df=21$, $n=24$). An all pairwise test showed the myoglobin content of adult muscle was significantly greater than muscle from all chick age classes ($P<0.05$). The myoglobin content of the 136-day age class was significantly greater than all of the younger chick age classes (0–6, 8–29, 33–51, and 59–84-day age classes at $P<0.05$). The 8–29-day age class had the lowest mean myoglobin content, 0.27 ± 0.04 g (100 g wet muscle)$^{-1}$. The relationship between age and muscle myoglobin content was described by two linear regressions with a breakpoint between 43–46 days. For penguin chicks $\leq$43-days-old, myoglobin content was negatively correlated with age. For older chicks, muscle myoglobin content increased with age (Fig. 1B). The highest myoglobin content for king penguins measured in this study was 1.06 g (100 g wet muscle)$^{-1}$ for the 136-day-old chick. This is considerably lower than the adult level of 4.3 g (100 g wet muscle)$^{-1}$ reported by Kooyman (1989).

Development of skeletal muscle in seals (terrestrial-born marine mammals)

For the purpose of comparison, previous reports of myoglobin content from the *m. longissimus dorsi* of different age classes of northern elephant seals [7, 30, 60,

Table 1 Body length and myoglobin content of cetaceans at different age classes ($n=$ sample size; length and Mb reported as means $\pm$ 1SEM)

| Cetacean species	Age class (n)	Length (cm)	% Adult length	[Mb] [g (100 g wet muscle^{-1})]	% Adult [Mb]	Reference
Bottlenose dolphin	Neonate (3)	110.5 ± 2.6	49%	0.27 ± 0.02	10%	Present study
(*Tursiops truncatus*)	Juvenile (4)	161 ± 11.1	72%	1.58 ± 0.34	57%	Present study
	Adult (5)	223.9 ± 9.6	100%	2.76 ± 0.15	100%	Present study
Pacific white-sided dolphin	Fetus (1)	70.4	34%	0.15	4%	Present study
(*Lagenorhynchus obliquidens*)	Juvenile (1)	185	90%	2.93	85%	Present study
	Adult (2)	205 ± 2.0	100%	3.45 ± 0.25	100%	Noren and Williams (2000)
Common dolphin	Neonate (2)	95.5 ± 5.3	45%	0.70 ± 0.44	20%	Present study
(*Delphinus capensis*)	Adult (3)	211.1 ± 7.7	100%	3.58 ± 0.32	100%	Noren and Williams (2000)
Striped dolphin	Juvenile (1)	153	66%	3.94	68%	Present study
(*Stenella coeruleoalba*)	Adult (1)	233	100%	5.78	100%	Noren and Williams (2000)
Gray whale	Neonate (1)	–	–	0.13	–	Present study
(*Eschrichtius robustus*)	Juvenile (1)	–	–	0.22	–	Castellini and Somero (1981)
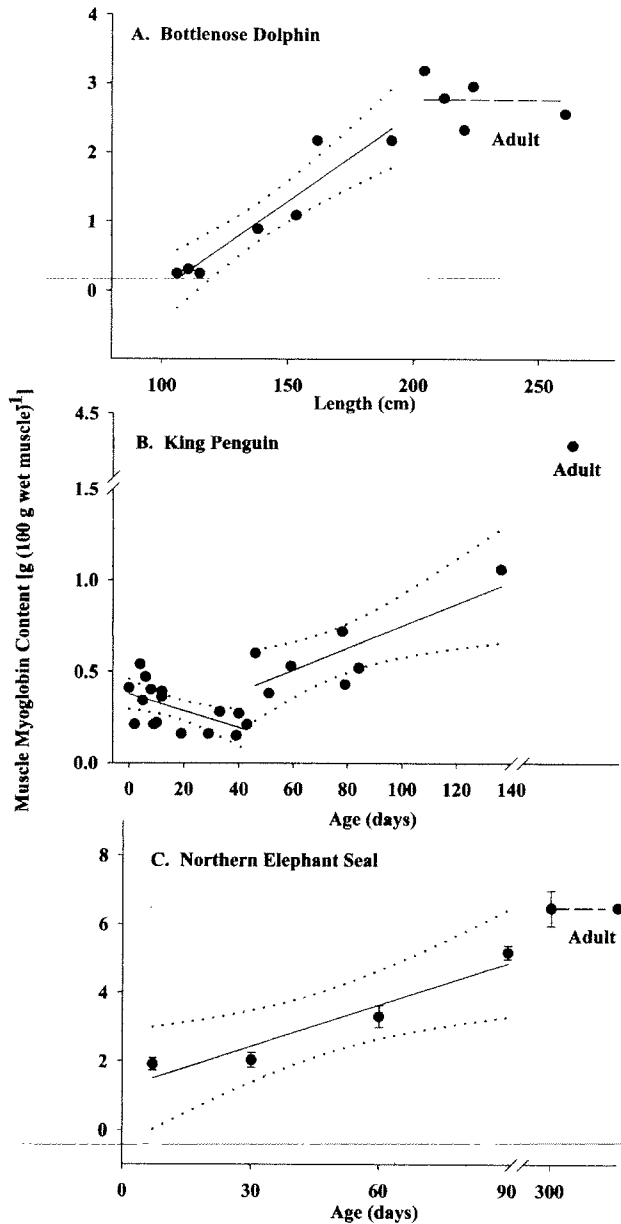
Fig. 1 Myoglobin content [g (100 g wet muscle)$^{-1}$] in relation to length (cm) as an index of age for bottlenose dolphins (**A**), and age (days) for king penguins (**B**) and northern elephant seals (**C**). *Solid lines* represent linear regressions determined by least squares method. *Dotted lines* represent the 95% confidence intervals. *Horizontal dashed lines* represent the average adult value for the muscle myoglobin content. **A** For dolphins, myoglobin content increased linearly with length up to 191 cm. This relationship is described by [Mb] = 0.03 length − 2.55 (r^2 = 0.89, F = 39.42, P = 0.002, df = 6, n = 7). **B** For penguins, myoglobin content was negatively correlated with age from 0 days to 43 days according to the relationship [Mb] = −0.005 age + 0.38 (r^2 = 0.32, F = 6.48, P = 0.02, df = 15, n = 16). For older chicks, muscle myoglobin content is positively correlated with age according to the relationship: [Mb] = 0.006 age + 0.14 (r^2 = 0.65, F = 9.10, P = 0.03, df = 6, n = 7). The adult value is from Kooyman (1989). **C** For seals, myoglobin content increased linearly with age for animals ≤90-days-old, as described by [Mb] = 0.04 age + 1.22 (r^2 = 0.92, F = 23.16, P = 0.041, df = 3, n = 4). Values for 0–300 -days-old seals are from Kohin (1998). The average adult value was calculated from sub-adult and adult categories from Thorson (1993)

