The Journal of Experimental Biology 213, 740-748

doi:10.1242/jeb.037929

Development of aerobic and anaerobic metabolism in cardiac and skeletal muscles from harp and hooded seals

J. M. Burns[1,*], N. Skomp[1], N. Bishop[1], K. Lestyk[1] and M. Hammill[2]

[1]Department of Biological Sciences, University of Alaska, Anchorage, AK 99508, USA and [2]Department of Fisheries and Oceans, Mont-Joli, Québec, Canada

*Author for correspondence (afjmb4@uaa.alaska.edu)

Accepted 17 November 2009

SUMMARY

In diving animals, skeletal muscle adaptations to extend underwater time despite selective vasoconstriction include elevated myoglobin (Mb) concentrations, high acid buffering ability (β) and high aerobic and anaerobic enzyme activities. However, because cardiac muscle is perfused during dives, it may rely less heavily on Mb, β and anaerobic pathways to support contractile activity. In addition, because cardiac tissue must sustain contractile activity even before birth, it may be more physiologically mature at birth and/or develop faster than skeletal muscles. To test these hypotheses, we measured Mb levels, β and the activities of citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (HOAD) and lactate dehydrogenase (LDH) in cardiac and skeletal muscle samples from 72 harp and hooded seals, ranging in age from fetuses to adults. Results indicate that in adults cardiac muscle had lower Mb levels (14.7%), β (55.5%) and LDH activity (36.2%) but higher CS (459.6%) and HOAD (371.3%) activities (all *P*<0.05) than skeletal muscle. In addition, while the cardiac muscle of young seals had significantly lower [Mb] (44.7%) β (80.7%) and LDH activity (89.5%) than adults (all *P*<0.05), it was relatively more mature at birth and weaning than skeletal muscle. These patterns are similar to those in terrestrial species, suggesting that seal hearts do not exhibit unique adaptations to the challenges of an aquatic existence.

Key words: cardiac muscle, marine mammal, metabolism.

INTRODUCTION

Marine mammals have a suite of biochemical and physiological adaptations that allow them to efficiently exploit underwater prey resources. For example, deep divers have large blood volume, high hemoglobin, increased muscle mass and high muscle myoglobin (Mb) loads, all of which increase the amount of oxygen (O_2) that can be carried to depth (Butler and Jones, 1997). To conserve available O_2 stores during the dive, marine mammals can reduce their heart rate (bradycardia) and restrict blood flow to skeletal muscles and non-essential organs (vasoconstriction) (Blix et al., 1983; Butler and Jones, 1997; Hochachka, 1981). However, perfusion is maintained to the brain, which relies exclusively on aerobic respiration, and to the heart, which relies primarily on aerobic respiration, but can survive short periods of low partial pressures of O_2 ($\dot{P}_{O_2}$) (Blix et al., 1983; Elsner et al., 1985; Hochachka, 1981; Kjekshus et al., 1982). As a result of differences in perfusion patterns, many physiological traits such as size of the O_2 reserves, enzyme activities and preferred metabolic substrates differ between cardiac and skeletal muscle tissues (Driedzic et al., 1987; Hochachka, 1981; Neely and Morgan, 1974).

For example, during terrestrial and surface activities when perfusion supplies working skeletal muscle with metabolic fuels and O_2, the large Mb reserves facilitate O_2 diffusion from blood to mitochondria (Davis and Kanatous, 1999; Wittenberg, 1970). Then, when muscle blood flow is reduced during diving, the resultant drop in muscle $\dot{P}_{O_2}$ causes oxy-Mb to release its stored O_2, allowing for continued aerobic respiration (Davis and Kanatous, 1999). As a result, the skeletal tissues of diving mammals contain more oxidative fibers and higher levels of aerobic enzymes, such as citrate synthase (CS) than might be expected given the lack of freely available O_2 (Kanatous et al., 2008; Polasek et al., 2006). In addition, even during periods of reduced blood flow, large endogenous lipid reserves provide the primary metabolic fuel for working muscles, as indicated by high levels of enzymes involved in the β-oxidation of fatty acids, such as β-hydroxyacyl-CoA dehydrogenase (HOAD) (Kanatous et al., 2008; Polasek et al., 2006; Reed et al., 1994). However, while the skeletal muscles of marine mammals are adapted for lipid-based aerobic endurance exercise, at times dives extend so long that tissue O_2 stores are exhausted, and swimming activity is fueled primarily by anaerobic respiration (Kanatous et al., 2008; Kooyman et al., 1980). This occasional reliance on glycolytic metabolism is reflected by high levels of lactate dehydrogenase (LDH) and substantial tissue acid buffering (β) capacity to counteract the resulting accumulation of hydrogen ions (Castellini et al., 1981; Castellini and Somero, 1981).

Cardiac muscle experiences more constant conditions due to its regular contractions and direct coronary perfusion. At the biochemical level, the heart has the highest capacity for lipid-based aerobic metabolism of any muscle tissue, as indicated by very high mitochondrial (MT) content, CS and HOAD activities (Stanley et al., 2005; Widner et al., 1974). While cardiac tissue typically has lower [Mb], β and lipid reserves than skeletal tissue, lipids provide 60–90% of ATP production, with pyruvate oxidation accounting for much of the remainder (Kodde et al., 2007; Stanley et al., 2005). Because a significant proportion of pyruvate is provided by lactate oxidation, cardiac tissue is a net consumer of circulating lactate, even when working at rates close to the maximum rate of O_2 uptake ($\dot{V}_{O_{2,max}}$) (Hochachka, 1981; Kodde et al., 2007; Stanley et al., 2005). However, marine mammal hearts can experience large reductions in blood flow due to diving-associated bradycardia, and may

therefore be better adapted to withstand low O_2 conditions than hearts of terrestrial species, should workload exceed the ability of endogenous reserves and vascular supply to meet O_2 demands (Blix et al., 1983; Henden et al., 2004; Kjekshus et al., 1982; Zapol et al., 1979).

While large tissue O_2 stores, high acid buffering (β) ability and the ability to use a mixture of respiratory pathways allow seal skeletal and cardiac muscles to function during diving-induced hypoxia, these adaptations are unlikely to be fully mature at birth. In developing terrestrial species, the skeletal muscles of neonates are typically less differentiated than those of adults, and even in very precocial species, such as guinea pigs, postnatal development includes changes in fiber size, capillarization, myosin heavy chain isoforms, protein content and enzyme levels (Briand et al., 1993; Glatz and Veerkamp, 1982; Ostadal et al., 1999; Stanley et al., 2005). While many of these aspects have yet to be examined in marine mammals, studies have revealed that pinniped pup skeletal muscles have lower [Mb], β and altered enzyme profiles than adults (Burns et al., 2007; Kanatous et al., 2008; Lestyk et al., 2009; Prewitt, 2008). In addition, there is little change in skeletal muscle mass, [Mb] or β during the nursing period (Burns et al., 2007; Lestyk et al., 2009). While slow skeletal muscle development may be due to low activity levels and limited hypoxic challenge (Brooks et al., 2005; Hoppeler and Fluck, 2007), the functional consequence for young pups is that, at weaning, their skeletal muscles are not as able to support aerobic underwater exercise as adults; thus, limiting their ability to dive and forage (Burns, 1999).

In contrast to the limited demands placed on skeletal muscle early in life, the heart begins rhythmic contractions *in utero*, and therefore may mature at an earlier age (Burggren, 2004; Liggins et al., 1980). However, because of the low $\dot{P}_{O_2}$ *in utero*, the fetal heart relies almost entirely on glycolysis and monocarboxylate oxidation for ATP production, and has limited ability to oxidize fatty acids (Stanley et al., 2005). Postnatal development is therefore characterized by a rapid upregulation of lipoloytic ability and a decline in carbohydrate utilization. This is accompanied by changes in enzymes such as CS and HOAD, whose levels are proportional to the maximum flux through the Krebs and β oxidation cycle, respectively (Lopaschuk et al., 1992; Ostadal et al., 1999; Winder et al., 1974), and in LDH levels, which in the heart are proportional to lactate oxidation rates (Driedzic et al., 1987).

