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NERI Technical Report No. 307

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An epidemiological study of possible cadmium induced nephropathy and osteodystrophy in ringed seals *(Phoca hispida)* from Qaanaaq in Northwest Greenland

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1999

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Data sheet

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Abstract: Cadmium concentrations in kidneys from ringed seals (*Phoca hispida*) from North West Greenland (Qaanaaq) are high. Concentrations range at level known to induce renal toxic effects (mainly tubulopathy) and demineralisation (osteopenia) of the skeletal system (Fanconi's Syndrome) in humans as well as laboratory mammals. We have studied possible cadmium induced histopathological changes in the kidneys as well as a demineralisation of the skeletal system (DXA-scanning of lumbal vertebraes). No obvious cadmium induced toxic changes were found. Food composition and physiological adaptations may explain the absence of toxic effects of cadmium in ringed seal

Key words:	Cadmium, North West Greenland, ringed seal (*Phoca hispida*), renal toxicology, skeletal demineralisation (osteopenia), food composition, physiologic adaptation
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Summary

Cadmium

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.

Purpose

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.

Cadmium levels related to renal toxicology

The cadmium concentrations in the kidney cortex ranged from 0 to 248 µg/g wet weight (mean = 44.5 µg/g w.w., n=100) in the 99 kidneys examined. Experience from cadmium poisoned humans and laboratory mammals indicates that concentrations above 50-200 µg/g w.w. may induce histopathological changes. Overall, 31 of the ringed seals had cadmium concentrations in the kidney cortex above 50 µg/g w.w, 11 had concentrations above 100 and 1 had concentrations above 200 µg/g w.w. Obvious histopathological changes (categorized mainly as glomerulonephritis) were found in 10 of the seals, however, none of these changes could be atrributed to cadmium induced renal damage (mainly tobulopathy) as described for other species.

Skeletal mineralisation

Damage to the proximal kidney tubules is known to induce demineralisation of the skeletal system (Fanconi's syndrom) 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.

Conclusions

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 females estrogen hormonal activity throughout life.

1 Introduction

AMAP

This report presents the results from an epidemiological study on possible cadmium induced kidney and bone effects in ringed seals (*Phoca hispida*) from Qaanaaq, Northwest Greenland as recommended by AMAP (Arctic Monitoring and Assessment Programme) in the Arctic Assessment Report (Dietz et al. 1998b).

Institutions involved

A number of people at different Danish research institutes in Copenhagen and Århus have been involved in the project. The program was initiated by the National Environmental Research Institute, Department of Arctic Environment (DAE), but has to a large entent relied on toxicologists and pathologists from the Royal Veterinary and Agricultural University in Copenhagen (KVL) and Århus University, and endocrinologists from the University Hospital of Hvidovre.

Background

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.

Accumulation

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).

Pilot study

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, this aspect was also examined in the investigation.

Sampling

Kidneys and bones (lower lumbar vertebraes 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

Locality

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 the shooting of the seal and less than 24 hours after the catch. The isolating effect of the blubber counteracted freezing of the internal organs prior to sampling.

Samples

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 PE plastic bags. Small fragments of the kidney (2 x 2 x 2 cm) were stored in an antifreezing fixation liquid (details below 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 with the seals identification number, and kept at outdoor temperature (-5 to -20 ºC) until frozen storage (-10 to -20 ºC). Samples were shipped as frozen goods from Qaanaaq to Copenhagen, where further storage were at -20 ºC.

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 was counted using the method described by e.g. Dietz et al. (1991).

2.4 Renal Histopathology

2.4.1 Preparation

The tissue samples were prepared in a Sakura TissueTek® VIP where following steps were conducted:

- 1 x 70% alcohol for 1 hour
- 2 x 96% alcohol for 1 hour
- 4 x 99% alcohol for 1 hour
- 2 x 100% xylene for 1 hour
- 4 x paraffin for 45 min

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

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

2.4.2 Tissue staining

The tissue staining was done manually in HE, PAS and van Gieson solutions. To avoid unequal staining all sections were stained simultaneously.

HE

Haematoxylin (Al-Haematein)-Eosin (HE) staining is the most 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).

PAS

Periodic Acid-Schiff (PAS) stains carbohydrates as homoglycanes (ex. glycogen), glycoproteins and neutral proteoglycanes. This results in a red colouring of collagen fibers, basement membranes and the brush-border basement membranes in the proximal tubules, cell membranes, cytoplasm and nucleus. Carbohydrates are stained dark red and nucleus dark blue (Lyon et al. 1991).

Van Gieson

Van Gieson is used to stain connnective tissue. The picrine acid stains the fibers red (kidney capsule, vessels and bassement membranes) (Lyon et al. 1991).

These three methods were used in the histopathological examination of all kidney samples.

PAS-M

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 x magnification.

2.5 X-ray analysis

2.5.1 Preparation

Preparation

The mandibles and the 3 lowest lumbar vertebrae were macerated and boiled at the Zoological Museum, University of Copenhagen so that muscles and tendons could be removed before examination and X-ray analysis. The bones were macerated for 96 hours, boiled for 15 minutes and dried in the air for a minimum of 72 hours.

2.5.2 X-ray (osteodensitometry)

X-ray

Osteodensitometry is a technique developed to determine osteoporosis (demineralisation) in the skeleton system of primarily postmenopause women. A Norland XR 26 X-ray bone densitometer was used to determine the mineralisation of the bones (calcium-phosphate content) at the University Hospital in Hvidovre. The principle in the

osteodensitometer is dual X-ray absorptiometry (DXA) where a high 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 was analysed using a software program (XR software 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 concent per square unit (g/cm^2) (see Fig. 2.1).

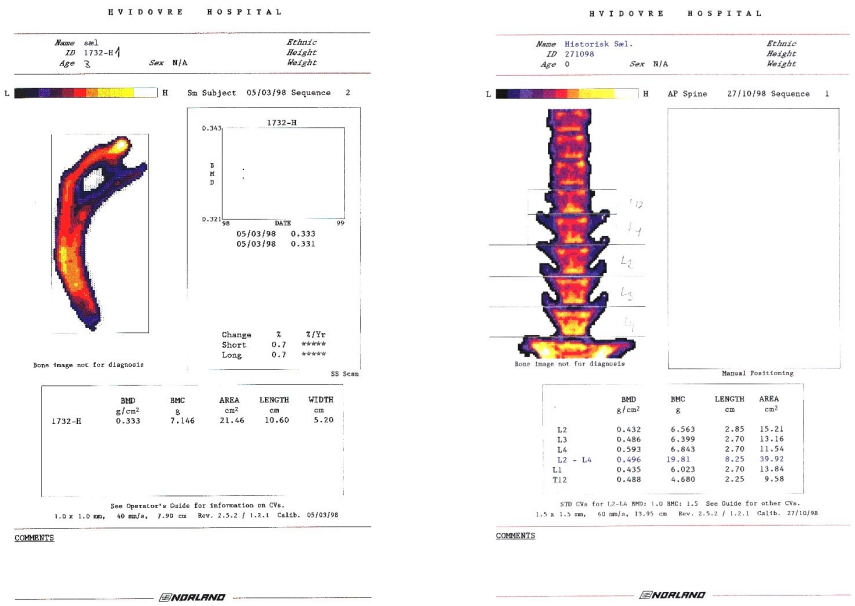

Fig. 2.1
DXA scanning of the right part of the mandible (left) and the lumbar vertebraes (right). Blue areas are low in calcium-phosphate and bright yellow areas are high in calcium-phosphate.