and 90-day age classes from Kohin (1998) and 300-day, sub-adult and adult age classes from Thorson (1993)] were evaluated statistically. The combined data set for the seals demonstrated an increase in skeletal muscle myoglobin content with age. Myoglobin content differed significantly between age classes (F = 33.48, P < 0.001, df = 33, n = 34). An all pairwise test showed that the myoglobin contents for the 7-day and 30-day age classes were significantly less than those reported for the 60, 90, 300-day, sub-adult, and adult age classes (P < 0.05). The myoglobin content for the 60-day age class was significantly less than older age classes (P < 0.05). Like the bottlenose dolphin and king penguin, muscle myoglobin content showed a biphasic relationship with age for the northern elephant seal (Fig. 1C). Muscle myoglobin content for northern elephant seals increased in a linear manner with age up to 90-days-old. A breakpoint occurred between 90-days-old and 300-days-old, suggesting that adult myoglobin values are obtained within this range of ages for northern elephant seals. For the range of animals studied in Thorson (1993) and Kohin (1998), myoglobin reached a plateau for animals that were 300-days and older. Because there were no statistical differences in the myoglobin contents of the sub-adult and adult age classes from Thorson (1993), these age classes were combined to represent the overall adult age class. Mean myoglobin content for the combined 300-day and adult age class at the plateau was 6.48 g (100 g wet muscle)$^{-1}$.

Discussion

Previous studies examining the development of the oxygen stores and cardiorespiratory control for diving have focused on terrestrial-born, marine endotherms such as seabirds (Weber et al. 1974; Merino and Barbosa 1997; Ponganis et al. 1999) and pinnipeds (Thorson 1993; Castellini et al. 1994; Horning and Trillmich 1997a; Burns et al. 2000). These studies demonstrated the necessity of a postnatal development period to acquire the enhanced whole body oxygen stores and heart rate control of adults. These developmental changes were critical for increasing dive duration with age in both seabirds (Ponganis et al. 1999) and pinnipeds (Thorson 1993; Horning and Trillmich 1997b; McCafferty et al. 1998).

