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Associations between complex OHC mixtures and thyroid and cortisol hormone levels in East Greenland polar bears

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ABSTRACT

The multivariate relationship between hair cortisol, whole blood thyroid hormones, and the complex mixtures of organohalogen contaminant (OHC) levels measured in subcutaneous adipose of 23 East Greenland polar bears (eight males and 15 females, all sampled between the years 1999 and 2001) was analyzed using projection to latent structure (PLS) regression modeling. In the resulting PLS model, most important variables with a negative influence on cortisol levels were particularly BDE-99, but also CB-180, -201, BDE-153, and CB-170/190. The most important variables with a positive influence on cortisol were CB-66/95, α-HCH, TT3, as well as heptachlor epoxide, dieldrin, BDE-47, *p,p′*-DDD. Although statistical modeling does not necessarily fully explain biological cause–effect relationships, relationships indicate that (1) the hypothalamic–pituitary–adrenal (HPA) axis in East Greenland polar bears is likely to be affected by OHC-contaminants and (2) the association between OHCs and cortisol may be linked with the hypothalamus–pituitary–thyroid (HPT) axis.

1. Introduction

The Arctic is a sink for long-range transportable persistent organohalogen contaminants (OHCs), all of which are produced and released into wind and ocean currents in the industrialized world (AMAP, 2004). Many of these pollutants and their metabolites have been shown to possess biological activities within the context of the broad classification of endocrine disrupting chemicals (EDCs). That is, they have an ability on some biochemical or level of biological organization basis to disrupt the balance of the hypothalamus–pituitary–thyroid (HPT) and the hypothalamic–pituitary–adrenal (HPA) axis, as well as the hypothalamus–pituitary–gonadal (HPG) axis. This effect can occur even at chronic low-level EDC exposure, causing detrimental effects on development, behavior, reproduction, immunology and general survival of the affected organism (Colborn et al., 1993; Vos et al., 2000; Mendes, 2002; Chalubinski and Kowalski, 2006; Prasanth et al., 2010; Hamlin and Guillette, 2011). OHCs are often lipophilic, accumulating in the fatty tissues of e.g. ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), the preferred prey of polar bears (*Ursus maritimus*) (Ramsay and Stirling, 1988; Derocher et al., 2002). Hence, East Greenland and Barents Sea polar bears have been found to carry some of the highest OHC loads of any Arctic mammal species (Norstrom et al., 1998; Dietz et al., 2004,2007; Verreault et al., 2005; Muir et al., 2006; Gebbink et al., 2008; Letcher et al., 2010). Chronic exposure to OHCs in polar bears have been associated with afflictions such as endocrine disruption, impaired immune system, reduced size of sexual organs, and organ histopathology (Gutleb et al., 2010; Letcher et al., 2010; Sonne, 2010; Villanger et al., 2011a; Bechshøft et al., 2012).

Several studies of wildlife species, including polar bears, indicate the vitally important corticoid and thyroid hormone systems are potentially vulnerable to disruption by OHCs (Braathen et al., 2004; Odermatt et al., 2006; Verboven et al., 2010; Villanger et al., 2011a,b). Cortisol is the major corticosteroid hormone in most mammals and participates in the physiological regulation of most body tissues (McDonald and Langston, 1995), including the regulation of metabolism, growth, and development, as well as responses to stress influencing the physiology and

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endocrinology of the reproductive and immune systems (Moberg, 1991; Dobson and Smith, 2000; Sjaastad et al., 2003; von Borell et al., 2007; Schmidt and Soma, 2008). Polar bear cortisol levels have traditionally been measured in blood plasma (Tryland et al., 2002; Haave et al., 2003; Oskam et al., 2004), but were reported recently in hair of East Greenland polar bears (Bechshøft et al., 2011; Bechshøft et al., 2012). Hair provides a much more stable matrix which reflects long-term stress levels instead of rapid fluctuations brought on by acute stress or inherent variation (Koren et al., 2002; Davenport et al., 2006).

