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# Shifts in thermoregulatory strategy during ontogeny in harp seals (*Pagophilus groenlandicus*)

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ABSTRACT

Heat balance can be difficult for young and/or small animals in polar regions because environmental conditions in combination with small body size or physiological immaturity can increase heat loss. We investigated how thermoregulatory patterns change with ontogeny in 5 age classes of harp seal (*Pagophilus groenlandicus*) from birth to post-molt to further understand the timing of thermoregulatory development in relation to their potential vulnerability to ongoing fluctuations in the extent and stability of Arctic pack ice. We measured changes in the amount, conductivity, and resistance of the seal pups' insulative layers (blubber and fur), the potential for endogenous heat-generation by shivering (muscle enzyme activity), and nonshivering thermogenesis (NST; brown adipose tissue (BAT) uncoupling protein 1 (UCP1) expression and mitochondrial density). There was no significant difference in blubber conductivity among age classes, though the amount of blubber insulation significantly increased from birth to weaning. Pelage conductivity was low ($0.12 \pm 0.01$ W m$^{-1}$ °C$^{-1}$) except in 9-day old pups ($0.40 \pm 0.08$ W m$^{-1}$ °C$^{-1}$); the significantly higher conductivity may signal the beginning of the molt, and this age group may be the most vulnerable to early water entry. Citrate synthase activity significantly increased ($49.68 \pm 3.26$ to $75.08 \pm 3.52$ μmol min$^{-1}$ g wet weight$^{-1}$) in the muscle; however it is unlikely that increasing a single enzyme greatly impacts heat generation. BAT of younger pups contained UCP1, though expression and mitochondrial density quickly declined, and the ability of pups to produce heat via NST was lost by weaning. While total thermal resistance did not differ, neonatal and early nursing animals gained the majority of their thermal resistance from lanugo ($82.5 \pm 0.03$%); however, lanugo is not insulative when wet, and NST may be important to maintain euthermia and dry the coat if early immersion in water occurs. By late nursing, blubber seems sufficient as insulation ($75.87 \pm 0.01$% of resistance after 4 weeks), but high conductivity of fur may be responsible for retention of UCP1 expression. Weaned animals rely on blubber insulation, and no longer need NST, as wetted fur is no longer a threat to euthermia.



## 1. Introduction

All mammals must balance metabolic heat production and heat loss to maintain thermoregulatory homeostasis (Scholander et al., 1950; Scholander, 1955). Relative to terrestrial mammals, marine mammals face potential elevated rates of heat loss because of the high thermal conductivity and heat capacity of water compared with air (Scholander et al., 1950). Adult marine mammals have evolved a suite of morphological and physiological adaptations to counteract elevated heat transfer when immersed (Irving and Hart, 1957; Scholander et al., 1950). Adults reduce heat loss to the surrounding environment by having a lower surface area to volume ratio (SA:V) as compared to terrestrial mammals of similar mass

*Abbreviations:* BAT, brown adipose tissue; COX, cytochrome c oxidase; CS, citrate synthase; HOAD, β-hydroxyacyl CoA dehydrogenase; LD, *longissimus dorsi*; LCT, lower critical temperature; MR, metabolic rate; MT, mitochondria; NST, nonshivering thermogenesis; PWF, post-weaning fast; RMR, resting metabolic rate; SA:V, surface area to volume ratio; UCP1, uncoupling protein 1; TEM, transmission electron microscope; TNZ, thermal neutral zone

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(Innes et al., 1990), as well as increased amounts of insulation in the form of blubber and/or fur (Ryg et al., 1993; Scholander et al., 1950). Vasoconstriction of the periphery and countercurrent heat exchangers conserve heat in the water; conversely vasodilation in conjunction with countercurrent heat exchangers can dissipate heat when individuals are highly active or hauled out (resting on a terrestrial substrate; Irving and Hart, 1957; Scholander, 1955). As in other endotherms, marine mammals can increase heat production by raising their metabolism and/or initiating shivering thermogenesis. However, metabolic heat production is energetically expensive, and the broad thermal neutral zones (TNZs) of most adult marine mammals indicate that these mechanisms are rarely required (Gallivan and Ronald, 1979; Hart and Irving, 1959; Lavigne et al., 1986).

The thermoregulatory challenges faced by young marine mammals differ from those of adults because of habitat, use patterns, and physiological differences (Dunkin et al., 2005; Liwanag et al., 2009; Noren et al., 2003). Most phocids are born on land or ice and do not face the thermoregulatory challenges of an aquatic lifestyle until they begin independent foraging at some point after weaning. However, even when on land, the smaller size and greater SA:V (Blix and Steen, 1979), and poor vasocontrol (Lapierre et al., 2004) in young pups result in a greater potential for heat loss to the environment than experienced by adults (Blix and Steen, 1979). In addition, most phocid pups are born without a thick subcutaneous blubber layer and instead rely on their lanugo, or natal pelage. When dry, lanugo is a more effective insulator than an equivalent thickness of blubber and is also lightweight, so small pups are able to carry a thicker fur layer than they could blubber (Ryg et al., 1993). However, lanugo loses its insulative capacity when wet and pups must replace it with blubber before beginning foraging activities (Kvadsheim and Aarseth, 2002). In contrast, blubber is an internal insulator and retains its insulative capacity regardless of ambient environmental conditions (Kvadsheim and Aarseth, 2002; Liwanag et al., 2012b). Pups must develop thermoregulatory capabilities of 'aquatic' adults from a 'terrestrial' starting point in a relatively short period (days–weeks), because prolonged immersion during early independent foraging without the physiological capabilities to defend against higher rates of heat loss may energetically compromise young animals (Liwanag et al., 2009).

Despite the fact that fur can provide better insulation than blubber, even a thick lanugo coat may not be sufficient insulation to maintain euthermia in polar environments where animals regularly face gradients of $>40\,^{\circ}C$ between core and ambient temperatures (Blix and Steen, 1979; Grav et al., 1974; Øritsland and Ronald, 1978). When insulation is no longer sufficient, nonshivering thermogenesis (NST) or shivering thermogenesis may be required to provide the additional heat needed for homeothermy, particularly if the lanugo becomes wet. While NST may provide a mechanism for core warming and for drying saturated lanugo, the use of NST for these purposes results in a substantial increase in resting metabolic rate (RMR) (Cannon and Nedergaard, 2008). As the blubber layer develops, similar environmental conditions are much less likely to require NST (Davydov and Makarova, 1964; Liwanag et al., 2012b). As pups mature, switching insulation from lanugo to blubber is likely essential early survival, especially in the event of early ice break up and immersion, and to reduce thermoregulatory costs once weaned pups foraging.

Harp seals are born on pack ice with a very thin blubber layer and a thick lanugo coat. During the 12-day-nursing period, pups gain mass at a rate of 2–2.5 kg $d^{-1}$, most of which is deposited as blubber (Kovacs and Lavigne, 1986; Oftedal et al., 1989; Stewart and Lavigne, 1980; Worthy, 1991). While early work that located apparent brown adipose tissue (BAT) deposits suggested that neonatal harp seals are capable of NST, the presence of uncoupling protein 1 (UCP1) has yet to be confirmed (Blix et al., 1979; Grav et al., 1974). The utility of BAT would likely be short lived because by weaning, pups have developed a thick ($>5$ cm) blubber layer and have begun to molt their lanugo coat, and therefore have functional insulation upon immersion. Pups then remain hauled out 3–4 weeks for the duration of the postweaning fast (PWF) before beginning to forage independently (Stewart and Lavigne, 1980; Worthy, 1987; Worthy and Lavigne, 1987). The loss of lanugo during this period is likely reflective of the increased blubber insulation and decreased SA:V from nursing, the need to prepare for diving, and the probability that lanugo would not be effective insulation as the ice thins and immersion becomes more likely. During the PWF, metabolic costs are met by catabolizing lipid in the blubber layer and protein from the body core, as animals must balance metabolic needs while fasting and the cost of thermoregulation (Worthy and Lavigne, 1987). Fatter animals or those that lose weight more slowly, typically fast for longer periods (Noren et al., 2003, 2008), potentially reducing protein catabolism, and allowing more time for physiological development of the oxygen stores, muscle structure, and biochemistry necessary for sustained diving and foraging (Burns et al., 2007; Lestyk et al., 2009; Noren et al., 2008).

