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Concentrations of vitamin A, E, thyroid and testosterone hormones in blood plasma and tissues from emaciated adult male Arctic foxes (*Vulpes lagopus*) dietary exposed to persistent organic pollutants (POPs)

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ABSTRACT

The aim of the present study was to investigate the relationships and effects of oral POP exposure on retinol (vitamin A), α-tocopherol (vitamin E), thyroid hormones and testosterone in emaciated adult farmed Arctic foxes. Eight brother-pairs were exposed to either a diet containing naturally POP-contaminated minke whale blubber (*Balaenoptera acutorostrata*) (n=8), or a control diet containing pig (*Sus scrofa*) fat as the primary fat source (n=8) for 22 months. In the whale blubber containing feed the ∑POPs concentration was 802 ng/g w.w. and it was 24 ng/g w.w. in control feed. The liver mass was significantly higher and the ratio of FT4 (free thyroxine):FT3 (free triiodothyronine) was significantly lower in the POP exposed group as compared to the control group given feed with pig fat (both $p < 0.05$). The exposed group revealed lower plasma and liver concentrations of α-tocopherol compared to the control group (both $p < 0.05$). These results indicate that plasma FT4:FT3 ratio and plasma and liver α-tocopherol are valuable biomarker endpoints for chronic oral POP exposure in wild Arctic foxes. Based on this we suggest that plasma FT4:FT3 ratio and plasma and liver α-tocopherol are valuable biomarker endpoints for chronic POP exposure in wildlife Arctic foxes and that these perturbations may affect their health status.

1. Introduction

The Arctic fox (*Vulpes lagopus*) population in Svalbard (Norway) has a profound seasonal cycle of fattening and emaciation (Prestrud and Nilssen, 1992). Deposition of subcutaneous and visceral fat takes place during autumn when food availability is in excess. Their body mass reaches a maximum in November, when body fat stores may constitute more than 20% of the total body mass. Lipid stores are mobilized during the winter period and become depleted from March through May, and are lowest in June-July, when they constitute 6% of total body mass (Prestrud and Nilssen, 1992).

High concentrations of persistent organic pollutants (POPs) and in particular organochlorine contaminants (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordanes (CHLs), hexachlorocyclohexanes (HCHs) and chlorobenzenes (CBzs) have been reported in arctic top predator species such as Arctic fox, polar bear (*Ursus maritimus*), glacous gull (*Larus hyperboreus*) and seals spp. (*Phocidae*) (Brunstrom and Halldin, 2000; Fuglei et al., 2007; Letcher et al., 2010). High exposure levels to POPs are known to exert adverse biological effects and associations between POP levels and biological parameters have been reported in several populations of these species (Letcher et al., 2010; Pedersen et al., 2015; Sonne, 2010). When the Arctic foxes, due to low food availability, utilise lipids stored during spring time, the POP concentrations become bioavailable and redistributed to other tissues (Helgason et al., 2013). In these foxes, POP concentrations increased by 20, 5 and 3 fold, respectively, in blood, liver and adipose tissue, during a feed restricted period simulating natural fasting lasting from

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November to June (Helgason et al., 2013). Thus, body fat mobilization processes appear to be associated with a significant increase in blood and tissue POP concentrations which is likely also a problem for wild foxes at e.g. Svalbard (Pedersen et al., 2015). The end of the feed restricted period (March-June), when blood and tissue POP concentrations in wild Arctic foxes are high, coincides with their mating and breeding periods which raises concerns about the health and reproductive effects (Pedersen et al., 2015). There is a particular concern about endocrine disrupting effects from POP exposure on the thyroid and sex hormone homeostasis and fat soluble vitamin A and E status as shown for Arctic marine mammals including e.g. polar bears, seal spp. and beluga whales (*Delphinapterus leucas*) (Bytingsvik et al., 2013; Gabrielsen et al., 2015; Letcher et al., 2010; Simon et al., 2013; Sonne, 2010; Villanger et al., 2011a, 2011b). POP exposure is also known to induce oxidative stress through the production of peroxides and free radicals that may damage components of the cell, including proteins, lipids, and DNA (Palace et al., 1996b; Twaroski et al., 2001a, 2001b).