While the basic physiological changes that take place postnatally in young seals are likely to be similar to those in terrestrial species, two factors suggest that the development may need to occur more rapidly. First, neonatal seals are nursed on an extremely high fat (>50%), low carbohydrate (<10%) diet (Oftedal, 1993), and may therefore need to rely more heavily on lipoloysis at an earlier age. Second, young seals born in arctic environments rely on high rates of metabolic heat production to meet thermoregulatory demands, and therefore have very high metabolic rates (Blix and Steen, 1979; Ostadal et al., 1999). Conversely, immature ability to reduce diving blood flow and heart rate during apnea and diving may favor maintenance of cardiac glycolytic abilities (Greaves et al., 2005; Kodde et al., 2007). However, immature cardiac O_2 stores, enzyme levels and fuel-use patterns could limit the heart's ability to function during dives, and therefore impact diving and foraging activities of young pups.

The objective of this study was to characterize indices of aerobic and anaerobic metabolism in seal cardiac muscle to determine if O_2 stores, buffering ability and the activities of CS, HOAD and LDH potentially limit performance to a similar degree as in skeletal muscle, or if early development of cardiac contractile function equates to early metabolic maturity. This work addresses three hypotheses: (1) that due to constant perfusion during diving, seal cardiac muscle relies more exclusively on aerobic respiration than skeletal muscle, and therefore has higher aerobic enzyme activities; (2) that despite early contractile activity, the cardiac tissue of neonatal seals has lower aerobic and anaerobic enzyme activities than in mature seals; and (3) that due to early functional demands cardiac tissue matures more rapidly than skeletal tissue.

Muscle development rates differ in precocial and altricial species (Dietz and Ricklefs, 1997; Ostadal et al., 1999; Shea et al., 2007), and this study focuses on cardiac and skeletal muscle maturation in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals, two pelagic pack-ice-breeding species that differ in their life history and foraging strategies. Hooded seal pups are highly precocial (12.3% maternal mass at birth), are born with substantial insulative subcutaneous blubber reserves, have the shortest nursing period of any mammal (3.6 days) and gain mass at a remarkable $7\,kg\,day^{-1}$. Following weaning, pups fast on the ice for approximately one month, during which time they lose approximately a third of the mass gained during the nursing period (Lavigne and Kovacs, 1988). By contrast, newborn harp seal pups are smaller (8% maternal mass), and rely on shivering and non-shivering thermogenesis to maintain core temperature until their blubber layer develops (Blix and Steen, 1979). The more altricial status of harp seals is also reflected in the longer period of maternal care (12 days) and a postweaning fast of 5–6 weeks (Lavigne and Kovacs, 1988). Thus, harp seal pups have a terrestrial period that is ~30% longer than hooded seals. As a result, this study will also address a fourth hypothesis, i.e. that cardiac and skeletal muscle development occurs more extensively prenatally, and/or proceed more rapidly postnatally, in hooded than in harp seal pups.

MATERIALS AND METHODS

Skeletal *longissimus dorsi*, used to power swimming activities [and consisting of >50% Type I fibers (J.M.B., unpublished)] and heart (left ventricle) muscle samples were collected from 30 hooded [*Cystophora cristata* (Erxleben, 1777)] and 42 harp (*Pagophilus groenlandicus*, Erxleben, 1777) seals from the Gulf of St Lawrence stock near Prince Edward Island, Canada. In addition, total muscle mass and heart mass were measured in 30 animals collected from the Greenland Sea (for details, see Burns et al., 2007; Lestyk et al., 2009). Hooded seal pups were categorized as neonatal (1 day), nursing (2–4 days) or weaned following Bowen et al. (Bowen et al., 1987). Harp seal pups were aged based on appearance, and categorized as fetal, neonates (1–3 days), early weaned (~12 days) and late weaned (>25 days) (Table 1). Samples were collected in the field and stored frozen (–80°C) until analyzed. Animal handling and experimental protocols were approved by Department of Fisheries and Oceans, Canada, and the University of Alaska Anchorage Institutional Animal Care and Use Committee. Samples were imported into the United States under Marine Mammal permit 782-1399.

The Mb content ($mg\,g^{-1}$ wet tissue) and β (measured in slykes, the moles of NaOH required to increase the pH level 1 unit per gram of wet tissue) of skeletal and cardiac muscles were determined following previously published methods (Reynafarje, 1963; Castellini and Somero, 1981; Lestyk et al., 2009). Total protein content (TP, mg protein g^{-1} wet tissue mass) was determined using Pierce Coomassie Blue 'The Better Bradford' Total Protein Assay (Pierce Chemicals, Rockford, IL, USA). For these assays, frozen muscle samples were weighed, sonicated at 0°C in homogenization buffer ($50\,mmol\,l^{-1}$ imidazole, $1\,mmol\,l^{-1}$ EDTA, $2\,mmol\,l^{-1}$ $MgCl_2$,

Table 1. Age and mass (mean±s.e.m.) of hooded and harp seals handled in Canada in 2005 and 2008, and used in this study

Species	Age class	Sample size (M:F)	Mean mass (kg)	Muscle mass (% total mass)*	Cardiac mass (% total mass)
Harp	Fetus	6 (2:4)	8.4±0.5		
	Neonates	11 (3:8)	9.6±0.7	18.4±0.8	0.61±0.02 (4)
	Early weaned	6 (6:0)	41.5±2.0		
	Late weaned	9 (5:4)	32.6±1.4		
	Adults	10 (0:10)	114.9±5.1	25.6±0.6	0.69±0.04 (4)
Hooded	Neonates	10 (7:3)	23.6±0.9	19.6±1.0	0.67±0.07 (2)
	Weaned	10 (5:5)	48.4±1.2	20.1±1.2	0.59±0.03 (11)
	Adult	10 (3:7)	271.3±17.0	28.0±0.6	0.68±0.01 (9)

Relative cardiac and skeletal muscle mass data come from seals handled in 1999 and 2000 in Norway, with sample size provided in parentheses. *Initially reported in Lestyk et al., 2009.

pH 7.0) and centrifuged at 10,000 ***g*** for 5 min at 4°C. Then, 10 µl of the supernatant was diluted to 300×, mixed with dye, incubated at room temperature for 10 min and read at λ=595 nm. Total protein was calculated from plate-specific standard curves derived from bovine serum albumin standards.

To evaluate the ability of cardiac tissue to produce ATP under aerobic and anaerobic conditions, CS, HOAD and LDH activities (µmol min^{-1} g^{-1} wet tissue mass) were determined under substrate saturating conditions at experimentally determined dilution factors following published protocols (Polasek et al., 2006; Prewitt, 2008; Reed et al., 1994). All kinetic assays were performed on the supernatant of crude whole-muscle homogenates in a Molecular Devices (Sunnyvale, CA, USA) SpectraMax 340 microplate reader held at 37°C, using aliquots of the above supernatant. The assay formulae were as follows: CS (EC 4.1.3.7): 0.25 mmol l^{-1} DTNB, 0.4 mmol l^{-1} acetyl coA, 0.5 mmol l^{-1} oxaloacetate, 50 mmol l^{-1} imidazole buffer, pH 7.5 at 37°C, ΔA_{412}, millimolar extinction coefficient ε_{412}=13.6; HOAD (EC 1.1.1.35): 0.3 mmol l^{-1} NADH, 1 mmol l^{-1} EDTA, 0.2 mmol l^{-1} acetoacetyl coA, 50 mmol l^{-1} imidazole buffer, pH 7.0 at 37°C, ΔA_{340}, millimolar extinction coefficient ε_{340}=6.22; and LDH (EC 1.1.1.27): 0.3 mmol l^{-1} NADH, 1 mmol l^{-1} pyruvate, 50 mmol l^{-1} imidazole buffer, pH 7.0 at 37°C, ΔA_{340}, millimolar extinction coefficient ε_{340}=6.22. Absolute activities (i.u. g^{-1} wet tissue mass) were calculated from the change in absorbance at the maximal linear slope of the assay. The ratio of CS:HOAD was used to estimate the reliance on aerobic oxidation of lipids to fuel ATP production, and the ratio of LDH:CS was used to estimate the relative importance of aerobic *vs* anaerobic metabolism (Winder et al., 1974).

