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Persistent organic pollutants, skull size and bone density of polar bears (*Ursus maritimus*) from East Greenland 1892–2015 and Svalbard 1964–2004

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ABSTRACT

We investigated skull size (condylobasal length; CBL) and bone mineral density (BMD) in polar bears (*Ursus maritimus*) from East Greenland (n = 307) and Svalbard (n = 173) sampled during the period 1892–2015 in East Greenland and 1964–2004 at Svalbard. Adult males from East Greenland showed a continuous decrease in BMD from 1892 to 2015 (linear regression: $p < 0.01$) indicating that adult male skulls collected in the early pre-pollution period had the highest BMD. A similar decrease in BMD over time was not found for the East Greenland adult females. However, there was a non-significant trend that the skull size of adult East Greenland females was negatively correlated with collection year 1892–2015 (linear regression: $p = 0.06$). No temporal change was found for BMD or skull size in Svalbard polar bears (ANOVA: all $p > 0.05$) nor was there any significant difference in BMD between Svalbard and East Greenland subpopulations. Skull size was larger in polar bears from Svalbard than from East Greenland (two-way ANOVA: $p = 0.003$). T-scores reflecting risk of osteoporosis showed that adult males from both East Greenland and Svalbard are at risk of developing osteopenia. Finally, when correcting for age and sex, BMD in East Greenland polar bears increased with increasing concentrations of persistent organic pollutants (POPs) i.e. ΣPCB (polychlorinated biphenyls), ΣHCH (hexachlorohexane), HCB (hexachlorobenzene) and ΣPBDE (polybrominated diphenyl ethers) while skull size increased with ΣHCH concentrations all in the period 1999–2014 (multiple linear regression: all $p < 0.05$, n = 175). The results suggest that environmental changes over time, including exposure to POPs, may affect bone density and size of polar bears.

1. Introduction

Despite their remoteness, arctic environments are polluted by heavy metal and organic contaminants from industrial areas (AMAP, 2009). Toxic industrial chemicals are long-range transported via ocean and atmospheric currents from industrialized regions at southern latitudes to the Arctic, where they condensate and precipitate (AMAP, 2009). Numerous persistent organic pollutants (POPs) have been shown to

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biomagnify in the lipid-rich Arctic food chains with apex predators such as polar bears (*Ursus maritimus*) having particularly high concentrations of numerous POPs in adipose and/or liver tissues (AMAP, 2009; Dietz et al., 2013a, 2013b; Letcher et al., 2010). Long-range transported POPs that have been measured are used as categorized into pesticides, flame retardants, sealants and coolants (AMAP, 2009). These chemicals have been produced and used in high quantities since the 1930s and throughout the 20th century. Despite many of these substances being phased out or banned, they are still found in high concentrations in e.g. polar bears (AMAP, 2009; Letcher et al., 2010). Due to the nature of the atmospheric and oceanic transport pathways, Greenland and Svalbard are among the polar regions with the most POP-contaminated species on the planet (Letcher et al., 2010). The consequences of contaminants and particularly POPs on polar bear health have been investigated since the 1990s and a wealth of information is available (Dietz et al., 2015; Letcher et al., 2010; Jenssen et al., 2015; Sonne, 2010; Sonne et al., 2012). It has been suggested that systems such as the immune and endocrine systems are affected by POP exposure (Bechshøft et al., 2012; Desforges et al., 2016, 2017; Jenssen et al., 2015). Such perturbations affect bone growth and density due to disruption of vitamin and hormone homeostasis that may have fatal consequences for polar bear health and survival (Sonne, 2010; Sonne et al., 2006, 2013, 2015).

Here we investigate bone composition and size and compare parameters from East Greenland and Svalbard polar bears spanning 1892–2015. The period 1892–1936 is defined to be the "pre-pollution" reference period while 1966–2015 is viewed as being the "pollution" period according to Sonne et al. (2004) and Sonne (2010). A previous study has shown that skull bone mineral density (BMD) represents the mineral status of the skeletal system in general, and we therefore used skulls as a proxy for general skeletal mineralisation (Sonne et al., 2004). Skull BMD was measured as a quantitative bone quality endpoint, while skull size (condylobasal length, CBL) was used as an indirect measure of growth and size (Derocher and Wiig, 2002). In addition, adipose tissue concentrations of POPs, available from a subsample of the East Greenland bears sampled 1999–2014, were used to test for a direct statistical link between BMD/skull size and concentrations of environmental pollutants at the individual level. T-score, a measure for calculating whether there is a risk of osteoporosis, was done for bears in the pollution-period using the pre-pollution period as normal reference values.