Studies of effects of pollutants on wildlife are usually correlative and it is therefore difficult to document clear cause-effect relationship between POP compounds and possible harmful effects. To study effects of POP compounds in carnivores, several pioneering studies on exposed wild or domesticated carnivores kept in captivity to contaminants have been carried out (Backlin and Bergman, 1992; Brouwer et al., 1989; de Swart et al., 1995, 1994; Håkansson et al., 1992; Kirkegaard et al., 2011, 2010; Letcher et al., 2010; Ross et al., 1996; Sonne, 2010). These studies include mink (*Neovison vision*), harbour seals (*Phoca vitulina*) and Greenland sledge dogs (*Canis familiaris*) that have been exposed to POPs by adding pollutants to the diet, or by giving them a diet containing naturally high POP concentrations.

To investigate the effects of POPs in Arctic fox, an experimental longitudinal study was conducted in 2003–2005. From these foxes, a large number of tissues, and blood samples were taken for measurements of a variety of biological endpoints, of which many have been published already (Hallanger et al., 2012; Helgason et al., 2013; Sonne et al., 2008, 2009a, 2009b). Eight brothers-pairs of farmed Arctic fox (blue fox) were exposed to either a diet containing naturally high POP-contaminated minke whale blubber (*Balaenoptera acutorostrata*) (the exposed group; n=8), or a low POP diet containing pig (*Sus scrofa*) fat as the primary fat source (the control group, n=8) for 22 months. The whale-containing feed summed concentration of all measured POPs up to 802 ng/g wet weight, whereas the concentration of all measured POPs was 24 ng/g wet weight in the control feed (Helgason et al., 2013). The foxes were given to these respective diets from weaning and until they were two years of age. Effects of the exposure were then examined in juvenile foxes after 4.5 months of exposure, in adult well-nourished “winter-foxes” exposed for 15 months, and finally in the present study in emaciated adult “summer foxes” that had been exposed for a total of 22 months (Hallanger et al., 2012; Helgason et al., 2013; Sonne et al., 2008). The aim of the present study was to investigate the relationships and effects of POP exposure on retinol (vitamin A) , α-tocopherol (vitamin E), thyroid hormones and testosterone in adult emaciated foxes that had been exposed to a high POP containing diet for 22 months through comparison to control groups. The foxes received restricted amounts of feed for 6.5 months to mimic the normal energy restricted period that wild Arctic foxes experience during spring. Since the circulating testosterone were reported to be lower in juvenile foxes exposed to the high POP containing diet during the first 4.5 months in the present exposure study (Hallanger et al., 2012), it was hypothesised that this effect on plasma testosterone concentrations would persist in emaciated adult foxes also after 22 months of exposure to high POP levels. Based on reports of thyroid gland lesions in the emaciated adult foxes (Sonne et al., 2009a), we also hypothesise that the plasma thyroid hormone homeostasis would be disrupted in the emaciated adult high POP exposed foxes. Given the higher hepatic Cyp1A activity in the emaciated adult exposed foxes indicating oxidative stress (Helgason et al., 2013) we furthermore tested if plasma concentrations of retinol and α-tocopherol were lower in liver, kidney and blood plasma from exposed foxes compared to controls (Palace et al., 1996b).

2. Material and methods

2.1. Animals, exposure and sampling

The experiments were conducted in accordance with national and international guidelines for animal research, and the experiments were approved by the Norwegian Animal Research Authority (www.fdu.no). Eight newly weaned brother-pairs of foxes (54 days old) were separated into two groups, one POP exposed group (n=8) and one control group (n=8). Thus, the groups were balanced with respect to body mass and genotype (brother-pairs). The foxes were individually housed in outdoor cages (1.5×1.2×1.0 m) at the University of Life Sciences and Agriculture, Ås, Norway, and exposed to natural photoperiod and ambient temperature. The exposed group received wet feed containing minke whale blubber as main fat source, whereas the control group was given feed added pig fat as main fat source. Information on the composition of the two diets with respect to various ingredients and POP concentrations is presented elsewhere (Helgason et al., 2013; Sonne et al., 2008).