All samples were assayed in quadruplicate, and runs included a tissue control from harbor seals. Samples were reanalyzed if the coefficient of variance exceeded 10%, and data were screened for outliers and normality prior to analysis. General Linear Models (SPSS v.14.0, SPSS, Inc., Chicago, IL, USA) were used to compare Mb, TP, β, enzyme activities and enzyme ratios in muscle between age classes (pup and adult), muscle type (cardiac *vs* skeletal) and species (harp *vs* hood). In addition, to control for the potentially confounding effect of age-related increases to tissue TP content, the ratios of Mb, β, CS, HOAD and LDH to TP content were also examined (Mb_{TP}, etc.). Due to significant three-way interactions (ANOVA, P<0.05, Bonferroni *post-hoc* tests), age-related differences in all parameters were analyzed separately for each

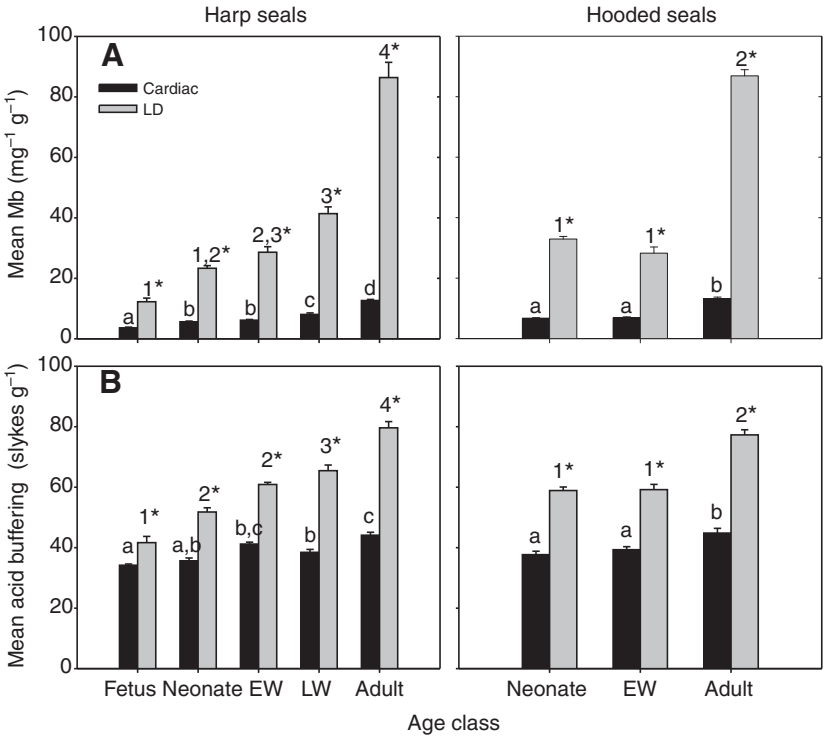

Fig. 1. Age-related changes in mean (±1 s.e.m.) (A) Myoglobin (Mb) values (mg g^{-1} wet tissue) and (B) acid buffering ability (β) in cardiac and skeletal muscles for harp and hooded seals. Significant differences between muscles within each age class (fetus, neonate, early weaned, late weaned and adult) are indicated by *. Similar superscript letters (cardiac) or numbers (skeletal) indicate like values across age classes within each muscle type. EW, early weaned; LW, late weaned.

species and muscle type whereas differences due to muscle type were analyzed for each age class and species separately. To assess the relative maturity of measured parameters, the absolute value of the difference between individual pup values and the mean adult value was calculated, and then scaled to the mean adult value. These values were then compared first between harp and hooded seal pups within each muscle type and then second between muscle types within each species using one-way ANOVA. Significant differences were assumed at $P<0.05$.

RESULTS

Differences by tissue type and age

In both harp and hooded seals, there were significant differences in all measured parameters, both between muscle types within age classes and across age classes. As expected based on perfusion and use patterns, [Mb] and β were significantly lower in cardiac tissue than in skeletal tissue for all age classes in both species (Fig. 1; Mb harp: $F_{1,83}=489.5$, $P<0.001$; Mb hooded: $F_{1,58}=388.1$, $P<0.001$; β harp: $F_{1,83}=512.0$, $P<0.001$; β hooded: $F_{1,58}=434.8$, $P<0.001$). Similarly, the higher metabolic rate and increased reliance on aerobic metabolic pathways in cardiac tissue was reflected in significantly elevated CS and HOAD activities and lower LDH activity as compared with skeletal muscle, in all ages and both species (Fig. 2; CS harp: $F_{1,83}=392.7$, $P<0.001$; CS hooded: $F_{1,59}=299.2$, $P<0.001$; HOAD harp: $F_{1,83}=156.6$, $P<0.001$; HOAD hooded: $F_{1,59}=73.9$, $P<0.001$; LDH harp: $F_{1,83}=177.3$, $P<0.001$; LDH hooded: $F_{1,59}=169.1$ $P<0.001$). Muscle TP was also consistently lower in cardiac muscles than in skeletal muscle (Fig. 3; harp: $F_{1,83}=67.4$, $P<0.001$; hooded: $F_{1,58}=166.4$, $P<0.001$).

Despite early contractile activity and mature size (~0.65% body mass, no effect of age, Table 1), the TP content in cardiac muscle was lower in pups than in adults (harp: $F_{4,41}=5.6$, $P<0.001$; hooded: $F_{2,29}=4.3$, $P=0.024$, see Fig. 3). In addition, the ability of cardiac tissue to store O_2 and produce ATP aerobically was not mature at birth in either species, as indicated by significantly lower Mb, CS and HOAD activities in neonatal pups as compared with adults, and the significant

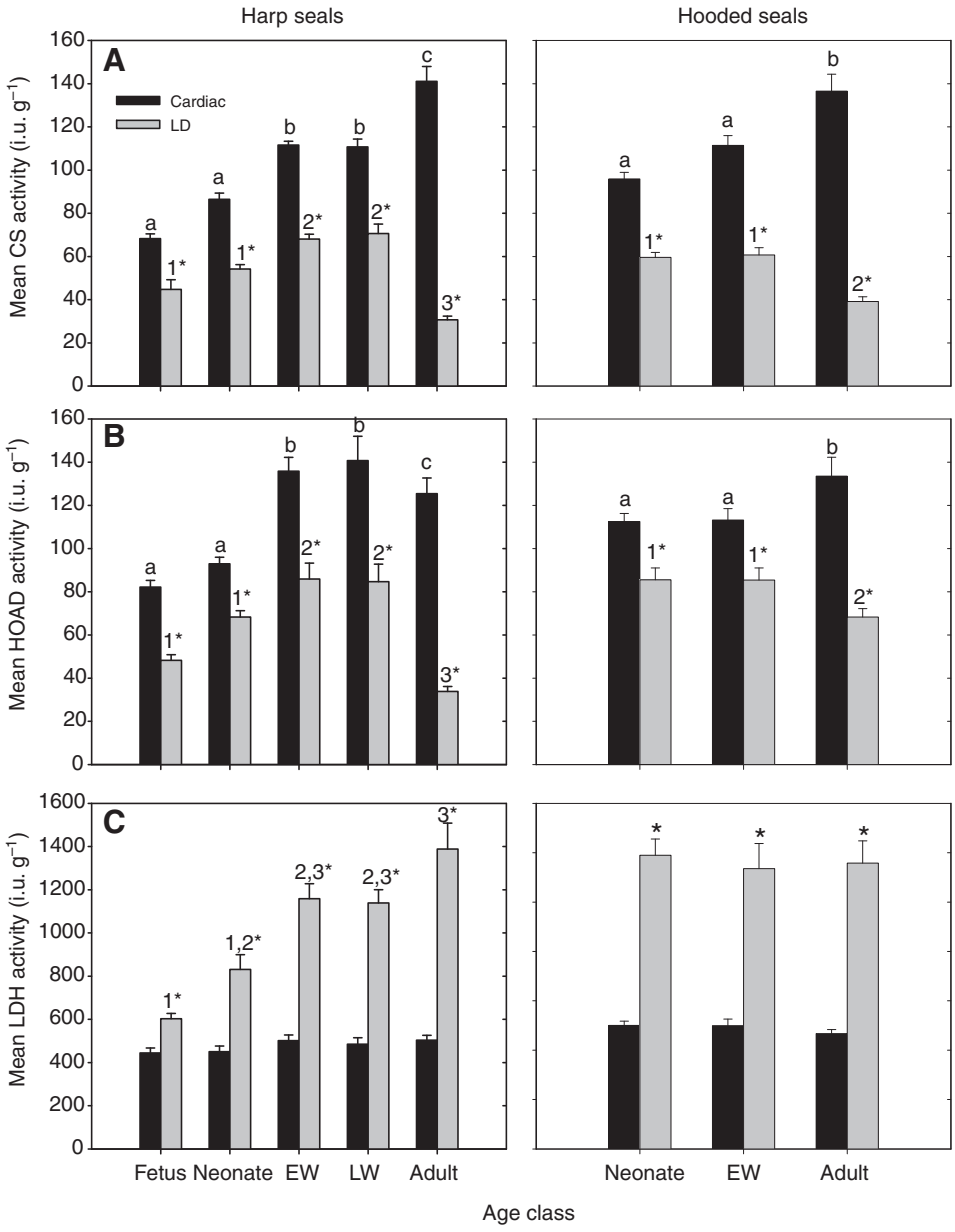

Fig. 2. Age-related changes in mean (±1 s.e.m.) (A) citrate synthase (CS), (B) β-hydroxyacyl-CoA dehydrogenase (HOAD) and (C) lactate dehydrogenase (LDH) enzyme activities (i.u. g^{-1} wet tissue) in cardiac and skeletal muscles for harp and hooded seals. Annotation as in Fig. 1. EW, early weaned; LW, late weaned.