Quality assurance

The University Hospital in Hvidovre controlled the method daily by carrying out a standard calibration using a bone phantome with a known mineral density (a double determination of two mandibles was done as well). This showed a reproducibility of > 99% and an accuration between +/- 2SD (see Fig. 2.2).

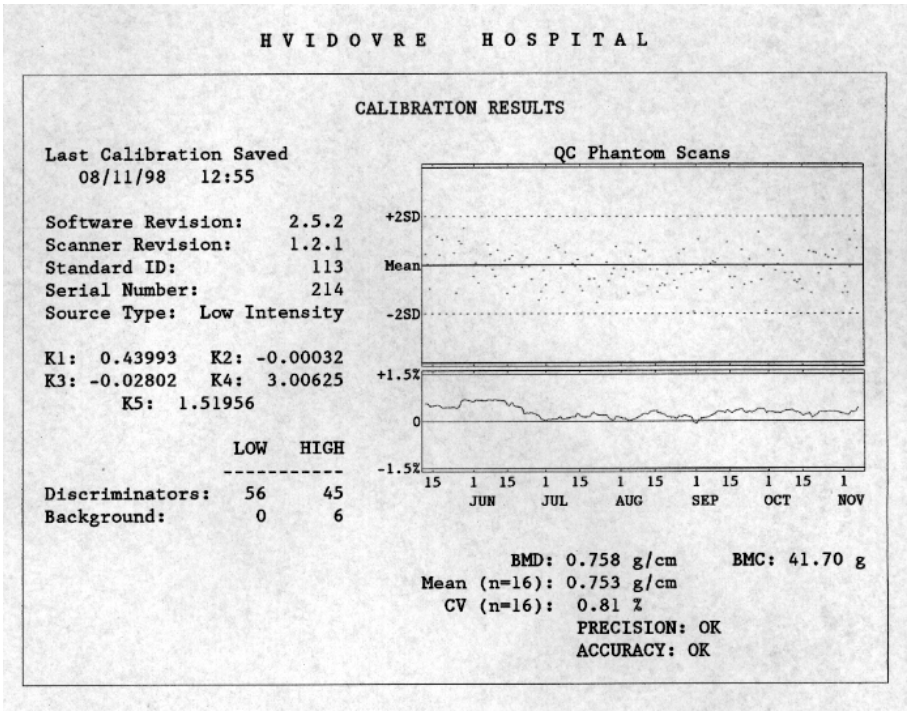

Fig. 2.2
Calibration of the Norland® Densitometer with the accuracy (top) and the reproducability (buttom).

2.6 Cadmium analyses

The metal analyses were performed at the Department of Arctic Environment laboratory. 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 the tarred Teflon liner of a Berghof stainless steel bomb. After the addition of 3 ml of 65% HNO_3 (Merck Suprapur®), the bombs were closed and incubated for 12 hours at 120° - 150°C. Following a cooling period, the digests were transferred quantitatively to 50 ml screw-cap polyethylene bottles and adjusted to c. 25 g weight using metal-free, deionized water (Millipore®). Approximately 8% HNO_3 was used for all further dilutions.

All cadmium analyses were carried out by flame AAS (Perkin-Elmer 3030), however, the graphite furnace technique (Perkin-Elmer 3030 with Zeeman background correction) was used for the final analysis of samples with less than 2.5 µg/g wet weight cadmium.

The lower limit of detection for laboratory analyses of cadmium was 0.015 µg/g wet weight (w.w.) All concentrations are reported as µg/g w.w.. For recalculation into µg/g dry weight (d.w.), a correction factor of 3.67 was calculated on the basis of the means 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 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

Excel (7.0®) was used as database and Systat 7.0® and SAS® PC-version was used to carry out statistical analyses.

Pearson

Pearsons correlation coefficient is used to determine correlations between variables; length, weight, bone mineral density (BMD) and cadmium concentration in the kidney cortex (CdK).

The BMD and CdK data were logarithmic transformed to meet the assumption of normal distribution and equal variance before data handling was carried out.

GLM (SS3)

The principle in the data handling is a model of covarianse (SAS® GLM-procedure (SS3)) with logBMD and logCdK as the dependent varibles, 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.

X^2 and logistic regression

The distribution of nephropathy (kidney damage) among the sex was tested with a X^2-test and logistic regression.

3 Results and Discussion

Variables

Basic statistics, correlation coefficients and significant levels of the continuous variables are shown in Table 3.1.

Table 3.1
*Basic statistics and correlation coefficients for the variables in the sample. CdK is the cadmium concentration of the kidney cortex (µg/g w.w.) and BMD is the Bone Mineral Density of calciumphosphate (g/cm^2) where BMDm is the BMD of the mandible (BMDm,r indicates the right and BMDm,l indicates measurements of the left part of the mandible) and BMDb is the BMD of the lower three lumbar vertebrae. ***: P ≤ 0.001, **: 0.001 < P≤ 0.01, *: 0.01 < P ≤ 0.05.*

Variable	Count	Mean	Std.dev.	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 w.w.)	100	44.5	40.8	0-248

Variable	Age	Length	Weight	BMDm	BMDb	CdK
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 w.w.)	n.s.	n.s.	n.s.	n.s.	***	1.000

3.1 The sample

Basic statistics

The distribution of sex, age, weight and length is shown in Fig. 3.1 and Table 3.2. It appears that the standard deviation (SD) is highest in CdK, intermediate in weight, length and age and lowest in BMD, and that weight and length is highly correlated (r= +0,85***, see Table 3.1).