To simulate the annual feeding regime of free-ranging arctic foxes, both groups were given high-energy feeds during 13 Aug 2003 to 4 Jan 2004, low-energy feeds during 5 Jan 2004 to 7 Aug 2004 and high-energy feeds during 8 Aug 2004 to 28 Nov. 2004 and finally low-energy feeds during 29 Nov 2004 to 16 June 2005. Because of different energy content of the feed and different daily feed allowance in these periods, the body mass of the blue foxes was highest in November-January and lowest in June–July. The two groups received identical rations of feed throughout the study. Feed was given once a day, and water was supplied *ad libitum*. Following the 22 months period of feeding, the sixteen lean blue foxes (24 months old) were fasted for 24 h and sacrificed using electrocution on June 16. Blood samples were taken by heart puncture using a 10 mL vacutainer tube containing heparin as anticoagulant. Haematocrit was determined using a microspin centrifuge (Bayer Diagnostics, Germany). Plasma was separated by centrifugation at 2520 G (90 mm ø; 5000 rpm) for 20 min and transferred to cryotubes, wrapped in aluminium foil to prevent photogenic degradation of vitamins, and immediately frozen in liquid nitrogen. The animals were dissected by the same person (group blind) and the mass of the liver, kidneys, adrenals, thyroid, brain and fat omentum (fat and nourishment store in the abdomen) were recorded together with biometric data. Samples of liver and kidney were wrapped in aluminium foil and frozen in liquid nitrogen.

The concentrations of all measured POP compounds in the blood and adipose tissue of the emaciated exposed foxes were approximately 91 μg/g lipid weight and 6.5 μg/g lipid weight, respectively (Helgason et al., 2013). More detailed information on the concentrations of PCBs, DDTs, CHLs and CBzs in liver, adrenal, brain, blood and adipose tissue of the exposed adult emaciated foxed as well as the chemical analyses are given elsewhere (Sonne et al., 2008; Helgason et al., 2013). Unfortunately, contaminant concentrations were not analysed in the control foxes in the present study. However, based on the low POP concentrations in the control feed which was only 3% of that in the contaminated feed (802 vs 24 ng/g wet weight) we assume that body burdens of POPs in the control foxes were negligible.

2.2. Vitamin measurements

The concentrations of retinol and α-tocopherol in plasma, liver and kidney were determined by high-performance liquid chromatography (HPLC) using auto sampler with a peltier sample tray, LC pump, vacuum degasser and fluorescence detector: Elmer 200 series, Massachusetts, USA. Column: Chrompack Intersil, ODS-3,

150×4.6 mm, 5 µm, Varian, Inc., California, USA. Computer: Dell Optiplex GX 280, Dell Computer CO., Texas, USA. Software: TurboChrom Workstation 6.1.2, Perkin Elmer, Massachusetts, USA. Sample injection volume was 80 µl and flow was 1.5 mL/min at 800–1000 PSI. Concentrations of vitamins were determined by comparison with standards (Sigma Chemical, CO, Missouri, USA). Further details are found in Supplementary Information.

2.3. Hormone measurements

Concentrations of the thyroid hormones total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3), free triiodothyronine (FT3) and testosterone (T) in plasma were determined using radio-immunoassay (RIA) and commercially available RIA kits with coated tubes (Coat-A-Count Total T4, Coat-A-Count Free T4, Coat-A-Count Total T3, Coat-A-Count Free T3; Diagnostic Product Corporation, DPC, Los Angeles, California, USA, and Spectria Testosterone [^{125}I]; Orion Diagnostica, Espoo, Finland). The amount of bound radioactive antigen was quantified using a gamma counter (Cobra Auto-Gamma; Packard Instrument Company, Dowers, IL, USA). Further details are found in Supplementary Information.