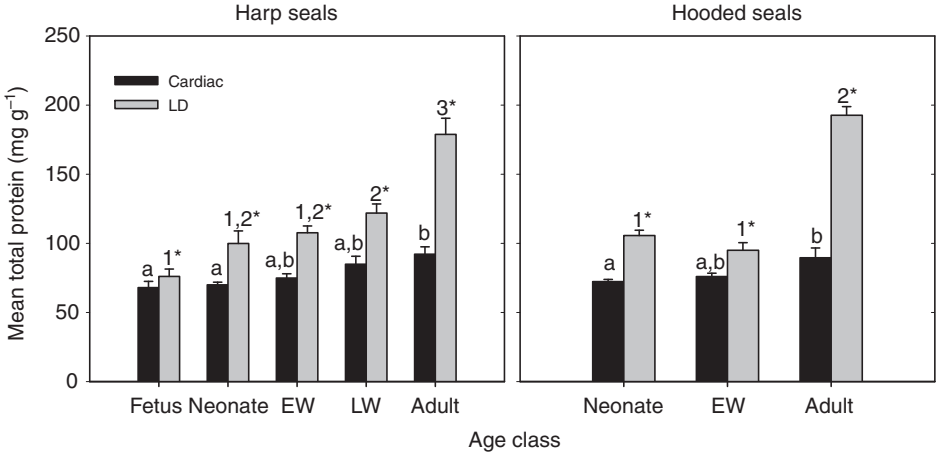

Fig. 3. Age-related changes in mean (±1 s.e.m.) total protein content (mg g^{-1}) in cardiac and skeletal muscle for harp and hooded seals. Annotation as in Fig. 1. EW, early weaned; LW, late weaned.

increase with age during the nursing period (Mb harp: $F_{4,41}$=100.3, P<0.001; CS harp: $F_{4,41}$=36.8, P<0.001; HOAD harp: $F_{4,41}$=11.1, P<0.001; Mb hooded: $F_{2,28}$=92.3, P<0.001; CS hooded: $F_{2,29}$=13.7, P<0.001; HOAD hooded: $F_{2,29}$=3.6, P=0.041, see Fig. 1A and Fig. 2A,B). By contrast, cardiac LDH activity did not differ significantly by age in either harp or hooded seals (Fig. 2C). While the β ability in the cardiac tissue of young seals was significant lower than that in adult hearts, the absolute difference was small (<20%; harp: $F_{4,41}$=20.0, P<0.001; hooded: $F_{2,28}$=8.0, P<0.05, see Fig. 1B).

Conversely, despite limited exercise and no diving activity, there were marked age-related increases in the mass (from <20% of total body mass in neonates to >25% in adults; see Table 1), TP content (harp: $F_{4,41}$=18.1, P<0.001; hooded: $F_{2,28}$=100.6, P<0.001, see Fig. 3) and metabolic characteristics of skeletal muscle. Muscle Mb loads were very low at birth and increased only slightly during the nursing period, such that pups were weaned with values only ~30% those of adults (harp: $F_{4,41}$=96.9, P<0.001; hooded: $F_{2,29}$=346.3, P<0.001, see Fig. 1A). Remarkably, despite these low O_2 stores, aerobic enzyme levels were markedly higher in pup skeletal muscles than in samples from adults, and values increased, rather than declined, during the nursing period (CS harp: $F_{4,41}$=30.8, P<0.001; HOAD harp: $F_{4,41}$=20.3, P<0.001; CS hooded: $F_{2,29}$=20.6, P<0.001; HOAD hooded: $F_{2,29}$=3.9, P=0.033, see Fig. 2A,B). By contrast, LDH levels were lower than adults at birth, but increased during the nursing period so that by the time pups were weaned, LDH activity in skeletal muscles was similar to adults (Fig. 2C). Buffering ability was also fairly mature at birth and increased significantly during the nursing and postweaning periods (harp: $F_{4,41}$=62.1, P<0.001; hooded: $F_{2,29}$=45.9, P<0.001, see Fig. 1B).

As a result of the differing trends in the aerobic and anaerobic enzymes among muscles and age classes, the metabolic profiles of cardiac and skeletal tissues diverged with age. In cardiac tissue, the CS:HOAD ratio increased with age while the LDH:CS ratio declined significantly with age (CS:HOAD harp: $F_{4,41}$=5.97, P<0.001; CS:HOAD hooded: $F_{2,29}$=2.53, P=0.098; LDH:CS harp: $F_{4,41}$=17.6, P<0.001; LDH:CS hooded: $F_{2,29}$=11.7, P<0.001); both largely due to increases in cardiac CS activity. By contrast, in skeletal tissue the CS:HOAD ratio did not vary with age while the LDH:CS ratio increased significantly with age in both harp and hooded seals (harp: $F_{4,40}$=62.3, P<0.001; hooded: $F_{2,29}$=9.7, P=0.001). As a result, mature skeletal LDH:CS ratios were nearly 10× those in cardiac tissue (Table 2).

Maturation rates by tissue type and species

At birth, the cardiac muscle of both harp and hooded seal pups had Mb, β, TP, CS, HOAD and LDH values that were significantly more similar to those of adults than did the skeletal muscle. This difference was still apparent at weaning, for all parameters except LDH and CS:HOAD ratios (Table 3). In addition, in both cardiac and skeletal muscles, markers of anaerobic metabolism (LDH, β) were more similar to adult values at birth and weaning than were markers of aerobic metabolism (CS, Mb). Interspecific differences in the absolute values, relative maturity at birth or weaning, and maturation rates were far more prevalent in skeletal muscle than cardiac muscle, in aerobic than anaerobic markers and in neonatal rather than weaned pups. In those cases where there were differences, hooded seal muscles were consistently more mature than in harp seal pups. For example, the hearts of hooded seal neonates had Mb, CS and HOAD levels much more similar to those of adults than

Table 2. Mean (±s.e.) cardiac and skeletal CS:HOAD and LDH:CS activities for harp and hooded seals

Species	Age class	CS:HOAD cardiac	CS:HOAD skeletal	LDH:CS cardiac	LDH:CS skeletal
Harp	Fetus	**0.84±0.05^{a}**	0.93±0.10	**6.53±0.37^{a}**	**14.40±1.99^{a,*}**
	Neonates	**0.94±0.05^{a,b}**	0.81±0.05	**5.19±0.20^{b}**	**15.41±1.22^{a,*}**
	Early weaned	**0.83±0.05^{a}**	0.82±0.08	**4.49±0.20^{b,c}**	**17.14±1.24^{a,*}**
	Late weaned	**0.82±0.06^{a}**	0.86±0.06	**4.42±0.31^{b,c}**	**16.43±0.86^{a,*}**
	Adults	**1.14±0.06^{b}**	0.93±0.06*	**3.60±0.14^{c}**	**41.61±2.03^{b,*}**
Hooded	Neonates	**0.86±0.04^{a,b}**	0.73±0.07*	**5.25±0.21^{a}**	**20.17±1.21^{a,*}**
	Weaned	**1.01±0.08^{b,c}**	0.73±0.05*	**4.57±0.34^{a}**	**19.06±1.58^{a,*}**
	Adult	**1.04±0.05^{c}**	0.59±0.05*	**3.51±0.20^{b}**	**30.13±2.75^{b,*}**

Significant differences between muscles within each age class are indicated by *. Bold values differ by age class within each species with similar superscript letters indicating like values across age classes within each muscle type. CS, citrate synthase; HOAD, β-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase.