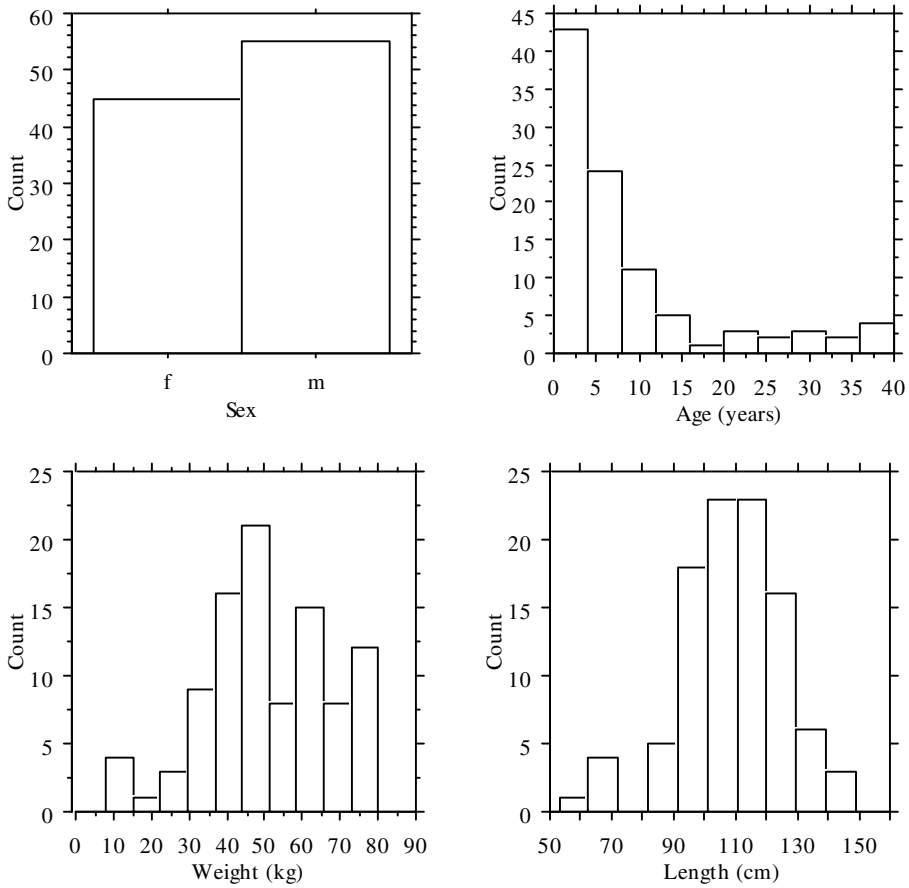

Fig. 3.1
Frequencies of the investigated sample presented for categories sex (f=female; m=male), age (years), weight (kg) and length (cm).

The sample consisted of 55 male and 45 female ringed seals. The sample mainly consisted of subadult and adult seals (see Table 3.2 and Fig. 3.1) 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.

Table 3.2
The distribution of age and sex in the investigated sample.

Age (year)	Female	Male	Sum
$y < 1$	4	4	8
$1 \leq x < 5$	33	29	62
$x \geq 5$	8	22	30
Sum	45	55	100

3.2 Osteodensitometry

Diagnosis

The skeletal content of calcium-phosphate 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 upon not only the DXA examination, but also on clinical and paraclinical examination and, if possible, also histopathological examination (autopsy/biopsy).

Number examined

It was possible to determine the BMD on 96 mandibles and 91 lumbar vertebrae (L_2-L_4). Four of the mandibles were damaged by shooting, and 9 of the lumbar vertebrae were damaged during maceration and boiling.

Macroscopic examination

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 years). Their kidney cortex concentration of cadmium (CdK) were not high, the BMD were very high (mean BMD = 0.939; range: 0.85-1.000); 2 had kidney damage (age related) which was a significantly higher percentage (40%) than the overall mean (10%) (see Table 3.3). These changes were therefore classified as age related anatomical changes.

Table 3.3.
Measurements of the five ringed seals with macroscopic changes in the mandibles. Abbreviations used: IdNo: Identi-fication number; CdK: The cadmium concentration in the kidney cortex (µg/g w.w.); BMDb: Bone mineral density of the lower three lumbar vertebrae (g/cm^2); f: female; m: male.

IdNo	20708	20709	20716	20759	20797
Sex	f	m	m	m	m
Age (years)	38	34	33	40	29
CdK (µg/g w.w.)	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

Earlier samples

Prior to the sampling in Thule in 1998, the BMD in ringed seal mandibles (N = 50) from earlier samples from Greenland were determined. This was done to expand the range of the CdK 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 Table 3.1). 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.

Lumbar vertebraes and mandibles

The BMD in the lower lumbar vertebrae (BMDb) and in the mandibles (BMDm) were also strongly correlated (r = +0.80***, see Table 3.1). 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 1986, WHO 1992, Hyldstrup pers. com.).

Both mandibles and the lumbar vertebrae consist of spongious bone tissue, and changes in the metabolism should therefore be reflected in both. The National Environmental Research Institute, Department of Arctic Environment in Denmark keeps mandibles and heavy metal analysis also from earlier ringed seal samplings in Greenland and these could be used for further studies concerning osteopenia.

Constant growth

In Fig. 3.2 the calcium phosphate content in the three lower lumbar vertebrae (BMDb) is shown as a function of the seals age. It can be seen that the BMDb increases with age in the same way as the length, because the growth continuous even after sexual maturity (McLaren 1958). This reflects the constant skeletal mineralisation of the ringed seals, which means that their skeletal system is mineralised throughout their lifetime.

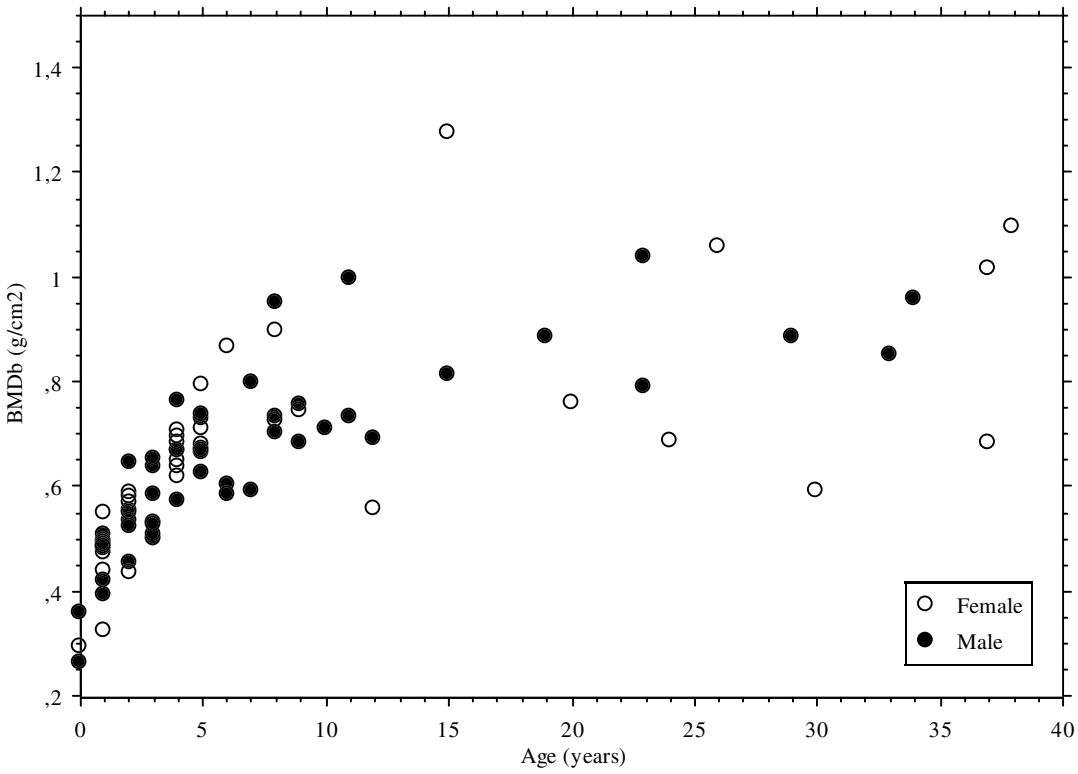

Fig. 3.2
Mineralisation of the lumbar vertebrae (BMDb, g/cm^2) of the ringed seals as a function of the age (years) and sex.