This study examines how the thermoregulatory strategy of harp seal pups changes as pups grow and prepare for independent foraging. The morphological transition from lanugo to blubber in harp seals, like other phocids, has been well documented (Kvadsheim and Aarseth, 2002; Oftedal et al., 1996; Worthy, 1991). However, we were interested in quantifying insulative properties of the fur and blubber during ontogeny, examining potential capacity of heat production through NST and shivering, and determining if changes in insulation were correlated with changes in the thermogenic capacity. We hypothesized that neonatal pups with lanugo coats require or possess additional heat generating mechanisms like NST, but as pups grow and the blubber layer thickens to provide equivalent internal insulation, additional heat generating mechanisms should no longer be necessary. Understanding the timing of thermoregulatory development in harp seal pups is important for understanding their potential vulnerability to ongoing changes in the extent and stability of Arctic pack ice (Post et al., 2013). Declines in pack ice and increases in storm events in the Arctic may increase the chance young pups enter the water earlier or more frequently than in the past (Friedlaender et al., 2010; Hansen et al., 2013; Post et al., 2013). If these changes occur before pups have developed insulation that is capable of dealing with the increased thermal capacity of water, they may reduce pup survival in the days and weeks post-weaning.

## 2. Materials and methods

### 2.1. Animals and sample collection

To assess potential changes in thermoregulatory strategies with development, neonatal (within $\sim$24 h of birth; $n=6$), newly weaned ($\sim$12–15 days old; $n=5$), and post-weaned ($\sim$21 days old; $n=5$) harp seal pups, and 4 adult harp seal females were hand-captured in March 2008 in the Gulf of St. Lawrence, Canada (47°36' N, 62°13' W). Additionally, neonatal ($n=4$), early nursing ($\sim$4 days old; $n=3$), and late nursing ($\sim$9 days old; $n=4$) animals were hand-captured in March 2011 in the "West Ice" off Greenland (72°24' N, 14°15' W). Together, these two sampling periods represent 5 developmental age classes from birth to late weaning. Animals were aged following Stewart and Lavigne (1980), sacrificed using methods approved for scientific harvest in Canada or Norway, and samples were imported to the United States under NMFS Permit #782-1694-02 (Canada) and 15510 (Norway). The

University of Alaska Anchorage Animal Care and Use Committee approved all sampling protocols (#Burns2005 and #149278-1).

### 2.2. Morphometrics and body condition

To assess changes in body condition and blubber thickness, all pups ($n=27$) were weighed to the nearest 0.5 kg using a spring scale. Post-mortem measurement of straight length was taken with a tape measure and blubber thickness was measured using a ruler to the nearest 0.1 cm at 4 locations (neck, axillary umbilicus, and pelvis) along the dorsal and ventral axis of the body. Mean blubber depth was calculated as the average of all measurements taken from an individual. The mass:length ratio was calculated for all animals, and a greater mass:length ratio was assumed to be indicative of better condition. For the pups collected in Greenland ($n=11$), girth and curvilinear length measurements were taken between each blubber depth measurement location, and surface area, body volume, and % blubber by volume calculated using equations published by Gales and Burton (1987). Lean tissue volume was calculated as the difference between total volume and blubber volume.

### 2.3. Assessment of insulation, conductivity, and heat flux

We assessed the quality of insulation provided by blubber and fur by measuring the thermal conductivity ($k$) of the pelt according to the methods outlined by Kvadsheim et al. (1994), Dunkin et al. (2005) and Liwanag et al. (2012a, 2012b). Sculp samples (full blubber thickness+skin+fur) were collected from all 5 pup age classes and the adults ($n=31$ total) within 30 min post-mortem from the mid-trunk region and stored at −20 °C. Thermal conductivity of the sculp, blubber (alone), and pelt (fur with skin) was measured on square (~10 cm × 10 cm) samples using the 'standard materials method' described by Dunkin et al. (2005) and Liwanag et al. (2012a, 2012b). An elastomer (Plastisol vinyl, Carolina Biological Supply, Burlington, NC, USA) was used as the standard material ($k=0.109\pm0.0006$ W m$^{-1}$ °C$^{-1}$), and was placed in series with the sculp sample. Three thickness measurements to the nearest 0.01 mm from each side of the sample were taken of the blubber, skin, and dry fur using digital calipers (ABSO-LUTE Digimatic Caliper Series 500, Mitutoyo, Aurora, IL, USA); mean values were used for calculations. Thermal conductivity was calculated across the sculp, blubber, and pelt, using the Fourier equation (Kreith, 1958):

$$k = H \times L/A \times \Delta T \quad (1)$$

where $k$ is conductivity (W m$^{-1}$ °C$^{-1}$), $H$ is heat transfer (J s$^{-1}$), $L$ is the thickness of the material (m), $A$ is the area (m$^2$) through which the heat is moving, and $\Delta T$ is the temperature differential (°C) across the material. As heat transfer is assumed to be equal across the standard material and sample, the equations were set equal and solved for the thermal conductivity of the sample. To account for changes in insulation due to changes in the thickness of blubber and fur with development, thermal resistance ($R$; m$^2$ °C W$^{-1}$), was calculated for the sculp, blubber, and pelt using the equation

$$R = L/k \quad (2)$$

The rate of heat loss or gain from the environment was measured at 6 locations along the body (ears, neck, axillary, sternum, mid, umbilicus, and ankles) for 11 live pups from Greenland, using a heat flux sensor (Thermonetics Inc, San Diego, CA, USA) attached to a digital multimeter. All measurements were taken within 12 h of capture and while animals were resting in outdoor enclosures aboard the ship. Ambient air temperature was measured during all heat flux measurement periods and averaged $-1.0\pm0.5$ °C. Pups were protected from wind by their enclosures. After completion of heat flux measurements, animals were sacrificed and samples collected as described in Sections 2.1, 2.4, and 2.5. Heat flux measurements were converted to W m$^{-2}$ using the calibration factor provided by the manufacturer. Mean heat flux was calculated as the mean of the heat flux values from all body locations.

### 2.4. Assessment of the potential for metabolic heat production

To assess the capacity for metabolic heat production by the *longissimus dorsi* (LD) muscle through either shivering thermogenesis or futile cycling, we measured the activity of three metabolic enzymes which play important roles in providing reducing substrates for heat production: citrate synthase (CS) as an estimate of TCA cycle activity, β-hydroxyacyl CoA dehydrogenase (HOAD) as a measure of lipid β-oxidation, and cytochrome c oxidase (COX) as a measure of electron transport chain activity. We measured all enzyme activities under substrate saturating conditions following previously published protocols (Kanatous et al., 2008; Prewitt et al., 2010). Post-mortem samples of LD (100 mg) were immediately frozen in liquid nitrogen upon collection and stored at −80 °C until assayed. Samples were homogenized at 0 °C in buffer (1:20 wt:vol) containing phosphate buffered saline, 1% Tween 20, 20% glycerol, and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA) and centrifuged at 10,000$g$ for 10 min at 4 °C. The supernatant was used for the enzyme assays, which were run in a Molecular Devices SpectraMax 340 microplate reader (Sunnyvale, CA, USA) held at body temperature (37 °C). Assay conditions were as follows: CS (EC 4.1.3.7): 50 mmol l$^{-1}$ imidazole, 0.25 mmol l$^{-1}$ 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 0.4 mmol l$^{-1}$ acetyl CoA and 0.5 mmol l$^{-1}$ oxaloacetate, pH 7.5 at 37 °C; $\Delta A_{412}$, $\in_{412}=13.6$. HOAD (EC 1.1.1.35): 50 mmol l$^{-1}$ imidazole, 1 mmol l$^{-1}$ EDTA, 0.1 mmol l$^{-1}$ acetoacetyl CoA, and 0.15 mmol l$^{-1}$ NADH, pH 7.0 at 37 °C; $\Delta A_{340}$, $\in_{340}=6.22$. COX: 0.1 mmol l$^{-1}$ DTT, 0.22 mmol l$^{-1}$ ferrocytochrome-c, 10 mmol l$^{-1}$ Tris–HCl pH 7.0 with 120 mmol l$^{-1}$ KCl; $\Delta A_{550}$ $\in_{550}=21.84$ (Sigma Aldrich #CYTOCOX100). Samples were run in triplicate; values were only accepted if replicate coefficient of variations (CVs) < 10%. Activity values for the entire assay plate were rejected and rerun if values for a control muscle with known enzyme activity fell outside a previously determined normal range (Prewitt et al., 2010). Specific enzyme activities (IU g$^{-1}$ wet tissue mass) were calculated from the change in absorbance at the linear slope of the assay.

### 2.5. Assessment of potential for nonshivering thermogenesis

To determine if harp seals were capable of NST we examined animals for the presence of BAT in previously described locations: the *venus plexus* of the neck, subcutaneously in the blubber, and superior to the kidneys (Blix et al., 1979; Grav et al., 1974). We collected samples of a tissue that matched the gross morphological descriptions of BAT (Afzelius, 1970; Grav et al., 1974) from the *venus plexus* of the neck from all 5 age classes of pups ($n=27$). No other locations had BAT-like tissue. All tissue samples were stored in Whirlpaks® at −20 °C for up to 2 weeks before being transferred to −80 °C until analysis. Blubber and muscle (LD) samples were also collected for use as negative controls in Western blot analysis.

