Ecotoxicology and Environmental Safety 104 (2014) 403–408

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Altered vitamin D status in liver tissue and blood plasma from Greenland sledge dogs (*Canis familiaris*) dietary exposed to organohalogen contaminated minke whale (*Balaenoptera acuterostrata*) blubber

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ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form
12 November 2013
Accepted 18 November 2013
Available online 13 April 2014

Keywords:
25-Hydroxyvitamin D_3
Sledge dogs
Organohalogen contaminants
PCBs
Polychlorinated biphenyls
Vitamin D_3

ABSTRACT

This study compared vitamin D_3 ($vitD_3$) and 25-OH vitamin D_3 ($25OHD_3$) status in Greenland sledge dogs (*Canis familiaris*) given either minke whale (*Balaenoptera acuterostrata*) blubber high in organohalogen contaminants (OHCs) or clean porcine (*Suis scrofa*) fat for up to 636 days. A group of six exposed and six control sister bitches (maternal generation) and their three exposed and four control pups, respectively, were daily fed 112 g whale blubber (193 μg ∑PCB/day) or porcine fat (0.17 μg ∑PCB/day). Mean level of ∑PCB in adipose tissue of exposed bitches and their pups was 3106 and 2670 ng/g lw, respectively, which was significantly higher than the mean concentration of 53 ng/g lw for all controls ($p<0.001$). The vitamin analyses showed that $25OHD_3$ in liver of maternal exposed bitches were significantly lower than in controls ($p=0.004$) while $vitD_3$ was significantly highest in liver of exposed pups ($p<0.003$). Regarding blood plasma concentrations, exposed *F* generation pups had significantly higher concentrations of $25OHD_3$ than controls ($p=0.009$). Correlation analyses showed that blood $25OHD_3$ decreased significantly with increased adipose tissue concentrations of ∑PCB in exposed dogs ($R^2=0.64$, $p=0.005$) and a similar trend was found for liver $25OHD_3$ ($R^2=0.32$, $p=0.08$). The results indicate that the homeostasis and metabolism of vitamin D compounds may respond differently to the dietary composition of fatty acids and OHC exposure. It is unknown if the lower level of $25OHD_3$ in the liver of exposed dogs would have any negative effects on immunity and reproduction and more focus should be conducted on this compound in Arctic wildlife.

1. Introduction

Vitamin D (vitD) is categorized as a fat soluble seco-steroid hormone rather than a vitamin and it has several important functions in the organism, including regulation of mineral and calcium metabolism hereunder bone mineralization. Other functions of the hormone include regulation of blood pressure, immune functions, cell proliferation and differentiation, gonadal functions, apoptosis and cancer protection (Kinuta et al., 2000; Li et al., 2004; Lou et al., 2004; Uitterlinden et al., 2004; Tuohimaa et al., 2005; Norman, 2008). $VitD_3$ exists in two main forms: vitamin D_2 ($VitD_2$, ergocalciferol) and vitamin D_3 ($VitD_3$, cholecalciferol) (Feldman et al., 2005; Norman, 2008). $VitD_3$ is absorbed in the intestine or produced in skin from 7-dehydrocholesterol during UV-light exposure. It is metabolized first in the liver to 25-OH vitamin D_3 ($25OHD_3$) and then further to 1,25-dihydroxyvitamin D_3 ($1,25OHD_3$) mainly in the kidney but also in many other tissues. The latter is the active hormonal form of $vitD_3$ in tissues and binds to the nuclear vitamin D receptor (VDR) (Feldman et al., 2005). The liver hydroxylation of $vitD_3$ to $25OHD_3$ is, as opposed to hydroxylation of $25OHD_3$ to $1,25OHD_3$, unregulated, and $25OHD_3$ is accepted as a good indicator for $vitD_3$ status (Hollis, 2005). $25OHD_3$ and $1,25OHD_3$ circulate in the blood stream as complexes with Vitamin D-binding protein (DBP), albumin and lipoproteins (Kalueff et al., 2004). There is, however,

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http://dx.doi.org/10.1016/j.ecoenv.2013.11.017

species difference and for example domestic cats (*Felis catus*) and dogs (*Canis familiaris*) are not able to synthesize $vitD_3$ adequately in the skin following sunlight exposure why $vitD_3$ is an essential dietary vitamin for these two species (How et al., 1994; Kenny et al., 2004). Additionally, studies have also linked alterations in the vitD-VDR system to behavioral disorders in mice such as anxiety, motor behavior, maze performance and exploration, suggesting that $vitD_3$ and VDR are important factors in the central nervous system and imbalance may significantly affect emotional behavior (Kalueff et al., 2004).