The thyroid hormones (THs) thyroxine (T4, 3,5,3′,5′-tetraiodothyronine) and triiodothyronine (T3, 3,5,3′-triiodothyronine) are of critical importance to neurodevelopment in young animals (fetus, neonate, and juvenile) and for the development and function of somatic cells and gonads (Cooke et al., 2004). THs also influence the circulating levels of sex steroids, and are involved in the regulation of metabolism, thermoregulation, reproduction, and in maintaining the general physiological homeostasis (Hadley, 1996; McNabb, 1992; Cooke et al., 2004; Zoeller et al., 2007). An increased level of cortisol can result in an inhibition of the production of THs in the thyroid gland itself, or lead to a decrease in the deiodination of T4 to T3 (Chastain and Panciera, 1995). Thus, high cortisol levels can lead to lower serum T4 or T3 or both. However, clinical studies of hyperthyroid patients and animals have shown that (sub)clinical hyperthyroidism may be associated with adrenocortical hyperactivity, i.e. increased cortisol concentrations (Johnson et al., 2005 and references there-in). Therefore, increased thyroid levels may actually in some cases lead to increased cortisol concentrations.

The connection between cortisol and THs has been studied extensively in controlled experiments involving birds, fish, and humans (Geris et al., 1999; Douyon and Schteingart, 2002; Kitaysky et al., 2005; Walpita et al., 2007; Peter, 2011). However, wildlife ecotoxicology studies tend to examine the effect of contaminants on either cortisol or the THs (Jenssen et al., 1994; Skaare et al., 2001; Routti et al., 2010; Corlatti et al., 2011), or actually measure both but without correlating them (Bubenik and Brown, 1989; Saeb et al., 2010). The objective of the present study was therefore to investigate the multivariate relationship between hair cortisol, whole blood circulating thyroid hormone, and tissue complex mixtures of organohalogen contaminant levels measured in subcutaneous adipose of East Greenland polar bears.

2. Materials and methods

2.1. The sample

A total of 23 East Greenland polar bears were included in this study. Of these eight were males (mean age: 6.7 years, range: 5–9) and 15 were females (mean age: 9.8 years, range: 3–22). All bears were sampled between the years 1999 and 2001. Age estimation had been done according to Dietz et al. (1991). The individual polar bears and their cortisol and TH levels have previously been included in, respectively, the studies presented in Bechshøft et al. (2011, 2012) and Villanger et al. (2011a), which also give further details on the samples used in the present study. Biological measurements were taken in the field when sampling the bear. Detailed descriptions of tissue sampling and biological measurements are given elsewhere (Dietz et al., 2004; Sandala et al., 2004). Body mass (BM) was calculated based on measured body length and girth according to Derocher and Wiig (2002), and date of capture was calculated into a numerical value between 1 and 365.

2.2. OHC analysis of adipose tissue

The OHC analysis methods and subsequent quantitative results for the present polar bears have been reported in Dietz et al. (2004,2007). The individual or co-eluted compounds measured in subcutaneous adipose tissue included in the present study were: CB-31/28, -52, -60, -64/71, -66/95, -74, -97, -99, -101/84, -118, -128, -129/178, -138, -146, -151, -153, -170/190, -171/202/156, -172, -177, -180, -182/187, -183, -194, -195, -200, -201, -203/196, and -206; BDE-47, -99, -100, -153, α- and β-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), trichlorobenzene (TCB), pentachlorobenzene (QCB), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p′*-DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene, (*p,p′*-DDE),1,1,dichloro-2,2-bis(4-chlorophenyl) ethane (*p,p′*-DDD), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor epoxide, dieldrin and octachlorostyrene (OCS). All contaminant concentrations are given in nanograms per gram lipid weight (ng/g l.w.).