Western blot analyses were performed on 12% SDS-PAGE gels with tissue homogenates of the BAT-like tissue to determine if UCP1 was expressed from all age classes sampled ($n=27$). Samples were homogenized in the same buffer used for enzyme assays and centrifuged at 10,000$g$ for 10 min at 4 °C. Total protein content of the homogenate was determined using Pierce Coomassie Blue 'The Better Bradford' Total Protein Assay (Pierce Chemicals, Rockford, IL, USA). Thirty micrograms protein per sample were mixed with

loading dye, loaded onto 12% SDS-PAGE gels, run at 100 V for 1 h, and transferred onto nitrocellulose membrane. Membranes were then stained with Ponceau S Solution (0.2% v/v in 5% acetic acid; Alfa Aesar, Ward Hill, MA, USA) to ensure proper protein transfer. Western blot development was done on a SNAP i.d. (EMD Millipore, Billerica, MA, USA). The primary antibody (rabbit anti-UCP1, IgG, 1:3000; #ab10983, Abcam, Cambridge, MA, USA) was detected using an Alexa Fluor 680 goat anti-rabbit (IgG, 1:5000) secondary antibody (Invitrogen, Carlsbad, CA, USA). Additionally, β-Actin (rabbit anti-β-Actin, IgG, 1:3000, #ab8227, Abcam, Cambridge, MA, USA) was detected on the same membranes, to ensure equal protein loading across samples and gels. Protein bands were visualized using a LI-COR Odyssey imaging system (LI-COR, Lincoln, NE, USA) and band intensity was quantified using a digital analysis program (Image J, Schneider et al., 2012). Samples were run in triplicate, and expression of UCP1 and β-actin were calculated for each individual. UCP1 expression relative to β-actin expression was calculated for each individual and averaged for each age class. Arctic ground squirrel (*Urocitellus parryii*) BAT was used as a positive control for antibody reactivity to UCP1. We tested for cross-reactivity of the antibody with other UCPs by performing Western blot analyses using the UCP1 antibody on muscle and blubber tissues. Peptide competition assays were also performed to ensure that binding was not due to antibody cross-reactivity. In this assay, UCP1 antibody (1:1500) was pre-incubated with UCP1 peptide (1:60; #ab24282, Abcam, Cambridge, MA, USA) for 60 min at 37 °C. Incubation and development proceeded as above using the primary antibody–peptide mix in place of the primary antibody. Peptide competition resulted in complete inhibition of antibody activity, indicating the protein bound by the UCP1 antibody in the Western blot was UCP1 and not antibody cross-reactivity.

The mitochondrial (MT) volume density in BAT was measured to assess tissue ultrastructure and potential for thermogenesis in the harp seal samples collected in Greenland, representing pups during the nursing period ($n=11$). In the field, 15 mg sub-samples of BAT-like tissue were immediately fixed in a 2% gluteraldehyde, 2% paraformaldehyde, 0.1 M sodium cacodylate buffer (pH 7.4) and stored at 4 °C. Subsequently, at the Electron Microscopy Lab at the University of Maine (Orono, ME), samples were cut into 1 mm blocks, and rinsed 3 × with and held overnight in 0.1 M cacodylate buffer (pH 7.4). Samples were then post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h while on ice, rinsed in di$H_2O$, and dehydrated with increasing concentrations of acetone (50–100%). Samples were infiltrated with 50/50 mix of 100% acetone and epon-araldite resin overnight, moved to fresh resin under vacuum (2 × 30 min) the following day, embedded in fresh epon-araldite resin, and cured for 48 h at 65 °C. Thin sections (2 μm) were cut on a Leica UC6 Ultramicrotome (Leica Biosystems, Buffalo Grove, IL, USA) and placed onto G200 copper grids (Electron Microscopy Sciences, Hatfield, PA, USA). Grids were counter-stained with 1% uranyl acetate in water for 20 min followed by 0.5% lead citrate in water for 4 min. Sections were photographed on a JEOL 1200 Transmission Electron Microscope (TEM; JEOL, Peabody, MA, USA) in the Advanced Instrument Lab at the University of Alaska Fairbanks. Carbon grating replica calibration was performed on the TEM to confirm that magnification was within 5% of nominal magnification. Digital images were captured at 3000 ×. Twenty images were taken per sample for analysis. The volume fraction of mitochondria was estimated using standard point counting procedures (Weibel, 1979) modified for digital photography (Watson et al., 2007). Adobe Photoshop CS5 (Adobe Systems Inc., San Jose, CA, USA) with the 'grid' feature enabled was used to generate a grid of appropriate point density for each tissue based on the relative size of the mitochondria (Weibel, 1979). All points falling on mitochondria were counted. The relative standard errors (RSEs) of the volume density of each sample were calculated by pooling counts from all images for a sample, and applying the RSE equation for binomial counts (Mathieu et al., 1981). RSE was 7.69 ± 1.31% for all samples.

We evaluated the potential for metabolic flux through mitochondria in BAT from all 5 age classes of pups sampled ($n=27$), by measuring the aerobic enzyme activities (μmol $ml^{-1}$ $g^{-1}$ wet tissue) of CS, HOAD, and COX using the same protocols used for LD muscle described in Section 2.4.

### 2.6. Statistical analysis

Data were tested for normality and transformations were used as necessary. No data points were identified as outliers (mean ± 2 SD), so all data points were retained. One-way ANOVAs and Bonferroni post-hoc tests were used to determine differences among age classes, and significance was considered at the 95% level ($P<0.05$). Age-related changes in mean and location-specific heat flux measurements were analyzed using a non-parametric Kruskal–Wallis test. To determine the correlation between insulation and NST in nursing pups, step-wise linear regression models were used. In this analysis, mean blubber depth, fur thickness, thermal resistance of blubber, and mass:length ratio were used as metrics of insulation and condition, and UCP1 expression was used as a metric of NST potential. UCP1 data were transformed using a Box–Cox power transformation with lambda=0.2 prior to regression analysis. All analyses were completed in SPSS Software (v 21, IBM, Armonk, NY, USA).

**Table 1**
Mean morphometric and body condition values (± SEM) for harp seal pups during nursing and early postweaning fast.

| | Neonate | Early nursing | Late nursing | Early weaned | Late weaned |
|---|---|---|---|---|---|
| Sample size | 10 | 3 | 4 | 5 | 5 |
| Mass (kg) | $9.8 \pm 0.65^{a}$ | $16.5 \pm 0.29^{b}$ | $28.6 \pm 1.60^{c}$ | $42.0 \pm 2.37^{d}$ | $29.8 \pm 1.55^{c}$ |
| Standard length (cm) | $82.8 \pm 2.79^{a}$ | $87.3 \pm 3.48^{a,b}$ | $104.7 \pm 2.73^{b}$ | $101.2 \pm 2.22^{a}$ | $89.6 \pm 5.50^{a,b}$ |
| mass/length | $0.10 \pm 0.01^{a}$ | $0.19 \pm 0.01^{b}$ | $0.28 \pm 0.01^{c}$ | $0.41 \pm 0.02^{d}$ | $0.34 \pm 0.02^{c}$ |
| Total volume (L) | $11.03 \pm 0.89^{a}$ | $15.72 \pm 1.53^{a}$ | $30.13 \pm 3.50^{b}$ | | |
| Surface area ($m^2$) | $325.21 \pm 13.89^{a}$ | $388.25 \pm 26.09^{a}$ | $576.63 \pm 41.42^{b}$ | | |
| SA:V | $29.63 \pm 0.99^{a}$ | $24.87 \pm 1.03^{b}$ | $20.73 \pm 1.17^{c}$ | | |
| Lean tissue volume (L) | $9.56 \pm 0.68^{a}$ | $11.16 \pm 1.04^{a,b}$ | $17.14 \pm 2.24^{b}$ | | |
| Blubber volume (L) | $1.49 \pm 0.17^{a}$ | $4.55 \pm 0.62^{b}$ | $12.99 \pm 1.47^{c}$ | | |
| % Blubber by volume | $13.75 \pm 1.03^{a}$ | $29.00 \pm 2.08^{b}$ | $43.50 \pm 1.84^{c}$ | | |
| Blubber thickness (cm) | $0.80 \pm 0.14^{a}$ | $1.50 \pm 0.10^{a}$ | $2.00 \pm 0.20^{b}$ | $4.90 \pm 0.21^{c}$ | $3.90 \pm 0.14^{d}$ |
| Fur thickness (cm) | $2.081 \pm 0.371^{a}$ | $2.425 \pm 0.163^{a}$ | $2.598 \pm 0.145^{a}$ | $0.312 \pm 0.030^{b}$ | $0.192 \pm 0.032^{b}$ |

Superscripts indicate statistically significant differences ($P<0.05$) between mean values for which there was effect of age. Measurements for volume calculations in early and late weaned animals were not obtained in this study.

