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Cadmium toxicity to ringed seals (*Phoca hispida*): an epidemiological study of possible cadmium-induced nephropathy and osteodystrophy in ringed seals (*Phoca hispida*) from Qaanaaq in Northwest Greenland

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Abstract

The Greenland marine food chains contain high levels of cadmium, mercury and selenium. Concentrations of cadmium in the kidney of ringed seals (*Phoca hispida*) from the municipalities of Qaanaaq and Upernavik (Northwest Greenland) are among the highest recorded in the Arctic. The purpose of the study was to determine whether cadmium-induced damage in the kidneys and the skeletal system could be detected among 100 ringed seals from Northwest Greenland. The cadmium concentrations in the kidney cortex ranged from 0 to 248 $\mu\text{g/g}$ wet weight (mean = 44.5, $N=100$) in the 99 kidneys examined. Experience from cadmium-poisoned humans and laboratory mammals indicates that concentrations above 50–200 $\mu\text{g/g}$ wet wt. may induce histopathological changes. Overall, 31 of the ringed seals had cadmium concentrations in the kidney cortex above 50 $\mu\text{g/g}$ wet wt., 11 had concentrations above 100 and one had a concentration above 200 $\mu\text{g/g}$ wet wt. Obvious histopathological changes (categorised mainly as glomerulonephritis) were found in 10 of the seals; however, none of these changes could be attributed to cadmium-induced renal damage (mainly tubulopathy) as described for other species. Damage to the proximal kidney tubules is known to induce demineralisation of the skeletal system (Fanconi's syndrome). Therefore, the three lowest lumbar vertebrae were scanned in 91 seals to measure the content of calcium. The 10 cases of nephropathy could neither be linked to the degree of mineralisation of the skeleton nor to the cadmium concentrations. Furthermore, the degree of mineralisation of the skeleton was not correlated with the cadmium concentration, age or sex. It can therefore be concluded that despite high levels of cadmium, none of the ringed seals showed any signs of cadmium-induced nephropathy or osteodystrophy. This might be explained by the composition of the ringed seals diet, which contains high levels of vitamin D, calcium, phosphorus, zinc, selenium and protein. These elements are all likely to counteract cadmium-induced damage. It is speculated that ringed seal are not particularly vulnerable to osteodystrophy, due to their continuous growth (bone mineralisation) throughout life and the oestrogen hormonal activity of females throughout life. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Northwest Greenland; Ringed seals (*Phoca hispida*); Cadmium; Nephropathy; Osteopathy

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1. Introduction

As documented in the Arctic Assessment Report, high concentrations of mercury, cadmium and selenium are found in higher trophic levels of the Arctic marine food chains (Dietz et al., 1998b). This review reveals that ringed seals in Northwest Greenland hold the highest cadmium levels in the Arctic. The cadmium concentration observed in the kidneys of ringed seals are high enough to pose a risk of kidney damage based on results from human groups and laboratory mammals. The high concentrations of cadmium in the higher trophic levels of the Arctic food chains are believed to be a result of long food chains, slow growth processes and crustaceans which accumulate significant amounts of cadmium (Dietz et al., 1996, 1998b).

The present study is a first attempt to elucidate the potential presence of cadmium-induced nephropathy. As it is well documented that cadmium-induced nephropathy can induce osteopenia (demineralisation) of the skeleton system (WHO, 1992), this aspect was also examined in the investigation. Kidneys and bones (lower lumbar vertebrae and mandible) from 100 ringed seals sampled in the Qaanaaq district Northwest Greenland in early May to mid-June 1998 were examined. In addition, samples from muscle, liver, blubber, reproductive organs, blood, urine, bile, stomach and claws were also taken to provide the basis for additional studies.

2. Materials and methods

2.1. Sampling

Samples were collected from 100 ringed seals in the Qaanaaq area from early May to mid-June 1998. The tissue samples were taken as soon as possible after shooting of the seal and less than 24 h after the catch. The isolating effect of the blubber counteracted freezing of the internal organs prior to sampling.

Samples from the kidney, liver, muscle, blubber, stomach, claws, reproductive organs, mandible, lower lumbar vertebrae, blood, urine and bile were taken from each seal and stored in separate poly-

ethylene (PE) plastic bags. Small fragments of the kidney ($2 \times 2 \times 2 \text{ cm}^3$) were stored in an anti-freeze fixation liquid (details in Section 2.2) to prevent freezing damage. Blood samples were taken from the heart, the aorta or the caval vein and stabilised in heparin to prevent coagulation. All samples were collected in a PE bag labelled with the seal identification number, and kept at outdoor temperature (-5 to $-20 \text{ }^\circ\text{C}$) until frozen storage (-10 to $-20 \text{ }^\circ\text{C}$). Samples were shipped as frozen goods from Qaanaaq to Copenhagen, where further storage was at $-20 \text{ }^\circ\text{C}$.

2.2. Anti-freeze fixative

A combination of formaldehyde and alcohol (10% of a 35% formaldehyde solution and 90% of a 96% ethanol solution) was used to avoid freeze damage to the kidney samples.

2.3. Age determination

The age determination was carried out by the Canadian Wildlife Service in Edmonton, Canada, where the cementum growth layer groups (GLG) of the lower left canine were counted using the method described by Dietz et al. (1991).

2.4. Renal histopathology

2.4.1. Preparation

The tissue samples were prepared in a Sakura TissueTek[®] VIP, with the following steps conducted:

- 1 × 70% alcohol for 1 h;
- 2 × 96% alcohol for 1 h;
- 4 × 99% alcohol for 1 h;
- 2 × 100% xylene for 1 h; and
- 4 × paraffin for 45 min.

This treatment resulted in dehydration of the tissue and subsequent replacement of the water with paraffin.

The tissue was then cut into 2–4- μm thin slices on a Zeiss HM 440E microtome.

