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Persistent organic pollutants and penile bone mineral density in East Greenland and Canadian polar bears (*Ursus maritimus*) during 1996–2015

Tobias Daugaard-Petersen[a], Rikke Langebæk[b], Frank F. Rigét[a], Markus Dyck[c], Robert J. Letcher[d], Lars Hyldstrup[e], Jens-Erik Bech Jensen[e], Rune Dietz[a], Christian Sonne[a,*]

[a] Aarhus University, Faculty of Science and Technology, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark

[b] University of Copenhagen, Faculty of Health and Medical Sciences, Department of Veterinary Clinical and Animal Sciences, Dyrlægevej 16, 1-72, DK-1870 Frederiksberg C, Denmark

[c] Wildlife Management Division, Department of Environment, Government of Nunavut, PO Box 209, Igloolik, NU X0A 0L0, Canada

[d] Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment and Climate Change Canada, National Wildlife Research Centre, Carleton University, Ottawa, ON K1A 0H3, Canada

[e] University Hospital of Hvidovre, Kettegaards Allé 30, DK-2650 Hvidovre, Denmark

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ABSTRACT

Persistent organic pollutants (POPs) are long-range transported to the Arctic via atmospheric and oceanic currents, where they biomagnify to high concentrations in the tissues of apex predators such as polar bears (*Ursus maritimus*). A major concern of POP exposure is their physiological effects on vital organ-tissues posing a threat to the health and survival of polar bears. Here we examined the relationship between selected POPs and baculum bone mineral density (BMD) in the East Greenland and seven Canadian subpopulations of polar bears. BMD was examined in 471 bacula collected between years 1996–2015 while POP concentrations in adipose tissue were determined in 67–192 of these individuals collected from 1999 to -2015. A geographical comparison showed that baculum BMD was significantly lowest in polar bears from East Greenland (EG) when compared to Gulf of Boothia (GB), Southern Hudson (SH) and Western Hudson (WH) Bay subpopulations (all $p < 0.05$). The calculation of a T-score osteoporosis index for the EG subpopulation using WH bears as a reference group gave a T-score of −1.44 which indicate risk of osteopenia. Concentrations of ΣPCB_{74} (polychlorinated biphenyls), ΣDDT_3 (dichlorodiphenyltrichloroethanes), *p,p′*-DDE (dichlorodiphenyldichloroethylene), ΣHCH_3 (hexachlorohexane) and α-HCH was significantly highest in EG bears while ΣPBDE (polybrominated diphenyl ethers), BDE-47 and BDE-153 was significantly highest in SH bears (all $p < 0.04$). Statistical analyses of individual baculum BMD vs. POP concentrations showed that BMD was positively correlated with ΣPCB_{74}, CB-153, HCB (hexachlorobenzene), ΣHCH, β-HCH, ClBz (chlorobenzene), ΣPBDE and BDE-153 (all $p < 0.03$). In conclusion, baculum density was significantly lowest in East Greenland polar bears despite the positive statistical correlations of BMD vs. POPs. Other important factors such as nutritional status, body mass and body condition was not available for the statistical modelling. Since on-going environmental changes are known to affect these, future studies need to incorporate nutritional, endocrine and genetic parameters to further understand how POP exposure may disrupt bone homeostasis and affect baculum BMD across polar bear subpopulations.

1. Introduction

Climatic changes as well as infectious diseases and persistent organic pollutants (POPs) are considered the most substantial environmental stressors of the Arctic ecosystem (AMAP, 2015; Jenssen et al., 2015; Letcher et al., 2010; Sonne et al., 2012). The presence of POPs in the Arctic marine environment is the result of long-range atmospheric and oceanic transport, which has occurred since the 1940s from lower latitude sources in the industrialized parts of the world (AMAP, 1998, 2004). Due to the lipophilic nature of many POPs, these chemicals persist in the slow-growing and lipid-rich Arctic food chains (Letcher et al., 2010). Consequently, high POP concentrations are found in the

* Corresponding author at: Dipl. ECZM (Wildlife Health), Aarhus University, Faculty of Science and Technology, Department of Bioscience, Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark.

E-mail addresses: ril@sund.ku.dk (R. Langebæk), ffr@bios.au.dk (F.F. Rigét), MDyck1@GOV.NU.CA (M. Dyck), Robert.Letcher@canada.ca (R.J. Letcher), larshyldstrup@dadlnet.dk (L. Hyldstrup), jebj@dadlnet.dk (J.-E.B. Jensen), rdi@bios.au.dk (R. Dietz), cs@bios.au.dk (C. Sonne).

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Inuit populations and in marine top predators that consume large amounts of high trophic level marine mammals with East Greenland and Hudson Bay as particular hotspots (AMAP, 2015; Dietz et al., 2013a, 2013b; Letcher et al., 2010, 2018; McKinney et al., 2013).

Recently, polar bears have received considerable attention as a vulnerable Arctic species that is highly influenced by climatic change (Jenssen et al., 2015; Wiig et al., 2015). Thus, it is argued that the projected sea ice loss across the Arctic Ocean will restrict polar bears' access to principal prey such as ringed seals (*Phoca hispida*) during autumn (Durner et al., 2009; Molnár et al., 2011; Stirling and Derocher, 2012). In addition, polar bears are top predator species and therefore at greater risk of severe population declines due to POP exposure (Jenssen et al., 2015; Letcher et al., 2010; Sonne, 2010). The East Greenland ecosystem carry the highest loads of POPs in the Arctic and therefore polar bears from these subpopulations are among the most contaminated (Letcher et al., 2010). Increases in biomagnification of POPs has occurred in East Greenland polar bears over the last decades mainly because changes in ice dynamics that has led to dietary changes and toward more highly polluted prey i.e. hooded (*Cystophora cristata*) and harp (*Pagophilus groenlandicus*) seals (McKinney et al., 2013). The consequence of these dietary changes and elevated POP exposure is an increase in the risk for effects on growth and development of, for example, reproductive organs and the immune and skeletal system (Desforges et al., 2016; Dietz et al., 2015; Letcher et al., 2010; Sonne, 2010; Sonne et al., 2006, 2012).