2.4. Statistics

All statistical analysis was conducted using SPSS, Version 13.0 (SPSS Inc., Chicago, USA). The variables were tested for normality (skewness, curtosis, QQ-plot, Shapiro-Wilk's test) and for equality of variances (Levene's test), and outliers were identified. Due to the relatively low number of observations, the presence of outliers and extremes, marked skewness and disparity of variance, nonparametric test were employed. Mann-Whitney test (exact, two-sided) was used to examine differences between the exposed group and the control group. The level of significance was set to α=0.05.

3. Results

The liver mass was significantly higher in the POP exposed group than in the control group (Table 1; $p < 0.01$). There were no group differences with respect to body mass, body length, kidney mass, adrenal gland mass, thyroid gland mass, brain mass, omentum fat mass or haematocrit (Table 1). Plasma concentrations of TT4, TT3, FT4 and FT3 and testosterone did not differ between the two groups (Table 2). However, the FT4:FT3 ratio was significantly lower in the POP exposed compared group to the control group (p=0.04, Table 3). No other differences were found for the thyroid hormone ratios of for the two groups (Table 3). There were no differences in testosterone concentrations (p=0.44, Table 2). The POP exposed foxes had lower concentrations of α-tocopherol in plasma (p=0.05) and in liver ($p < 0.01$, Table 4 and Fig. 1) compared to control foxes. There were no group differences in concentrations of α-tocopherol in the kidney samples (Table 4). Retinol concentrations in plasma, liver and kidney levels did not differ between the groups ($p > 0.05$, Table 4 and Fig. 1).

4. Discussion

Complementary results from this controlled contaminant exposure study on farmed juvenile and adult Arctic foxes have previously been reported elsewhere (Hallanger et al., 2012; Helgason et al., 2013; Sonne et al., 2009a, 2008, 2009b). The concentrations of PCBs in the adipose tissues of the emaciated animals that had been exposed to the POP containing diet for 22 months (4.3 µg/g lw; Helgason et al., 2013) were somewhat lower than reported in wild Arctic foxes in Svalbard in the period 1973–1999 (9.7–20.5 µg/g l.w.; Fuglei et al., 2007), but within the range of those reported in wild Arctic foxes from Iceland, Alaska and Canada (Hoekstra et al., 2003; Klobes et al., 1998; Pedersen et al., 2015).

Table 1
Biometric measurements of adult farmed Arctic foxes fed with a diet containing minke whale blubber presenting high concentrations of persistent organic pollutants (POP; exposed) and in foxes fed with a non-contaminated diet (control). Values are given as mean ± standard deviation (SD) and median, minimum - maximum.

Variable	POP exposed	Control
Body mass (kg)	5.39 ± 0.44 5.35, 4.80–6.10	5.54 ± 0.50 5.55, 4.80–6.20
Body length (m)	69.9 ± 1.9 70.3, 66.5–72.5	69.9 ± 2.8 69.1, 65.5–74.5
Liver mass (g)*	189.1 ± 20.0 189.8, 162–215.2	154.8 ± 8.6 156.8, 164.5–239.7
Kidney mass (g)	15.5 ± 1.4 15.3, 13.7–18.0	15.1 ± 1.4 15.1, 12.7–16.9
Adrenal mass (g)	0.30 ± 0.05 0.30, 0.22–0.38	0.30 ± 0.05 0.30, 0.24–0.38
Thyroid mass (g)	0.68 ± 0.11 0.70, 0.52–0.82	0.87 ± 0.30 0.86, 0.42–1.35
Brain mass (g)	30.7 ± 1.3 30.6, 29.0–32.7	30.0 ± 3.0 30.8, 25.7–33.1
Fat omentum (g)	29.5 ± 8.1 29.0, 17.6–41.0	34.2 ± 13.4 34.5, 14.3–60.4
Hematocritt (%)	50.3 ± 3.4 49.5, 45.5–55.5	51.7 ± 2.3 53.0, 49.0–54.0

* Significant differences between the POP exposed and control groups at $p < 0.01$ (Mann-Whitney test).

