Report

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Bone Mineral Density in Male Baltic Grey Seal (*Halichoerus grypus*)

Bone mineral density (mg cm^{-3}) was studied in male Baltic grey seals (4–23 years of age) by noninvasive computed tomography (pQCT). The material was grouped according to year of collection. Group A: 1850–1955, a period before the main introduction of organochlorines (OCs); Group B: 1965–1985, a period with very high OC contamination; and Group C: 1986–1997, a period with decreasing concentrations of OCs. The reproducibility of the measurements was good with a Coefficient of Variation (CV) ranging from 0.1% to 2.1%. Trabecular bone mineral density of the radius was significantly higher in specimens collected 1986–1997 than in those collected 1965–1985 ($p < 0.05$). Cortical bone mineral density of the mandible was significantly lower in specimens collected 1986–1997 compared with those collected 1850–1955 ($p < 0.05$). These results indicate different responses over time in trabecular and cortical bone. During the period of very high OC contamination (1965–1985), trabecular bone density was lowest, whereas cortical bone density was lowest in specimens collected 1985–1997, representing a period of fairly low OC contamination. The mechanisms behind these effects are not known. However, it can be assumed that OCs are involved. Information about residue levels of OCs in the studied individuals is lacking and, therefore, it was not possible to evaluate the impact of OCs in this respect.

Rarefaction and loss of teeth in left maxillodental bone of an adult Baltic grey seal. Photo: G. Frisk, Swedish Museum of Natural History.

INTRODUCTION

In the past, the Baltic became seriously contaminated by organochlorines (1). The most dramatic increase occurred in the levels of the 2 major organochlorines, namely dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB) occurred after 1955 (2). A rapid decrease in concentrations of DDT compounds started during the beginning of the 1970s, whereas concentrations of PCB compounds did not decrease until the late 1970s (3). The annual decrease since the beginning of the 1970s has been 9–12% for DDT-compounds. For PCB, the annual decrease since 1975 has been 4–7% (4). In addition, concentrations of dioxins have decreased in the Baltic over the last three decades, although slightly less than the concentrations of PCBs (5).

During the 20th century the 3 Baltic seal species, the ringed seal (*Phoca hispida botnica*), the harbor seal (*Phoca vitulina*) and the grey seal (*Halichoerus grypus*) have decreased in number (6). At the beginning of the 20th century the Baltic grey seal population was estimated to include 88 000–100 000 individuals, while, by 1980, the estimated number was not more than 750 (7). After the mid-1950s the decrease is mainly believed to be related to organochlorine pollution of the Baltic and poor reproduction (Härkönen, pers. comm.).

High frequencies of uterine stenoses and occlusions among Baltic ringed and grey seals, correlated with high concentrations of DDT and PCB, were reported in the 1970s (8). Further studies in Baltic grey and ringed seals (9, 10) revealed a high frequency of uterine leiomyoma in grey seals and a disease complex in both species, comprising severe chronic lesions of claws, arterial walls, large intestine, adrenals, (9–11), and kidneys (9, 12).

Lesions of skull bones are part of the disease complex and are most evident in Baltic grey seals. These lesions include loss of alveolar bone, widening of teeth sockets, porosity, and frequent severe erosions and perforations of masticatory bones, resembling severe periodontitis (11). Skull bone lesions were found in old as well as in fairly young adult animals of both sexes (11). Also skull asymmetry was observed (13). Accordingly, results from a study on harbor seal skulls from the Swedish west coast and Danish and Swedish waters of the southern Baltic, collected 1835–1988, indicated an influence by environmental pollutants (14). Lesions in harbor seals of the same character as in Baltic grey seals have increased in prevalence during the late 20th century (14).

Skeletal lesions and malformations, which might be linked to environmental pollutants, have also been reported in other wild mammalian and nonmammalian species (15–17) as well as in experimental animals (18–23) and in humans (24, 25). Mandibular and maxillar lesions in mink given 3,3',4,4',5-pentachlorobiphenyl (PCB126) or 2,3,7,8-tetrachlorobibenzo-p-dioxin (TCDD) (22, 23) resemble those reported for marine mammals, Baltic seals (11–14) and Beluga whale (16).

The aim of the present study was to compare mineral density in mandibular and radius bones of male Baltic grey seals from various time periods by using noninvasive peripheral quantitative computed tomography (pQCT).

MATERIAL AND METHODS

Material

Mandibular (lower jaw-bone) and radius bones from male grey seals were used to determinate bone characteristics. Age was determined by counting annual rings of cementum zones in undecalcified tooth sections according to Johnston and Watt (26).

In total, bones from 43 individuals were analyzed (43 mandibular and 15 radius bones). The bone material, belonging to the Section of Vertebrate Zoology at the Swedish Museum of

Natural History, was divided into 3 groups, according to year of collection.