Unlike these other groups of divers, cetaceans encounter the demands of swimming and diving immediately after birth. The only two studies to examine postpartum development of diving characteristics in cetaceans suggest that the locomotor muscles of immature dolphins have low aerobic capacity (Dolar et al. 1998; Dearolf et al. 2000). The results of the present study provide further support for these findings. In view of the similarity in pattern for pinnipeds, penguins and cetaceans it appears that diving marine endotherms require a period of postnatal development before myoglobin contents reach adult levels, regardless of whether development takes place on land or at sea (Fig. 1A–C).

Table 2 Myoglobin content of seabirds at different age classes (n = sample size: Mb reported as means ± 1SEM for this study)

Seabird species	Age class (n)	[Mb] [g (100 g wet muscle^{-1})]	% Adult [Mb]	Reference
King penguin (*Aptenodytes patagonicus*)	0- to 6-day-old chick (5)	0.39 ± 0.13	9%	Present study
	136-day-old chick (1)	1.06	25%	Present study
	Adult	4.3	100%	Kooyman (1989)
Pigeon guillemot (*Cepphus columba*)	Chick	Not detectable	0%	Haggblom et al. (1988)
	Fledgling	0.52	24%	
	Adult	2.16	100%	
Emperor penguin (*Aptenodytes forsteri*)	Pre-molt Chicks	1.55	24%	Ponganis et al. (1999)
	Post-molt juveniles	2.0	31%	
	Adult	6.4	100%	
Adelie penguin (*Pygoscelis adeliae*)	Chick (one-third fledged)	0.10	3%	Weber et al. (1974)
	Young adult	1.16	40%	
	Adult	2.88	100%	
Gentoo penguin (*Pygoscelis papua*)	Young chick	0.05	1%	Weber et al. (1974)
	Adult	4.42	100%	

Factors influencing the maturation of muscle myoglobin content

Factors such as changes in physical activity, thermal demands, and exposure to hypoxia have been shown to increase myoglobin content in endotherm locomotor muscles. For example, treadmill-conditioned bar-headed geese (Saunders and Fedde 1991) and dive-conditioned tufted ducks (Stephenson et al. 1989) showed increases of 31% and 57% in myoglobin contents in the primary locomotor muscles from pre-conditioned levels, respectively. Morrison et al. (1966) demonstrated that myoglobin contents in red-backed voles in the winter show a 2.5-fold increase associated with increased metabolic output from the shivering muscles. Likewise, diving muskrats experienced an 18% increase in myoglobin content in the locomotor muscles during winter that was associated with an increased dependence on diving and the hypoxic conditions of winter lodges (MacArthur 1990). These findings indicate the malleable nature of myoglobin and these factors may explain the changes we observed in myoglobin content for the skeletal muscles of immature marine mammals and birds. Throughout their early lives, marine mammals and birds experience changes in physical activity, thermal demands, and time spent in apnea during swimming and diving that may influence the postnatal enhancement of myoglobin concentrations in the muscle.

From the moment of birth, cetaceans experience the demands of thermoregulation, swimming, and diving in the marine environment. Changes in swimming style and increased time spent in apnea during swimming and diving throughout postpartum development undoubtedly influence myoglobin development in dolphin calves. The swimming style of newborn dolphins is qualitatively different from that of older animals. The predominate position for neonate dolphins swimming with their mothers is the "echelon" position, flanking the mother near the dorsal fin region (Gubbins et al. 1999). In this swimming position, an infant dolphin has a decreased cost of transport because it is carried by the pressure wave created by its mother's larger body (Williams et al. 1992). This position enables the calf to maintain the group speed with a decreased tailbeat frequency (Norris and Prescott 1961). Calves 1 week of age spend 67% of their time swimming in "echelon" position when swimming in association with their mothers; by the time calves are 1 year of age this decreases to 23% (Gubbins et al. 1999). Dolphins also increase diving and breath-holding capacities with age. Wild dolphins begin foraging between the ages of 4 months and 11 months (Perrin and Reilly 1984), and detailed observations of a captive dolphin calf showed the most dramatic increase in breath-hold ability at 6 months (Peddemors 1990). Heightened demands on the skeletal muscle from increased independent swimming and increased time spent in apnea during swimming and diving could be associated with the development of higher muscle myoglobin content observed in the juvenile age class (Table 1, Fig. 2A).