2.4.2. Tissue staining

The tissue staining was manually carried out in haematoxylin–eosin (HE), periodic acid–Schiff

(PAS) and van Gieson solutions. To avoid unequal staining, all sections were stained simultaneously. Haematoxylin (Al–haematein)–eosin (HE) staining is the most commonly used staining technique. The Al–haematein colour complex stains the acidophilic cell components (nucleus) blue, whereas the eosin stains the basophilic cell components (cytoplasm and matrix proteins) red (Lyon et al., 1991). Periodic acid–Schiff (PAS) solution stains carbohydrates as homoglycans (e.g. glycogen), glycoproteins and neutral proteoglycans. This results in a red colouring of collagen fibres, basement membranes and the brushborder basement membranes in the proximal tubules, cell membranes, cytoplasm and nucleus. Carbohydrates are stained dark red and nuclei dark blue (Lyon et al., 1991). Van Gieson solution is used to stain connective tissue. The picric acid stains the fibres red (kidney capsule, vessels and basement membranes) (Lyon et al., 1991). These three methods were used in the histopathological examination of all kidney samples. A few of the slides were also stained with periodic acid silver methenamine (PAS-M) to accentuate the basement membranes, as described by Lyon et al. (1991).

2.4.3. Examination

Microscopical examination was performed on a Leica DMLB microscope with 50×, 100×, 200×, 400×, 630× and 1000× magnification.

2.5. X-Ray analysis

2.5.1. Preparation

The mandibles and the three lowest lumbar vertebrae were macerated and boiled so that muscles and tendons could be removed before examination and X-ray analysis. The bones were macerated for 96 h, boiled for 15 min and dried in air for a minimum of 72 h.

2.5.2. X-Ray (osteodensitometry)

Osteodensitometry is a technique developed to determine osteoporosis (demineralisation) in the skeleton system of primarily postmenopausal women. A Norland XR26 X-ray bone densitometer was used to determine the mineralisation of the bones (calcium phosphate content) at the Univer-

sity Hospital in Hvidovre. The principle of the osteodensitometer is dual X-ray absorption (DXA), in which a highly stable X-ray tube generates a broad spectrum of photons which are subsequently filtered (K-edge filtration) into two distinct peaks, as described by the Norland Corporation (1993).

The data were analysed using a software program (XR[®] revision 2.4) which generates a picture of the bone segment and calculates the bone mineral content (BMC), the area and the bone mineral density (BMD). The BMC is the calcium phosphate content of the bone (g) and the BMD is the calcium phosphate content per unit squared (g/cm^2).

The method was controlled daily by carrying out a standard calibration using a bone phantom with a known mineral density (a double determination of two mandibles was also carried out). This showed a reproducibility of >99% and an accuracy of $\pm 2 \times \text{S.D.}$

2.6. Cadmium analyses

The metal analyses were performed at the Department of Arctic Environment laboratory (accredited under DANAK by DS/EN ISO/IEC 17025, No 435 for analysing Hg, Cd, Pb, Zn, Cu, Cr, Ni and As in biological tissues and sediments). After removal from the freezer, the tissue samples were lightly thawed and the outer exposed tissue layer was cut away to minimise possible contamination and changes in water content due to handling and storage. Stainless steel scalpels, polyethylene gloves and cutting boards were used. Approximately 0.5 g of tissue was transferred to a tared Teflon liner of a Berghof stainless steel bomb. After the addition of 3 ml of 65% HNO₃ (Merck Suprapur[®]), the bombs were closed and incubated for 12 h at 120–150 °C. Following a cooling period, the digests were quantitatively transferred to 50-ml screw-cap polyethylene bottles and adjusted to ca. 25 g weight using metal-free deionized water (Millipore[®]). Approximately 8% HNO₃ was used for all further dilutions.

All cadmium analyses were carried out by flame atomic absorption spectrometry (AAS) (Perkin Elmer 3030); however, the graphite furnace tech-

Table 1
Basic statistics and correlation coefficients for the variables in the sample

Variable	Count	Mean	S.D.	Range			
Age	98	8.08	10.1	0–40			
Length (cm)	100	109	17.4	53–149			
Weight (kg)	100	50.6	16.4	8–80			
BMDb (g/cm ²)	91	0.65	0.19	0.26–1.27			
CdK (μg/g wet wt.)	100	44.5	40.8	0–248			
	<i>Age</i>	<i>Length</i>	<i>Weight</i>	<i>BMDm</i>	<i>BMDb</i>	<i>CdK</i>	
Age	1.000	+0.55	+0.47	+0.87	+0.69	+0.23	
Length (cm)	***	1.000	+0.85	+0.73	+0.69	+0.19	
Weight (kg)	***	***	1.000	+0.73	+0.69	+0.19	
BMDm (g/cm ²)	***	***	***	1.000	+0.8	+0.10	
BMDb (g/cm ²)	***	***	***	***	1.000	+0.43	
CdK (μg/g wet wt.)	NS	NS	NS	NS	***	1.000	

CdK, cadmium concentration of the kidney cortex; BMD, bone mineral density of calcium phosphate; BMDm is the BMD of the mandible (BMDm,r indicates the right and BMDm,l indicates measurements of the left part of the mandible); BMDb, BMD of the lower three lumbar vertebrae; *** $P \leq 0.001$; NS, not significant.

nique (Perkin Elmer 3030 with Zeeman background correction) was used for the final analysis of samples with less than 2.5 μg/g wet wt. cadmium.

The lower limit of detection for laboratory analyses of cadmium was 0.015 μg/g wet wt. of kidney. All concentrations are reported as μg/g wet wt. For recalculation into μg/g dry wt., a correction factor of 3.67 was calculated on the basis of the mean of weight percentages routinely recorded in the DAE laboratory.

The analytical quality was checked by repeating analyses, and by the frequent use of various reference standards, especially Tort-1 (lobster hepatopancreas) supplied by the National Research Council of Canada (Marine Analytical Chemistry Standards Programme) and the dried tuna internal standard of the National Food Agency of Denmark. The DAE laboratory participates in the international intercalibration exercises conducted by the International Council for the Exploration of the Sea (ICES), EEC (QUASIMEME), National Research Council, Canada and by the Department of Fisheries and Oceans, Winnipeg, Canada.