## 3. Results

### 3.1. Animal growth and changes in insulation

Mass, length, and mass:length ratio of pups increased from birth through the nursing period (mass: $F_{4,20}=97.32$, $P<0.001$; length: $F_{4,20}=6.29$, $P=0.002$; mass/length: $F_{4,22}=75.67$, $P<0.001$; Table 1). As pups increased in mass, their body volume and surface area also increased (body volume: $F_{2,8}=17.75$, $P=0.001$; surface area: $F_{2,8}=20.01$, $P=0.001$), primarily due to an increase in blubber from $13.75\pm1.03\%$ in neonates to $43.50\pm1.84\%$ in newly weaned pups. However, because pup volume increased more rapidly than surface area, the SA:V ratio significantly decreased over the nursing period ($F_{2,8}=18.42$, $P=0.001$, Table 1). Body mass, but not length, decreased during the post-weaning fast, likely causing a small secondary increase in SA:V, but this was not measured in this study because no weaned pups were sampled in Greenland.

The increase in relative % blubber with age was paralleled by the increase in blubber thickness at all locations along the body (neck $F_{4,20}=45.46$, $P<0.001$; axillary $F_{4,20}=80.03$, $P<0.001$; umbilicus $F_{4,20}=95.26$, $P<0.001$; pelvis $F_{4,20}=58.67$, $P<0.001$; Fig. 1), and mean blubber thickness quintupled between birth and weaning ($F_{4,22}=104.45$, $P<0.001$; Table 1). Fur thickness averaged $2.410\pm0.020$ cm in neonates and did not change during the nursing period. Thickness declined as pups began to molt; in weaned pups, fur thickness decreased by nine fold ($F_{4,23}=26.09$, $P<0.001$; Table 1).

The mean conductivity of harp seal blubber was $0.2100\pm0.0183$ W m$^{-1}$ °C$^{-1}$. There was no significant difference in blubber conductivity among the 5 age classes of pups or between the pups and adults (Table 2), indicating the material properties of blubber did not change with ontogeny. However, the material properties of the pelt did change with age: pelt conductivity was highest in pups late in the nursing period just before the lanugo was shed ($F_{5,27}=12.35$, $P<0.001$; Table 2). As a result, the thermal conductivity of the sculp (blubber+fur+skin) was highest in late-nursing pups compared to other pups and adults ($F_{5,27}=4.71$, $P=0.005$). Once weaned, sculp conductivity declined, largely due to a 67% decrease in the conductivity of the pelt component (Table 2).

While there were no significant changes in the overall thermal resistance of the sculp with age class or between pups and adults, there were significant differences in the relative contribution of blubber and fur to the overall thermal resistance. As blubber layer thickness increased, the proportion of the overall thermal resistance due to blubber also increased ($F_{4,27}=21.09$, $P<0.001$). Similarly, as fur thickness decreased, there was a significant decrease in the resistance of the pelt ($F_{5,27}=11.42$, $P=0.001$; Fig. 2). In weaned pups, both the overall thermal resistance and the relative contributions of fur and blubber were similar to adult values (Fig. 2). Remarkably, for the heat flux measurements on live pups, there was little effect of age class or body location on overall heat flux rates in nursing animals and mean heat flux was not significantly different among age classes (Fig. 3), with the exception of slightly higher rates of heat flux in neonatal animals.

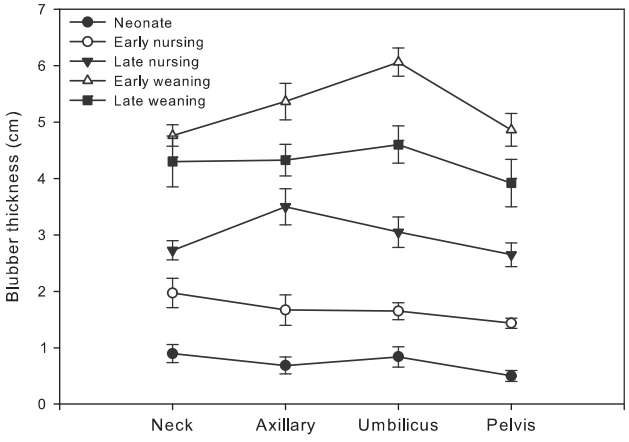


**Fig. 1.** Blubber thickness (cm ± SEM) of harp seals during ontogeny across the body measured along the dorsal midline at the neck, axillary, umbilicus, and pelvis. At each location along the body, there was a significant difference in thickness between each age class ($P<0.05$).

### 3.2. Potential for metabolic heat production

The potential for metabolic heat production through shivering or futile cycling, as indicated by enzyme activity in the LD muscle, generally increased with age class, although these increases were not always statistically significant, nor the patterns linear (Table 3). For example, COX activity was significantly higher in both neonates and weaned pups as compared to nursing pups ($F_{4,20}=3.91$, $P=0.017$). In contrast, CS activity was significantly lower in all nursing pups as compared to all weaned pups ($F_{4,20}=12.01$, $P<0.001$), and HOAD activity did not differ significantly among age classes ($F_{4,20}=2.85$, $P=0.051$).

### 3.3. Potential for nonshivering thermogenesis

BAT-like tissue was found in, and collected from, the dorsal intrascapular region near the *venus plexus* of pups of all age classes. BAT was not found above subcutaneously, or superior to the kidneys. Expression of UCP1 (relative to β-actin) in this tissue was highest in neonates and declined precipitously with age class, such that it could not be detected in any weaned pups ($F_{4,23}=37.12$, $P<0.001$; Fig. 4A–D). While the BAT of neonatal harp seals had multilocular lipid droplets and high MT density, as UCP1 expression declined with age, MT density in BAT decreased 86% by weaning, from a high of $13.88\pm2.00\%$ in neonates to $4.50\pm2.80\%$ by early nursing, and $1.95\pm0.87\%$ in late weaned pups ($F_{2,10}=11.00$, $P<0.003$; Fig. 5A–C). Concomitant with the decline in mitochondrial density, enzyme activity (COX, CS, HOAD) in BAT also declined over this period, and by early weaning COX activity was approximately 20% of activity in neonates, and CS and HOAD declined by 50% (Table 3; COX: $F_{4,20}=5.80$, $P=0.003$; CS: $F_{4,20}=10.07$, $P<0.001$; HOAD: $F_{4,20}=7.00$, $P=0.001$). The decline in CS and COX (82% and 85% decline by weaning respectively) closely match the 86% decline in MT density. Combined, these further suggest that the ability of BAT to produce heat via NST was functionally absent by early weaning. Step wise regression analysis revealed that in nursing animals, UCP1 expression was negatively correlated with mean blubber depth ($F_{1,16}=14.603$, $P=0.002$, $R^2=0.493$; Fig. 6); UCP1 was not expressed once blubber depths were greater than 3.2 cm, and not expressed in any weaned pup (Fig. 6). Mean blubber depth was significant ($t=-3.821$, $P=0.002$); no other variables were included in the regression model because of multicolinearity, and when included individually alone with mean blubber thickness, they did not improve model fit.

## 4. Discussion

The results of this research show that neonatal harp seals have equivalent thermal resistance as older, fatter pups. But because this insulation largely comes from a wettable lanugo coat, they possess the potential for additional thermogenesis by NST through

**Table 2**
Mean conductivity values ( ± SEM) for sculp (fur+skin+blubber), blubber, and pelt (fur with skin) of harp seal pups during nursing and early postweaning fast and from harp seal adults.

| | Neonate | Early nursing | Late nursing | Early weaned | Late weaned | Adult |
|---|---|---|---|---|---|---|
| Conductivity ($k$: W m$^{-1}$ °C$^{-1}$) | | | | | | |
| Sculp | 0.14 ± 0.02$^{a}$ | 0.17 ± 0.02$^{a,b}$ | 0.28 ± 0.03$^{b}$ | 0.18 ± 0.02$^{a,b}$ | 0.16 ± 0.02$^{a,b}$ | 0.19 ± 0.02$^{a,b}$ |
| Blubber | 0.21 ± 0.05 | 0.22 ± 0.01 | 0.24 ± 0.02 | 0.19 ± 0.02 | 0.18 ± 0.02 | 0.19 ± 0.02 |
| Pelt (fur with skin) | 0.12 ± 0.02$^{a}$ | 0.15 ± 0.02$^{a}$ | 0.40 ± 0.08$^{b}$ | 0.12 ± 0.01$^{a}$ | 0.08 ± 0.01$^{a}$ | 0.09 ± 0.01$^{a}$ |

Superscripts indicate statistically significant differences ($P < 0.05$) between mean values for which there was effect of age on conductivity.