Bone formation and resorption is controlled by multiple physiological factors such as hormones, vitamins and micronutrients (Barret et al., 2010; Herlin et al., 2010). POPs are known to disrupt bone homeostasis (Lind et al., 2003, 2004) and in polar bears there has previously been reported inverse relationships between bone density and several POP compounds (Sonne, 2010; Sonne et al., 2004, 2006). A study by Sonne et al. (2015) even suggested that changes in the baculum density of East Greenland polar bears may lead to population declines as reduced strength could lead to fractures and inability to successfully mate.

Canadian polar bear subpopulations are, with the exception of Hudson Bay, less contaminated with POPs compared the East Greenland subpopulation (Norstrom et al., 1998; McKinney et al., 2011; Verreault et al., 2005). A comparative study of baculum bone density and POPs in East Greenland and Canadian polar bears is therefore warranted (Sonne et al., 2015). The aim of the present study was to investigate possible geographical differences in baculum BMD, as well as correlations between POP concentrations and baculum BMD.

2. Materials and methods

2.1. The sample

BMD was measured in 471 bacula collected in the period 1996–2015. These were from eight management areas including East Greenland (EG) (n = 137), Baffin Bay (BB) (n = 32), Davis Strait (DS) (n = 28), Lancaster Sound (LS) (n = 62), Gulf of Boothia (GB) (n = 31), Foxe Basin (FB) (n = 80), Southern Hudson Bay (SH) (n = 74) and Western Hudson Bay (WH) (n = 27) (Fig. 1). All bacula were collected as part of research projects based on local Inuit subsistence hunting (Letcher et al., 2010, 2018; Sonne, 2010; Sonne et al., 2012, 2015). The bacula were stored frozen at −20 °C and manually cleaned, macerated and dried at room temperature prior to analyses. POP concentrations was available from SH (n = 72–74), WH (n = 27) and EG (n = 15–91) as part of previous studies conducted 1999–2015 (Dietz et al., 2013a, 2013b, Unpubl. data; Letcher et al., 2010, 2018; McKinney et al., 2010). The individual age estimations was performed by counting the cementum growth layer groups (GLG) 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. Bone mineral density (BMD) measurements

X-Ray osteodensitometry was applied to determine baculum bone mineral density. The X-ray bone densitometer (model XR 26; Norland Corporation, Fort Atkinson, WI, USA) determined BMD (calcium phosphate, hydroxyapatite; g/cm^2) using dual X-ray absorptiometry (DXA). The bacula were scanned in "research" mode (speed, 60 mm/s; resolution, 3.0 × 3.0 mm; width, 24.9 cm) and analysed using XR software (revision 2.4; Norland Corporation), which generated a picture of the bone segment and calculated the BMD of hydroxyapatite in grams per square centimetre. The DXA scanner was calibrated daily using a phantom with known bone 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 99.88% ([1 – (SD/mean) × 100%]). T-score, a measure used in human medicine for calculating whether patients are in risk of osteoporosis (WHO, 2007) was estimated for East Greenland polar bears according to Sonne (2010): $\text{T-score}_{EG} = M_{EG}\text{-}M_{WH}/SD_{WH}$. M = mean, SD = standard deviation, EG = East Greenland polar bears, WH = Western Hudson polars used as reference group holding the highest BMD values. Normal bone: T-score > −1; Osteopenia: −2.5 < T-score < −1; Osteoporosis: T-score < −2.5.

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. 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 74 polychlorinated biphenyl congeners (ΣPCB_{74}) including 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 (ΣDDT) including *p,p'*-DDE (dichlorodiphenyldichloroethylene), *p,p'*-DDD (dichlorodiphenyldichloroethane) and *p,p'*-DDT, hexachlorocyclohexanes (ΣHCH) including α-, β- and γ-HCH, hexachlorobenzene (HCB), chlorobenzene (ΣClBz), chlordanes including oxychlordane, *trans*-chlordane, nonachlor III (MC6), *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide, 14 polybrominated diphenyl ethers ($\Sigma PBDE_{14}$) including the congeners BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −190, −209 and hexabromocyclododecane (HBCDD). The POP concentrations are expressed in lipid weight (lw). Quantification methods have been reported in detail by e.g. Letcher et al. (2018) and McKinney et al. (2010, 2011).

2.4. Statistical analyses

First, a regression analysis was conducted to investigate the relationship between age and BMD. To secure comparability among the polar bear subpopulations and to take sample size into account, only individuals being 3–9 years of age were used to investigate the growth pattern and differences in BMD between subpopulations by analysis of covariance models (ANCOVA). The explanatory variables were age and age^2 assuming that growth followed a polynomial curve. A test of least square means (LSM) of significant factors was applied for pairwise comparisons in BMD among polar bear subpopulations. LSM (or marginal means) are estimates of the means when controlled for co-variables (age). Analyses of covariance (ANCOVA) with Tukey's post hoc was used to analyse differences in ΣPCB concentrations among EG, SH and WH subpopulations taking age into consideration. To obtain