Sex differences

Mammals mobilise large amounts of calcium and phosphate during

pregnancy and the suckling period, where it is used for both skeletal production in the fetus, and maintenance its own and the offspring's calcium-phosphate homeostasis.

As the female ringed seals mobilise calcium-phosphate to the foetus and later on also to the weaning pub lower bone mineral density (BMD) could be expected in the females. In addition endocrinologic changes in the postmenopausale period of women have proven to affect the calcium homeostase 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:

- The BMD of the lower lumbar vertebrae is increasing by age
- The females are significantly lower in the BMD than the males

Test results

BMD appears to increase significant with age ($P < 0.001$) and that 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 postmenopausale period as women and that could explain the maintained calcium homeostasis (Smith 1987, Reeves 1998).

3.3 Histopathology

Macroscopic appearance

Macroscopic examination of the kidneys did not show any histopathological changes.

Fixation

Prior to the sampling the properties of the fixative in a low temperature environment were tested. Samples from bovine kidneys were stored in a freezer (-18 °C) for 48 hours 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.

Autolysis

One animal (a juvenile female) was not included in the histological examination due to autolysis caused by a suboptimal preparation.

Classification

The kidneys were divided into four groups on the basis of histopathological findings (see Table 3.3). Group 1 and 2 represent kidney tissue without obvious histopathological changes and group 3 and 4 represents kidney tissue with clear histopathological changes. In Fig. 3.3^{A-G} is the histopathological findings shown.

Table 3.3
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. 3.3[A]).

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. 3.3[B]) .

Group 3: Obvious histopathological changes (N = 6). Generalized 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 Fig. 3.3[C-F]).

Group 4: Intense histopathological changes (N = 4). Generalized 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 postive deposits (humps). Arteriosclerotic changes in the afferent and efferent arterioles leading to sclerosis (atrophy and fibrosis) of the glomeruli. Fibrous peritubular necrotic tubules (see Fig. 3.3[D-G]).

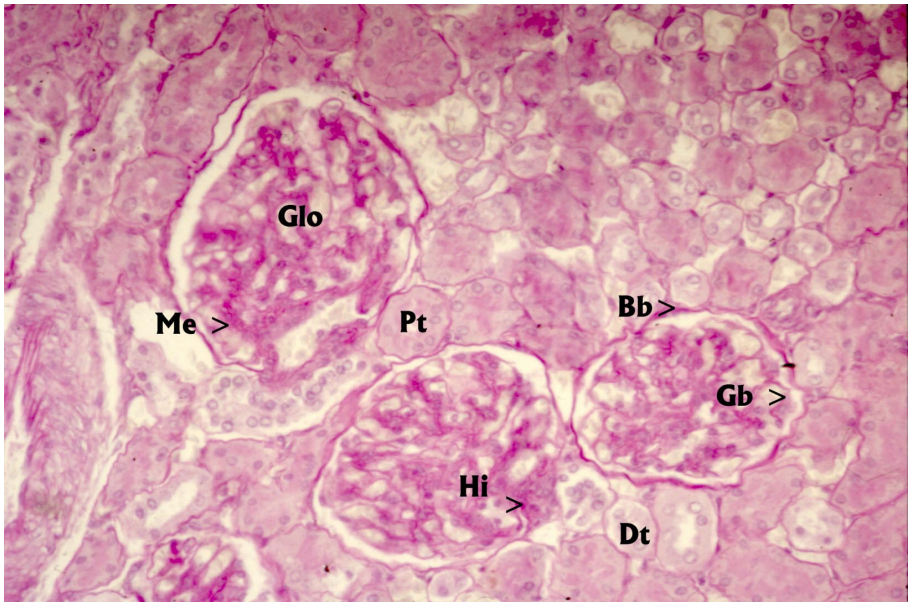

Fig. 3.3[A]
Histological findings in Group 1. No changes are seen neither in the mesangium nor in the basement membranes, the glomeruli or the tubules. Abbreviations used: Glo: glomerulus, Me: mesangium, Pt: proximale tubules, Hi: hilus, Dt: distale tubules, Gb: glomerulare basale membrane, Bb:Bowmann's basale membrane (PAS, 250x).

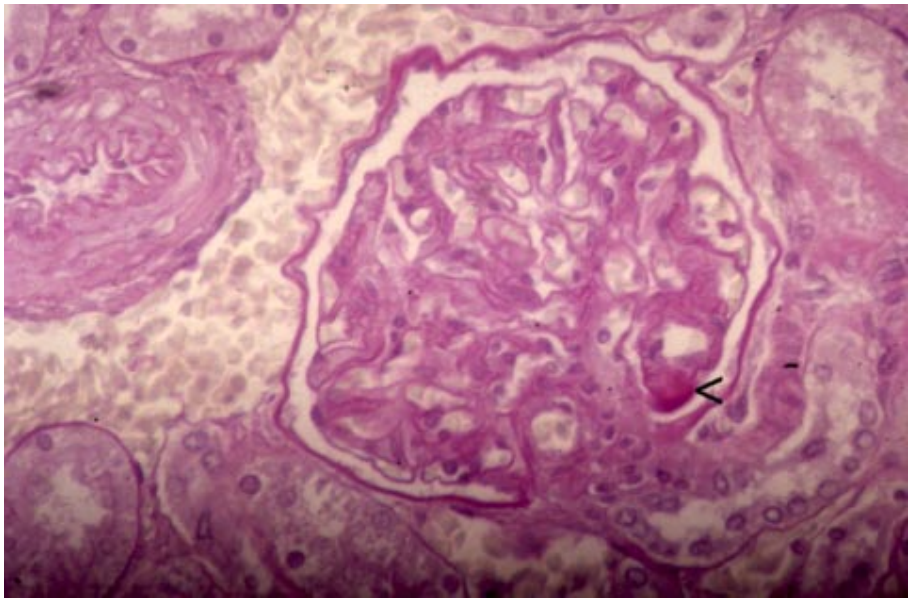

Fig. 3.3[B]
Histological findings in Group 2. Note the minor PAS-positive deposit in the glomerular basement membrane on the right (arrow) (PAS, 400x).