Table 3. Relative maturity of cardiac and skeletal muscle physiological parameters, shown as the mean ± s.e. proportionate difference between values in neonatal pups compared with those in adults

	Harp seal pups Cardiac		Harp seal pups Skeletal	Hooded seal pups Cardiac		Hooded seal pups Skeletal
Mb	–55.3±1.7 (–51.4±2.1)	> >	–73.0±1.0 (–66.8±2.1)	**–48.8±1.5** (–47.9±2.5)	> >	**–62.1±1.0** (–67.5±2.4)
TP	–24.1±2.1 (–18.8±3.2)	> >	–44.1±5.1 **(–39.8±2.7)**	–19.3±1.8* (–15.2±2.6)	> >	–45.2±2.1 (–50.7±2.9)
β	–19.3±2.1 (–6.8±1.5)	> >	–34.9±1.7 (–23.5±0.8)	–15.9±2.5 (–12.2±2.2)	> >	**–23.8±1.5** (–23.4±2.2)
LDH	–10.5±5.2 (–0.3±5.3)	> =	–40.1±4.9 (–16.5±5.0)	7.1±3.7 (6.9±5.8)	= =	**2.7±5.6** (2.0±8.8)
CS	–38.7±2.1 (–20.9±1.3)	> >	76.8±6.5 (121.9±7.2)	**–29.8±2.3** (–18.4±3.3)	> >	**52.1±5.9 (54.9±8.6)**
HOAD	–25.9±2.6 (8.2±5.1)	> >	102.0±8.6 (154.1±21.7)	**–15.7±2.8** (–15.2±3.9)	= >	**25.3±8.0 (25.2±8.1)**
CS:HOAD	–17.9±4.5 (–26.9±4.7)	= =	–12.6±6.5 (–11.4±9.0)	–17.3±3.8 (–2.8±7.6)	= =	–24.2±12.5 (–24.1±9.1)
LDH:CS	44.4±5.5 (24.7±5.6)	> >	–66.7±2.6 (–63.0±2.7)	49.5±5.9 (30.2±9.6)	< =	**–33.1±4.0 (–36.7±5.2)**

Values in parentheses are the mean ± s.e. proportionate difference between weaned pup and adult values.

Values closer to zero indicate greater similarity between pups and adults, with positive values indicating that pups values were larger than those of adults. Within each species, symbols indicate whether the cardiac muscle was significantly (ANOVA, $P<0.05$) similar to (=) or more (>) or less (<) mature than skeletal muscle. Within each muscle and age group (i.e. cardiac tissue, neonates), the species highlighted in bold was significantly more mature (ANOVA, $P<0.05$).

Mb, myoglobin; TP, total protein content; CS, citrate synthase; HOAD, β-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase.

did harp seal neonates, although most differences were gone by weaning (Table 3). By contrast, the skeletal muscles of hooded seal neonates had aerobic and anaerobic metabolic indicators more similar to adults than did harp seal skeletal muscles, and this pattern persisted to weaning (Table 3).

DISCUSSION

Both the skeletal and cardiac muscles of adult seals exhibit adaptations to diving hypoxia primarily at the levels of elevated tissue O_2 stores (Mb) and enzyme ratios that reflect a heavy reliance on lipid-based oxidative metabolism. Together with studies indicating that work load drops in parallel with heart rate and blood flow during dives (Blix et al., 1983; Elsner et al., 1985; Hochachka, 1981; Murphy et al., 1980; Zapol et al., 1979), these findings suggest that peripheral vasoconstriction and central bradycardia maintain cardiac $\dot{P}_{O2}$ at levels that do not require significant increases in tissue O_2 reserves or substantial alterations of aerobic metabolic pathways. By contrast, the immature Mb reserves, reduced β ability and altered enzyme levels found in the cardiac and skeletal muscles of pups suggests that both tissues are less able to support aerobic metabolism during dives; thus, providing a potential explanation for the long postweaning fasts seen in these species (Lavigne and Kovacs, 1988). Physiological maturity was achieved at a younger age in cardiac tissue than in skeletal tissue, and in hooded seals as compared with harp seals, as expected due to activity levels, perfusion patterns, metabolic substrate preferences and life-history traits. Therefore, the most notable finding of this work was the overall similarity between muscle development in seals and other mammals, suggesting that limits on phenotypic plasticity and developmental rates can and do constrain behavioral options in young pinnipeds.

As in all terrestrial species, seal cardiac muscle has lower Mb and β and higher aerobic enzyme activities than skeletal muscle (Bishop et al., 1995; Gondret et al., 2004; Polasek et al., 2006; Stewart et al., 2005; Zonderland et al., 1999). However, seal cardiac Mb levels are more similar to terrestrial values than are skeletal muscle Mb values (Table 4), suggesting that the heart does not require large endogenous O_2 stores to continue functioning during dives. That seal cardiac muscle relies primarily on aerobic pathways for ATP production is also indicated by cardiac CS and HOAD activities that were slightly higher than those reported in other large terrestrial and marine mammals (Fuson et al., 2003; Murphy et al., 1980; Ohtsuka and Gilbert, 1995). While the high CS levels are likely to be due to cardiac tissue's high metabolic rates and MT content (Driedzic et al., 1987; Sordahl et al., 1983; Winder et al., 1974), muscle metabolic rate was not measured in this study. HOAD levels are likely to be elevated due to cardiac tissue's heavy reliance on lipid oxidation and the large dietary lipid intake. Indeed, the CS:HOAD ratio in seal hearts is much lower than is typically seen in terrestrial species (Table 4), and suggests that the ability of β-oxidation of lipids to produce acetyl-CoA for oxidative metabolism is nearly equivalent to the ability of the citric acid cycle to consume it (Stanley et al., 2005; Winder et al., 1974). Together all of these

Table 4. Myoglobin levels in locomotory skeletal muscle and left ventricle cardiac muscle from adult terrestrial and marine mammals, and cardiac CS:HOAD and LDH:CS ratios

Species	Sources	Skeletal Mb (mg g^{-1})	Cardiac Mb (mg g^{-1})	Cardiac CS:HOAD	Cardiac LDH:CS
Shrew	McIntyre et al., 2002; Stewart et al., 2005	11.4	9.2	10	0.8
Rat	Stewart et al., 2005; Daneshrad et al., 2000	3.1	1.9	5	4.9
Pika	Sheafor, 2003	–	–	1.7	0.7
Rabbit	Daneshrad et al., 2000; Sheafor, 2003	0.9	1.7	5	1.2
Dog	Polasek et al., 2006	3.5	1.8	3.3	0.7
Sheep	Griffiths et al., 1994; Ohtsuka and Gilbert, 1995	6.0	4.0	–	1.5
Pig	O'Brien et al., 1992; Castellini and Somero, 1981	1.7	1.0	–	17.5
Ox	Murphy et al., 1980	–	–	2.5	9.0
Weddell seal	Castellini and Somero, 1981; Murphy et al., 1980	44.6	–	1.7	35.8
Harbor seal	Polasek et al., 2006; Fuson et al., 2003	37.0	18.4	0.7	9.3
Harp seal	This study	86.4	12.7	1.1	3.6
Hooded seal	This study	86.9	13.2	1.0	3.5

Mb, myoglobin; CS, citrate synthase; HOAD, β-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase.

findings suggest that seal hearts are an extremely oxidative tissue, just as in their terrestrial counterparts.

From the perspective of the indicators of anaerobic and glycolytic metabolism measured in this study, seal hearts do not appear to be any better adapted to either produce ATP anaerobically, or withstand anaerobic byproducts, than hearts from terrestrial species. This can be seen by the generally much lower β ability (~50%) and LDH activity (~40%) in seal hearts as compared with skeletal muscle, and the similar, or even lower, LDH:CS ratios in seal hearts as compared with terrestrial mammals (Table 4). These findings suggest that in adult seals, as in other mammals, cardiac muscle rarely relies on anaerobic metabolism, instead oxidizing lipids and monocarboxylate fuels, such as lactate, for metabolic heat production (Hochachka, 1981; Murphy et al., 1980). However, seal hearts do differ from those of terrestrial mammals, in that they contain large glycogen stores (Henden et al., 2004; Hochachka, 1981; Kerem et al., 1973), and are able to withstand long periods of anaerobic respiration and reduced coronary blood flow without evidence of ischemic damage (Elsner et al., 1985; Kjekshus et al., 1982), indicating that seal hearts possess additional adaptations to withstand the progressive hypoxemia that accompanies diving that were not measured in this study.