Weaning, the attainment of fully independent foraging, occurs at 18–20 months (Perrin and Reilly 1984), corresponding to a body length of 170–180 cm in bottlenose dolphins (Barros and Odell 1990). Because this length is approximately 10 cm longer than the average length of our juvenile age class, it is likely that the animals in this age class were not weaned. According to their body length, our adult age class consists of dolphins that were most likely weaned. Muscle myoglobin contents for the juveniles were lower than those observed for adults (Table 1, Fig. 2A), suggesting that suckling juvenile dolphins require further muscle development before they attain the aerobic capacities of adults and can be successfully weaned (Fig. 2A). Previous studies have suggested that echolocation and other foraging-related behaviors are learned during the prolonged period of nursing in cetaceans (Leatherwood and Reeves 1983). The present study indicates that the pro-
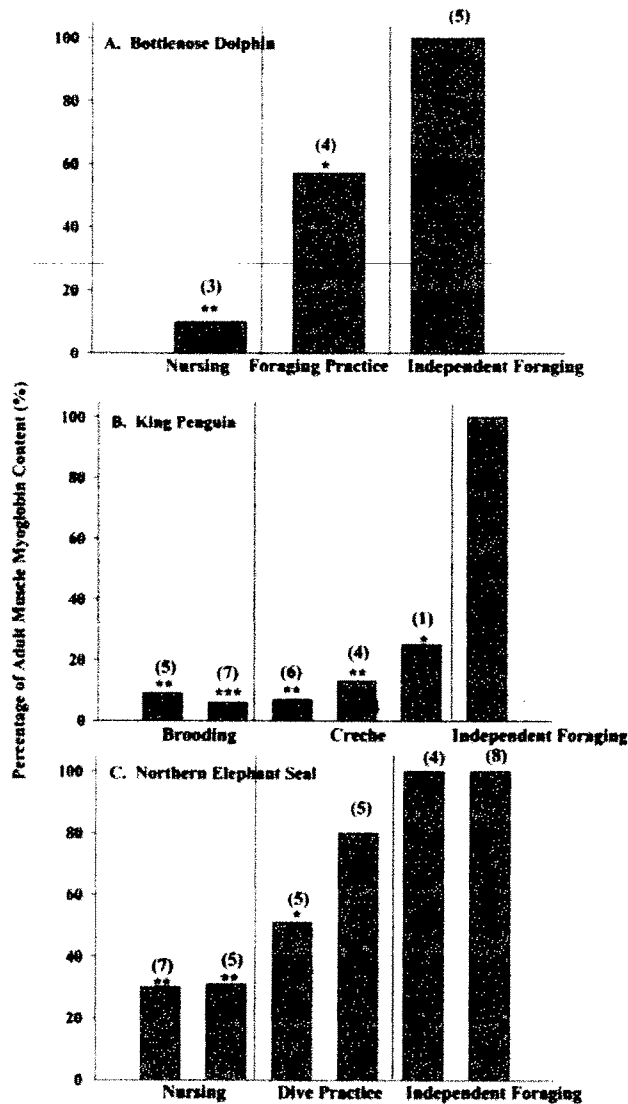
Fig. 2 Muscle myoglobin content as a percentage of adult values for bottlenose dolphins (A), king penguins (B), and northern elephant seals (C) at different activity states during development. *Solid vertical lines* represent transitions between stages. *Numbers in parentheses* indicate the sample size for each age class. *Heights of the bars* show the mean value for each age category. Age classifications used in this study are written in the bars. A Bottlenose dolphins. (**significantly less than juvenile and adult age classes, *significantly less than adult age class, $P < 0.05$). B King penguins. The adult value is from Kooyman (1989). (***significantly less than 59–84 day, 136 day, and adult age classes, **significantly less than 136 day and adult age classes, *significantly less than adult age class, $P < 0.05$). C Northern elephant seals. Pup age classes (7–300 days) from Kohin (1998). Adult value calculated from sub-adult and adult categories from Thorson (1993). (**significantly less than 60 day, 90 day, 300 day, and adult age classes, *significantly less than 90 day, 300 day, and adult age classes, $P < 0.05$)

longed nursing period may also allow time for important physiological changes to occur within the developing skeletal muscles that will enhance dive capacity and ensure successful foraging at weaning.

