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Effects of biometrics, location and persistent organic pollutants on blood clinical-chemical parameters in polar bears (*Ursus maritimus*) from Svalbard, Norway

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ABSTRACT

In the present study, blood clinical-chemical parameters (BCCPs) were analysed in 20 female and 18 male Svalbard polar bears (*Ursus maritimus*) captured in spring 2007. The aim was to study how age, body condition (BC), biometrics, plasma lipid content and geographical location may confound the relationship between persistent organic pollutants (POPs) including PCBs, HCB, chlordanes, DDTs, HCHs, mirex and OH-PCBs and the concentrations of 12 specific BCCPs (hematocrit [HCT], hemoglobin [HB], aspartate aminotransferase [ASAT], alanine aminotransferase [ALAT], γ-glutamyltransferase [GGT], creatine kinase [CK], triglycerides [TG], cholesterol [CHOL], high-density lipoprotein [HDL], creatinine (CREA], urea, potassium (K]), and to investigate if any of these BCCPs may be applied as potential biomarkers for POP exposure in polar bears. Initial PCA and O-PLS modelling showed that age, lipids, BC and geographical location (longitude and latitude) were important parameters explaining BCCPs in females. Following subsequent partial correlation analyses correcting for age and lipids, multiple POPs in females were still significantly correlated with HCT and HDL (all $p < 0.05$). In males, age, BM, BC and longitude were important parameters explaining BCCPs. Following partial correlation analyses correcting for age, biometrics, lipids and longitude in males, multiple POPs were significantly correlated with HCT, ASAT, GGT and CHOL (all $p < 0.05$). In conclusion, several confounding parameters has to be taken into account when studying the relations between BCCPs and POPs in polar bears. When correcting for these, in particular HCT may be used as a simple cost-efficient biomarker of POP exposure in polar bears. Furthermore, decreasing HDL concentrations and increasing CHOL concentration with increasing POP concentrations may indicate responses related to increased risk of cardiovascular disease. We therefore suggest to further study POP exposure and lipidome response to increase knowledge of the risk of cardiometabolic syndrome in polar bears.

1. Introduction

Persistent organic pollutants (POPs) are a group of organic compounds that primarily originate from anthropogenic sources and include pesticides, industrial chemicals, and by-product from combustion or industrial processes. Examples of POPs include polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE), hexachlorobenzene (HCB), chlordanes, and DDT (El-Shahawi et al., 2010). Although POPs are structurally a diverse group of chemicals, most of them have low water solubility, they are highly lipophilic, and resistant to physical, chemical and biochemical degradation (AMAP, 2004; Borgå et al., 2004). Their toxic effects, persistence and capacity for long-range transport and bioaccumulation have raised concern about their environmental impact, and have led to restrictions or even

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complete ban on the use of these chemicals in many countries (Godduhn and Duffy, 2003; El-Shahawi et al., 2010; Letcher et al., 2010). Due to their physical-chemical properties, they can reach high concentration in top predators, such as polar bears (Letcher et al., 2010; Riget et al., 2016). Despite the restrictions and bans, POPs may remain at significant (and potentially toxic) levels in biota for decades due to their persistent nature (Brown et al., 2018; Dietz et al., 2013a, 2013b). In mammals, POPs may be transferred from mothers to their offspring *in utero* and through lactation (Bytingsvik et al., 2012a, 2012b; Polischuk et al., 2002). The Arctic is characterized by low temperatures, limited nutrient availability, and pronounced seasonality with short growing seasons (Sobek et al., 2010). Due to the seasonal absence of sea ice polar bears have adapted to the lack of access to marine mammal prey, and go through a seasonal period of fasting, with a preceding period of feeding in which they must obtain sufficient fat reserves for reproduction and/or later fasting. These adaptations do also influence on the accumulation and dynamics of POPs in the Arctic biota. During the fasting period, lipid stores are metabolised. This often leads to mobilization of lipophilic contaminants stored in fat depot, and the blood levels of contaminants will increase. Contaminants in the blood can be distributed throughout the body, exposing various vital organs to the toxic compounds, and hence, increase the animal's susceptibility for adverse effect with potential negative physiological effects (Cherry et al., 2009; Routti et al., 2010; Tartu et al., 2017a). In polar bears, the hormone and vitamin concentrations, organ morphology, as well as reproductive and immune systems are likely to be influenced by PCB exposure (Dietz et al., 2015; Letcher et al., 2010; Sonne, 2010).

In most cases, biotransformation processes lead to detoxification of POPs, which will protect the organisms. However, in some cases biotransformation of parent compounds may result in metabolites being more toxic than the parent compound (Walker et al., 2006). For instance, toxic and endocrine disrupting hydroxylated PCB metabolites (OH-PCBs) are formed by the oxidative metabolism of PCBs by cytochrome P450 (CYP P450) monooxygenase enzyme systems (Grimm et al., 2015). As the ultimate predator in Arctic food chains, polar bears are especially at risk of accumulating lipophilic compounds. Although recent temporal decreases in POP concentrations in polar bears from Svalbard have been reported (Bytingsvik et al., 2012a), another study has shown that the concentrations in polar bears are increasing on Greenland (Riget et al., 2016), presumably due to climate change related alterations in Arctic food web structures (Brown et al., 2018; McKinney et al., 2013). Other recent studies have indicated that the concentrations of PCBs in polar bears may be above toxic threshold levels for immune, reproductive and carcinogenic effects (Dietz et al., 2015), and possible effects of PCBs on the population level in polar bears have been discussed (Pavlova et al., 2016; Nuijten et al., 2016).