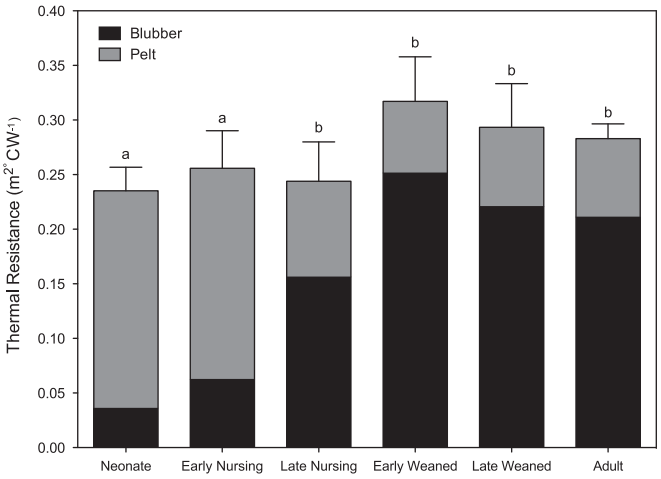


**Fig. 2.** Mean ( ± SEM) total thermal resistance of harp seals. Total thermal resistance is the sum of the thermal resistance of the blubber and pelt. There was a significant increase ($P < 0.05$) in the resistance of blubber with age, and an equivalent decrease in pelt resistance ($P < 0.05$) with age. Because significant changes in the resistance of the blubber and pelt resulted in the same groupings, letters indicate statistically significant differences ($P < 0.05$) between mean values for which there was an effect of age on both the resistance of blubber and pelt.

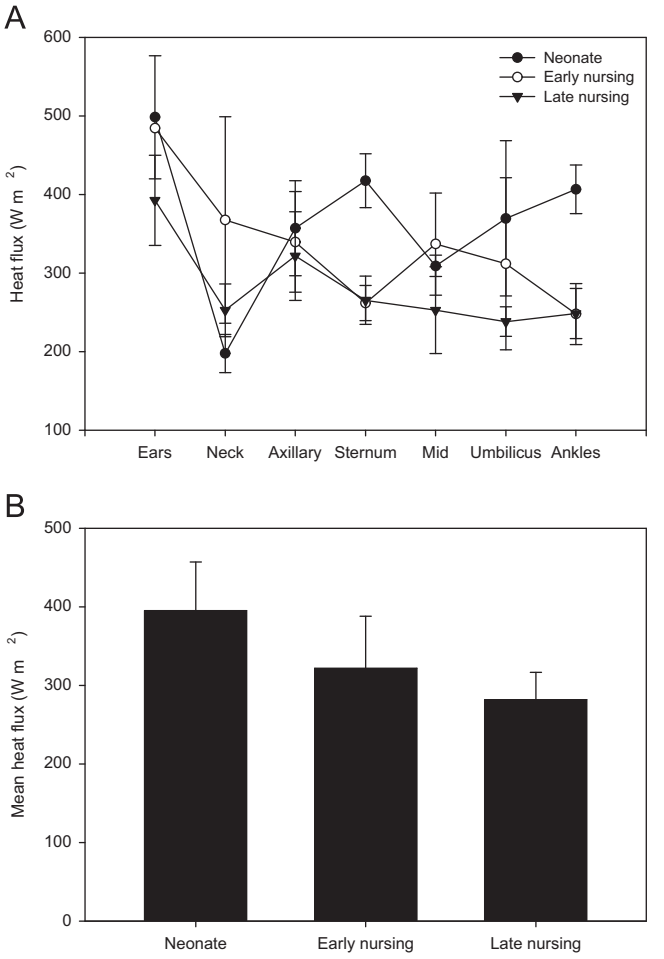


**Fig. 3.** (A) Heat flux ( ± SEM) of nursing harp seals at 6 dorsal midline locations: ears, neck, axillary, sternum, mid, umbilicus, and ankles. There were no significant differences between age classes in heat flux at any location along the body ($P > 0.05$). (B) Mean heat flux ( ± SEM), averaged from all dorsal heat flux measurements across the body, did not differ significantly between age classes ($P > 0.05$).

the expression of UCP1 in BAT. As animals progress through the nursing period and gain substantial blubber volume, this forms their main insulative layer and source of thermal resistance. As older pups no longer rely on a wettable coat for insulation, additional thermogenesis is not necessary, and the ability for NST is lost. The potential for shivering thermogenesis does not appear to change across ontogeny, and is thus not an important source of heat generation in smaller animals.

The nursing and early developmental period is critical for animals, and for phocids this is the time when all energy reserves to fuel the PWF and initial foraging activities are accumulated (Oftedal et al., 1989; Stewart and Lavigne, 1980; Noren et al., 2008). Pups that can maximally reduce metabolic expenditure during the nursing period are able to allocate the largest fraction of milk energy to blubber, and thus enter the PWF with larger energy reserves to withstand PWF and initiate independent foraging. Maintaining thermoregulatory homeostasis through insulative mechanisms and reducing reliance on excess heat production via NST or shivering is essential to reducing metabolic costs. By refraining from swimming and diving throughout the nursing period (Kovacs and Lavigne, 1986) harp seals decrease the chance the lanugo becomes wet, decreasing the need for NST, and their thermoregulatory strategy reflects of this behavior. Unlike species that enter the water soon after birth such as hooded seals (*Cystophora cristata*; Oftedal et al., 1991), neonatal harp seals rely on their lightweight lanugo for insulation, and young harp seals do not have sufficient body size to support the amount of blubber necessary to provide adequate insulation in air (Liwanag et al., 2012a; Ryg et al., 1993). Based on our thermal resistance data, ~4 cm of blubber provides equivalent insulation as ~2.4 cm of lanugo. If pups were born with equivalent blubber insulation instead of lanugo, they would be over 75% blubber, which is quite unrealistic. However, should their lanugo become saturated with water, their high SA:V and thin subcutaneous blubber layer may lead to high rates of heat loss and therefore necessitate additional heat generation in the form of NST. As pups grow and deposit blubber reserves during the nursing period, their SA:V declines and primary insulation shifts from lanugo to blubber, and the potential need for NST is lost. Thus it appears that the large increase in blubber (volume and % total body volume) during the nursing period is essential for both the accumulation of energy reserves to fuel the PWF, and the formation of a thermal barrier that is effective when wet (Liwanag et al., 2012b; Noren et al., 2008). Indeed, fasting harp seal pups (post-weaning) have the

**Table 3**
Mean enzyme activity ( ± SEM; μmol $min^{-1}$ g wet $weight^{-1}$) of cytochrome c oxidase (COX), citrate synthase (CS), and β-hydroxyacyl CoA dehydrogenase (HOAD) in the *longissiumus dorsi* muscle and brown adipose tissue. Mean mitochondrial density ( ± SEM) of brown adipose tissue in harp seal pups was determined from images obtained by transmission electron microscopy that are represented in Fig. 5.

| | Neonate | Early nursing | Late nursing | Early weaned | Late weaned |
|---|---|---|---|---|---|
| Enzyme activity | | | | | |
| Muscle | | | | | |
| COX | $6.25 \pm 1.27^{a,b}$ | $1.45 \pm 0.43^{a,b}$ | $1.05 \pm 0.38^{a}$ | $5.29 \pm 0.78^{a,b}$ | $7.61 \pm 1.53^{b}$ |
| CS | $49.68 \pm 3.26^{a}$ | $48.30 \pm 2.22^{a}$ | $47.38 \pm 3.13^{a}$ | $67.37 \pm 0.80^{b}$ | $75.08 \pm 3.52^{b}$ |
| HOAD | $70.61 \pm 3.04$ | $64.02 \pm 15.65$ | $83.39 \pm 7.62$ | $87.80 \pm 3.01$ | $98.42 \pm 10.54$ |
| Brown adipose tissue | | | | | |
| COX | $15.47 \pm 2.17^{a}$ | $3.70 \pm 0.28^{a}$ | $2.63 \pm 0.30^{b}$ | $2.15 \pm 0.57^{b}$ | $3.74 \pm 0.74^{b}$ |
| CS | $102.23 \pm 15.75^{a}$ | $94.15 \pm 17.26^{a}$ | $67.27 \pm 10.20^{a,b}$ | $13.51 \pm 1.42^{b}$ | $17.44 \pm 1.65^{b}$ |
| HOAD | $193.14 \pm 31.44^{a}$ | $114.57 \pm 8.50^{a,b}$ | $101.57 \pm 21.34^{a,b}$ | $17.09 \pm 8.99^{b}$ | $32.70 \pm 8.79^{b}$ |
| Mitochondrial density (%) | $13.88 \pm 2.00^{a}$ | $4.50 \pm 2.00^{b}$ | $1.95 \pm 0.87^{c}$ | | |

Data are reported as mean ± SEM. Superscripts indicate statistically significant differences ($P < 0.05$) between mean values for which there was effect of age.