Only few studies have investigated the effects of organohalogen contaminant (OHC) on $vitD_3$ status in mammals. Lilienthal (2000) studied PCB exposed rat (*Rattus rattus*) dams (5–40 mg ∑PCB/kg in the diet), and reported decreased levels of both $25OHD_3$ and $1,25OHD_3$ using OHC reconstituted congener patterns similar to those found in human breast milk, even for the lowest dose. In addition to this, Routti et al. (2008) studied wild gray (*Halichoerus grypus*) and ringed (*Pusa hispida*) seals from the Baltic region revealing a relationship between circulating $1,25OHD_3$, calcium, phosphate and thyroid hormone levels and hepatic PCB and DDT concentrations.

In the present study, Greenland sledge dog bitches were fed minke whale (*Balaenoptera acuterostrata*) blubber containing high OHC concentrations or clean porcine (*Suis scrofa*) fat low in OHC for 656 days. This composition and daily oral intake mimic the effects from chronic OHC exposure of Arctic mammals. We report on OHC exposure and subsequent tissue residue levels and the potential effects on concentrations of $vitD_3$ (liver) and $25OHD_3$ (liver and plasma).

2. Materials and methods

2.1. Experimental design

The maternal sledge dog generation (*P*) was composed of six exposed and six control sister bitches from Aasiaat in Disco Bay, West Greenland (N 68°42′, W 52°51′, Fig. 1) obtained at two months of age. One bitch from each sister-pair was assigned randomly to either the exposed or control group in order to minimize age and genetic differences between the two groups (Table 1). The bitches were mated with the same unexposed male which resulted in the pup generation (*F*) that was composed of three exposed and four control pups all siblings within the group (Table 2). After weaning at 6–8 weeks of age, they were fed the same diet as their mother. The exposed dogs were fed whale blubber without epidermis from a West Greenland minke whale, rich in OHCs, polyunsaturated lipids and vitamins obtained by native subsistence hunting while control dogs were fed porcine fat (lard) low in OHCs and polyunsaturated fatty acids (Sonne et al., 2006, 2007a, 2007b, 2008a, 2008b, 2008c). All dogs were fed 50–200 g/day of either blubber or porcine fat which was equivalent to twenty percent of the daily total energy intake. All dogs were also fed 50–200 g/day of standardized Royal Canine Energy 4300/4800 dry dog pellets to cover basic nutrients, vitamins and microelements (www.RoyalCanin.com). The energy intake was balanced in order to achieve comparable weights among siblings in the two groups. Concentrations of vitamins A, E and D were higher in the whale blubber given the exposed dogs (Table 3). Description and detailed discussion of concentrations of pollutants, lipids and nutrients in the diet has been published previously (Sonne et al., 2008b, 2008c). The dogs were subjected to various treatments during the study as described elsewhere (Sonne et al., 2006, 2007a, 2007b, 2008a, 2008b, 2008c) and were euthanized upon experiment completion at adulthood for the maternal generation at an age of 1.5 ± 0.1 years (range 1.5–2 years) while offspring were all one year old. The animal experiment was performed on a license granted by the Home Rule Government Chief Veterinarian in Greenland and conducted in accordance with national and institutional guidelines for the protection of animal welfare.

2.2. Blood and tissue sampling

Blood was sampled from the cava vein using a Vacutainer™ blood collection system with an eighteen gauge needle and sterile Lithium-Heparin Greiner 9 mL Vacuette and centrifuged at 3600 rpm at 1450g for 10 min. Subsequently 4 mL blood plasma from each dog was sampled in 10 mL sterile Nunc 348224 cryo tubes and frozen to −18 °C immediately. A total of 50 exposed and 50 control blood plasma samples were taken from the six sister pairs of the P generation between

