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Mineral density and biomechanical properties of bone tissue from male Arctic foxes (*Vulpes lagopus*) exposed to organochlorine contaminants and emaciation

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ABSTRACT

We investigated the impact from dietary OC (organochlorine) exposure and restricted feeding (emaciation) on bone mineral density (BMD; g hydroxy-apatite cm^{-2}) in femoral, vertebrate, skull and baculum osteoid tissue from farmed Arctic blue foxes (*Vulpes lagopus*). For femur, also biomechanical properties during bending (displacement [mm], load [N], energy absorption [J] and stiffness [N/mm]) were measured. Sixteen foxes (EXP) were fed a wet food containing 7.7% OC-polluted minke whale (*Balaenoptera acutorostrata*) blubber in two periods of body fat deposition (Aug–Dec) and two periods of body fat mobilisation (Jan–July) in which the food contained less energy and only 2% blubber. ΣOC food concentration in the food containing 7.7% whale blubber was 309 ng/g wet mass. This corresponded to a ΣOC exposure of *ca.* 17 µg/kg body mass/d and a responding ΣOC residue in subcutaneous adipose tissue of *ca.* 1700 ng/g live mass in the 8 EXP fat foxes euthanized after 16 months. A control group (CON) composed of 15 foxes were fed equal daily caloric amounts of clean pork (*Sus scrofa*) fat. After 16 months, 8 EXP and 7 CON foxes were euthanized (mean body mass = 9.25 kg) while the remaining 8 EXP and 8 CON foxes were given restricted food rations for 6 months resulting in a body weight reduction (mean body mass = 5.46 kg). The results showed that only BMD_{skull} vs. $BMD_{vertebrae}$ were significantly correlated ($R = 0.68$; $p = 0.03$; $n = 10$) probably due to a similar composition of trabecular and cortical osteoid tissue. No difference in any of the BMD measurements or femoral biomechanical properties was found between EXP and CON foxes although BMD baculum was 1.6-folds lower in the EXP group. However, lean summer foxes had significantly lower femoral biomechanical properties measured as displacement (mm), energy absorption (J) and time (s) biomechanical properties than fat winter foxes (all $p < 0.004$). This indicates lower stiffness and softer bones from fasting which is in agreement with previous studies. Further, it should be kept in mind when studying bone tissues in Arctic mammals also in order to avoid confounding effects from body condition.

1. Introduction

The integrity of the skeletal system is vital for mammals and serves as a multi-purpose tissue. Primarily, bones are essential in the maintenance of the anatomical and physical properties of the organism due to its rigidity and touch properties. Secondly, osteoid tissue is linked up to a dynamic endocrine micro-nutrient homeostatic equilibrium reservoir of calcium, phosphorus and magnesium (Ganong, 1997). Bone formation of collagen and minerals is a result of a complex set of inter-related mechanisms that include various hormones (sex steroids, cortisol, PTH, T3/T4 and calcitonin) mediated via the hypophyseal–adrenal/thyroid/gonadal-axis, vitamins (D, A

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and C) and minerals (calcium, phosphate and zinc) (Ganong, 1997). Further, the highly specialised composition of the organic collagen matrix mineralised through a deposition of calcium hydroxy-apatite crystals makes it resistant toward fracturing (Ganong, 1997).

Bone is continuously remodelled according to a complicated cascade of hypophyseal hormone feed-back mechanisms, vitamin activations and a continuous physical osteocytic mechano-transduction from daily loadings. In cases of insufficient mineralisation there is an increased fracture risk (Van Langendonck et al., 2002; Turner, 2006; Tung and Iqbal, 2007 and references therein). These fine mechanisms make bone tissue susceptible to endocrine disrupting chemicals in the environment and insufficient dietary composition. For instance, high levels of OCs such as PCBs (polychlorinated biphenyls) have been associated with deficiencies in bone structures of a wide range of wildlife species (Bergman et al., 1992; Fernie et al., 2003; Ford and Holliday 2005; Lind et al., 2003; Lundberg et al., 2007; Sonne et al., 2004, 2006; Thompson et al., 2006) and humans (Alveblom et al., 2003; Wallin et al., 2005). Specific mechanisms are unknown, but there are several indications that OCs influence bone structure via *e.g.* disruptive effects of the thyroid system and on vitamin D homeostasis (Routti et al., 2008 and references therein). Regarding dietary composition, bone tissue is also influenced by nutritional components and periods of starvation (Talbott et al., 2001) as well as temperature extremes (Brandt and Siegel, 1978; Siegel and Mooney, 1987; Siegel et al., 1992).

Arctic animals are both exposed to endocrine disrupting pollutants and face large amplitudes in dietary composition. Especially top predators like polar bears (*Ursus maritimus*) and Arctic foxes (*Vulpes lagopus*) are heavily exposed to long-range transported OCs such as PCBs, DDTs (dichloro diphenyl trichloroethane), chlordanes and their derived metabolites (AMAP, 1998, 2004 and references therein; Verreault et al., 2005; Fuglei et al., 2007). Furthermore, these species experience a yearly cycle of limited access to food resources and temperature extremes leading to starvation and energetic stress (*e.g.* Fuglei and Øritsland, 1999; Polischuk et al., 2002; Mustonen et al., 2006). As previously suggested by Sonne et al. (2004), seasonal lipid cycles are likely to result in a release of accumulated contaminant residues from peripheral adipose tissue. Such a release of bioavailable contaminants enhances the risk of sensitive periods in which the individual becomes more susceptible to adverse health effects such as osteoporosis and alterations in the biomechanical properties (Sonne et al., 2004, 2006). However, not only the skeletal system may suffer, as effects on immune and endocrine (Skaare et al., 2001; Haave et al., 2003; Braathen et al., 2004; Lie et al., 2004, 2005 and reproductive systems (Sonne et al., 2006) also occurs.