Table 2
Plasma concentrations of thyroid hormones (total thyroxine [TT4]), free thyroxine [FT4], total triiodothyronine [TT3], free triiodothyronine [FT3]) and testosterone (T) in adult farmed Arctic foxes fed with a diet containing minke whale blubber presenting high concentrations of persistent organic pollutants (POP; exposed) and in foxes fed with a non-contaminated diet (control). Values are given as mean ± standard deviation (SD) and median, minimum - maximum.

Hormone	POP exposed	Control
TT4 (nmol/L)	25.3 ± 5.8 28.0, 15.2–31.6	23.7 ± 5.0 24.3, 16.6–31.0
FT4 (pmol/L)	3.75 ± 0.98 3.91, 2.47–4.82	3.93 ± 0.69 3.81, 2.83–4.89
TT3 (nmol/L)	0.66 ± 0.09 0.63, 0.60–0.88	0.61 ± 0.13 0.63, 0.43–0.83
FT3 (pmol/L)	0.30 ± 0.18 0.24, 0.11–0.58	0.18 ± 0.08 0.18, 0.10–0.32
T (nmol/L)	1.59 ± 1.94 0.85, 0.01–5.32	0.77 ± 1.25 0.33, 0.01–3.71

4.1. Retinol

Several studies have reported that hepatic and plasma concentrations of retinol may be affected by exposure to POPs (Brouwer and van den Berg, 1986; Jenssen et al., 2003; Kirkegaard et al., 2010; Mos et al., 2007). However, in the present study, there were no differences in retinol concentrations in plasma, liver and kidney between the two groups of adult foxes. This is in accordance with the results reported in the six-month old farmed juvenile arctic male foxes that had been exposed to the current POP containing diet for 4.5 months (Hallanger et al., 2012). The lower hepatic concentrations in the adult males in the present study may partly be due to that these animals were lean and

Table 3
Ratios between plasma concentrations of thyroid hormones (total thyroxine [TT4]), free thyroxine [FT4], total triiodothyronine [TT3], free triiodothyronine [FT3]) in adult farmed Arctic foxes fed with a diet containing minke whale blubber presenting high concentrations of persistent organic pollutants (POP; exposed) and in foxes fed with a non-contaminated diet (control). Values are given as mean ± standard deviation (SD) and median, minimum - maximum. To simplify the presentation of the ratios, the free concentrations are multiplied with 1000 prior to calculation of the ratios.

Ratio	POP exposed	Control
TT4:FT4	6.83 ± 0.90 6.61, 5.89–8.21	6.04 ± 0.75 6.09, 5.00–7.19
TT3:FT3	2.84 ± 1.44 2.57, 1.13–5.56	3.73 ± 1.33 3.41, 2.00–6.16
TT4:TT3	38.5 ± 8.1 42.4, 25.3–46.6	39.2 ± 6.9 39.5, 26.1–48.9
FT4:FT3*	15.0 ± 5.4 14.5, 8.0–23.9	24.3 ± 8.9 23.6, 12.0–38.2

* Significant differences between the POP exposed and control groups at p=0.04 (Mann-Whitney test).

Table 4
Plasma, liver and kidney concentrations of retinol and α-tocopherol in adult farmed Arctic foxes fed with a diet containing minke whale blubber presenting high concentrations of persistent organic pollutants (POP; exposed) and in foxes fed with a non-contaminated diet (control). Values are given as mean ± standard deviation (SD) and median, minimum - maximum.