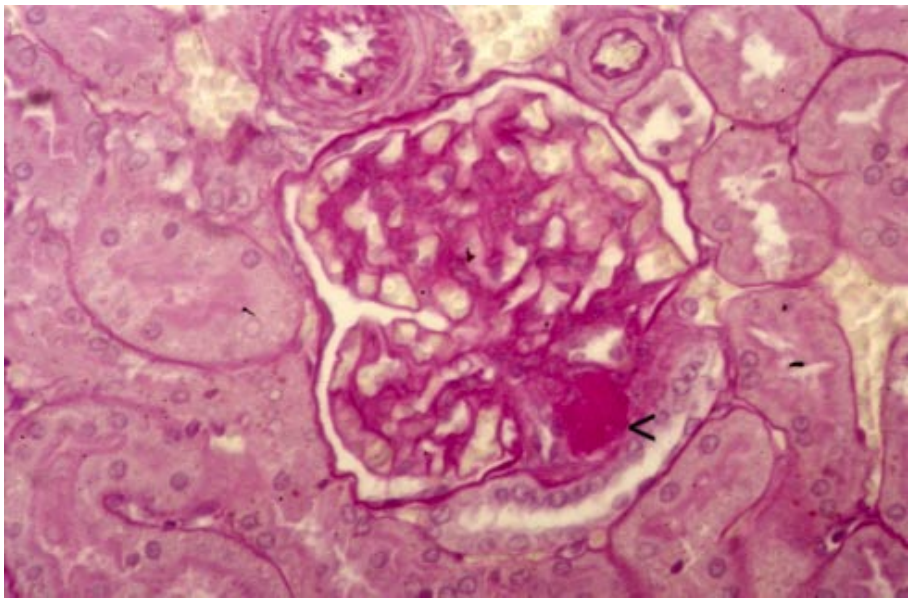

Fig. 3.3[C]
Histopathological findings in Group 3. Note the obvious PAS-positive deposits in the hilus (arrow) (PAS, 400x).

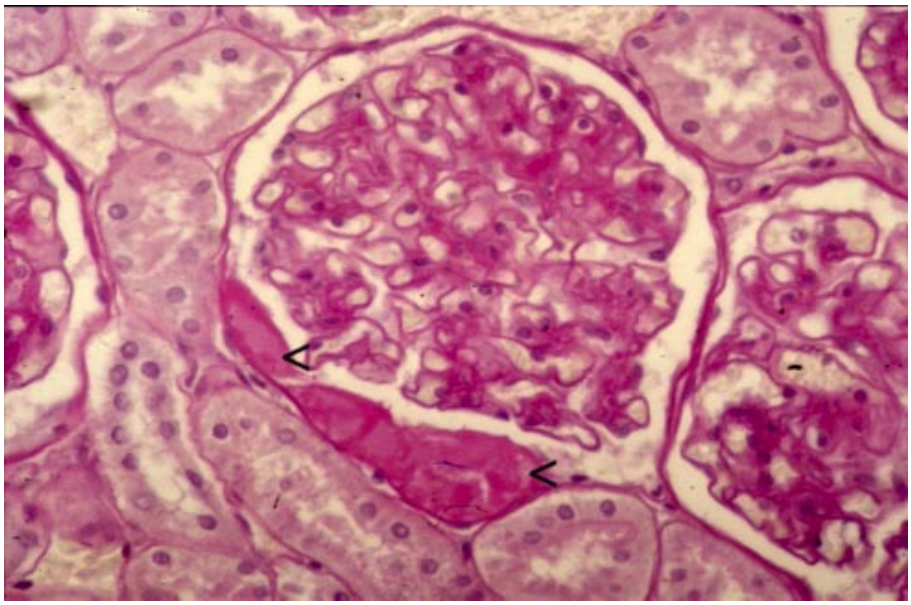

Fig. 3.3[D]
Histopathological findings in Group 4. Note the intense PAS-positive deposits in Bowmann's basale membrane (humps) (arrows). Deposits are also seen in the mesangium (PAS, 400x).

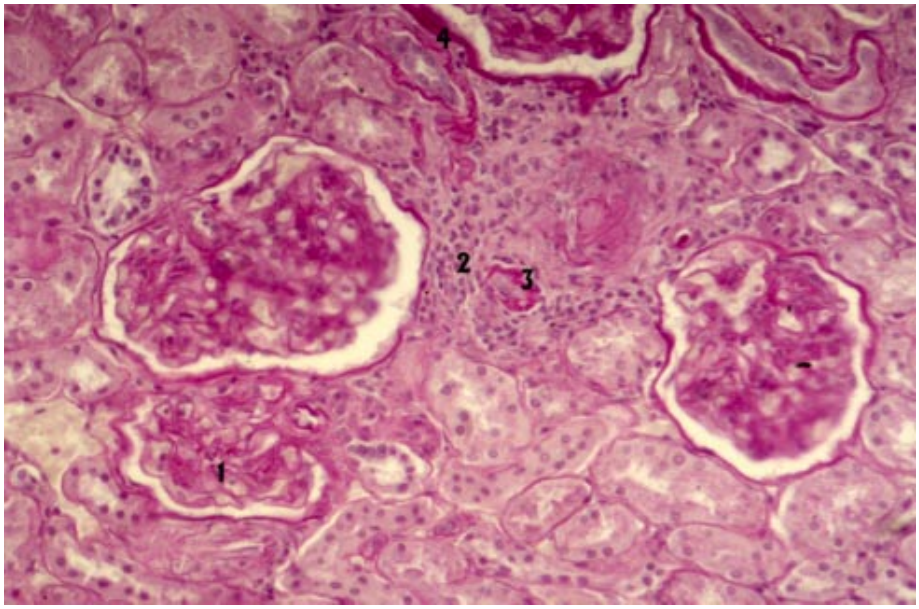

Fig. 3.3[E]
Histopathological findings in Group 3 and 4. Note the glomerular fibrosis (1), the infiltration of mononuclear cells (2), the necrotic tubules (3) and the segmental thickening of the basement membrane (4) (PAS, 250x).