Analysis of blood clinical-chemical parameters (BCCPs) provides valuable information for evaluating the health and physiological status, as well as identifies target organs for toxicity in organisms (Castellanos et al., 2010; Firat and Kargin, 2010). Blood clinical-chemical parameters have previously been applied as biomarkers showing that POP exposure may affect liver, kidney and bone metabolism in free-living wildlife (Sonne et al., 2010, 2012, 2013). Using BCCPs integrate changes in physiological status across organs-systems and tissue reflecting perturbations in biochemical pathways, cellular integrity and overall homeostasis (Klaassen, 2013). Specifically, for polar bears the lipidome may be affected by POP exposure, as may the overall metabolome (Tartu et al., 2017a). Therefore, BCCPs related to cholesterol and triglyceride metabolism as well as liver, kidney and bone metabolism may be affected as an indication of changes in the overall metabolism, homeostasis and organ functioning (Sonne, 2010). The aim of this study was therefore to examine 1) how age, body condition, plasma lipid content, geographical location and body mass may confound the relationships between POPs and BCCPs in 20 female and 18 male Svalbard polar bears captured in spring 2007 and 2) based on this evaluate if any of the analysed BCCPs may be applied as potential biomarkers for POP exposure in polar bears.

2. Material and methods

2.1. Field sampling

Field sampling procedures are described in details in Bytingsvik et al. (2012a, 2012b). Briefly, blood samples were collected from 38 polar bears (20 females and 18 males, all independent bears aged > 3 years) captured at Spitsbergen and Edgeøya, Svalbard, Norway (76.7 – 79.8°N, 11.8 – 21.3°E) in March/April 2007. Sampling location, capture day (Julian day) and a selection of biometric data were recorded. Age was estimated by either counting annual growth layers in cementum of an extracted vestigial premolar tooth (Calvert and Ramsay 1998) or was known if a bear was first time captured as a cub. Capture and handling procedures followed standard protocols (Stirling et al., 1989; Derocher and Wiig, 2002), and were approved by the National Animal Research Authority (NARA), Norway. Blood was collected from the femoral vein. Within 8 h after sampling, the samples were separated into plasma and blood by centrifugation (3500 rpm, 10 min) and the BCCPs were analysed on the fresh whole blood or plasma samples. Further, plasma samples were stored at − 20 °C in the field and then at − 70 °C in the lab freezer until analysis of POPs.

2.2. Analyses of BCCPs

Previously it has been shown that variations in concentrations of several BCCPs, such as ALAT, GGT, alkaline phosphatase (ALKP), urea, cholesterol, lactate dehydrogenase, glucose, creatinine kinase may be linked to exposure of POPSs/OHCs in reptiles, birds, and mammals (including humans) (Edqvist et al., 1992; Sonne et al., 2008a, 2010, 2013; Camacho et al., 2013; Singh and Chan, 2018). In this study the plasma samples were analysed for BCCPs, using a “dry” clinical-chemistry analyser with test-strip devices (Reflotron®, Boehringer-Mannheim, Mannheim, Germany). Prior to the analysis, the samples were kept at ca. 5 °C. The BCCPs subjected for analysis were two hematologic parameters (HCT, HB), four enzymes (ASAT, ALAT, GGT and CK), five metabolites (TG, CHOL, HDL, CREA, and UREA), and one mineral (K). Two or three parallels were analysed for each animal and for each parameter.

Hematocrit (HCT) is the volume fraction of erythrocytes in whole blood while hemoglobin (HB) is the iron-containing pigment of the erythrocytes being responsible for oxygen and CO_2 binding and transport so measurements of these may help finding anaemia, blood loss, or dehydration (D'Orazio and Meyerhoff, 2008; Kirk et al., 2010; Nuttal and Klee, 2001). Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) are enzymes widely distributed in animal tissues, and elevated blood levels are a nonspecific indicator of liver and kidney dysfunction with ALAT being the most liver-specific (Evans, 2009; Franson, 1982; Marshall and Bangert, 2008; Panteghini and Bais, 2008). Gamma-glutamyl transferase (GGT) is an enzyme found in liver, kidney and pancreas and is used as a sensitive indicator for hepatobiliary diseases (Krefetz and Mcmillin, 2005; Marshall and Bangert, 2008). Creatine kinase (CK) is an enzyme with highest activity in muscle and brain and elevated levels of CK indicate either growth or high metabolism (muscle) or physical activity. CK may also help in diagnosis of central nervous system and thyroid gland diseases (Krefetz and Mcmillin, 2005; Panteghini and Bais, 2008). Creatinine (CREA) is synthesized in the muscle, mainly from the turnover of creatine, and is a marker of growth and metabolism (Newman and Price, 2001). Triglycerides (TG) and cholesterol (CHOL) are some of the major lipids in blood and physiological changes in these are related either to recent diet or to mobilization of lipid from fat to blood. However, it may e.g. also be an indicator of liver or intestinal disease (Burnett, 2010; Marshall and Bangert, 2008; Rifai et al., 2001; Van den Steen et al., 2010). High-density lipoprotein (HDL) is a complex mixture of lipoproteins inversely related to major adverse cardiovascular events in humans (Lüscher et al., 2014). The kidney secretes CREA while UREA is