Despite hatching and developing on land, king penguins show a surprisingly similar pattern in myoglobin development to that of cetaceans. King penguin chicks do not go to sea until fledging at approximately 350 days after hatching (Adams and Klages 1987). As the penguin chick ages, there is an overall increase in muscle myoglobin content (Fig. 2B). Several factors may facilitate the increase in myoglobin content that occurs in chicks older than 43 days (Fig. 1B). First, chicks at this age join other chicks in a crèche for a 4-month overwintering fast. At this stage, the chicks are considerably more active (Moore et al. 1999). Consequently, conditioning associated with the increased activity could enhance myoglobin content. Second, the climate extreme of the overwintering fast may also enhance skeletal muscle myoglobin content. Skeletal muscle has been proposed as a possible site of nonshivering thermogenesis (NST) in king penguin chicks (Duchamp et al. 1989). Furthermore, cold-acclimated king penguin chicks have increased muscle oxidative capacity (Duchamp et al. 1991) which is likely accompanied by increases in myoglobin content. Finally, birds also increase the mass specific pectoral muscle mass during ontogeny to increase their capacity for NST (Aulie 1976; Marsh and Wickler 1982). There is an increase in mass-specific pectoral muscle mass with age in king penguins between 46 days and 136 days (oldest specimen examined; T.M. Williams unpublished data), suggesting that king penguin chicks do rely on muscles for thermoregulation.

Unfortunately, there were insufficient samples from king penguins to determine whether myoglobin development is complete before the first foraging trip. The oldest chick examined in this study was 136-days-old, while fledging requires 350 days (Adams and Klages 1987). The myoglobin content of the 136-day-old chick was only 25% of the value for adult king penguins [4.3 g (100 g wet muscle)$^{-1}$, Kooyman 1989]. We would expect further increases in myoglobin content in the 200 days before the first foraging trip, however, other penguin species show only 24–40% of adult values at the time of fledging (Table 2). It is possible that the first foraging trips by king penguins occur before myoglobin development is complete. Increased time spent in apnea during swimming and diving may then contribute to the completion of the development of the muscle oxygen store.

Like the penguins, elephant seals are born on land and experience a terrestrial, postnatal developmental period. During the relatively inactive period of nursing (0–30 days), myoglobin content remains low (Fig. 2C). Over the subsequent 2.5-month post-weaning fast, pups become more active on the beach and myoglobin content increases significantly from 30 days to 60 days of age (Kohin 1998). Further increases occur from 60 days to 90 days of age (Kohin 1998) as pups increase the proportion of time spent in water (Thorson 1993). Ninety-day-old pups have approximately 80% of adult myoglobin content prior to departing on their first foraging trip, while 300-day-old northern elephant seals return from their first trip to sea with 100% of the adult

value (Thorson 1993; Kohin 1998). This suggests that ocean experience and the associated increased time spent in apnea during swimming and diving catalyzes the final stage in muscle oxygen store development.

In addition to increased activity levels and the demands of diving as plausible factors that may increase myoglobin concentration in northern elephant seals, thermoregulatory factors, as we suggested for king penguins, may also play a role. Because increased myoglobin content enhances overall aerobic capacity, it is important for NST and shivering thermogenesis. In harp seals, nonshivering thermogenic capacity reaches a maximum at the time of transition to aquatic life approximately 30 days after birth (Blix and Steen 1979). For northern elephant seal pups, NST or shivering thermogenesis may be particularly beneficial for the pup at the end of the post-weaning fast as the pup enters the highly conductive marine environment with its depleted blubber layer.