Tissue	Vitamin	POP exposed	Control
Plasma#	Retinol (μg/g)	0.30 ± 0.09 0.32, 0.14–0.39	0.36 ± 0.15 0.34, 0.19–0.63
	α-tocopherol* (μg/g)	4.80 ± 1.31 10.7, 6.4–17.3	13.90 ± 3.78 12.8, 9.5–21.7
Liver	Retinol (μg/g)	1.03 ± 0.82 0.74, 0.30–2.35	0.93 ± 0.39 0.83, 0.51–1.70
	α-tocopherol(μg/g)**	21.2 ± 5.12 22.8, 13.0–27.9	36.0 ± 5.2 36.8, 30.1–42.0
Kidney	Retinol (μg/g)	38.8 ± 23.3 29.4, 17.0–78.0	49.8 ± 18.1 53.7, 24.3–70.4
	α-tocopherol (μg/g)	92.9 ± 21.6 86.9, 69.5–131.5	107.1 ± 16 106.8, 85.9–136.7

To convert to μg /mL, assume 1 mL plasma=1.025 g.
* Significant differences between the POP exposed and control groups at $p < 0.05$ (Mann-Whitney test).
** Significant differences between the POP exposed and control groups at $p < 0.01$ (Mann-Whitney test).

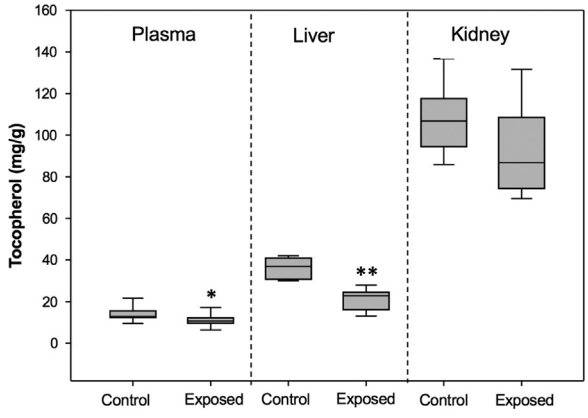

Fig. 1. Box plot showing plasma, liver and kidney concentrations of α-tocopherol in adult farmed Arctic foxes fed with a non-contaminated diet (control) and foxes fed a diet containing minke whale blubber presenting high concentrations of persistent organic pollutants (exposed). Vitamin concentrations are given in Table 4. *: significant differences ($p < 0.05$). **: significant differences ($p < 0.01$).

had received foodstuff with lower energy and fat content containing less retinol than the juvenile foxes (Hallanger et al., 2012).

4.2. Tocopherol

The hepatic levels of tochopherol in our adult control foxes seemed to be similar to levels previously reported in the juvenile 2–5 months old blue foxes (Ahlstrom and Skrede, 1995), and apparently also similar to the plasma tochopherol concentrations in our former study on juvenile exposed foxes (Hallanger et al., 2012). The negative effects of the POP exposure on hepatic α-tocopherol concentrations reported in the present study are in accordance with results from previous studies of mink and Greenland sledge dogs exposed to POPs in their diet (Kirkegaard et al., 2010; Käkelä et al., 1999). Furthermore, inverse associations between PCB burdens and hepatic α-tocopherol have been documented in experimental studies with rodents (Banudevi et al., 2006; Mantyla and Ahotupa, 1993; Twaroski et al., 2001a). In a field study on beluga whales (*Delphinapterus leucas*), inverse relationships between total PCB concentrations and hepatic α-tocopherol concentrations have been reported (Desforges et al., 2013). High levels of POPs have also been reported to be associated with reduced hepatic α-tocopherol concentrations in various fish species (Palace et al., 2001, 1998, 1996a, 1996b).