2.7. Statistics

Pearson's correlation coefficient was used to determine correlations between the variables length, weight, bone mineral density (BMD) and

cadmium concentration in the kidney cortex (CdK). The BMD and CdK data were log-transformed to meet the assumption of normal distribution and equal variance before data handling was carried out. The principle in the data handling is a model of covariance [SAS[®] GLM procedure (SS3)] with logBMD and logCdK as the dependent variables, sex as class variables, the age as covariable and the interaction link between these. The model is successively reduced to non-significant interactions ($P > 0.05$) and a test on significant differences between the means of age, corrected sex and preage groups (LSMean) was carried out. The distribution of nephropathy (kidney damage) among the sexes was tested with a χ^2 test and log regression.

3. Results and discussion

The basic statistics, correlation coefficients and significant levels of the continuous variables are shown in Table 1.

3.1. The sample

The distribution of sex, age, weight and length is shown in Fig. 1 and Table 1. The standard deviation (S.D.) is highest in CdK, intermediate in weight, length and age, and lowest in BMD; weight and length are highly correlated ($r = +$

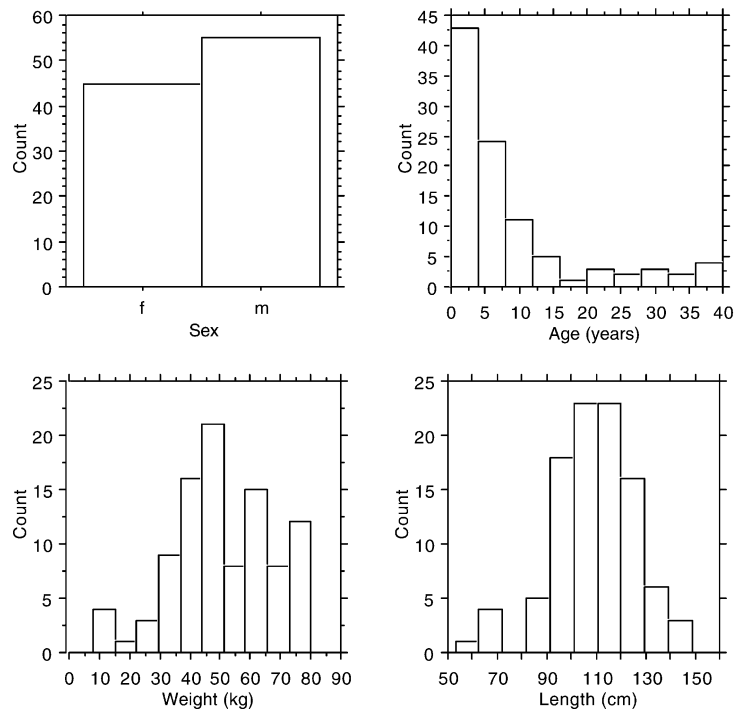


Fig. 1. Frequencies of the sample investigated presented for categories sex (f=female; m=male), age (years), weight (kg) and length (cm).

0.85***; see Table 1). The sample consisted of 55 male and 45 female ringed seals. The sample mainly consisted of subadult and adult seals (Table 1 and Fig. 1) because of the hunting technique, which involves shooting seals at their breathing holes. The sex distribution was almost equal, and the weight and length of the seals corresponded well with their age.

3.2. Osteodensitometry

The skeletal content of calcium phosphate (BMD) was determined by the DXA procedure. This investigation alone is inadequate to decide if the skeleton could be demineralised due to cadmium-induced osteodystrophy, because the diagnosis is usually based not only upon the DXA examination, but also on clinical (fractures, pains) and paraclinical (blood chemistry, regular X-ray) examination and, if possible, histopathological examination (autopsy/biopsy).

It was possible to determine the BMD on 96 mandibles and 91 lumbar vertebrae (L₂–L₄). Four of the mandibles were damaged by shooting, and nine of the lumbar vertebrae were damaged during maceration and boiling.

Macroscopic examination of the lumbar vertebrae did not reveal any pathological changes, whereas macroscopic changes were evident in mandibles from five individuals. These individuals were among the oldest (mean age 34.8 years; range 29–40). Their kidney cortex concentration of cadmium (CdK) was not high and the BMD was very high (mean BMD 0.939; range 0.85–1.000). Two had kidney damage (age-related) which was a significantly higher percentage (40%) than the overall mean (10%) (Table 2). These changes were therefore classified as age-related anatomical changes.

Prior to the sampling in Qaanaaq in 1998, the BMD in ringed seal mandibles ($N=50$) of earlier samples from Greenland were determined. This

was carried out to expand the range of the CdK values and to correlate BMD to Cd in muscle, liver and kidney. During this work, experience was obtained using the DXA procedure and the correlation between the left and right part of the mandible was estimated ($r = +0.987^{***}$; see Fig. 1). Based on this finding, the BMD of the mandibles from Qaanaaq 1998 was calculated as the average of the left and right part of the mandible. The BMD in the lower lumbar vertebrae (BMDb) and in the mandibles (BMDm) were also strongly correlated ($r = +0.80^{***}$; Fig. 1). It is known that cadmium-induced metabolic dysfunctions can induce osteopenia (demineralisation) of the lumbar vertebrae in humans, and our investigation therefore also used the BMDb to reflect the skeletal mineralisation conditions (Friberg et al., 1986; WHO, 1992; Hyldstrup, 1998).