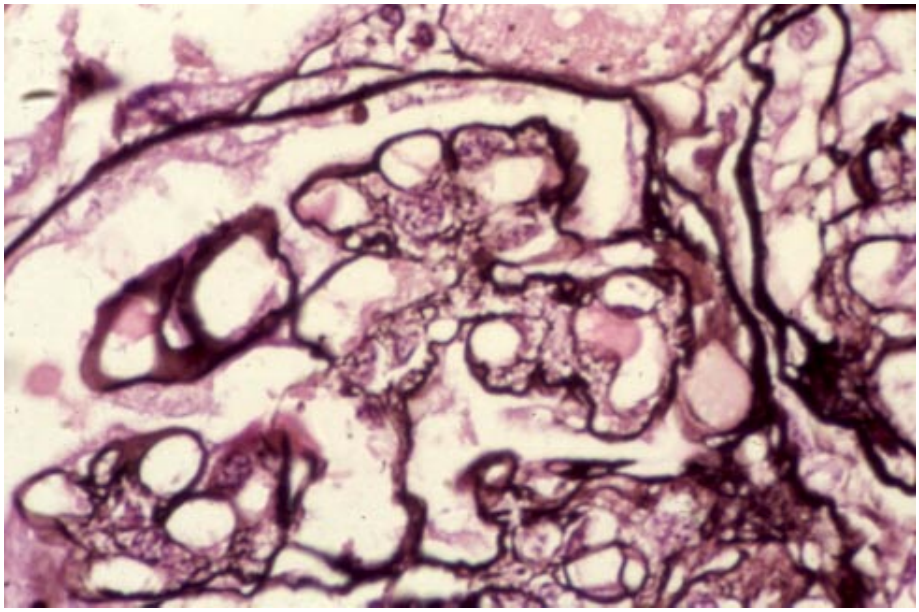

Fig. 3.3[F]
An example of obvious thickening of the basement membrane (black) (PASM, 1000x).

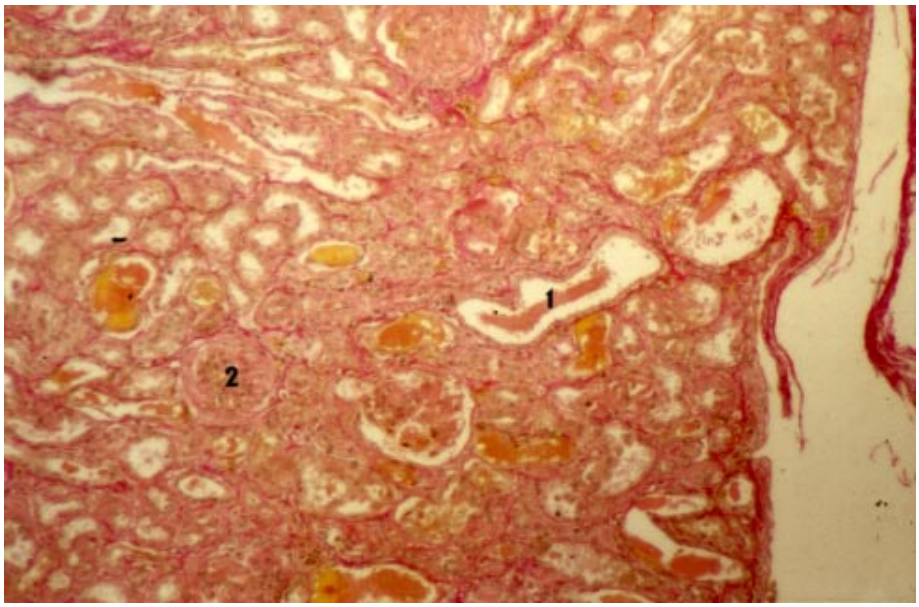

Fig. 3.3[G]
Histopathologic findings in group 4. Interstitial fibrosis (coloured red), dilatation and atrophy of the proximale tubules with luminal hyaline deposits (1) and glomerular sclerosis (atrophy and fibrosis) (2) (Van Gieson, 100x).
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Nephritis in group 3 and 4

In group 3 and 4 the diagnosis of the histopathological changes are glomerulonephritis, interstitial nephritis and arteriosclerosis (see Fig. 3.3[E, G]) (Confer and Panciera 1995).

Glomerulonephritis

Glomerulonephritis is ususally connected to infections caused by bacteria or viruses in another 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 it is seen in Itai-itai patients chronic low exposed cadmium poisoned women in Japan after the 2nd World War (Friberg et al. 1986, WHO 1992, Confer and Panciera 1995).

Arteriosclerosis

Interstitial fibrosis

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 of the kidney. On the other hand it is also known that the cadmium concentration in the kidney can stay high although damage has occured. None of these animals were high or low in their cadmium concentration in the kidneys or showed low BMD levels. Hence the fibrosis is explained as being age related without connection to the glomerulonephritis (see Table 3.4 and Fig. 3.3[E, G]) (Confer and Panciera 1995).

Table 3.4.
Measurements of the three ringed seals with interstitial fibrosis. Abbreviations as in Table 3.3.

IdNo	20708	20709	20756
Sex	f	m	m
Age (years)	38	34	9
CdK (µg/g w.w.)	30.8	6.35	32.8
BMDb (g/cm^2)	1	0.958	1
Kidney damage	yes	yes	yes

Cadmium induced changes

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 proxi-

male 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. 1982, 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 (see Fig. 3.3[E]).

HDD and protein casts

HDD (hyaline droplet degeneration) 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 occurence of HDD and protein casts, but no significant connection was found between histopathological findings and HDD ($P = 0.58$ for the X^2-test), or between histopathological findings and protein casts ($P = 0.64$ for the X^2-test). A possible explanation is that the tubular protein casts can be found before the glomerular lesions can be observed.

Sex and age differences

As for the bone mineral density (BMD), histopathological changes were tested for sex related differences. The difference is tested with a X^2-test and a logistic regression analysis. The test did not show any significant difference in the occurence of histopathological changes between the sexes ($P = 0.34$ for the X^2-test and $P = 0.4$ for the log. reg. 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 (see Fig. 3.4).

Time of exposure

It is therefore assumed that the occurence of histopathological changes are equal between the two sexes as well as all ages. From studies of orally low administered cadmium in humans and laboratory mammals it is known that the appearance of cadmium induced damage in the kidney is seen 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 have not been induced by cadmium (see Fig. 3.4).