The ability for young pups to regulate heart rate during dives emerges *in utero*, as fetal heart rates decline following maternal bradycardia (Bacon et al., 1985; Liggins et al., 1980), and although not fully mature, harbor seal pups demonstrate significant bradycardia during long dives, even at a very young age (Greaves et al., 2005). Despite this, the hearts of young seals are not mature at birth. As in terrestrial species (Baldwin et al., 1977; Griffiths et al., 1994; Ostadal et al., 1999; Stanley et al., 2005), aerobic indicators in seal hearts are much less mature at birth than are anaerobic indices. For example, compared with adult values, pup heart muscle has ~50% lower Mb, ~33% lower CS and ~20% lower HOAD levels, and ~15% lower β and ~5% lower LDH levels. Thus, postnatal cardiac development is characterized by the rapid upregulation of aerobic metabolism, as expected following the transition from a hypoxic intrauterine environment with its associated heavy reliance on glycolytic metabolism, to a well oxygenated environment where lipid is the primary fuel (Baldwin et al., 1977; Ohtsuka and Gilbert, 1995; Ostadal et al., 1999). In addition, increases in cardiac metabolic rate, as indicated by CS levels, are accompanied by increases in TP, probably due to fiber hypertrophy and increases in MT volume density (Glatz and Veerkamp, 1982; Morris et al., 1995). That these increases occur while pups are facing significant thermoregulatory but limited hypoxic challenge suggests that seal hearts are responding primarily to the dramatic increases in resting metabolic rates and lipid intake that accompany birth (Blix and Steen, 1979; Lopaschuk et al., 1992; Ostedal et al., 1999).

However, in contrast to terrestrial species, where postnatal upregulation of oxidative pathways is frequently accompanied by upregulation of glycolytic pathways (Baldwin et al., 1977; Bishop et al., 1995; Glatz and Veerkamp, 1982; Morris et al., 1995), the postnatal increases in anaerobic indices in seal hearts are much smaller (β) or non-existent (LDH). In the heart, LDH activity can reflect either the heart's need to reduce pyruvate under hypoxic conditions and/or its ability to oxidize lactate under aerobic conditions, with the LDH:CS ratio being significantly greater in tissues that rely more heavily on pyruvate reduction (Driedzic et al., 1987; Kodde et al., 2007; Ostadal et al., 1999). In cardiac muscle, the LDH:CS ratio is lower than in skeletal tissue and declines with age, suggesting that cardiac LDH activity is more indicative of reliance on pyruvate oxidation than sustained or increased anaerobic glycolysis (Driedzic et al., 1987; Hochachka, 1981). Therefore, just as adult hearts do not appear uniquely adapted for hypoxic functioning, neither do those of pups. Indeed, due to their lower blood and muscle O_2 stores [this study; (Burns et al., 2007)], higher resting metabolic rates and poor cardiovascular control (Greaves et al., 2005), similar anaerobic indices may translate into less tolerance for underwater metabolism in the hearts of young seal pups than in adults.

Early development of the heart's ability to function both on land and underwater is critical, as highlighted by the heart's greater relative maturity both at birth and weaning than the highly oxidative *longissimus dorsi* skeletal muscle. At the gross scale, this is reflected by the mature size and much higher protein content of hearts whereas at the biochemical level, both enzyme levels and fuel-use patterns (as indicated by enzyme ratios) are more similar to adult values in cardiac than skeletal muscles (see Table 2). In addition, postnatal cardiac muscle growth is accompanied by much smaller increases in TP content, suggesting that less tissue hypertrophy is required to reach mature status. These differences mirror those of terrestrial species (Table 5) (Briand et al., 1993; Griffiths et al., 1994; Ostadal et al.,

Table 5. Relative maturity of cardiac (left ventricle) and a major locomotory skeletal muscle at birth in a variety of vertebrates, expressed as a percentage of adult values

Muscle/species	CS activity (% adult)	HOAD activity (% adult)	LDH activity (% adult)	Source
Cardiac muscle				
Barnacle goose	55	26	70	Bishop et al., 1995
Sheep	39	–	80	Gondret et al., 2004
Harp seal	61	74	89	This study
Hooded seal	70	84	107	This study
Rat	45	42	66	Baldwin et al., 1977; McGuire et al., 1990
Skeletal muscle				
Barnacle goose	12	13	6	Bishop et al., 1995
Rabbit	58	–	24	Gondret et al., 2004
Dog	30	–	93	Castellini and Somero, 1981
Harp seal	177	202	60	This study
Hooded seal	152	125	103	This study
Rat	43	12	40	Glatz and Veerkamp, 1982; McGuire et al., 1990; Nemeth et al., 1989

CS, citrate synthase; HOAD, β-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase.

1999) and probably reflect the much higher contractile activity of young hearts. However, differences between the two tissues may also reflect the much higher myocytes proliferation and turnover rates in postnatal skeletal, as compared with cardiac, muscle (Bergmann et al., 2009; Terman and Brunk, 2004). Lower cardiac myocyte turnover postnatally may require more extensive prenatal cardiac development for timely emergence of the mature phenotype (Ostadal et al., 1999).

By contrast, skeletal muscle is known to undergo significant remodeling in response to a variety of stimuli, potentially allowing for more of the require changes to take place postnatally (Dietz and Ricklefs, 1997; Hoppeler and Fluck, 2007; Shea et al., 2007). However, there is little development of skeletal muscle during the nursing periods in seal pups (see Table 2), even though rapid development might improve its ability to function during diving. For example, skeletal muscle Mb loads are 75% lower in pups than in adults, indicating relatively poor tissue O_2 reserves during a period when the pups' CS and HOAD levels are 25–154% higher [probably due to their much higher mass-specific metabolic and lipid intake rates (Emmett and Hochachka, 1981)] and CS:HOAD ratios similar to those of adults. Thus, skeletal muscles of young seals appear to have a similar capacity to utilize O_2 for aerobic, lipid-based respiration as adults but lower ability to store O_2 for this metabolism, should blood flow be restricted. At the same time, the much lower β and LDH:CS ratio suggests that the ability of skeletal muscle to sustain anaerobic metabolism, if so required, is also reduced. Because skeletal muscle perfusion is sustained during the nursing period, neither immature O_2 reserves nor reduced anaerobic metabolic potential are likely to pose significant challenges to the maintenance of metabolic homeostasis. However, once seal pups begin diving and foraging, vasoconstriction may isolate skeletal muscles from vascular fuel sources (Davis and Kanatous, 1999; Guyton et al., 1995) at a time when endogenous O_2 reserves are low and likely rapidly consumed by tissues with limited glycolytic capacity.

While both the cardiac and skeletal muscles of newly weaned harp and hooded seal pups seem poorly adapted to support the challenges of underwater activities, it may be that prenatal and terrestrial development can only prepare muscle so far, and exercise and/or hypoxic challenge is required for final maturation (Brooks et al., 2005). Certainly, pre- and postnatal maturation rates are responsive to life-history strategies, with cardiac and skeletal muscles of both seals being quite mature at birth, particularly as compared with terrestrial species (Table 5). In addition, hooded seals were slightly more mature at birth than harp seals, as predicted based on the shorter period of time available for postnatal development prior to the initiation of underwater foraging activities. However, interspecific differences were less evident in cardiac tissue, perhaps for reasons already discussed.

Because cardiac and skeletal muscles respond differently to hypoxic and endurance exercise challenges (Daneshrad et al., 2000; Kainulainen et al., 1984; Ohtsuka and Gilbert, 1995; Sheafor, 2003; Winder et al., 1974; Zonderland et al., 1999), it is possible to speculate about the relative importance of these two stimuli on final tissue maturation. In general, endurance exercise causes much larger increases in CS, HOAD, MT density, Mb and lipid stores than in the glycolytic potential of skeletal muscle, but has little to no impact on these parameters in the heart (Brooks et al., 2005; Driedzic et al., 1987; Stanley et al., 2005; Zonderland et al., 1999). By contrast, hypoxic challenge is associated with reductions in aerobic lipolysis and increases in Mb and glycolytic potential in both skeletal and cardiac muscles (Hoppeler and Vogt, 2001; Ohtsuka and Gilbert, 1995; Reynafarge, 1962; Sheafor, 2003). However, because CS levels increase in the heart, but not in skeletal muscle, hypoxic challenge causes increases in the CS:HOAD ratio only in the heart (Daneshrad et al., 2000; Ohtsuka and Gilbert, 1995; Winder et al., 1974). In this study, the changes observed in the skeletal muscle of seal pups from weaning to maturity more closely parallel those associated with hypoxic challenge than with (perfused) exercise, while changes in cardiac tissue seem to reflect both stimuli.