In Fig. 2, the calcium phosphate content of the three lower lumbar vertebrae (BMDb) is shown as a function of seal age. It is evident that the BMDb in males increases with age in the same way as the length, because of continuous growth, even after sexual maturity (McLaren, 1958). The same trend is not as clear for the females, as these mobilise large amounts of calcium and phosphate during pregnancy and the suckling period, where they are used for both skeletal production in the foetus and to maintain calcium phosphate homeostasis in both the mother and the offspring. In addition, endocrinological changes in the postmenopausal period of women have been proved to affect calcium homeostasis, and thereby induce a negative calcium balance, which results in osteopenia (Friberg et al., 1986; WHO, 1992; Simonsen, 1998). As this process is reinforced by the presence of cadmium, two hypotheses were tested:

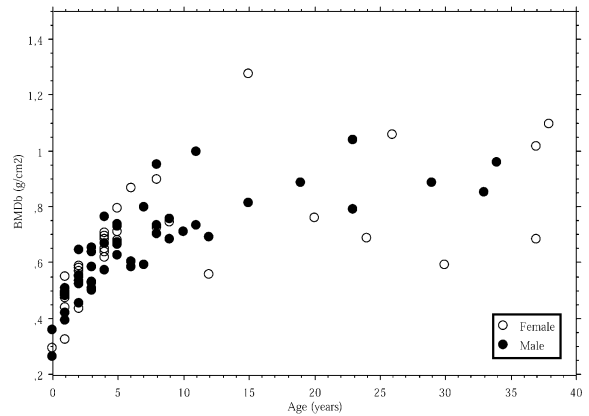


Fig. 2. Mineralisation of the lumbar vertebrae (BMDb, g/cm^2) of the ringed seals as a function of the age (years) and sex.

- The BMD of the lower lumbar vertebrae increases with age; and
- The females are significantly lower in BMD than the males.

BMD appears to significantly increase with age ($P < 0.001$) and there is no difference between sexes ($P = 0.68$), nor is there any interaction between age and sex ($P = 0.26$). Hence, no effects on the calcium metabolism could be detected as functions of age, pregnancy or endocrinological changes. Female ringed seals do not go into a postmenopausal period as in humans, and this could explain the maintenance of calcium homeostasis (Smith, 1987; Reeves, 1998).

3.3. Histopathology

Macroscopic examination of the kidneys did not show any histopathological changes. Prior to sam-

Table 2

Measurements of the five ringed seals with macroscopic changes in the mandibles

Identification number	20708	20709	20716	20759	20797
Sex	F	M	M	M	M
Age (years)	38	34	33	40	29
CdK ($\mu\text{g}/\text{g}$ wet wt.)	24.6	6.35	7.08	78.4	14.9
BMDb (g/cm^2)	1.000	0.958	0.850	1.000	0.886
Kidney damage	Yes	Yes	No	No	No

Abbreviations used: CdK, cadmium concentration in the kidney cortex; BMDb, bone mineral density of the lower three lumbar vertebrae; F, female; M, male.

Table 3
Classification of the histopathological findings into four groups

Group 1	No apparent histopathological changes ($N=75$); lesser PAS positive deposits focally spread in the mesangial matrix (see Fig. 3)
Group 2	Minimal changes ($N=14$); focally spread PAS-positive matrix deposits in the mesangium and a limited segmental thickening of the glomerular basement membrane (see Fig. 4)
Group 3	Obvious histopathological changes ($N=6$); generalised distinct PAS-positive deposits in the mesangium leading to a thickening of the hilus; segmental distinct thickening of the glomerular basement membrane with PAS-positive deposits (humps); varying degrees of arteriosclerotic changes in the efferent and afferent arterioles (see Figs. 5–8)
Group 4	Intense histopathological changes ($N=4$); generalised distinct PAS-positive deposits in the mesangium leading to an obvious thickening of the hilus; segmental distinct thickening of the glomerular basement membrane with PAS-positive deposits (humps); arteriosclerotic changes in the afferent and efferent arterioles leading to sclerosis (atrophy and fibrosis) of the glomeruli; fibrous peritubular necrotic tubules (see Figs. 6–9)

pling, the properties of the fixative were tested in a low-temperature environment. Samples from bovine kidneys were stored in a freezer ($-18\text{ }^{\circ}\text{C}$) for 48 h and the formaldehyde–alcohol fixation liquid was compared to the regular fixation in 4% formaldehyde liquid. It was found that the formaldehyde–alcohol combination gave fewer artefacts and less freeze damage. One animal (a juvenile female) was not included in the histological examination due to autolysis caused by a suboptimal preparation.

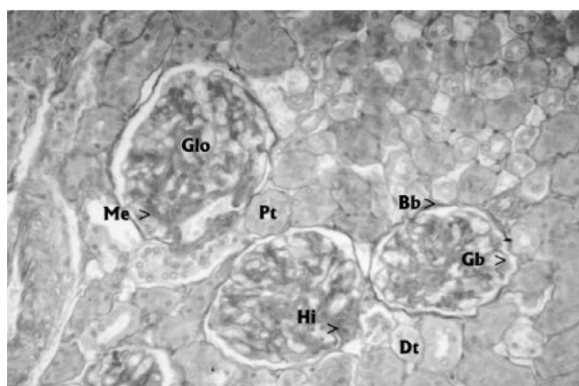


Fig. 3. Histological findings in group 1. No changes are evident, neither in the mesangium nor in the basement membranes, the glomeruli or the tubules. Abbreviations used: Glo, glomerulus; Me, mesangium; Pt, proximal tubules; Hi, hilus; Dt, distal tubules; Gb, glomerular basal membrane; and Bb, Bowmann's basal membrane (PAS, $250\times$).

The kidneys were divided into four groups on the basis of histopathological findings (Table 3). Groups 1 and 2 represent kidney tissue without obvious histopathological changes, and groups 3 and 4 represents kidney tissue with clear histopathological changes. In Figs. 3–9, the histopathological findings are shown.

In groups 3 and 4, diagnosis of the histopathological changes shows glomerulonephritis, interstitial nephritis and arteriosclerosis (Figs. 7–9) (Confer and Panciera, 1995).