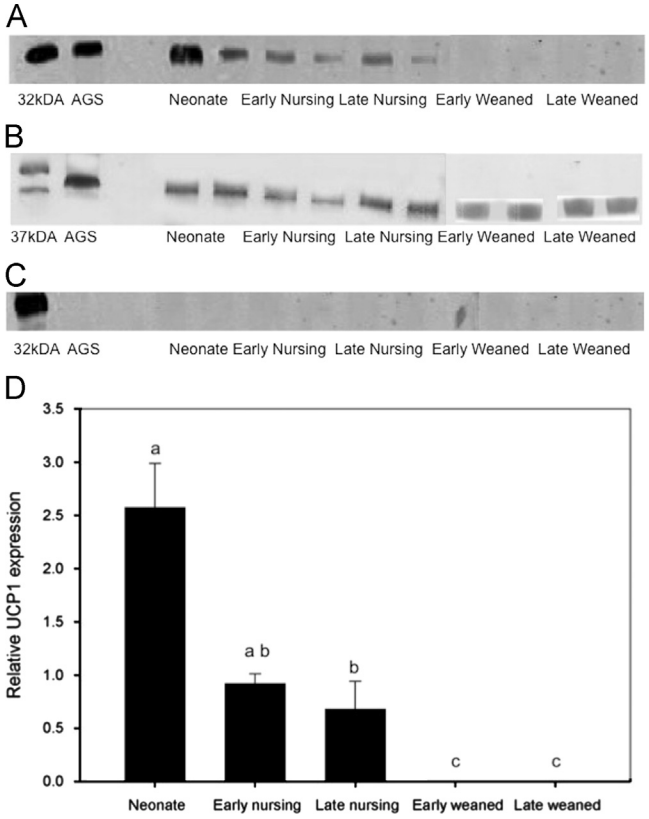


**Fig. 4.** (A) Representative Western blot of uncoupling protein 1 expression in brown adipose tissue from 2 individuals from each of the 5 age classes of harp seal pup. Each band represents an individual pup. (B) Representative Western blot of the loading control, β-actin, in brown adipose tissue from 2 individuals from each of the 5 age classes of harp seal pup. Each band represents the same pup shown in (A). (C) Complete blockage of antibody binding in the peptide inhibition assay. Each band represents an individual pup, and are the same pups as in (A) and (B). (D) Relative UCP1 protein expression ( ± SEM) in different age classes of harp seals during ontogeny, as determined by digital analysis of band intensities from Western blots. Letters indicate statistically significant differences ($P < 0.05$) between mean values for which there was an effect of age.

same MR whether they are fasting in water or air (Worthy and Lavigne, 1987).

For species that are born with lanugo, the thick fur allows the outer skin to be maintained close to core body temperature while the blubber develops, and even once the blubber is thick, the lanugo likely facilitates maintaining warmer skin temperatures during the post-natal molt. This is a critical period when a juvenile pelage is grown, and replaces the lanugo fur, which is shed. As the skin must be kept warm during molting (Ling, 1970), beginning the molt while lanugo is still present and naturally facilitates warm skin temperatures may be beneficial in lowering metabolic costs associated with the postnatal molt. We observed initial growth of juvenile pelage in 3-day old animals (Liwanag and Pearson, unpublished data). Additionally, in this study, though late nursing animals' coats were not visually different from younger animals, pups had much higher pelt conductivity than all other age classes, and this may signify the true beginning of the molt before the adult hair has fully grown.

In adult marine mammals, inter-species comparisons indicate the thermal conductivity of blubber is inversely proportional to the blubber % lipid (Liwanag et al., 2012a, 2012b; Worthy and Edwards, 1990). However, this trend is seen across animals of varying health and condition, and lower conductivity values were reported in animals of poor condition with less than 50% lipid in the blubber (Liwanag et al., 2012a, 2012b; Dunkin et al., 2005). Dunkin et al. (2005) reported no change in conductivity despite increases in blubber lipid content for bottlenose dolphins (*Tursiops truncatus*) during development. The thermal conductivity of harp seal blubber did not change during ontogeny and was not different between pups and adults, but increased blubber thickness resulted in a concomitant increase in the proportion of thermal resistance from blubber as the animals aged. Similar findings in both dolphins and harp seals suggest young animals that are quickly depositing lipid, thus accruing blubber with high lipid content (Kovacs and Lavigne, 1985), have potentially already minimized the conductivity of the blubber. As a result, additional lipid serves only to increase depth and thus resistance of the layer.

The TNZ of harp seal pups is determined by overall conductivity and resistance, but previous studies have only considered the contribution from blubber. Our work suggests, especially in very young pups, the fur is important in determining overall thermal resistance, and low conductivity. While direct measurements of harp seal pup TNZ in air do not exist, model estimates based solely on blubber conductivity measurements predict the lower critical temperature (LCT) of neonates and early nursing animals (blubber depths = 1 cm) to be −1 °C in air, whereas the predicted LCT of weaned and fasting animals (blubber depths = 10 cm) are −59 °C and −85.4 °C (Øritsland and Ronald, 1978; Worthy, 1991). In the Arctic, average temperatures during the March nursing period range from −20 °C to 10 °C, suggesting that neonatal and nursing pups may face environmental conditions below their LCT. However, the insulative value of lanugo is not included in these models, as models are based on the conductivity of blubber alone rather than the sculp. This study showed that the conductivity of the sculp is lower than the conductivity of blubber only (Table 1), and

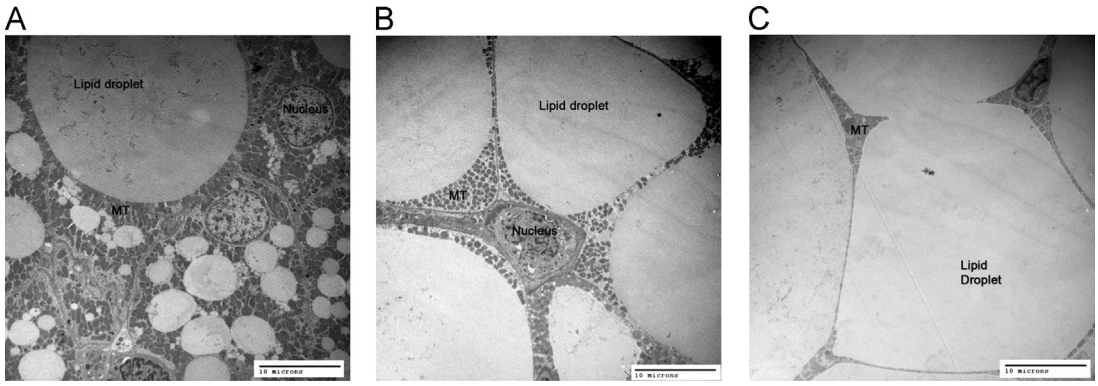


**Fig. 5.** Transmission electron microscopy images (3000× magnification) of brown adipose tissue in neonates (A), early nursing (B), and late nursing (C) harp seal pups. The brown adipose tissue of neonates contains multilocular lipid droplets, and has a high mitochondrial density, but the brown adipose tissue of late nursing pups is characterized by few mitochondria and cells are filled with a single large lipid droplet. *MT* represents mitochondria.

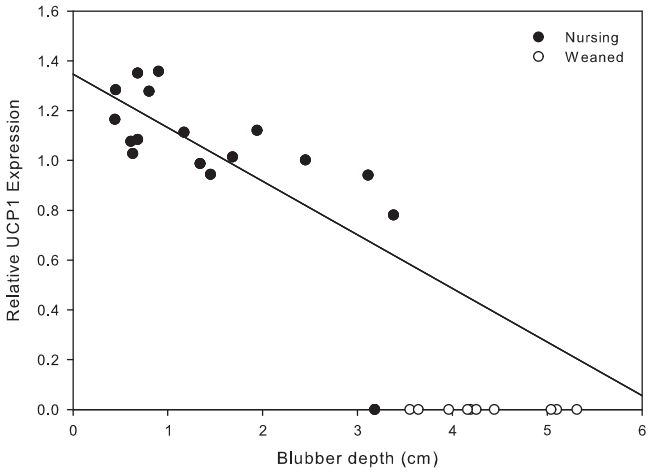


**Fig. 6.** Regression of uncoupling protein 1 expression in relation to mean blubber depth of nursing harp seals (black circles) during ontogeny. There is a significant negative linear correlation between the decline in uncoupling protein 1 expression and mean blubber thickness in nursing harp seals ($R^2$=0.493, $P$=0.002). Weaned harp seals (open circles) did not express any UCP1, and were not included in the regression analysis.

it may be that the LCT of young pups is lower than reported by Øritsland and Ronald (1978).

