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Relationships between POPs, biometrics and circulating steroids in male polar bears (*Ursus maritimus*) from Svalbard☆

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ABSTRACT

The aim of this study was to determine the effects of persistent organic pollutants (POPs) and biometric variables on circulating levels of steroid hormones (androgens, estrogens and progestagens) in male polar bears (*Ursus maritimus*) from Svalbard, Norway (n = 23). Levels of pregnenolone (PRE), progesterone (PRO), androstenedione (AN), dehydroepiandrosterone (DHEA), testosterone (TS), dihydrotestosterone (DHT), estrone (E1), 17α-estradiol (αE2) and 17β-estradiol (βE2) were quantified in polar bear serum by gas chromatography tandem mass spectrometry (GC-MS/MS), while POPs were measured in plasma. Subsequently, associations between hormone concentrations (9 steroids), POPs (21 polychlorinated biphenyls (PCBs), 8 OH-PCBs, 8 organochlorine pesticides (OCPs) and OCP metabolites, and 2 polybrominated diphenyl ethers (PBDEs)) and biological variables (age, head length, body mass, girth, body condition index), capture date, location (latitude and longitude), lipid content and cholesterol levels were examined using principal component analysis (PCA) and orthogonal projections to latent structures (OPLS) modelling. Average concentrations of androgens, estrogens and progestagens were in the range of 0.57–83.7 (0.57–12.4 for subadults, 1.02–83.7 for adults), 0.09–2.69 and 0.57–2.44 nmol/L, respectively. The steroid profiles suggest that sex steroids were mainly synthesized through the Δ-4 pathway in male polar bears. The ratio between androgens and estrogens significantly depended on sexual maturity with androgen/estrogen ratios being approximately 60 times higher in adult males than in subadult males. PCA plots and OPLS models indicated that TS was positively related to biometrics, such as body condition index in male polar bears. A negative relationship was also observed between POPs and DHT. Consequently, POPs and body condition may potentially affect the endocrinological function of steroids, including development of reproductive tissues and sex organs and the general condition of male polar bears.

1. Introduction

The Arctic region is a pristine environment with few local pollution sources. Nevertheless, the ecosystems in this region are strongly affected by global pollution (Riget et al., 2010; Letcher et al., 2010). The contamination sources are most often located outside the Arctic region, and long-range atmospheric transport of pollutants delivers most of the persistent organic pollutants (POPs) to the region (Riget et al., 2010). Other major routes for contaminants are via north flowing rivers from mid-latitude areas (Wania and Mackay, 1993, 1995), such as Northern Eurasia and North America, which are flowing into the Arctic oceans where they enter the Arctic food chain (Lohmann et al., 2007). Since most POPs are lipophilic they accumulate in lipid-rich tissues of living organisms, and thereby biomagnify in the food web (Letcher et al., 2010; Blais

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et al., 2005). POPs include many different contaminant groups such as organochlorine pesticides (OCPs), including dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs) used as flame-retardants, and industrial organochlorines (OCs) such as polychlorinated biphenyls (PCBs).

Polar bears (*Ursus maritimus*), as top predators, generally have high levels of POPs (Letcher et al., 2010). The Svalbard (Norway) and East Greenland polar bears are reported to have the highest PCB concentrations, while lower levels are found in North American sub-populations of polar bears (Haave et al., 2003; Verreault et al., 2005; Letcher et al., 2010). The PCB congeners 99, 118, 138, 153, 156, 170, 180 and 194 have been reported to be the most abundant PCBs in Svalbard polar bears (Bernhoft et al., 1997; Bytingsvik et al., 2012a). However, polar bears have an efficient cytochrome P450 system, and can metabolize POPs (Letcher et al., 2010). This high metabolic capacity may lead to an accumulation of metabolites such as hydroxylated PCBs (OH-PCBs) (Verreault et al., 2005; Letcher et al., 2010; Bytingsvik et al., 2012a), which in some cases may be more toxic to polar bears than the parent PCB congener (Tehrani and Van Aken, 2014; Gustavson et al., 2015a). Most biotransformed OH-PCBs are easily excreted, while the remaining OH-PCBs are limited to 5–10 persistent single congeners and are mainly transformed from persistent penta-, hexa- and heptachlorinated PCBs (Letcher et al., 2000). The most commonly found OH-PCBs are the 4-OH-CB107, 4-OH-CB146, 4′-OH-CB172 and 4-OH-CB187 (Bytingsvik et al., 2012a; Gustavson et al., 2015a).

Exposure to PCBs and OH-PCBs may potentially affect the endocrine system, and certain OH-PCBs have been suggested to affect steroid homeostasis in female polar bears from Svalbard (Gustavson et al., 2015a). Pesticides such as DDT and HCB are also found in polar bears in high concentrations and may potentially interact with their reproductive hormones (Oskam et al., 2003; Gustavson et al., 2015a). PBDEs have been reported to act as agonists by binding to estrogen receptors (ERs) *in vitro* (Meerts et al., 2001). In the Arctic food chain, most PBDEs have been found to biomagnify in the lower trophic levels. In the upper part of the food chain, however, biomagnification appears to be lower, perhaps due to higher metabolism. For example, Only BDE-153 was reported to biomagnify in polar bears (Sørmo et al., 2006), although other BDEs were also present.