Glomerulonephritis is usually connected to infections caused by bacteria or viruses in another

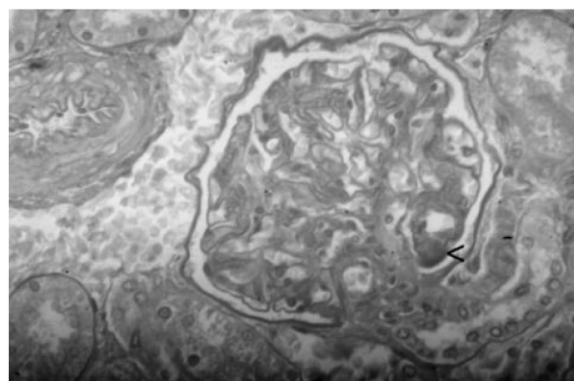


Fig. 4. Histological findings in group 2. Note the minor PAS-positive deposit in the glomerular basement membrane on the right (arrow) (PAS, $400\times$).

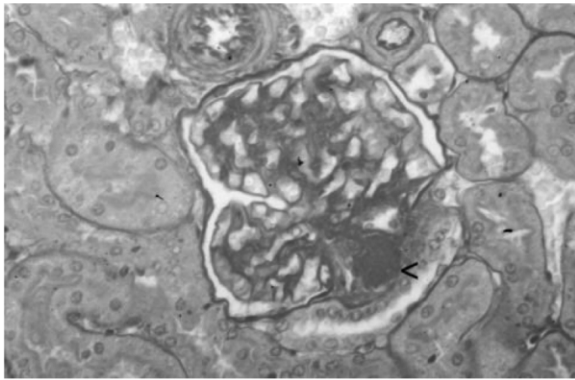


Fig. 5. Histopathological findings in group 3. Note the obvious PAS-positive deposits in the hilus (arrow) (PAS, 400×).

part of the body, which leads to deposition of PAS-positive immune complexes in the glomeruli. Glomerulonephritis has been reported as being an autoimmune reaction due to cadmium exposure, but it is more likely to be a result of infections caused by bacteria or viruses (Friberg et al., 1986; WHO, 1992). The arteriosclerosis, however, usually represents age-related changes, as observed in itai itai patients, women in Japan after World War II with cadmium poisoning due to chronic, low levels of exposure (Friberg et al., 1986; WHO, 1992; Confer and Panciera, 1995).

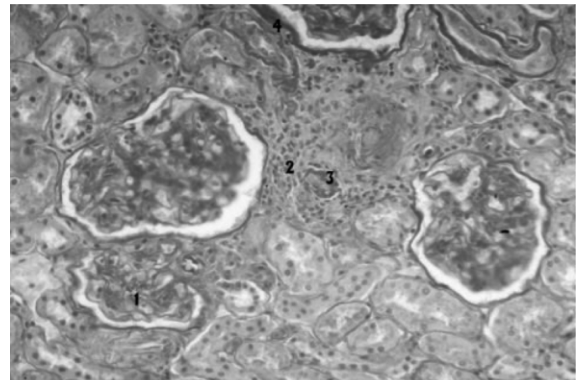


Fig. 7. Histopathological findings in groups 3 and 4. Note the glomerular fibrosis (1), the infiltration of mononuclear cells (2), the necrotic tubules (3) and the segmental thickening of the basement membrane (4) (PAS, 250×).

The three animals that had interstitial fibrosis were all adults. Exposure to cadmium is known to induce damage in the kidneys, which results in a flush of cadmium to the urine, leading to a drop in the cadmium concentration in the kidney. On the other hand, it is also known that the cadmium concentration in the kidney can remain high, although damage has occurred. None of these animals showed high or low cadmium concentration in the kidneys or showed low BMD levels. Hence, the fibrosis is explained as being age-related, without connection to the glomerulone-

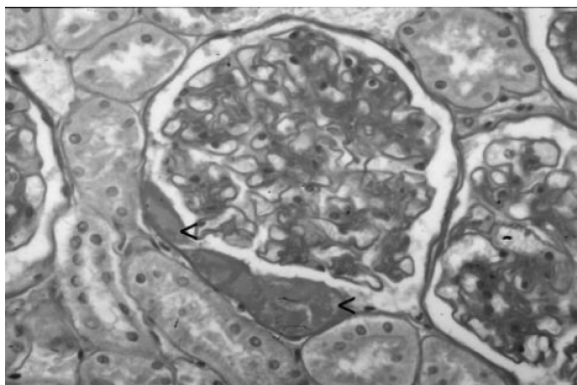


Fig. 6. Histopathological findings in group 4. Note the intense PAS-positive deposits in Bowman's basal membrane (humps) (arrows). Deposits are also evident in the mesangium (PAS, 400×).

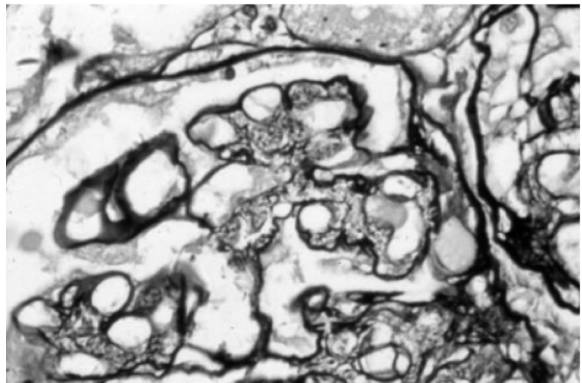


Fig. 8. An example of obvious thickening of the basement membrane (black) (PASM, 1000×).

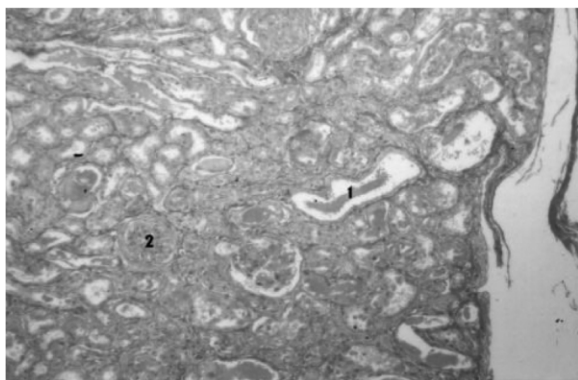


Fig. 9. Histopathological findings in group 4. Interstitial fibrosis (coloured red), dilatation and atrophy of the proximal tubules with luminal hyaline deposits (1) and glomerular sclerosis (atrophy and fibrosis) (2) (Van Gieson, 100 \times).

phritis (Table 4, Figs. 7 and 9) (Confer and Panciera, 1995).