Duration of muscle development

Although these three taxa show similar patterns in terms of increasing myoglobin content with age, the time required to attain adult levels varies. For example, bottlenose dolphin calves and king penguin chicks begin with similar proportions of the adult myoglobin content, at 10% and 9%, respectively. However, the time it takes for complete maturation of the muscle oxygen store varies between groups. Myoglobin content of a 90-day-old northern elephant seal is 80% of the adult myoglobin content. In comparison, a 136-day-old king penguin has only 25% of the adult myoglobin content. Although king penguin chicks were not studied to the point of fledging, based on conclusions from other penguins (Table 1), we can hypothesize that a 350-day-old newly fledged king penguin may still not have adult myoglobin contents. Meanwhile, it takes at least 1.5 years for bottlenose dolphins to reach adult myoglobin contents in the primary locomotor muscle. Interestingly, when both juvenile seals and dolphins begin to practice hunting, they show similar proportions of their adult values (64% and 57%, respectively), marking the final stage of myoglobin enhancement (Fig. 2). From these data, it appears that the duration of myoglobin development is dependent on the initiation of independent foraging by the young. Northern elephant seals, which start independent foraging at 3 months of age, show the shortest period of muscle myoglobin development, obtaining adult values sometime between 3-months-old and 10-months-old. In comparison, bottlenose dolphins, which start independent foraging at 18–20 months, experience the longest period of myoglobin development, obtaining adult values sometime between 18-months-old and 41-months-old.

To summarize, although the demands of locomotion, diving, and thermoregulation differ throughout maturation, cetaceans, penguins, and pinnipeds show similar patterns in myoglobin development from birth or hatching to adulthood. The possible effects of physical conditioning, thermal demands, and the increased time spent in apnea during swimming and diving on the postnatal development of muscle myoglobin content are apparent for all three taxa. The time it takes for complete maturation of the muscle oxygen store varies between groups depending on when the animal begins independent foraging. The final stage of postnatal development of myoglobin occurs during the initiation of independent foraging whether the animal is born at sea or born/hatched on land.

References

Adams NJ, Klages NT (1987) Seasonal variation in the diet of the king penguin (*Aptenodytes patagonicus*) at sub-Antarctic Marion Island. J Zool (Lond) 212: 303–324

Aulie A (1976) The pectoral muscles and the development of thermoregulation in chicks of willow ptarmigan (*Lagopus lagopus*). Comp Biochem Physiol A 53: 343–346

Barros NB, Odell DK (1990) Food habits of bottlenose dolphins in the southeastern United States. In: Leatherwood S, Reeves RR (eds) The bottlenose dolphin. Academic Press, San Diego, pp 309–328

Blix AS, Steen JB (1979) Temperature regulation in newborn polar homeotherms. Physiol Rev 59: 285–304

Burns JM, Blix AS, Folkow LP (2000) Physiological constraint and diving ability: a test in hooded seals, *Cystophora cristata*. FASEB J Abst 317: 8

Castellini MA, Somero GN (1981) Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. J Comp Physiol B 143: 191–198

Castellini MA, Rea LD, Sanders JL, Castellini JM, Zenteno-Savin T (1994) Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. Am J Physiol 267: R1294–R1301

Crockcroft VG, Ross GJB (1990) Observations on the early development of a captive bottlenose dolphin calf. In: Leatherwood S, Reeves RR (eds) The bottlenose dolphin. Academic Press, San Diego, pp 461–478

Dearoff JL, McLellan WA, Dillaman RM, Frierson D Jr, Pabst DA (2000) Precocial development of axial locomotor muscle in bottlenose dolphins (*Tursiops truncatus*). J Morphol 244: 203–215

Dolar MLL, Suarez P, Ponganis PJ, Kooyman GL (1998) Myoglobin in pelagic small cetaceans. J Exp Biol 202: 227–236

Duchamp C, Barré H, Delage D, Rouanet J-L, Cohen-Adad R, Minaire Y (1989) Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. Am J Physiol 257: R744–R751

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Duchamp C, Barré H, Rouanet J-L, Lanni A, Cohen-Adad R, Berne G, Brebion P (1991) Nonshivering thermogenesis in king penguin chicks. I. Role of skeletal muscle. Am J Physiol 261: R1438–R1445

Gubbins C, McCowan B, Lynn SK, Hooper S, Reiss D (1999) Mother-infant spatial relations in captive bottlenose dolphins, *Tursiops truncatus*. Mar Mamm Sci 15: 751–765

Haggblom L, Terwilliger RC, Terwilliger NB (1988) Changes in myoglobin and lactate dehydrogenase in muscle tissues of a diving bird, the pigeon guillemot, during maturation. Comp Biochem Physiol B 91: 273–277

Horning M, Trillmich F (1997a) Development of hemoglobin, hematocrit and erythrocyte values in Galapagos fur seals. Mar Mamm Sci 13: 100–113