2.3. Cortisol analysis of hair

Hair samples were analyzed for cortisol according to the procedure described in Bechshøft et al. (2011). In short, hair samples were washed, dried, and then ground to a fine powder in an MM 200 ball mill (Retsch, Newtown, USA). Approximately 50 mg of powdered hair was extracted for 24 h with HPLC-grade methanol (Fisher Scientific), dried down, reconstituted in assay buffer, and then analyzed for cortisol using a sensitive and specific enzyme immunoassay (Salimetrics, State College, PA, USA). Intra- and interassay coefficients of variation of this assay averaged less than 10%.

2.4. Thyroid hormone analysis of whole blood

Polar bear whole blood samples were analyzed for total T3 (TT3) and total T4 (TT4) at the Department of Biology, Norwegian University of Science and Technology (NTNU, Trondheim, Norway). The blood samples were extracted with ethanol prior to analysis using commercially available solid-phase ^{125}I radioimmunoassay (RIA) kits (Coat-A-Count Total T3 and Coat-A-Count Total T4, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The procedures described in the test protocols were followed (Siemens 2006a,b). A more detailed description of the analytical procedures and quality assurance are presented in Villanger et al. (2011a).

2.5. Data analysis

The data in this study consisted of 23 polar bears grouped according to age and sex (Rosing-Asvid et al., 2002); Subadults (Sub, consisting subadult females <5 yr and subadult males <6 yr, $n=5$); Adult males (AdM, consisting of males ≥ 6 yr, $n=6$), and adult females (AdF consisting of females ≥ 5 yr, $n=12$). Multivariate analyses were performed using the software Simca-P+ (Version 12.0, Umetrics AB, Umeå, Sweden). Projections to latent structures by means of partial least squares (PLS; Eriksson et al., 2006) was applied to investigate the multivariate relationships between the predictor (*X*)-variables (contaminants and biological data including circulating TH levels) and their unidirectional influence on the response (*Y*)-variable cortisol. This multivariate regression method has been applied in several recent wildlife studies (Murvoll et al., 2006; Jenssen et al., 2010; Villanger et al. 2011a, b). Since factors such as age and sex can influence TH levels and responses, effect studies should ideally consider these as covariates or perhaps separate statistical analyses based on these factors (Gochfeld, 2007; Zoeller et al., 2007; Abdelouahab et al., 2008). However, hair cortisol levels have been shown to be independent of age and sex Bechshøft et al., (2012). In any case, sample size in the various age and sex groups in the current study was considered too low for separate analyses. Thus, Sub, AdF and AdM were grouped together when investigating the multivariate influence of individual OHC load, age, sex, morphometric data, lipid content of adipose tissue, capture day, and circulating TT3 and TT4 on hair cortisol levels using PLS.

Since normality is not required for PLS regression, data were not transformed. All variables were centered and scaled (to variance 1), and significance level was set to 0.05 (Umetrics, 2008). PLS modeling was validated by the explained variation in the *X*-matrix (R^2X), explained variance of the *Y*-variables by the *X*-matrix (goodness of fit, R^2Y), goodness of prediction (Q^2) obtained by cross-validation and permutation analyses (20 permutations) and an analysis of variance testing of cross-validated predictive residuals (CV-ANOVA; see Eriksson et al., 2008; Umetrics, 2008). The importance of individual *X*-variables in explaining the *X*- and *Y*-matrix were evaluated using the variable importance for the regression (VIP) values. A VIP value >1 denotes high importance for the model, and a VIP <0.5 indicate low or no importance (Umetrics, 2008). Optimizing of the PLS model was done by successively removing *X*-variables with lowest VIP values. Another evaluation parameter for individual *X*-variables used herein is the regression coefficient (CoeffCS) value, which shows the correlative relationship (strength and direction) between each *X*-variable with *Y* (Umetrics, 2008). A more detailed description of PLS can be found elsewhere (Eriksson et al., 2006; Umetrics, 2008; Wold et al., 2001).