2. Materials and methods

2.1. Samples

The present study is an expansion of the East Greenland study of bone composition and size by Sonne et al. (2013) combined with a first-time comparison of bone density in the East Greenland and Svalbard subpopulations over the period 1892–2015. We analysed polar bear skulls from East Greenland (n = 307, sampled at the Natural History Museum of Denmark, University of Denmark) and Svalbard (n = 173, sampled at the Natural History Museum, University of Oslo). The skulls from East Greenland were sampled during four periods (1892, 1900–1936, 1966–1977 and 1986–2015). The Svalbard skulls were sampled during 1964–1969 and 1977–2004. Here, the period 1892–1936 is defined to be the "pre-pollution" reference period while 1966–2015 is viewed as the "pollution" period according to Sonne et al. (2004) and Sonne (2010). Furthermore, corresponding adipose tissue samples were sampled from 175 of the 307 East Greenland polar bears in the period 1999–2015. The individual age estimation of the bears was performed by counting the cementum growth layer groups of the lower right incisor (I_3) after decalcification, thin sectioning (14 µm) and staining with Toluidine Blue as described by Hensel and Sorensen (1980) and Dietz et al. (1991).

2.2. Skull size and bone mineral density (BMD) measurements

Skull size was measured as the condylobasal length (CBL) with callipers to the nearest 0.1 mm. It was measured on the left and right side of each skull according to Sonne et al. (2005). The mean of two measurements was used as skull size. Data on skull size and BMD from the period 1892–2010 were available from East Greenland and Svalbard from previous studies (Pertoldi et al., 2012; Sonne et al., 2013). X-ray osteodensitometry (DXA scanning) was applied to detect BMD (calcium phosphate, hydroxyapatite; g/cm^2) using DXA scanner Norland Corporation, XR 26 (Fort Atkinson, WI, USA) at Hvidovre Hospital's Osteoporosis Unit. For BMD, skulls were scanned in "research mode" (speed, 60 mm/sec; resolution, 3.0 × 3.0 mm; width, 24.9 cm) and analysed using XR software (version 2.4; Norland Corporation), which generated a picture of the bone segment and calculated BMD (hydroxyapatite). The DXA scanner was calibrated daily using a phantom sample with known mineral density. In addition, the precision was tested by a 10 × rescanning (mean ± SD, 521.96 ± 0.60 g/cm^2), which gave a precision of 99.9% ([1 – (SD/mean) × 100%]). T-score, a measure used to calculate risk of osteoporosis (WHO, 2007), was applied on BMD data. For East Greenland polar bears from the "pollution-period" (which we defined as post 1966), the T-score was calculated as: $\text{T-score}_{EG\text{-}pol} = (M_{EG\text{-}Pol} - M_{EG\text{-}Prepol})/SD_{EG\text{-}Prepol}$, where M = mean, SD = standard deviation, EG-Pol = East Greenland polar bears from the pollution period (1966–2015), EG-Prepol = East Greenland polar bears from the pre-polluted period (prior to 1937) acting as reference material. Normal bone: T-score > −1; Osteopenia: −2.5 < T-score < −1; Osteoporosis: T-score < −2.5 (Sonne, 2010). The mean and SD for pre-pollution East Greenland polar bears were also used to estimate T-scores for Svalbard polar bears from the pollution period following the similar algorithm of $\text{T-score}_{SV} = (M_{SV} - M_{EG\text{-}Prepol}/SD_{EG\text{-}Prepol})$.

2.3. Persistent organic pollutant (POP) determination

The determination of selected POPs in adipose tissue was conducted at the Organic Contaminant Research Laboratory at Environment and Climate Change Canada's National Wildlife Research Centre (Carleton University) in Ottawa, Canada. Data for POPs from the period 1999–2014 were available from East Greenland (Dietz et al., 2013a, 2013b). Contaminants were extracted from the tissue and determined by gas chromatography-single quadrupole mass spectrometry (GC-MS) as described in McKinney et al. (2010). As part of an on-going AMAP (Arctic Monitoring and Assessment Program) programme; all adipose tissue samples were analysed by the same laboratory for polychlorinated biphenyls (PCBs) including the congeners CB-18, −17, −16/32, −31, −28, −33/20, −22, −52, −49, −47/48, −44, −42/59, −64/41, −74, −70/76, −66, −56/60, −95, −92, −101/90, −99, −97, −87, −85, −110, −118, −114, −105, −151, −149, −146, −153, −141, −130, −137, −138, −158, −128, −167, −156, −157, −179, −176, −178, −187/182, −183, −174, −177, −171, −172, −180, −170/190, −189, −202, −200, −199, −196/203, −208, −195, −207, −194, −206 and −201, dichlorodiphenyltrichloroethanes (DDTs) including *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT, hexachlorocyclohexanes (HCH) including α-, β- and γ-HCH, hexachlorobenzene (HCB), chlorobenzene (ClBz), chlordanes including oxychlordane, *trans*-chlordane, nonachlor III (MC6), *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide, polybrominated diphenyl ethers (PBDEs) including the congeners BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154 (co-elutes with BB-153), −183, −190, −209, hexabromocyclododecane (HBCDD), perfluorooctane sulfonic acid (PFOS). All concentrations were calculated and expressed in lipid weight (lw). Quantification methods have been reported in detail by e.g. McKinney et al. (2010).