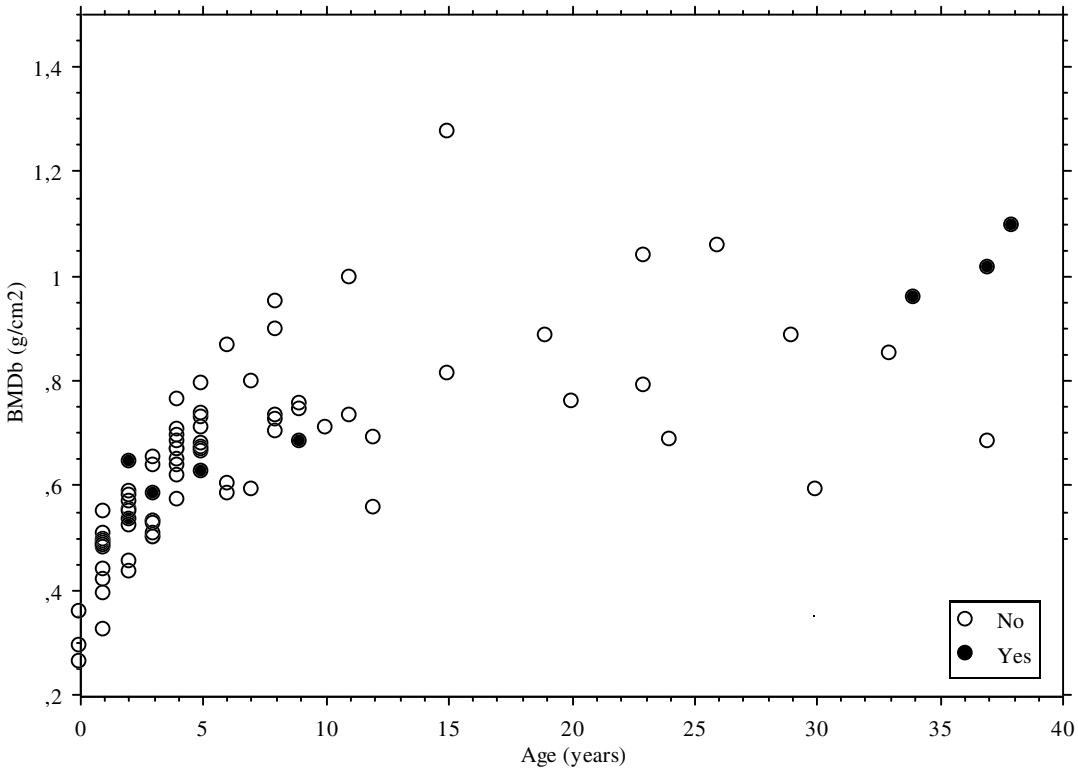

Fig. 3.4
Mineralisation of the lumbar vertebrae (BMDb, g/cm^2) as a function of the age (years) of the ringed seals. Presence of histopathological changes are indicated by: yes (•) and if not present by: no (o).

Fanconi's Syndrome

Cadmium induced renal damage can affect the calcium metabolism leading to osteopenia (decalcification such as osteomalacia and osteoporosis called Fanconi's Syndrome) (Friberg 1986, WHO 1992). If this is also the case with ringed seals delcalcification of the skeleton system would be evident in the older seals (> 10 years), and the ringed seals with kidney damage would have significantly lower measures of BMD. As seen in Fig. 3.4, it is obvious that this is not the case which once again indicates that the histopathological findings are not cadmium induced.

Diagnosis

A light microscope 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, GFR, protein in the urine and creatinin determination, which are normally compared with the clinical and histopathologic observations in patients (Friberg et al. 1986, WHO 1992).

3.4 Cadmium

CdK

The cadmium concentration in the kidney cortex (CdK) was determined for all 100 ringed seals. The cortex kidney concentration is known to be higher than the average level of the kidney, and can be extrapolated by multiplying the average concentration with 1.25

(Friberg 1986).

Levels

In Fig. 3.5 the cadmium concentration in the kidney cortex (CdK, µg/g w.w.) is shown as a function of the age (years). The suggested critical limits for damage to the kidney cortex of humans and laboratory mamals (50 and 200 µg/g w.w. respectively) is also indicated (Friberg 1986, WHO 1992, Elinder and Järup 1996). Thirtyone of the 100 ringed seals (31%) had cadmium concentrations in the kidney cortex ≥ 50 µg/g w.w. Only one individual (1%) had a kidney cortex concentration ≥ 200 µg/g w.w. 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 µg/g w.w. in their kidney cortex.

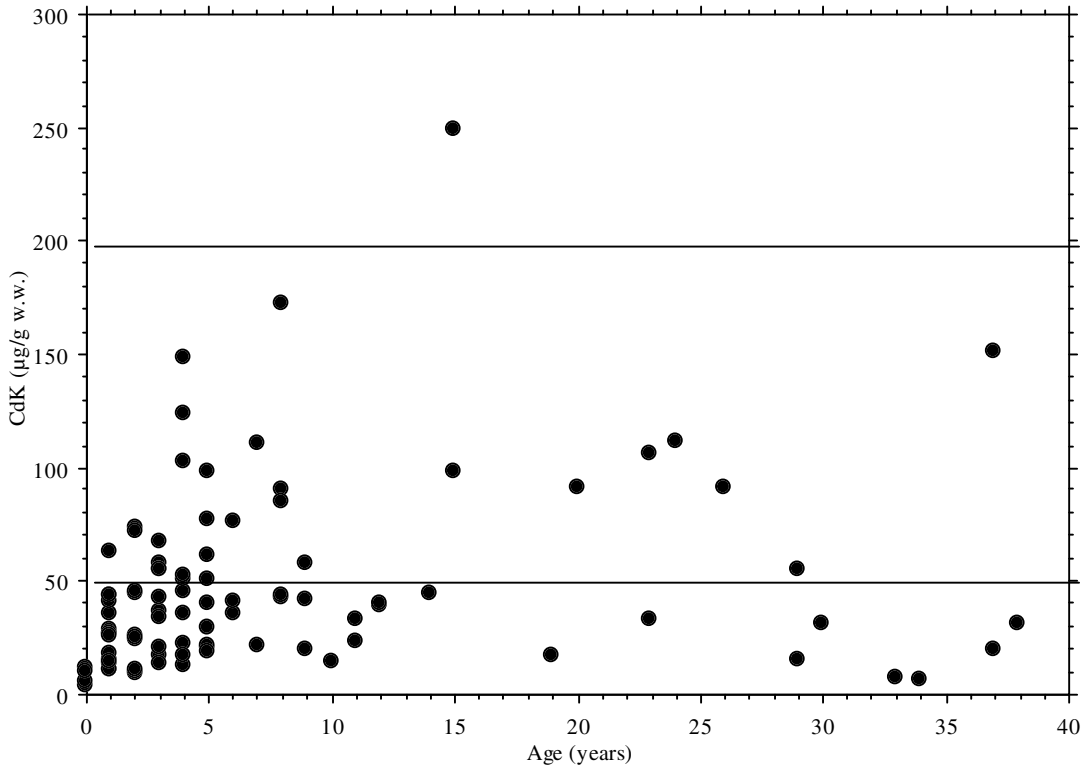

Fig. 3.5
The kidney cortex concentration of cadmium (CdK, µg/g w.w.) as a function of the age (years). Reported damage limits (50 and 200 µg/g w.w. respectively) by cadmium are shown.

CdK related to age and sex

Fig. 3.5 also shows that the juvenile and oldest ringed seals have the lowest cadmium concentration in the kidney cortex, while the age group inbetween is highest. It appeares that the cadmium concentration in the kidney cortex increases to a certain level with age (P = 0.05) and that there is no significant difference between sexes (P = 0.81). This has been shown previously for ringed seals in Greenland by Dietz et al. (1998a-c). The decrease in the kidney cortex concentration in the older seals can be explained by reduced renal funtion/metabolism, kidney damage, and a shift in food preferences (from crustaceans to fish) (Friberg 1986, WHO 1992, Dietz et al. 1998c).