In summary, this study found that skeletal and cardiac muscles of harp and hooded seals pups and adults exhibit different adaptations in response to the need to sustain work while seals forage underwater. Skeletal muscle adaptations (high Mb, β, LDH and low CS:HOAD ratios) primarily reflect evolution of highly efficient oxidative metabolism, despite the isolation from vascular O_2 supply that accompanies diving vasoconstriction. By contrast, measured parameters suggest that cardiac blood flow supplies sufficient O_2 to sustain lipolytic pathways even during dives. In addition, cardiac tissue was more mature than skeletal muscles at birth and weaning, probably due to its earlier workload and lower cellular turnover rate. Still, neither tissue was fully mature at weaning, with the differences suggesting that pup muscles are less able to sustain both aerobic and anaerobic ATP production during dives than adults; thus, providing a potential explanation for the postweaning fast. If seals must begin diving before final muscle maturation, initial foraging behaviors will probably reflect their reduced O_2 stores, and altered enzyme levels. Because the lower dive durations and increased recovery times would likely reduce foraging efficiency and thereby exert strong selective pressure on muscle maturation rates, the overall similarity of muscle development in seals and other mammals suggests that there are strong constraints on phenotypic plasticity and developmental rates in both cardiac and skeletal muscles.

LIST OF SYMBOLS AND ABBREVIATIONS

β	buffering ability (slykes g^{-1} wet tissue)
CS	citrate synthase activity (i.u. g^{-1} wet tissue)
HOAD	β-hydroxyacyl-CoA dehydrogenase activity (i.u. g^{-1} wet tissue)
LDH	lactate dehydrogenase activity (i.u. g^{-1} wet tissue)
Mb	myoglobin (mg Mb g^{-1} wet tissue)
O_2	oxygen
$\dot{P}_{O_2}$	partial pressure of oxygen
TP	total protein content (mg protein g^{-1} wet tissue)

ACKNOWLEDGEMENTS

We thank the Canadian Coast Guard helicopter pilots Harrison McRae and Bruce Kendall, the Château Madelinot and Roger Simon for providing laboratory space and Lena Measures, Sam Turgeon and Linnea Pearson for field assistance. Animal capture and experimental protocols were conducted under permits from the Department of Fisheries and Oceans, Canada and the University of Alaska Anchorage Institutional Animal Care and Use Committee. All samples were imported into the United States under Marine Mammal permits 782-1399 and 782-1694-02. This study was financed in part by contributions from the Alaska Heart Institute, UAA Office of Undergraduate Research and the Department of Fisheries and Oceans, Canada.

REFERENCES

Bacon, J. P., Mackay, R. S., Cohn, H. E. and Lee, A. F. S. (1985). Development of a biomodal heart rate pattern in undisturbed fetal harbor seals. *Mar. Mamm. Sci.* **1**, 294-303.

Baldwin, K. M., Cooke, D. A. and Cheadle, W. G. (1977). Enzyme alterations in neonatal heart muscle during development. *J. Mol. Cell. Cardiol.* **8**, 651-660.

Bergmann, O., Bhardwaj, R. D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicih, J., Alkass, K., Buchholz, B. A., Druid, H. et al., (2009). Evidence for cardiomyocyte renewal in humans. *Science* **324**, 98-102.

Bishop, C. M., Butler, P. J., Egginton, S., El Haj, A. J. and Gabrielsen, G. W. (1995). Development of metabolic enzyme activity in locomotor and cardiac muscles of the migratory barnacle goose. *Am. J. Physiol.* **269**, R64-R72.

Blix, A. S. and Steen, J. B. (1979). Temperature regulation in newborn polar homeotherms. *Physiol. Rev.* **59**, 285-304.

Blix, A. S., Elsner, R. W. and Kjekshus, J. K. (1983). Cardiac output and its distribution through capillaries and A-V shunts in diving seals. *Acta. Physiol. Scand.* **118**, 109-116.