The liver masses of the adult exposed foxes in the present study were significantly higher than in the control animals. Several studies on mammals have demonstrated that OCs may cause liver hypertrophy via up-regulation of hepatic cytochrome P450 activities (Safe, 1994). Indeed, an upregulation of the activity of the POP metabolizing CYP1A enzyme in the present, exposed foxes has been reported before (Helgason et al., 2013). POPs may also initiate formation of reactive oxygen species by inducing Cyp-enzymes and oxidation of both endogenous and exogenous substances (Palace et al., 2001, 1996b; Twaroski et al., 2001b). Vitamin E has a dominating role in the antioxidant system of vertebrates (Palace and Werner, 2006), and it has been shown that vitamin E protects against oxidative stress caused by PCB induction of the aryl hydrocarbon receptor and thus Cyp1A up-regulation (Banudevi et al., 2006; Slim et al., 1999). It is therefore possible that low hepatic and plasma α-tocopherol concentrations in the POP exposed foxes compared to the controls is due to increased utilisation of α-tocopherol to counteract (or neutralize) the POP induced free radicals (Kirkegaard et al., 2010).

In contrast to the apparent negative effects of POPs on hepatic α-tocopherol concentrations in the studies cited above and as found in the POP-exposed Arctic foxes, hepatic α-tocopherol concentrations were higher in seals from the highly polluted populations in the Baltic Sea as compared to in two reference populations in Svalbard and Canada (Nyman et al., 2003). Similar increases in α-tocopherol have also been found in blood plasma from POP-exposed rats, seals and humans (Belanger et al., 2008; Katayama et al., 1991; Nyman et al., 2003). It has been suggested that the higher tissue concentrations of α-tocopherol in seals with high body burdens of POPs were due to increased demands for antioxidants to deal with the is to counteract the POP-induced oxidative stress (Nyman et al., 2003; Routti et al., 2005). Such a response would, however, most likely depend on sufficient dietary intake of tocopherol. In the present study, the foxes received restricted amounts of feed for 6.5 months to mimic the normal energy restricted period that Arctic foxes experience during spring. Both diets were supplemented with identical vitamin E levels, 50 mg/kg feed (Sivertsen, 2005). Vitamin E analyses of feed showed that the whale blubber contained 19 and the pig fat 9 mg/kg vitamin E (Sivertsen, 2005). This means that POP exposed foxes received more vitamin E than the controls. The lower levels of α-tocopherol in plasma and liver could partly be due to POP exposure but also partly because the higher levels of dietary polyunsaturated fatty acids require increased antioxidant protection (Dierenfeld, 1994; Meydani et al., 1991; Olsen and Grahl-Nielsen, 2003). Furthermore, it has also been shown that a diet

containing high levels of unsaturated fatty acids may amplify cellular sensitivity for PCB-exposure (Hennig et al., 1999). Arctic fox populations that have high body burdens of POPs are also those that have a marine diet containing unsaturated fatty acids which may constitute toxic synergistic effects (Andersen et al., 2015; Fuglei et al., 2007; Pedersen et al., 2015).

Helgason et al. (2013) examined the effects of the 6.5 months restricted feeding period on tissue concentrations of the POPs in the present foxes, and reported that emaciation caused a significant 20.2-, 5.4- and 3.5-fold increase of POP concentrations in blood, liver and blubber, respectively. This was due to lipid catabolism during the winter/spring period which caused higher POP concentrations in the remaining lipid storage and a redistribution of POPs to the circulation and liver. We suggest that during feed restriction period, the combination of increasing levels of POPs and higher levels of dietary polyunsaturated fat than in controls resulted in the lower concentration of α-tocopherol levels in both blood and liver. A previous study on juvenile male foxes, which also formed part of the current experiment, found no differences in plasma α-tocopherol levels between the exposed and the control group (Hallanger et al., 2012). Those juvenile foxes had been exposed for a period of 6 months, whereas the current adult male foxes were exposed for 22 months. A similar result was reported by Kirkegaard et al. (2010) showing reduced plasma concentrations of α-tocopherol in adult Greenland dogs exposed to the POP containing diet, but reported that no differences were present in their 3–12 month old offspring. It is therefore likely that the different effects on plasma α-tocopherol in juveniles and adults are related to the fact that adults had gone through a period of body fat mobilization, while juveniles had not, or it is possible that the effects in the adults in the present study are due to the chronic long-term POP exposure of 22 months.