Group A: 1850–1955, cases representing the period before the main introduction of organochlorines in the environment (mandibles from 3 individuals from the Swedish west coast and 6 from the Baltic), 9–23-years old (mean age 15). No radius bones were obtained from these individuals.

Group B: 1965–1985, cases representing the period of very high OC contamination of the Baltic (mandibular and radius bones from 5 and mandibular bones from 17 individuals from the Baltic Sea, 4–15-years old (mean age 11).

Group C: 1986–1997, cases representing a period after which OC-concentrations decreased in the environment (mandibular and radius bones from 10 and mandibular bones from 22 individuals from the Baltic Sea, 4–15-years old (mean age 10). The selection of the time intervals was based on results from long-term monitoring of concentrations of environmental contaminants in the Baltic (4). Most skull bones of the individuals used in this study were previously examined macroscopically (11).

pQCT

The bones were examined by pQCT (Stratec XCT 960A, software version 5.20; Norland Stratec Medizintechnik, Pforzheim, Germany). Precision, long-term stability, linearity and accuracy of the pQCT bone scanner were evaluated once a day by using a validation phantom. For geometrical and densitometrical analysis of trabecular bone, peel mode 2, contour mode 1, threshold 0.660 cm^{-1} and inner threshold 0.700 cm^{-1} were used in order to establish a tomographic limit for the trabecular bone. For geometrical and densitometrical analysis of cortical bone, separation mode 1 and a threshold value of 0.930 cm^{-1} was set as a limit to define the cortical bone region. The bones were placed horizontally and scanned using voxel size C (0.295 mm). Trabecular area (Trab A, mm^2), trabecular content (Trab Cnt, mg mm^{-1}), trabecular bone mineral density (Trab BMD, mg cm^{-3}), total cross-sectional area (Tot A, including marrow cavity and cortical bone, mm^2), cortical area (Crt A, mm^2), cortical content (Crt Cnt, mg mm^{-1}), cortical thickness (Crt thk, mm) and cortical bone mineral density (Crt BMD, mg cm^{-3}) were used for the analyses.

Radius

The radius from the right or left forelimb was scanned at a point distanced 35% of the length from the proximal part of the bone (Fig. 1). This area was chosen because it is rich in trabecular bone.

Mandibular Bone

The width of the hindmost molar of the left mandibular bone was measured with an accuracy of 0.01 mm, using an electronic sliding caliper. The subsequent measurement of the bone mineral density was made at a point located twice this width value; behind the molar (Fig. 2). This point was chosen because the scanned area contains both trabecular bone and cortical bone.

Reproducibility

To evaluate the reproducibility of the pQCT measurements, the coefficient of variation (standard deviation/mean) was calculated from 10 repeated measurements with a single sample repositioning before each measurement.

Statistical Methods

The data recorded were evaluated by one-way ANOVA followed by post-hoc Fisher's PLSD. Differences were considered significant when $p < 0.05$. The possible influence by age was investigated by ANCOVA.

RESULTS

The reproducibility of the pQCT measurements of the radius and the mandible was good with a CV ranging from 0.1% to 2.1%.

The results from the measurements of the radius and the mandibular bones are presented in Tables 1 and 2, respectively. The significant results found by the post-hoc analysis are extracted as diagrams and presented in Figures 3 and 4. The trabecular bone mineral density (mg cm^{-3}) of the radius was significantly lower in bones collected between 1965 and 1985 (129.6 ± 16.9, mean ± SEM, n = 5; Fig. 3) compared with those collected between 1986 and 1997 (196.9 ± 14.5, n = 10, $p < 0.05$). This difference in trabecular bone mineral density was still significant ($p < 0.05$) when the effect of age hade been taken into account.

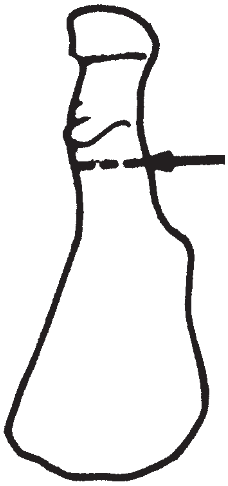

Figure 1. Drawing of seal radius bone showing the measuring point of the peripheral quantitative tomography (pQCT).

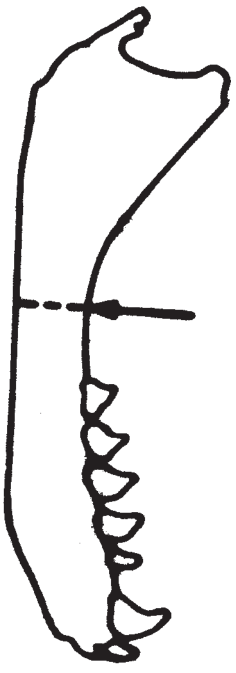

Figure 2. Drawing of seal mandibular bone showing the measuring point of the peripheral quantitative tomography (pQCT).