Bowen, W. D., Boness, D. J. and Oftedal, O. T. (1987). Mass transfer from mother to pup and subsequent mass loss by the weaned pup in the hooded seal, *Cystophora cristata*. *Can. J. Zool.* **65**, 1-8.
Burggren, W. (2004). What is the purpose of the embyronic heart beat? or How facts can ultimately prevail over physiological dogma. *Phys. Biochem. Zool.* **77**, 333-345.
Briand, M., Boissonnet, G., LaPlace-Marieze, V. and Briand, Y. (1993). Metabolic and contractile differentiation of rabbit muscles during growth. *Int. J. Biochem.* **25**, 1881-1887.
Brooks, G. A., Fahey, T. D. and Baldwin, K. M. (2005). *Exercise Physiology.* 4th ed. p. 876. New York, NY: McGraw-Hill.
Burns, J. M. (1999). The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. *Can. J. Zool.* **77**, 737-747.
Burns, J. M., Lestyk, K. C., Folkow, L. P., Hammill, M. O. and Blix, A. S. (2007). Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. *J. Comp. Physiol. B* **177**, 687-700.
Butler, P. J. and Jones, D. R. (1997). Physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837-899.
Castellini, M. A. and Somero, G. N. (1981). Buffering capacity of vertebrate muscle: correlations with potential for anaerobic function. *J. Comp. Physiol. B* **143**, 191-198.
Castellini, M. A., Somero, G. N. and Kooyman, G. L. (1981). Glycolytic enzyme activities in tissues of marine and terrestrial mammals. *Physiol. Zool.* **54**, 242-252.
Daneshrad, Z., Garcia-Riera, M., Verdys, M. and Rossi, A. (2000). Differential response to chronic hypoxia and dietary restriction of aerobic capacity and enzyme levels in the rat myocardium. *Mol. Cell. Biochem.* **210**, 159-166.
Davis, R. W. and Kanatous, S. B. (1999). Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. *J. Exp. Biol.* **202**, 1091-1113.
Dietz, M. W. and Ricklefs, R. E. (1997). Growth rate and maturation of skeletal muscles over a size range of Galliform birds. *Physiol. Zool.* **70**, 502-510.
Driedzic, W. R., Sidell, B. D., Stowe, D. and Branscombe, R. (1987). Matching of vertebrate cardiac energy demand to energy metabolism. *Am. J. Physiol.* **252**, R930-R937.
Elsner, R. W., Millard, R. W., Kjekshus, J. K., White, F. C., Blix, A. S. and Kemper, W. S. (1985). Coronary blood flow and myocardial segment dimensions during simulated dives in seals. *Am. J. Physiol.* **249**, H1119-H1126.
Emmett, B. and Hochachka, P. W. (1981). Scaling of oxidative and glycolytic enzymes in mammals. *Respir. Physiol.* **45**, 261-272.
Erxleben, J. C. P. (1777). Systema Regni Animalis per classes, ordines, genera, species, varietates cum synonymia et historia animalium. Classis I. Mammalia. p. xlviii-636. Lipsiæ [Leipzig]: Impensis Weygandianis.
Fuson, A. L., Cowan, D. F., Kanatous, S. B., Polasek, L. K. and Davis, R. W. (2003). Adaptations to diving hypoxia in the heart, kidneys, and splanchnic organs of harbor seals. *J. Exp. Biol.* **206**, 4139-4154.
Glatz, J. F. C. and Veerkamp, J. H. (1982). Postnatal development of pamitate oxidation and mitochondrial enzyme activities in rat cardiac and skeletal muscle. *Biochim. Biophys. Acta* **711**, 327-335.
Gondret, F., Damon, M., Jadhau, S. B., Houderbine, L.-M., Herpin, P. and Hocquette, J.-F. (2004). Age-related changes in glucose utilization and fatty acid oxidation in a muscle-specific manner during rabbit growth. *J. Muscle Res. Cell Motil.* **25**, 405-410.
Greaves, D. K., Schreer, J. F., Hammill, M. O. and Burns, J. M. (2005). Diving heart rate development in postnatal harbour seals, *Phoca vitulina*. *Phys. Bioch. Zool.* **78**, 9-17.
Griffiths, R. I., Baldwin, J. and Berger, P. J. (1994). Metabolic development of the sheep diaphram during fetal and newborn life. *Respir. Physiol.* **95**, 337-347.
Guyton, G. P., Stanek, K. S., Schneider, R. C., Hochachka, P. W., Hurford, W. E., Zapol, D. G., Liggins, G. C. and Zapol, W. M. (1995). Myoglobin saturation in free-diving Weddell seals. *J. Appl. Physiol.* **79**, 1148-1155.
Henden, T., Aasum, E., Folkow, L. P., Mjos, O. D., Lathrop, D. A. and Larsen, T. S. (2004). Endogenous glycogen prevents Ca2+ overload and hypercontracture in harp seal myocardial cells during simulated ischemia. *J. Mol. Cell. Cardiol.* **37**, 43-50.
Hochachka, P. W. (1981). Brain, lung and heart function during diving and recovery. *Science*. **212**, 509-514.
Hoppeler, H. and Fluck, M. (2007). Normal mammalian skeletal muscle and its phenotypic plasticity. *J. Exp. Biol.* **205**, 2143-2152.
Hoppeler, H. and Vogt, M. (2001). Muscle tissue adaptations to hypoxia. *J. Exp. Biol.* **204**, 3133-3139.
Kainulainen, H., Ahomaki, E. and Vihko, V. (1984). Selected enzyme activities in mouse cardiac muscle during training and terminated training. *Basic Res. Cardiol.* **79**, 110-123.
Kanatous, S. B., Hawke, T. J., Trumble, S. J., Pearson, L. P., Watson, R. R., Garry, D. J., Williams, T. M. and Davis, R. W. (2008). The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. *J. Exp. Biol.* **211**, 2559-2565.
Kerem, D., Hammond, D. D. and Elsner, R. (1973). Tissue glycogen levels in the Weddell seal (*Leptonychotes weddelli*): A possible adaptation to asphyxial hypoxia. *Comp. Biochem. Physiol. A. Physiol.* **45**, 731-736.
Kjekshus, J. K., Blix, A. S., Elsner, R. W., Hol, R. and Amundsen, E. (1982). Myocardial blood flow and metabolism in the diving seal. *Am. J. Physiol.* **242**, R97-R104.
Kodde, I. F., van der Stok, J., Smolenski, R. T. and de Jong, J. W. (2007). Metabolic and genetic regulation of cardiac energy substrate preference. *Comp. Biochem. Physiol. A. Physiol.* **146**, 26-39.
Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol. B* **138**, 335-346.
Lavigne, D. M. and Kovacs, K. M. (1988). *Harps and Hoods Waterloo.* Ontario: University of Waterloo Press.
Lestyk, K. C., Folkow, L. P., Blix, A. S., Hammill, M. O. and Burns, J. M. (2009). Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity. *J. Comp. Physiol. B* **179**, 985-996.
Liggins, G. C., Qvist, J., Hochachka, P. W., Murphy, B. J., Creasy, R. K., Schneider, R. C., Snider, M. T. and Zapol, W. M. (1980). Fetal cardiovascular and metabolic responses to simulated diving in the Weddell seal. *J. Appl. Physiol.* **49**, 424-430.
Lopaschuk, G. D., Collings-Nakai, R. L. and Itoi, T. (1992). Developmental changes in energy substrate use by the heart. *Cardiovasc. Res.* **26**, 1172-1180.
McGuire, B. S., Carroll, J. E., Chancey, V. F. and Howard, J. C. (1990). Mitochondrial enzymes responsible for oxidizing medium chain fatty acids in developing rat skeletal muscle, heart, and liver. *J. Nutr. Biochem.* **1**, 410-414.
McIntyre, I. W., Campbell, K. L. and MacArthur, R. A. (2002). Body oxygen stores, aerobic dive limits and diving behaviour of the star-nosed mole (*Condylura cristata*) and comparisons with non-aquatic talpids. *J. Exp. Biol.* **205**, 45-54.
Morris, G. S., Zhou, Q., Hegsted, M. and Keenan, M. J. (1995). Maternal consumption of a low vitamin D diet retards metabolic and contractile development in the neonatal rat heart. *J. Mol. Cell. Cardiol.* **27**, 1245-1250.
Murphy, B., Zapol, W. M. and Hochachka, P. W. (1980). Metabolic activities of heart, lung, and brain during diving and recovery in the Weddell seal. *J. Appl. Physiol.* **48**, 596-605.
Neely, J. R. and Morgan, H. E. (1974). Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu. Rev. Physiol.* **36**, 413-459.
Nemeth, P. M., Norris, B. J., Solanki, L. and Kelly, A. M. (1989). Metabolic specialization in fast and slow muscle fibers of the developing rat. *J. Neurosci.* **9**, 2336-2343.
O'Brien, P. J., Shen, H., McCutcheon, L. J., O'Grady, M., Byrne, P. J., Ferguson, H. W., Mirsalimi, M. S., Julian, R. J., Sargeant, J. M., Tremblay, R. R. et al., (1992). Rapid, simple and sensitive microassay for skeletal and cardiac muscle myoglobin and hemoglobin: use in various animals indicates functional role of myohemoproteins. *Mol. Cell. Biochem.* **112**, 45-52.
Oftedal, O. T. (1993). The adaptation of milk secretion to the constraints of fasting in bears, seals, and baleen whales. *J. Dairy Sci.* **76**, 3234-3246.
Ohtsuka, T. and Gilbert, R. D. (1995). Cardiac enzyme activities in fetal and adult pregnant and nonpregnant sheep exposed to high-altitude hypoxemia. *J. Appl. Physiol.* **79**, 1286-1289.
Ostadal, B., Ostadalova, I. and Dhalia, N. S. (1999). Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. *Physiol. Rev.* **79**, 635-659.
Polasek, L., Dickson, K. A. and Davis, R. W. (2006). Metabolic indicators in the skeletal muscles of harbor seals (*Phoca vitulina*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, R1720-R1727.
Prewitt, J. S. (2008). Developmental and environmental effects on the metabolic characteristics of harbor seal (*Phoca vitulina*) muscle. Thesis, University of Alaska Anchorage. p.102.
Reed, J. Z., Butler, P. J. and Fedak, M. A. (1994). The metabolic characteristics of the locomotory muscles of grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*), and Antarctic fur seals (*Arctocephalus gazella*). *J. Exp. Biol.* **194**, 33-46.
Reynafarje, B. (1962). Myoglobin content and enzymatic activity of muscle and altitude adaptation. *J. Appl. Physiol.* **17**, 301-309.
Reynafarje, B. (1963). Simplified method for the determination of myoglobin. *J. Lab. Clin. Med.* **61**, 138-145.
Shea, R. E., Olson, J. M. and Ricklefs, R. E. (2007). Growth rate, protein accumulation, and catabolic enzyme activity of skeletal muscles of Galliform birds. *Phys. Bioch. Zool.* **80**, 306-316.
Sheafor, B. A. (2003). Metabolic enzyme activities across an altitudinal gradient: an examination of pikas (genus Ochotona). *J. Exp. Biol.* **206**, 1241-1249.
Sordahl, L. A., Mueller, G. and Elsner, R. (1983). Comparative functional properties of mitochondria from seal and dog hearts. *J. Mol. Cell. Cardiol.* **15**, 1-5.
Stanley, W. C., Recchia, F. A. and Lopaschuk, G. D. (2005). Myocardial substrate metabolism in the normal and failing heart. *Physiol. Rev.* **85**, 1093-1129.
Stewart, J. M., Woods, A. K. and Blakely, J. A. (2005). Maximal enzyme activities, and myoglobin and glutathione concentrations in heart, liver, and skeletal muscle of the Northern short-tailed shrew (*Blarina brevicauda*; Insctivora: Soricidae). *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **141**, 267-273.
Terman, A. and Brunk, U. T. (2004). Myocyte aging and mitochondrial turnover. *Exp. Gerontol.* **39**, 701-705.
Winder, W. W., Baldwin, K. M. and Holloszy, J. O. (1974). Enzymes involved in ketone utilization in different types of muscle: adaptation to exercise. *Eur. J. Biochem.* **47**, 461-467.
Wittenberg, J. B. (1970). Myoglobin-facilitated oxygen diffusion: Role of myoglobin in oxygen entry into muscle. *Physiol. Rev.* **50**, 559-636.
Zapol, W. M., Liggins, G. C., Schneider, R. C., Qvist, J., Snider, M. T., Creasy, R. K. and Hochachka, P. W. (1979). Regional blood flow during simulated diving in the conscious Weddell seal. *J. Appl. Physiol.* **47**, 968-973.
Zonderland, M. L., Bar, P. R., Reijneveld, J. C., Spruijt, B. M., Keizer, H. A. and Glatz, J. F. C. (1999). Different metabolic adaptation of heart and skeletal muscles to moderate-intensity treadmill training in the rat. *Eur. J. Appl. Physiol.* **79**, 391-396.