4.3. Thyroid hormones

No differences were found in plasma concentrations of thyroid hormones between the POP exposed and the control group, however, a lower FT4:FT3 ratio was seen in the POPs exposed than in the control foxes (Table 3). This is in accordance with the results reported in the exposed six-month old, juvenile male foxes (Hallanger et al., 2012).

The apparent small effects on circulating concentrations of thyroid hormones in the present POPs exposed adult male foxes were in contrast to reports of lowered levels of FT3, TT3, FT4 and TT4 in adult female Greenland dogs that also were exposed to dietary consumption of POPs (Kirkegaard et al., 2011), and the associations between POPs and thyroid hormones reported in several other free-living Arctic mammals (Braathen et al., 2004; Gabrielsen et al., 2015; Knott et al., 2011; Villanger et al., 2011a, 2011b). In some of these studies, thyroid hormone levels in females appear to be more affected by POPs. It is, however, possible that the differences between the two studies are due to different species or sex specific effects of POPs on thyroid homeostasis. Interestingly, in Braathen et al. (2004), four thyroid hormone variables were associated with various POPs in female polar bears, whereas for males only two variables, FT3 levels and FT4:FT3 ratio, were significantly correlated with PCBs. This is somewhat similar to the findings in the present study on exposed adult male foxes where only FT4:FT3 seemed to be influenced. POPs could change the balance of free T4 levels in relation to T3, perhaps by increasing activity of deiodinase enzymes responsible for converting T4 to the more biological active form T3 by deiodination. Indeed, in a study of polar bears, deiodinase activity in muscle, liver and kidney tissues was positively associated with POP levels (Gabrielsen et al., 2015). Still, it is possible that the relatively limited effect of POPs on the thyroid hormones in the present study is linked to the foxes' nutritional status. It has previously been shown that plasma FT3 concentrations decreased during starvation in Arctic foxes (Fuglei et al., 2000), although the authors called for more investigation on the annual cycle of thyroid hormone levels in relation to physiological changes.

4.4. Testosterone

Testosterone levels in the foxes were in accordance with levels previously reported in wild blue foxes during June–July (Nieminen et al., 2004; Smith et al., 1985). In the present study there were no differences in the plasma levels of testosterone between POP exposed and control foxes. This apparent lack of effects is in contrast with our previous findings in the 6 months old juvenile male foxes that had been exposed to the current POP containing diet for 4.5 months in which testosterone levels in the POP exposed group was lower than in the control group (Hallanger et al., 2012). The cause of the differing results between these two fox studies may be related to the age of the animals and that they were sampled at different time of the year. In experimental animals and humans it has been reported that POP exposure may delay onset of puberty in males (Colciago et al., 2009; Grandjean et al., 2012; Korrick et al., 2011). It is possible that the lower testosterone concentrations in the POP exposed juvenile foxes is due to that these animals had a delayed pubertal onset that resulted in lower testosterone concentrations. The lack of difference in plasma testosterone concentrations between the POP exposed adult male foxes and the control males, suggest that the effects of POP did not persist into adulthood. As for the thyroid hormones, individual differences in testosterone levels due to preparation for mating, as shown previously for blue foxes where testosterone levels increase from November to May (Smith, 1987), could confound the relationship with contaminants.

5. Conclusions

Exposure to chronic oral POP exposure increased liver mass and decreased the ratio of FT4:FT3 in adult captive food restricted and emaciated Arctic foxes. Furthermore, the POP exposure seemed to decrease plasma and liver concentrations of α-tocopherol. All together this suggests that plasma FT4:FT3 ratio and plasma and liver α-tocopherol may be valuable biomarker endpoints for chronic POP exposure in wild Arctic foxes and that these perturbations may affect their health status.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2017.01.017.

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