Despite obvious potential interactions, not much is known about the relationship between steroids, and body size and condition, in male polar bears. In addition to controlling male characteristics such as reproductive organs, testosterone (TS) is also involved in the development of secondary male characteristics, and it is well established that both TS and dihydrotestosterone (DHT) have anabolic effects on the skeleton and muscles (Wiren, 2005; Clarke and Khosla, 2009; Thakur, 2016). The production and secretion of most sex hormones are controlled by neurons and negative hormone feedback regulation involving the hypothalamic–pituitary–gonadal axis (HPG axis) (Hill et al., 2008). This regulation of steroid hormones is linked to biological factors such as sexual maturity and body size, but toxic chemicals may impede the endocrine system (Klaassen, 2008). In mammals, TS is the primary androgenic steroid in males and is mainly secreted from the Leydig cells present in the testes. When TS has reached its target organ, it may be metabolized into DHT, or bind directly to the androgen receptor (AR). Other androgens, such as androstenedione (AN) and dehydroepiandrosterone (DHEA), have lower androgenic potency and function mainly as precursors for TS (Nieschlag and Behre, 2004). Both TS and DHT elicits their effects through binding to the AR. However, DHT amplifies the effect of TS due to a stronger affinity for AR (Hill et al., 2008; Kovacs, 2011). Testosterone and AN can be further metabolized into estrogens by the aromatase (Miller and Auchus, 2011). An overview of relevant steps in the gonadal steroidogenesis is illustrated in Supplement Material Fig. S1.

The aim of the present study was to investigate interactions between biometrics, POPs and circulating steroid levels in male polar bears. We therefore analysed 9 circulating steroid hormones and the steroid precursor cholesterol, along with biometric variables. Furthermore, we analysed circulating concentrations of 8 OCPs, 21 PCBs, 8 OH-PCBs and 2 PBDEs in 23 male polar bears between 3 and 21 years of age. The relationships between biometrics, steroids and POPs were investigated using principal component analysis (PCA) and bivariate correlations. To identify the most potent variables explaining the variation in androgen concentrations, the effects of biometric and POP variables on steroid concentrations were modelled using orthogonal projections to latent structures (OPLS).

2. Materials and methods

2.1. Sampling

The polar bear samples were collected at Svalbard in April 2008 as part of the International Polar Year project BearHealth. The sample location ranged from Vitovskybreen in the south (76.7 °N) to Waldenøya in the north (80.6 °N), and from Liefdefjorden in the west (12.6 °E) to Duvefjorden in the east (23.8 °E). Capture and handling procedures followed standard protocols (Derocher and Wiig, 2002; Stirling et al., 1989) and were approved by the National Animal Research Authority (NARA, Oslo, Norway). The bears were sedated for sampling by remote injection of a dart containing Zoletil (tiletamine/zolazepam, 200 mg/ml; Virbac Laboratories, Carros, France), fired from a helicopter. The rifle used was a Cap-Chur rifle with 5 or 7 ml metal darts and one barb needles of 20–42 mm in length, depending on the sex, age and condition of the bear. The date of each individual sampling event was recorded as the ordinal date (0–366). The age for each bear was estimated from the number of annual growth layer groups (GLGs) in a rudimentary premolar tooth for adults that had not been captured earlier (Christensen-Dalsgaard et al., 2010). Alternatively, age was calculated based on recapture of the bears captured as juveniles, of which age was known.

The sampled polar bears were divided into two age classes, those from 5 to 21 years ($n = 17$) were categorized as adults, while 3 and 4 year olds ($n = 6$) were termed subadults (Rosing-Asvid et al., 2002). The straight-line body length (SLBL) was measured as the dorsal straight line from the nose tip to the caudal end of the last tail vertebrae. The contour body length (CBL) was measured as the distance from the tip of the nose to the tip of the tail along the contour of the spine, when the bear was aligned laterally. Axial girth was measured as the circumference around the chest at the axilla. The head length was recorded as the straight-line length between the upper middle incisors at the gum line, to the most posterior dorsal scull process of the sagittal crest. Zygomatic width was the maximum width between the zygomatic arches. Body mass (BM) was estimated based on the body length and axial girth using the equation given by Derocher and Wiig (2002): body mass $= 0.00003377 \cdot \text{axial girth}^{1.7515} \cdot \text{body length}^{1.3678}$. To obtain an indication of the condition of the polar bears, we calculated the body condition index (BCI) according to Cattet et al. (2002) using the formula: $\text{BCI} = (\ln\text{BM} - 3.07 \cdot \ln\text{SLBL} + 10.76)/(0.17 + 0.009 \cdot \ln\text{SLBL})$.

Approximately 60 ml (6×10 ml) of blood was collected from the bears using 40 mm needles (21G) and stored at 4 °C. The collected blood samples were centrifuged with anti-coagulant in heparinised Venoject tubes (10 ml, Thermo Electron Corporation, Belgium) and without anti-coagulant for separation of plasma and serum,