The histopathological changes in the present study are not identical to the changes described in cadmium-poisoned laboratory mammals and humans. In the literature, the cadmium-induced histopathological changes described are mainly found in the proximal tubules, but glomerular sclerosis is found as well. The manifestations are typically desquamation and atrophy of the epithelium, dilatation of the proximal tubules with luminal hyaline casts, interstitial and tubular fibrosis, leucocyte infiltration of the interstitium, fusion of the parietal part of Bowmann's capsule and the glomerulus, and glomerular sclerosis (Scott et al., 1977; Squibb et al., 1979; Friberg et al., 1986; WHO, 1992; Yasuda et al., 1995; Liu et al., 1998).

In the present investigation, the few cases of dilatation and atrophy of the proximal tubules observed are ascribed to compromised perfusion because of arteriosclerosis, and are therefore not considered to be related to cadmium-induced tubulopathy (Fig. 7).

Hyaline droplet degeneration (HDD) was found in the proximal tubules and protein casts, primarily in the medulla of the kidney. The histopathological findings in the glomeruli were compared to the occurrence of HDD and protein casts, but no significant connection was found between histopathological findings and HDD ($P=0.58$ for the

Table 4

Measurements of the three ringed seals with interstitial fibrosis

Identification number	20708	20709	20756
Sex	F	M	M
Age (years)	38	34	9
CdK ($\mu\text{g/g}$ wet wt.)	30.8	6.35	32.8
BMDb (g/cm^2)	1	0.958	1
Kidney damage	Yes	Yes	Yes

Abbreviations as in Table 3.

χ^2 test), or between histopathological findings and protein casts ($P=0.64$ for the χ^2 test). A possible explanation is that the tubular protein casts can be found before the glomerular lesions can be observed.

Histopathological changes in bone mineral density (BMD) were tested for sex-related differences. The difference was tested with a χ^2 test and a log regression analysis. The test did not show any significant difference in the occurrence of histopathological changes between the sexes ($P=0.34$ for the χ^2 test and $P=0.4$ for the log regression test).

The histopathological findings do not appear to be age-related. Changes were observed in a total of 10 seals. Half of the observed changes ($N=5$) were found in animals between 0 and 5 years of age and the remainder in animals between 5 and 40 years of age (Fig. 10).

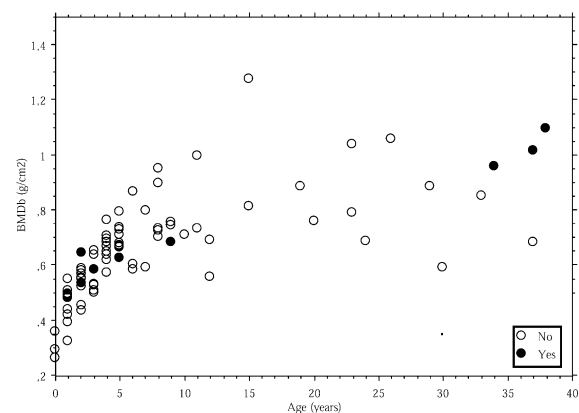


Fig. 10. Mineralisation of the lumbar vertebrae (BMDb, g/cm^2) as a function of the age (years) of the ringed seals. Presence of histopathological changes are indicated by (●) yes and by (○) no if not present.

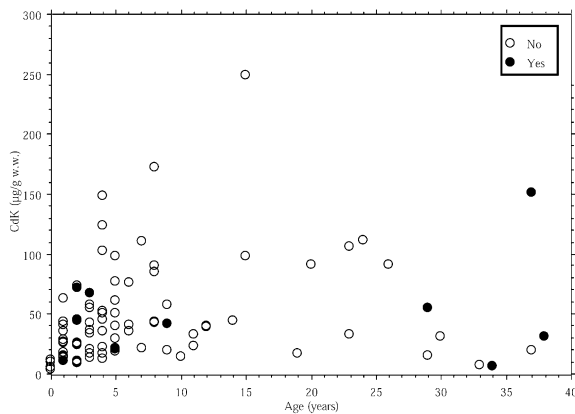


Fig. 11. The kidney cortex concentration of cadmium (CdK, $\mu\text{g/g}$ wet wt.) as a function of the age (years). Reported damage limits (50 and 200 $\mu\text{g/g}$ wet wt.) by cadmium are shown. Presence of histopathological changes are indicated by ● (present) and ○ (not present).

It is therefore assumed that the occurrence of histopathological changes is equal between the two sexes, as well as over all ages. From studies of low cadmium orally administered in humans and laboratory mammals, it is known that the appearance of cadmium-induced damage in the kidney is observed in adults only and first after at least 10 years of exposure (Friberg et al., 1986; WHO, 1992). The histopathological changes in the ringed seals were, however, equally distributed among all ages, which indicates that the renal damage observed in this study was not induced by cadmium (Fig. 11).

Cadmium-induced renal damage can affect calcium metabolism, leading to osteopenia (decalcification, such as osteomalacia and osteoporosis, called Fanconi's syndrome) (Friberg et al., 1986; WHO, 1992). If this is also the case with ringed seals, decalcification of the skeleton system would be evident in older seals (> 10 years), and ringed seals with kidney damage would have significantly lower measures of BMD. As observed in Fig. 10, it is obvious that this is not the case, which once again indicates that the histopathological findings are not cadmium-induced.

A light microscopy examination was carried out to detect possible cadmium-induced kidney damage. Only severe cases can be detected by this

method. Minor effects can be detected by use of % TRP, glomerular filtration rate (GFR), protein in the urine and creatinine determination, which are normally compared with the clinical and histopathological observations in patients (Friberg et al., 1986; WHO, 1992).