Table 1. Results obtained from pQCT (peripheral quantitative computed tomography) measurements of radius bone* from male Baltic grey seal (*Halichoerus grypus*), 4–15-years of age. The bone material was divided into groups according to year of collection (1965–1985 and 1986–1997). Values are mean ± SEM.

	Year of collection	
	1965–1985 (n = 5)	1986–1997 (n = 10)
Trabecular area (mm^2)	39.6 ± 7.2	45.0 ± 10.5
Trabecular content (mg mm^{-1})	4.7 ± 0.6	7.4 ± 1.2
Total cross-sectional area (mm^2)	173.1 ± 4.7	181.6 ± 5.4
Cortical area (mm^2)	126.0 ± 9.1	125.0 ± 10.9
Cortical content (mg mm^{-1})	158.9 ± 11.7	154.5 ± 14.0
Cortical BMD** (mg cm^{-3})	1262.0 ± 16.1	1231.8 ± 19.6
Cortical thickness (mm)	3.6 ± 0.4	3.5 ± 0.4

*) The measuring point was located at a point distanced 35% of the length from the proximal part of the bone (Fig. 1).
** Cortical BMD, cortical bone mineral density.

The cortical bone mineral density (mg cm^{-3}) of the mandible showed a continuous decline over time: 1174.8 ± 8.2, 1162.1 ± 5.5, and 1135.5 ± 8.2, respectively, for the 3 collecting periods ($p < 0.05$). The lowest density was observed in the material collected 1986–1997. It was significantly lower both compared with that of the specimens collected between 1880 and 1955 ($p < 0.01$) and that of the ones collected 1965–1985 ($p < 0.05$) (Fig. 4). This decline over time for cortical density was still significant ($p < 0.05$) when the effect of age had been taken into account.

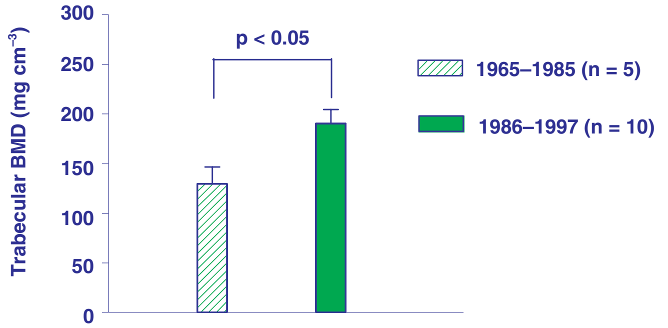

Figure 3. Trabecular bone mineral density (Trab BMD) of radius bone from male Baltic grey seal (*Halichoerus grypus*), 4–15-years of age, obtained by peripheral quantitative tomography (pQCT). The bone material belongs to the Swedish Museum of Natural History and was divided into 2 groups according to year of collection (1965–1985 and 1986–1997). Values are mean ± SEM.

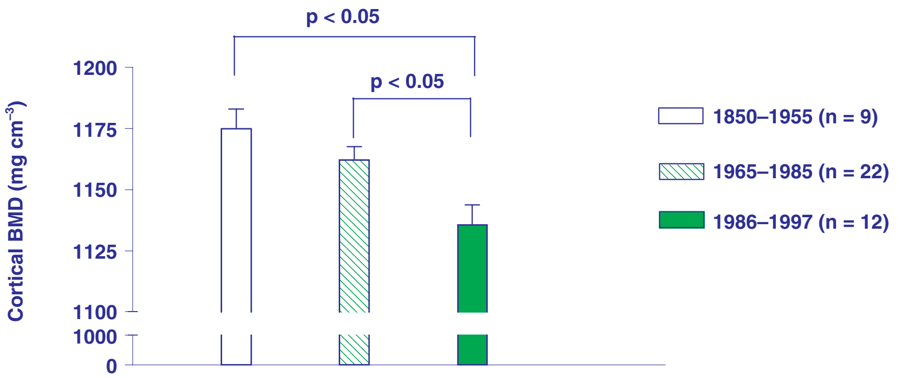

Figure 4. Cortical bone mineral density (Cort BMD) of mandibular bone from male Baltic grey seal (*Halichoerus grypus*), 4–23-years of age, obtained by peripheral quantitative tomography (pQCT). The bone material belongs to the Swedish Museum of Natural History and was divided into 3 groups according to year of collection (1850–1955, 1965–1985, and 1986–1997). Values are mean ± SEM; the numbers of animals are given in parentheses.