Horning M, Trillmich F (1997b) Ontogeny of diving behaviour in the Galapagos fur seal. Behaviour 134: 1211–157

Kohin S (1998) Respiratory physiology of northern elephant seal pups: Adaptations for hypoxia, hypercapnia, and hypometabolism. Ph.D. dissertation, University of California, Santa Cruz

Kooyman GL (1989) Diverse divers: physiology and behaviour. Springer, Berlin Heidelberg New York

Leatherwood S, Reeves RR (1983) The Sierra Club handbook of whales and dolphins. Sierra Club, San Francisco

MacArthur RA (1990) Seasonal changes in the oxygen storage capacity and aerobic dive limits of the muskrat (*Ondatra zibethicus*). J Comp Physiol B 160: 593–599

Marsh RL, Wickler SJ (1982) The role of muscle development in the transition to endothermy in nestling bank swallows, *Riparia riparia*. J Comp Physiol 149: 99–105

McBride AF, Kritzler H (1951) Observations on pregnancy, parturition, and post-natal behavior in the bottlenose dolphin. J Mamm 32: 251–266

McCafferty DJ, Boyd IL, Taylor RI (1998) Diving behavior of Antarctic fur seal (*Arctocephalus gazella*) pups. Can J Zool 76: 513–520

Merino S, Barbosa A (1997) Haematocrit values in chinstrap penguins (*Pygoscelis antarctica*): variation with age and reproductive status. Polar Biol 17: 14–16

Moore GJ, Wienecke B, Robertson G (1999) Seasonal change in foraging area and dive depths of breeding king penguins at Heard Island. Polar Biol 21: 376–384

Morrison P, Rosenmann M, Sealander JA (1966) Seasonal variation of myoglobin in the northern red-backed vole. Am J Physiol 211: 1305–1308

Noren SR, Williams TM (2000) Body size and skeletal muscle myoglobin of cetaceans: adaptations for maximizing dive duration. Comp Biochem Physiol A 126: 181–191

Norris KS, Prescott JH (1961) Observations on Pacific cetaceans of Californian and Mexican waters. Univ Calif Publ Zool 63: 291–402

Peddemors VM (1990) Respiratory development in a captive-born bottlenose dolphin *Tursiops truncatus* calf. S Afr J Zool 25: 178–184

Perrin WF, Reilly SB (1984) Reproductive parameters of dolphins and small whales of the family Delphinidae. Rep Inter Whaling Comm (Special Issue) 6: 97–133

Ponganis PJ, Starke LN, Horning M, Kooyman GL (1999) Development of diving capacity in emperor penguins. J Exp Biol 202: 781–786

Read AJ, Wells RS, Hohn AA, Scott MD (1993) Patterns of growth in wild bottlenose dolphins, *Turisops truncatus*. J Zool (Lond) 231: 107–123

Reynafarje B (1963) Simplified method for the determination of myoglobin. J Lab Clin Med 61: 138–145

Saunders DK, Fedde MR (1991) Physical conditioning: effect on the myoglobin concentration in skeletal and cardiac muscle of bar-headed geese. Comp Biochem Phys A 100: 349–352

Scholander PF (1940) Experimental investigation on the respiratory function in diving mammals and birds. Hvalradets Skr 22: 1–131

Stephenson R, Turner DL, Butler PJ (1989) The relationship between diving activity and oxygen storage capacity in the tufted duck (*Aythya fuligula*). J Exp Biol 141: 265–275

Tavolga RJ, Essapian FS (1957) The behavior of the bottle-nosed dolphin (*Tursiops truncatus*): mating, pregnancy, parturition, and mother-infant behavior. Zoologica 42: 11–31

Thorson PH (1993) Development of diving in the northern elephant seal. PhD dissertation, University of California, Santa Cruz

Weber RE, Hemmingsen EA, Johansen K (1974) Functional and biochemical studies of penguin myoglobin. Comp Biochem Physiol B 49: 197–214

Williams TM, Friedl WA, Fong ML, Yamada RM, Sedivy P, Haun JE (1992) Travel at low energetic cost by swimming and wave-riding dolphins. Nature 355: 821–823

Yeager DP, Ultsch GR (1989) Physiological regulation and conformation: a BASIC program for the determination of critical points. Physiol Zool 62: 888–907