In the present paper we test two hypothesis; 1) that there is an impact from dietary OC exposure on bone mineral density (BMD) and biomechanical properties in Arctic top predator mammals reflecting the risk of developing osteoporosis and 2) that the pronounced cycles of negative energy balance, which include severe emaciation, in Arctic top predators may change biomechanical properties and mineralisation status of the bone tissue. For that purpose, we used farmed Arctic fox males from the same genetic string which recently showed up to be a usable surrogate model for OC induced effects on internal organs of *e.g.* polar bears (Sonne et al., 2008a). The foxes were divided into an exposed group (EXP) fed minke whale (*Balaenoptera acutorostrata*) blubber as OC source and a control group (CON) that were fed OC clean pork (*Sus scrofa*) fat as main dietary fat source.

2. Materials and methods

2.1. Experimental setup and housing

The experiment was carried out in the research farm at the Department of Animal and Aquacultural Sciences, University of Life Sciences, Ås, Norway. There were two experimental groups of Arctic foxes; one group received a diet based on pork fat (CON group) and one group received a diet based on OC contaminated minke whale blubber (EXP group). The reason for using polluted whale blubber was to facilitate a cross-comparison with Arctic wildlife that relies on heavily polluted marine fatty tissues (*e.g.* AMAP 1998, 2004 and references therein). The minke whale blubber originated from animals caught in Norwegian waters. The contaminant concentrations in a test sample of the whale blubber were 2025 ng/g w.w. ΣPCB (average of two different whale blubber sources at 1850 and 2500 ng/g w.w., respectively) and 395 ng/g w.w. organochlorine pesticides (ΣOCP) while the pork fat contained insignificant levels (<0.001 ng/g). Thirty two farmed male Arctic foxes of the blue colour type comprised the experimental animals. The foxes were 16 brother pairs from litters born in the period May 23 to June 11 2003. The groups were balanced genetically by splitting the pairs to each of the experimental groups. The foxes were approximately 8 weeks old at the start of the experiment and about 1.5 years old at the end (Appendix B). One CON fox died early in the experiment (cause unknown) and the experiment comprised therefore a total of 15 CON foxes and 16 EXP foxes.

To mimic and support the natural yearly cycle of high dietary energy consumption during autumn, and low voluntary energy consumption during winter of which energy balance is negative, two

Table 1
Data on food composition and metabolizable energy (ME)

	High energy food		Low energy food	
	August 13 2003 to January 4 2004		January 5 2004 to August 7 2004	
	August 8 2004 to November 28 2004		November 29 2004 to June 16 2005	
Ingredients (%)	CON	EXP	CON	EXP
Whale blubber	–	7.7	–	2.0
Pork fat	7.7	–	2.0	–
Cod scraps	30.7	30.7	40.0	40.0
Poultry by-products	11.5	11.5	4.0	4.0
Fishmeal	2.5	2.5	2.0	2.0
Meat-and-bone meal	2.5	2.5	3.0	3.0
Slaughterhouse by-products	9.6	9.6	8.0	8.0
Precooked carbohydrates	11.5	11.5	12.0	12.0
Vitamin mineral mixture[1)]	0.1	0.1	0.2	0.2
Water	23.9	23.9	28.8	28.8
Sum	100.0	100.0	100.0	100.0
Chemical content				
Dry matter (%)	35.5	34.7	30.3	30.0
Ash (%)	3.8	3.8	4.4	3.9
Crude protein (%)	13.6	14.0	12.1	12.4
Crude fat (%)	10.8	9.0	4.5	4.5
% *n*3 FA	1.3	7.6	Not analysed	Not analysed
% *n*6 FA	16.4	10.0	"	"
*n*6:*n*3	12.6	1.3	"	"
Carbohydrates (by difference) (%)	7.3	7.9	9.3	9.2
ME content, MJ/kg food[2)]	7.46	6.93	5.04	5.09
ME content, MJ/kg dry matter	21.01	19.97	16.63	16.97
Energy distribution[3)]	31–56–13	34–51–15	40–35–25	41–34–25
PCBs and OCPs				
ΣPCB (ng/g w.w.)[a]	6	171	Not analysed	Not analysed
ΣDDT (ng/g w.w.)	0.7	70	"	"
ΣCHL (ng/g w.w.)	0.7	38	"	"
ΣHCH (ng/g w.w.)	0.1	6	"	"
ΣCBZ (ng/g w.w.)	0.7	1	"	"
Dieldrin a.o. (ng/g w.w.)[b]	1.8	24	"	"

Concentrations (ng/g wet mass) of PCBs and OCPs in the high energy food to control (CON) and exposed (EXP) farmed Arctic foxes are given. Please consult Appendix A for more PCB and OCP detailed information.
[1)]Norsk Mineralnæring, Hønefoss, Norway (please consult Appendix A for details). [2)]For calculating content of ME, digestibility values found in blue foxes and these factors were applied (kJ per g digestible nutrient): crude protein; 18.8, fat; 39.7, carbohydrates; 17.6. [3)]% of ME from protein, fat and carbohydrates. [a, b)]Please consult Appendix A.