Previous observations of young harp seal pups suggest they only rely on shivering thermogenesis during the first few hours after birth (Blix et al., 1979). Our examination of the activity of enzymes involved in muscle contraction and shivering thermogenesis support this conclusion, as activities were not any higher in nursing pups than in older animals. The exception to this was elevated COX in neonates only, which could reflect the observations of Blix et al. (1979). These results suggest avoidance of shivering thermogenesis, possibly because heat generation via muscle metabolism would be in conflict with rapid energy deposition during nursing and energy savings needed in preparation for the PWF. Alternatively, changes in muscle enzyme activity with age may reflect changes in muscle structure as pups develop the low oxygen use rates necessary for diving. The slight increase in enzyme activity post-weaning is more likely reflective of muscle development and remodeling that occurs in preparation for an aquatic lifestyle and independent foraging (Burns et al., 2007; Lestyk et al., 2009; Noren et al., 2008) than changes due to thermoregulatory constraints.

While young harp seals apparently do not rely heavily on shivering thermogenesis, they do possess the ability to use NST when ambient temperatures are below their LTC. Neonatal harp seals have BAT that expresses UCP1 and resembles the BAT of other species such as Arctic ground squirrels (*U. parryii*) and Syrian hamsters (*Mesocricetus auratus*) (Afzelius, 1970). We did not observe any of the additional BAT deposits described by Blix et al. (1979) and Grav et al. (1974) and found no other tissue expressing UCP1; thus it appears in neonatal harp seals the subscapular BAT deposit is the only tissue capable of generating heat via NST. This, in combination with the 86% total decrease in mitochondrial density and accompanying 82–85% decrease enzyme activity, suggests the need for NST declines quickly during the nursing period mirroring the increase in blubber, and by weaning, additional thermogenesis by NST is no longer necessary. The decline in thermogenic activity of BAT at 9 days in this study is later than reported by Blix et al. (1979). This also suggests that NST serves a dual role in young harp seals: (1) maintaining euthermia when pups are dry but ambient temperature is very low and (2) warming the body to dry the fur when the pelage becomes wet from storms or immersion. Though, to date, only harp and ringed seals (*Pusa hispida*) are known to express UCP1 (Taugbol, 1982), NST is likely an important mechanism for maintaining euthermia for other small species born with lanugo on pack ice such as spotted seals (*Phoca largha*) and ribbon seals (*Histriophoca fasciata*). For polar species born with a thicker blubber layer, such as hooded seals (*C. cristata*), NST may not be necessary; for species born on a more stable substrate such as Weddell seals (*Leptonychotes weddellii*), NST may not be necessary because the threat of early water entry is minimal.

In nursing harp seal pups, UCP1 expression is negatively correlated with the increase in blubber insulation, and expression is not present in pups with a blubber layer thicker than 3.2–3.5 cm, or any weaned pup (Fig. 6). This suggests that pups that are able to accumulate blubber more quickly may rely less extensively on NST and reserve more of the energy acquired during nursing for PWF. Such linkages between insulation and heat production offer mechanisms by which both maternal efforts and environmental conditions can influence pup growth rates and potential survival. For example in many species, smaller, less-experienced mothers transfer less energy to their pups during the nursing period, resulting in decreasing pup growth and blubber deposition rates (Iverson et al., 1993; Mellish et al., 1999; Stewart and Lavigne, 1984). Small pups would have higher thermoregulatory costs, and would lose mass and condition at a greater rate during the PWF, ultimately resulting in pups in poor condition entering into the initial foraging period (Kovacs and Lavigne, 1986; Mellish et al., 1999). Fuel use during the PWF is influenced by body composition at weaning, and preservation of the blubber layer while fasting is particularly important in species that may enter the water, which is reflected in harp seals' use of both muscle and blubber to fuel

the PWF (Worthy and Lavigne, 1987). Such effects on growth would be magnified during cold or stormy springs. Larger pups of better mothers may be buffered from environmental impacts on pup growth because of higher rates of energy transfer and blubber deposition (Iverson et al., 1993; Kovacs and Lavigne, 1986; Mellish et al., 1999).

Current environmental conditions in the Arctic are warming rapidly, resulting in reduced sea ice stability, depth, and duration (Friedlaender et al., 2010; Hansen et al., 2013; Post et al., 2013; Walsh, 2008), and a predicted increase in storm events (Schultz, 2013; Vermaire et al., 2013). Harp seals, like other Arctic phocids, are dependent on a stable sea ice substrate during the nursing and PWF periods (Bajzak et al., 2011; Friedlaender et al., 2010; Moore and Huntington, 2008) and poor ice conditions are known to increase pup mortality (Bajzak et al., 2011; Ferguson et al., 2005; Friedlaender et al., 2010; Johnston et al., 2012; Kovacs and Lydersen 2008). Our results suggest if pups are forced to enter the water early, nursing harp seals pups would have elevated thermoregulatory costs as compared to weaned pups, because of their lack of blubber. The increased thermoregulatory costs could have negative consequences on their survival (Davydov and Makarova, 1964; Smith and Harwood, 2001; Worthy, 1991). While harp seals gain blubber quickly (Kovacs and Lavigne, 1985) and thus have a relatively short period of vulnerability, this may not be the case in species with prolonged development periods and smaller birth size, such as ringed and spotted seals (Ferguson et al., 2005; Oftedal et al., 1996). As the pups of 7 of 11 polar phocids rely on lanugo when young, these finding suggest that vulnerability to changes in ice conditions may be widespread. While it may be possible for some species to shift from pupping on pack ice to nearby shorelines, such changes carry other risks such as predation, disturbance, and disease transmission. Ultimately, more research is needed to understand the mechanisms, costs, and timing associated with development of thermoregulatory strategies in young polar marine mammals and to predict the vulnerability to climate change of different ice-breeding seals during the critical time of early development.

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## References

Afzelius, B.A., 1970. Brown adipose tissue: its gross anatomy, histology, and cytology. In: Lindberg, O. (Ed.), Brown Adipose Tissue. American Elsevier, New York, pp. 1–31

Bajzak, C.E., Hammill, M.O., Stenson, G.B., Prinsenberg, S., 2011. Drifting away: implications of changes in ice conditions for a pack-ice-breeding phocid, the harp seal (*Pagophilus groenlandicus*). Can. J. Zool. 89, 1050–1062.

Blix, A.S., Grav, H.J., Ronald, K., 1979. Some aspects of temperature regulation in newborn harp seal pups. Am. J. Physiol. 236, R188–R197.

Blix, A.S., Steen, J.B., 1979. Temperature regulation in newborn polar homeotherms. Physiol. Rev. 59, 285–304.

Burns, J.M., Lestyk, K.C., Folkow, L.P., Hammill, M.O., 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.

Cannon, B., Nedergaard, J., 2008. Studies of thermogenesis and mitochondrial function in brown adipose tissue. In: Yang, K. (Ed.), Adipose Tissue Protocols, 2nd ed. Humana Press, London; Ontario, Canada, pp. 109–121

Davydov, A.F., Makarova, A.R., 1964. Changes in heat regulation and circulation in newborn seals on transition to aquatic from of life. Fed. Proc. 24, T563–T566.

Dunkin, R.C., McLellan, W.A., Blum, J.E., Pabst, D.A., 2005. The ontogenetic changes in the thermal properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus*. J. Exp. Biol. 208, 1469–1480.

Ferguson, S.H., Stirling, I., McLoughlin, P., 2005. Climate change and ringed seal (*Phoca hispida*) recruitment in western Hudson Bay. Mar. Mamm. Sci. 21, 121–135.

Friedlaender, A.S., Johnston, D.W., Halpin, P.N., 2010. Effects of the North Atlantic Oscillation on sea ice breeding habitats of harp seals (*Pagophilus groenlandicus*) across the North Atlantic. Prog. Oceanogr. 86, 261–266.

Gales, N.J., Burton, H.R., 1987. Ultrasonic measurement of blubber thickness of the Southern elephant seal, *Mirounga leonina* (Linn.). Aust. J. Zool. 35, 207–217.

Gallivan, G.J., Ronald, K., 1979. Temperature regulation in freely diving harp seals (*Phoca groenlandica*). Can. J. Zool. 57, 2256–2263.

Grav, H.J., Blix, A.S., Pasche, A., 1974. How do seal pups survive birth in arctic winter? Acta Physiol. Scand. 92, 427–429.

Hart, J.S., Irving, L., 1959. The energetics of harbor seals in air and in water with special consideration of seasonal changes. Can. J. Zool. 37, 447–457.

Hansen, J., Kharecha, P., Sato, M., Masson-Delmotte, V., Ackerman, F., Beerling, D.J., Hearty, P.J., Hoegh-Guldberg, O., Hsu, S., Parmesan, C., Rockstrom, J., Rohling, E. J., Sachs, J., Smith, P., Steffen, K., Van Susteren, L., von Schuckmann, K., Zachos, J.C., 2013. Assessing "dangerous climate change": required reduction of carbon emissions to protect young people, future generations and nature. PLOS One 8 (12), e81648.

Innes, S., Worthy, G.A.J., Lavigne, D.M., Ronald, K., 1990. Surface-areas of phocid seals. Can. J. Zool. 68, 2531–2538.

Irving, L., Hart, J.S., 1957. The metabolism and insulation of seals as bare-skinned mammals in cold water. Can. J. Zool. 35, 497–511.