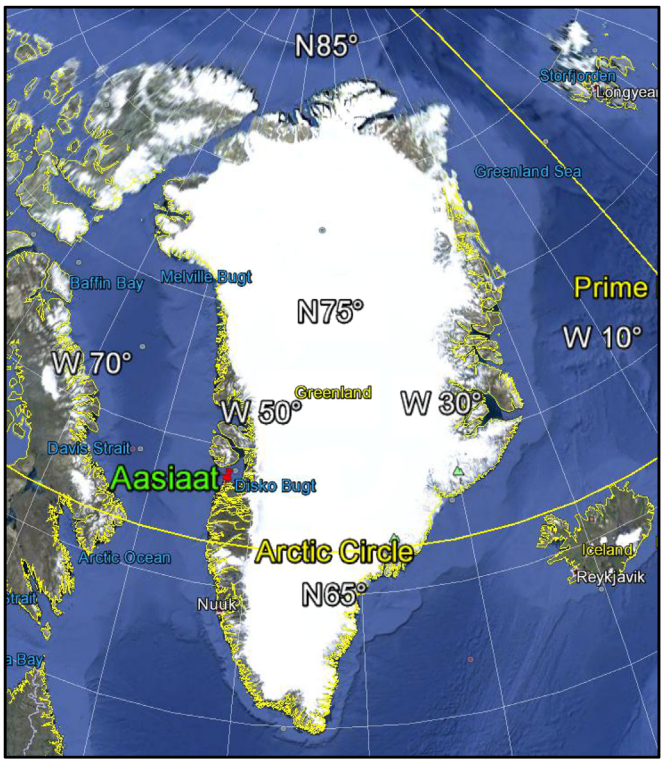

Fig. 1. Location of Aasiaat (Egedesminde; 68°42′N. 52°50′W) in West Greenland where the sledge dog study was conducted during year 2004–2005. Modified from www.google.com.

three and 21 months of age. From the seven (three exposed and four control siblings) F generation pups, nineteen blood plasma samples were taken between three and twelve months of age. Liver and adipose tissue was sampled from all bitches and pups immediately after euthanasia and transported and stored at −18 °C until chemical analysis in the respective laboratories as described below.

2.3. Analysis of tissue vitamin D_3 and $25OHD_3$

The vitamin D analyses were performed at the Technical University of Denmark, National Food Institute, Denmark. The analytical method and the equipment used to determine $vitD_3$ and $25OHD_3$ in whale blubber, porcine lard and dog livers has been previously described in detail (Jakobsen et al., 2004, 2007). Briefly, the internal standards of $vitD_2$ and 25 OHD_2 were added to the samples and saponified with ethanolic potassium hydroxide, and the unsaponifiable matter was extracted with diethylether–petroleum ether (1:1). This solution was purified on a silica solid-phase extraction column. Subsequently, clean-up was performed by preparative HPLC-procedure using a silica column combined with an amino column. Finally, the separation, detection and quantitation were performed on an analytical HPLC-system with a reversed phase column and diode array detector (DAD) and UV-detector. The limit of detection (LOD) and limit of quantification (LOQ) were for both D_3 and $25OHD_3$ 0.02 μg/100 g and 0.1 μg/100 g, respectively. The LOD, LOQ and analytical method including the equipment used to determine $25OHD_3$ in plasma has been previously described by Jakobsen et al. (2004, 2007, 2009). A minor modification was introduced by using $25OHD_2$ (Sigma-Aldrich, Buchs, Schwitzerland) as internal standard. Briefly, plasma proteins were precipitated with ethanol and the supernatant was cleaned by a MFC18 solid-phase extraction. Finally the separation, detection and quantitation of the $25OHD_3$ compounds were performed on an analytical HPLC-system using a cyano column and DAD- and a UV-detector. Quantification limits for $25OHD_3$ was 6 nmol/l. All samples from each sledge dog was analyzed in the same run, while the inter-assay variation was 12.4 percent ($n=62$), and the recovery efficiency was 94.1 ± 6.7 percent ($n=34$). The analyses were performed in a laboratory accredited according to ISO 17025.

2.4. Analyses of dietary vitamins, nutrients and fatty acids

In the dietary fat supplements, analyses for vitamin D ($vitD_3$ and $25OHD_3$), vitamin A (all-trans retinol) and vitamin E (α-tocopherol) were performed at Technical University of Denmark, National Food Institute, Søborg, Denmark. The method used for vitamin D is described above, and for vitamin A and E the methods applied were according to the EN12823-1 (2000) and EN12822 (2000),