3.4. Cadmium

The cadmium concentration in the kidney cortex (CdK) was determined for all 100 ringed seals. The cortex kidney concentration of cadmium is known to be higher than the average level of the entire kidney (cortex and medulla), and it has been shown that the cortex kidney concentration can be extrapolated from the cadmium concentration in the entire kidney by multiplying the average concentration by 1.25 (Friberg et al., 1986).

In Fig. 11, the cadmium concentration in the kidney cortex (CdK, $\mu\text{g/g}$ wet wt.) is shown as a function of age (years). The critical limits suggested for damage to the kidney cortex of humans and laboratory mammals (50 and 200 $\mu\text{g/g}$ wet wt., respectively) are also indicated (Friberg et al., 1986; WHO, 1992; Elinder and Järup, 1996), as well as the presence of histopathological damage. A total of 31 of the 100 ringed seals (31%) had cadmium concentrations in the kidney cortex ≥ 50 $\mu\text{g/g}$ wet wt. Only one individual (1%) had a kidney cortex concentration ≥ 200 $\mu\text{g/g}$ wet wt. Based on a larger sample size, Dietz et al. (1996, 1998c) found that as much as 2.4% (11 out of 463) of the ringed seals from Greenland waters had cadmium concentrations greater than 200 $\mu\text{g/g}$ wet wt. in the kidney cortex.

The cadmium concentration in the kidney cortex did not increase with age ($P=0.05$) and there was no significant difference between sexes ($P=0.81$). This has previously been shown for ringed seals in Greenland by Dietz et al. (1998a,b,c); a decrease in the kidney cortex concentration of older seals was detected and may be explained by reduced renal function/metabolism, kidney damage and a shift in food preferences (from crustaceans to fish) (Friberg et al., 1986; WHO, 1992; Dietz et al., 1998c).

Experience from humans and laboratory mammals (mouse, rat) indicate that cadmium concen-

trations in the kidney cortex of 200–220 $\mu\text{g/g}$ wet wt. can induce tubulopathy (damage to the proximal tubules), including proteinuria [rise in the urine concentration of proteins, especially low molecular weight (LMW) proteins, such as β_2 -microglobulin] (Friberg et al., 1986; WHO, 1992). Elinder and Järup (1996) found that a concentration of 50 $\mu\text{g/g}$ wet wt. in cortex was enough to induce renal dysfunction (proteinuria) in elderly humans and populations poisoned as a result of chronic environmental exposure.

It is known that individuals showing cadmium-induced histopathological changes in their kidneys are either low—because of damage to the proximal tubules, which leads to excretion of cadmium to the preurine—or high in kidney cadmium. This once again indicates that the histopathological changes found are not likely to be cadmium-induced (Friberg et al., 1986; WHO, 1992).

Histopathological examination of cadmium-poisoned laboratory mammals shows that the critical cadmium concentration in the kidney cortex that can induce histological damage lies between 45 and 575 $\mu\text{g/g}$ wet wt. (Friberg et al., 1986; WHO, 1992). Epidemiological studies of histopathological changes in the kidneys compared to cadmium concentration in the kidney have not been performed in humans. However, proteinuria is observed before histopathological changes in humans, which is the opposite to laboratory mammals, where histopathological findings are observed before proteinuria. Therefore, the critical cortex concentrations proposed in humans and laboratory mammals (50 and 200 $\mu\text{g/g}$ wet wt. cortex, respectively) could theoretically induce proteinuria both before and after histopathological changes in the ringed seals.

As 31 of the ringed seals had a cadmium concentration in the kidney cortex ≥ 50 $\mu\text{g/g}$ wet wt., these individuals were theoretically at risk of cadmium-induced kidney damage. The reason why the ringed seals did not show renal damage may be attributed to the fact that the limit of 50 $\mu\text{g/g}$ wet wt. is not critical to the Arctic ringed seals or the route of exposure.

Caution should be exercised when extrapolating data from humans and laboratory mammals to ringed seals. It seems that the food composition

and metabolism in seals differ from that of cadmium-poisoned terrestrial mammals, which could be the reason why no cadmium-induced damage was observed (see Section 3.5).

Experience from cadmium-poisoned humans indicates that individuals do not show histopathological changes until after at least 10 years of exposure. In our sample, only 24 of the ringed seals were older than 10 years. Given the small number of older seals, it would be surprising if any of the ringed seals showed renal histopathological changes, as only a minor percentage ($\geq 10\%$ at 200 $\mu\text{g/g}$ and $\geq 1\%$ at 50 $\mu\text{g/g}$ wet wt.) of cadmium-poisoned mammals develop renal damage (Friberg et al., 1986; WHO, 1992; Elinder and Järup, 1996).

Dietz et al. (1998a) examined 15 out of 462 ringed seals for cadmium-induced nephropathy, of which only five were in the group ≥ 200 $\mu\text{g/g}$ wet wt. The preparation was suboptimal (kept at -20 °C), and it was therefore difficult to carry out the light microscopy examination because of freeze damage. It was concluded that there was no evidence of cadmium-induced renal damage, and that the ringed seals could have adapted to the high cadmium levels (see Section 3.5).

If calcium metabolism in ringed seals is affected by cadmium concentrations, then the bone mineral density (BMD) should decrease with age. As observed in Fig. 2, this is not the case, and it is therefore very unlikely that the ringed seals suffer from osteodystrophy (osteopenia, i.e. demineralisation).

The cadmium intake of ringed seals is at least as high as the intake of itai itai patients and cadmium-poisoned laboratory mammals (Table 5). It is obvious that the cadmium concentration in the food of the seals is as high as the cadmium-poisoned rice known to cause osteopenia in Japanese women. The reason why this does not happen could be explained by a number of factors linked to sex, marine food chains and possible adaptations.