Iverson, S.J., Bowen, W.D., Boness, D.J., Oftedal, O.T., 1993. The effect of maternal size and milk energy output on pup growth in gray seals (*Halichoerus grypus*). Physiol. Zool. 66 (1), 61–88.

Johnston, D.W., Bowers, M.T., Friedlaender, A.S., Lavigne, D.M., 2012. The effects of climate change on harp seals (*Pagophilus groenlandicus*). Plos One 7 (1), e29158.

Kanatous, S.B., Hawke, T.J., Trumble, S.J., Pearson, L.E., Watson, R.R., Garry, D.J., Williams, T.M., Davis, R.W., 2008. The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. J. Exp. Biol. 211, 2559–2565.

Kovacs, K.M., Lavigne, D.M., 1985. Neonatal growth and organ allometry of Northwest Atlantic harp seals (*Phoca groenlandica*). Can. J. Zool. 63, 2793–2799.

Kovacs, K.M., Lavigne, D.M., 1986. Maternal investment and neonatal growth in phocid seals. J. Anim. Ecol. 55, 1035–1051.

Kovacs, K.M., Lydersen, C., 2008. Climate change impacts on seals and whales in the North Atlantic Arctic and adjacent shelf seas. Sci. Prog. 91, 117–150.

Kreith, F., 1958. Principles of Heat Transfer. Intext Educational Publishers, New York

Kvadsheim, P.H., Folkow, L.P., Blix, A.S., 1994. A new device for measurement of the thermal conductivity of fur and blubber. J. Theor. Biol. 19, 431–435.

Kvadsheim, P.H., Aarseth, J.J., 2002. Thermal function of Phocid seal fur. Mar. Mamm. Sci. 18, 952–962.

Lapierre, J.L., Schreer, J.F., Burns, J.M., Hammill, M.O., 2004. Developmental changes in cardiorespiratory patterns associated with terrestrial apnoeas in harbour seal pups. J. Exp. Biol. 207, 3891–3896.

Lavigne, D.M., Innes, S., Worthy, G.A.J., Kovacs, K.M., Schmitz, O.J., Hickie, J.P., 1986. Metabolic rates of seals and whales. Can. J. Zool. 64, 279–284.

Lestyk, K.C., Folkow, L.P., Blix, A.S., Hammill, M.O., 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.

Ling, J.K., 1970. Pelage and molting in wild mammals with special reference to aquatic mammals. Q. Rev. Biol. 45, 16–54.

Liwanag, H.E.M., Williams, T.M., Costa, D.P., Kanatous, S.B., Davis, R.W., Boyd, I.L., 2009. The effects of water temperature on the energetic costs of juvenile and adult California sea lions (*Zalophus californianus*): the importance of skeletal muscle thermogenesis for thermal balance. J. Exp. Biol. 212, 3977–3984.

Liwanag, H.E.M., Berta, A., Costa, D.P., Abney, M., Williams, T.M., 2012a. Morphological and thermal properties of mammalian insulation: the evolution of fur for aquatic living. Biol. J. Linn. Soc. 106, 926–939.

Liwanag, H.E.M., Berta, A., Costa, D.P., Budge, S.M., Williams, T.M., 2012b. Morphological and thermal properties of mammalian insulation: the evolutionary transition to blubber in pinnipeds. Biol. J. Linn. Soc. 107, 774–787.

Mathieu, O., Cruz-Orive, L.M., Hoppeler, H., Weibel, E.R., 1981. Measuring error and sampling variation in stereology: comparison of the efficiency of various methods for planar image analysis. J. Microsc. 121, 75–88.

Mellish, J.A., Iverson, S.J., Bowen, D.W., 1999. Variation in milk production and lactation performance in grey seals and consequences for pup growth and weaning characteristics. Physiol. Biochem. Zool. 72 (5), 677–690.

Moore, S.E., Huntington, H.P., 2008. Arctic marine mammals and climate change: impacts and resilience. Ecol. Appl. 118, S157–S165.
Noren, D.P., Crocker, D.E., Williams, T.M., Costa, D.P., 2003. Energy reserve utilization in northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. J. Comp. Physiol. [B] 173, 443–454.
Noren, S.R., Boness, D.J., Iverson, S.J., McMillan, J., Bowen, W.D., 2008. Body condition at weaning affects the duration of the postweaning fast in gray seal pups (*Halichoerus grypus*). Physiol. Biochem. Zool. 81, 269–277.
Oftedal, O.T., Bowen, W.D., Widdowson, E.M., Boness, D.J., 1989. Effects of suckling and the postsuckling fast on weight of the body and internal organs of harp and hooded seal pups. Biol. Neonate 56, 283–300.
Oftedal, O.T., Bowen, W.D., Widdowson, E.M., Boness, D.J., 1991. The prenatal molt and its ecological significance in hooded and harbor seals. Can. J. Zool. 69, 2489–2493.
Oftedal, O.T., Bowen, W.D., Bonness, D.J., 1996. Lactation perfromance and nutrient deposition in pups of the harp seal, *Phoca groenlandica*, on ice floes of Southeast Labrador. Physiol. Zool. 69 (3), 635–657.
Øritsland, N.A., Ronald, K., 1978. Aspects of temperature regulation in harp seal pups evaluated by in vivo experiments and computer simulations. Acta Physiol. Scand. 103, 263–269.
Post, E., Bhatt, U.S., Bitz, C.M., Brodie, J.F., Fulton, T.L., Hebblewhite, M., Kerby, J., Kutz, S.J., Stirling, I., Walker, D.A., 2013. Ecological consequences of sea-ice decline. Science 341 (6145), 519–524.
Prewitt, J.S., Freistroffer, D.V., Schreer, J.F., Hammill, M.O., Burns, J.M., 2010. Postnatal development of muscle biochemistry in nursing harbor seal (*Phoca vitulina*) pups: limitations to diving behavior? J. Comp. Physiol. [B] 180, 757–766.
Ryg, M., Lydersen, C., Knutsen, L.O., Bjorge, A., Smith, T.G., Oritsland, N.A., 1993. Scaling of insulation in seals and whales. J. Zool. 230, 193–206.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Scholander, P.F., Walters, V., Hock, R., Irving, L., 1950. Body insulation of some arctic and tropical mammals and birds. Biol. Bull. 99, 225–236.
Scholander, P.F., 1955. Evolution of climatic adaptation in homeotherms. Evol. Dev. 9, 15–26.
Schultz, C., 2013. Arctic climate warming leads to increased storm surge activity. Eos, Trans. Am. Geophys. Union 94, 156.
Smith, T.G., Harwood, L.A., 2001. Observations of neonate ringed seals, *Phoca hispida*, after early break-up of the sea ice in Prince Albert Sound, Northwest Territories, Canada, spring 1998. Polar Biol. 24, 215–219.
Stewart, R.E.A., Lavigne, D.M., 1980. Neonatal growth of northwest Atlantic Harp seals, *Pagophilus groenlandicus*. J. Mamm. 61 (4), 670–680.
Stewart, R.E.A., Lavigne, D.M., 1984. Energy transfer and female condition in nursing harp seals *Phoca groenlandica*. Ecography 7 (2), 182–194.
Taugbol, G., 1982. Ringed seal thermoregulation, energy balance and development in early life, a study on *Pusa hispida* in Kongsfj.. Svalbard, Institute of Zoophysiology. University of Oslo, Norway, Oslo p. 102
Vermaire, J.C., Pisaric, M.F.J., Thienpont, J.R., Courtney Mustaphi, C.J., Kokelj, S.V., Smol, J.P., 2013. Arctic climate warming and sea ice declines lead to increased storm surge activity. Geophys. Res. Lett. 40, 1386–1390.
Walsh, J.E., 2008. Climate of the arctic marine environment. Ecol. Appl. 18, S3–S22.
Watson, R.R., Kanatous, S.B., Cowan, D.F., Wen, J.W., Han, V.C., Davis, R.W., 2007. Volume density and distribution of mitochondria in harbor seal (*Phoca vitulina*) skeletal muscle. J. Comp. Physiol. [B] 177, 89–98.
Weibel, E.R., 1979. Stereological Methods: Practical Methods for Biological Morphometry. Academic Press, London
Worthy, G.A.J., 1987. Metabolism and growth of young harp and grey seals. Can. J. Zool. 65, 1377–1382.
Worthy, G.A.J., Lavigne, D.M., 1987. Mass loss, metabolic rate, and energy utilization by harp and gray seal pups during the postweaning fast. Physiol. Zool. 60, 352–364.
Worthy, G.A.J., Edwards, E.F., 1990. Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). Physiol. Zool. 63, 432–442.
Worthy, G.A.J., 1991. Insulation and thermal balance of fasting harp and gray seal pups. Comp. Biochem. Physiol. A – Physiol. 100, 845–851.