Table 2. Results obtained using peripheral quantitative computed tomography (pQCT) measurements of mandibular bone* from male Baltic grey seal (*Halichoerus grypus*), 4–15-years of age. The bone material was divided into groups according to year of collection (1850–1955, 1965–1985 and 1986–1997). Values are mean ± SEM.

	Year of collection		
	1850–1955 (n = 9)	1965–1985 (n = 22)	1986–1997 (n = 12)
Trabecular area (mm^2)	17.9 ± 1.5	14.4 ± 1.2	14.9 ± 1.5
Trabecular content (mg* mm^{-1})	6.8 ± 0.8	4.5 ± 0.8	4.6 ± 0.7
Trabecular BMD** (mg cm^{-3})	151.9 ± 10.2	119.6 ± 11.3	132.1 ± 14.9
Total cross-sectional area (mm^2)	245.9 ± 12.0	214.5 ± 9.3	224.8 ± 17.1
Cortical area (mm^2)	181.7 ± 10.8	164.2 ± 6.1	172.4 ± 15.0
Cortical content (mg mm^{-1})	214.0 ± 13.4	191.1 ± 7.2	196.4 ± 17.4
Cortical thickness (mm)	4.3 ± 0.2	4.3 ± 0.1	4.4 ± 0.3

* The width of the hindmost molar was measured to the nearest 0.01 mm, using an electronic sliding caliper and the subsequent measurement was taken at a point located at a distance equivalent to twice this width value; behind the hindmost molar of the left part of the mandibular bone (Fig. 2).

DISCUSSION

The results presented in Figures 3 and 4 show that the bones collected between 1965 and 1986 exhibit changes in trabecular bone mineral density similar to those present in bones of osteoporotic humans (normal chemical composition but thinning or loss of individual trabeculae) (27).

The trabecular and cortical bone mineral density in Baltic grey seals exhibited a somewhat different pattern over time. The trabecular bone mineral density was lower in the radius bones collected during 1965–1986, a period of very high PCB and DDT contamination in the Baltic, than in the radius bones collected 1987–1997. Unfortunately, this result is based on a small sample size due to a very limited number of available radius specimens at the bone collections of Swedish Museum of Natural History. The result is, however, strengthened by the results from the measurements of trabecular bone mineral density of the mandibular bones: 119.6 ± 11.3 mg cm^{-3} for the bones collected 1965–1985, and 132.1 ± 14.9 mg cm^{-3} for the bones collected 1986–1997.

The cortical bone mineral density in mandibles showed a continuous decline during the 3 collection periods: 1850–1955, 1965–1985 and 1986–1997. The cause of this difference is not known. We hypothesize that the difference observed might be due to the considerable change of composition and levels of contaminants in the Baltic, which have occurred during the last 3–4 decades. The concentrations of DDT and PCB in biota of the Baltic started to decrease during the first half of the 1970s and reached fairly low levels during the 1990s (3, 4). Contaminants introduced later, e.g. brominated flame retardants began to increase in Baltic biota during the late 1970s and did not start to decrease until the beginning of the 1990s (28).

The results presented in this study regarding trabecular bone are in accordance with the findings in an earlier macroscopic investigation of skull bones of the Baltic grey seal (11). The latter study showed a significantly higher prevalence of skull bone lesions in grey seals collected between 1960 and 1985 (60%) compared with samples collected before 1950 (5%). Some of the lesions observed were consistent with those present in severe periodontitis: loss of alveolar bone, widening of the teeth sockets and loss of teeth (11). The prevalence of these lesions has decreased during the last 20 years (Bergman, A. and Olsson, M. pers. comm.).

The bone lesions in Baltic grey seals are similar to those found in Beluga whales (*Delphinapterus leucas*) from the Gulf of St. Lawrence in eastern Canada. Forty-five Beluga whales, collected between 1983 and 1990, exhibited tooth loss, and periodontitis was a common finding. Less than 60% of the animals had the normal complement of 28–36 teeth (16). Like Baltic grey seals St. Lawrence Beluga whales are exposed to a variety of xenobiotics and the organochlorine body burden of the examined Beluga whales was significant (16).

The reproducibility of these measurements was good, indicating that pQCT is a suitable noninvasive method for this type of study.

In conclusion, the results of this study show different responses over time in trabecular and cortical bone mineral density of Baltic grey seal. During the period of very high OC contamination, the trabecular bone density was lowest, whereas the cortical bone density shows a continuous decline over time. The mechanisms behind these effects are not known, but organochlorines are likely to be involved. Unfortunately, no systematic organochlorine residue analysis has been performed in the grey seals used in this study. Therefore, it has not been possible to investigate the relationship between bone mineral density and organochlorine body burden.

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