3.5. Mechanisms of adaptation to counteract cadmium-induced nephropathy and osteopenia

Terrestrial mammals and ringed seals differ in a number of ways, including their intake of cadmi-

Table 5

Cadmium concentration in the food of the ringed seal, itai itai patients and laboratory mammals (rat, mouse and monkey)

Group	Ringed seal	Itai itai	Laboratory mammals
Administration source	Fish and crustaceans	Rice	Water, food, parenterals
Daily total intake ($\mu\text{g Cd}$)	40–16000 ^{c,d}	140–260 ^{a,b}	
Concentration in diet ($\mu\text{g Cd/g wet wt.}$)	0.02–8 ^{c,d}		1–300 000 ^{a,b}

^a Friberg et al. (1986).^b WHO (1992).^c Dietz et al. (1996).^d Dietz et al. (1998a,b,c).

um, proteins, calcium, vitamin D, zinc and selenium.

3.5.1. The kidneys

The kidney cortex concentration of cadmium (CdK) in the ringed seals is relatively low compared to the cadmium content in the food of ringed seals, which is high enough to induce tubulopathy (Table 5). As 24 of the seals were exposed to cadmium for more than 10 years, some of the individuals could be expected to show cadmium-induced damage. Several investigations have shown that only a minor proportion of individuals exposed ($\geq 10\%$ at 200 $\mu\text{g/g}$ and $\geq 1\%$ at 50 $\mu\text{g/g wet wt.}$) sustain tissue damage, and these are not necessarily visible under the light microscope at the time of histopathological examination (Friberg et al., 1986; WHO, 1992; Elinder and Järup, 1996). This, and the fact that the histopathological damage found in this study was obviously not cadmium-induced, indicates that the ringed seals were not affected by their high cadmium intake, because food composition affects cadmium uptake and toxicity.

Low concentrations of zinc and calcium will enhance cadmium absorption through the GI mucosa (Felley-Bosco and Diezi, 1992; Ohta and Cherian, 1995). However, the food of the ringed seals is very rich in both zinc and calcium, which reduces cadmium absorption (Riget et al., 1997; Dietz et al., 1998b). At the same time, zinc is able to induce synthesis of metallothionein (MT, a cysteine-rich LMW protein), which probably acts to detoxify Cd^{2+} by forming a Cd–MT complex (Felley-Bosco and Diezi, 1992; Ohta and Cherian, 1995).

The concentration of selenium in the kidney and liver of ringed seals, as well as in their food, is also high (Dietz et al., 1996, 1998b,c). Selenium is known to detoxify cadmium (and methylmercury) in insoluble selenide complexes (Goyer, 1996). It is not known whether the selenium is free or bound, but it could possibly contribute to the detoxification of cadmium (Dietz et al., 1998c). At least some of the selenium is believed to be bound to mercury, thereby detoxifying high mercury levels in marine mammals (Koeman et al., 1973). But in general, selenium is present in molar excess to mercury in most tissues of Arctic species, and could therefore contribute to the detoxifying of cadmium (Dietz, in press).

Dietz et al. (1998c) have shown that zinc and cadmium are positively correlated in the bile of ringed seals. Cadmium elimination through the bile in ringed seals is approximately 200-fold higher than found in terrestrial mammals (Friberg et al., 1986; WHO, 1992; Dietz et al., 1998c). As only approximately 5% of the cadmium is believed to be reabsorbed in the gastrointestinal channel, thereby contributing to the enterohepatic circulation, a substantial amount of cadmium is excreted through the bile. This could explain the low levels of cadmium in the kidney cortex, even though the intake is high.

3.5.2. The skeleton system

Cadmium-induced osteopenia (osteoporosis and osteomalacia) has been found in both humans and laboratory mammals (Friberg et al., 1986; WHO, 1992). Osteomalacia is usually induced by a deficiency of calcium, vitamin D, protein and phosphorus; cadmium is known to exacerbate this

(Friberg et al., 1986; WHO, 1992). Osteoporosis can also be caused by deficiency, but cadmium alone can also induce the disease. Humans with cadmium-induced osteopenia are hence treated with large amounts of vitamin D and anabolic steroids (Friberg et al., 1986; WHO, 1992).

Cadmium-induced damage is known to induce Fanconi's syndrome, which is a state of pathologically low vitamin D hydroxylation leading to osteoporosis and osteomalacia (Friberg et al., 1986; Hensyl, 1990; WHO, 1992). Ringed seals seem to avoid skeleton demineralisation, and thereby counteract the high cadmium levels. A substantial part of the ringed seal diet is comprised of fish rich in vitamin D, calcium, phosphorus, zinc and proteins, which counteract cadmium-induced osteopenia (Riget et al., 1997; Saxholt, 1998). Another vitamin D contribution is obtained in spring, when ringed seals emerge onto the ice for moulting and UV radiation from the Arctic midnight sun stimulates the synthesis of cholecalciferol (Vibe, 1981; Haarløv, 1986; Génsbøl, 1996).

4. Conclusions

Compared to terrestrial mammals, ringed seals are exposed to cadmium concentrations theoretically high enough to induce damage in the kidneys and the skeleton system.

In this investigation, no evidence of skeleton demineralisation (osteopenia) was found. 10% of the seals had clear and significant changes in the glomeruli in the kidneys, and a large proportion showed clear but minor mesangial deposits and thickening of the glomerular basement membrane. The conclusion was, however, that these changes were not caused by cadmium, due to their microscopic appearance, their occurrence related to age and to the cadmium and calcium levels measured.

The diagnosis and pathogenesis of cadmium-induced diseases are usually achieved by clinical, paraclinical and histopathological examinations. It is known from investigations on humans and laboratory mammals that elderly sensible individuals exposed to low concentrations of cadmium over several years (≥ 10 years) show signs of renal and skeleton damage. It seems that ringed

seals have adapted to the high cadmium levels through cadmium excretion and constant mineralisation of the skeleton system facilitated by their food composition.

5. Perspectives and recommendations

As the bone scanning and the pathological changes were not mutually related, and none of these appeared to be affected by the cadmium levels in the kidney, age or sex, further investigations are not suggested.

The lack of documented effects so far may indicate that the special Arctic marine ecosystem contains protective components against the effects of high cadmium exposure. Among these, the high intake of vitamin D, proteins, calcium and selenium, as well as zinc and phosphorus, may be of special importance. These clues should be pursued in human investigations and the treatment of osteoporosis and cadmium-induced effects.

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