Critical limits

Experience from humans and laboratory mammals (mouse, rat) indicate that cadmium concentrations in the kidney cortex of 200-220 µg/g w.w. can induce tubulopathy (damage to the proximale tubules) including proteinuria (rise in the urine concentration of proteins especially LMW as β_2-microglobulin) (Friberg 1986, WHO 1992). Elinder and Järup (1996) found that a concentration of 50 µg/g w.w. in cortex was enough to induce renal dysfunction (proteinuria) in elderly humans and populations poisoned as a result of chronic environmental exposure.

In Fig. 3.6 it is seen that the cadmium concentration in the kidney cortex of the individuals showing histopathological damage are neither high nor low. It is known that individuals who are showing cadmium induced histopathological changes in their kidneys are either low – because of damage to the proximale tubules which leads to an 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 1986, WHO 1992).

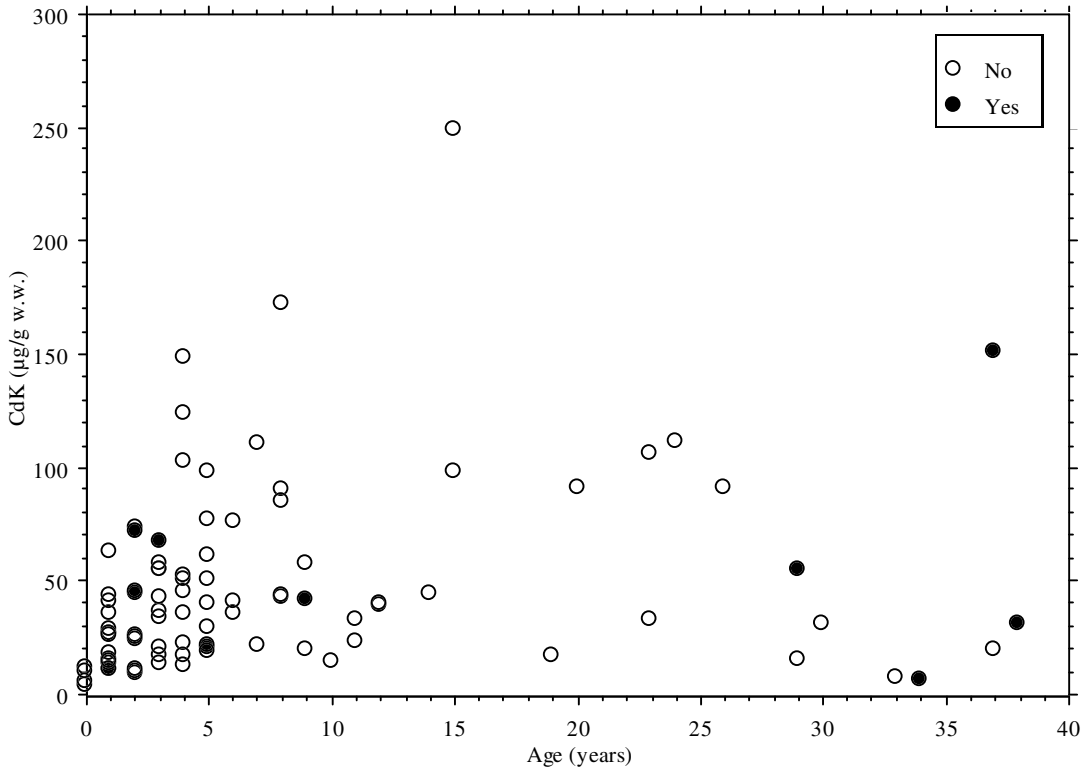

Fig. 3.6
The kidney cortex concentration of cadmium (CdK, µg/g w.w.) as a function of the age (years). Presence of histopathological changes are indicated by: yes (•) and if not present by: no (o).

Extrapolation

Histopathological examination of cadmium poisoned laboratory mammals shows that the critical concentration of cadmium in the

kidney cortex which can induce histological damage lies between 45-575 µg/g w.w. (Friberg 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 (50 and 200 µg/g w.w. cortex respectively) could theoretically induce proteinuria both before and after histopathological changes in the ringed seals.

As 31 of the ringed seals have a cadmium concentration in the kidney cortex ≥ 50 µg/g w.w., these individuals are theoretically in a risk of having cadmium induced kidney damage. The reason why the ringed seals do not show renal damage may be attributed to the fact that the limit of 50 µg/g w.w. is not critical to the arctic ringed seals.

Time of exposure

One should be cautious 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 have been observed (see section 3.5).

Experience from cadmium poisoned humans indicate 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 would show renal histopathological changes, as only a minor percentage (≥10% at 200 µg/g w.w. and ≥ 1% at 50 µg/g w.w.) of cadmium poisoned mammals, develop renal damage (Friberg et al. 1986, WHO 1992, Elinder and Järup 1996).

Adaptation

Dietz et al. (1998a) examined 15 out of 462 ringed seals for cadmium induced nephropathy of which only 5 were in the group ≥ 200 µg/g w.w. The preparation was suboptimal (kept at -20 °C) and it was therefore difficult to carry out the light microscope 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).

Osteodystrophy

If calcium metabolism in ringed seals is affected by cadmium concentrations, then the bone mineral density (BMD) should decrease with age. As seen in Fig. 3.2, this is not the case and it is therefore very unlikely that the ringed seals suffer from osteodystrophy (osteopenia = demineralisation).

Adaptation/regulation

The cadmium intake of the ringed seals are at least as high as the intake of the Itai-itai patients and laboratory cadmium poisoned mammals (see Table 3.5). 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 adaptions.

Table 3.5
The cadmium concentration in the food of the ringed seal, Itai-itai patients and laboratory mammals (rat, mouse and monkey) ([a)] Friberg et al. 1986, [b)] WHO 1992, [c)] Dietz et al. 1996, [d)] Dietz 1998a-c).

Group:	Ringed seal	Itai-itai	Laboratory mammals
Way of adm. (food):	Fish and crustaceans	Rice	Water, food, parenterale
Daily total intake:	40-16000 µg Cd[c&d)]	140-260 µg Cd[a&b)]	
Concentration in diet:	0.02-8 µg Cd[c&d)] per gram w.w.		1-300000 µg Cd[a&b)] per gram w.w.

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

3.5.1 The kidneys

Cortex levels of Cd

The kidney cortex concentration of cadmium (CdK) in the ringed seals are relatively low compared to the cadmium content in the ringed seals food which is high enough to induce tubulopathy (see Table 3.5). 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 show that it is only a minor proportion of the exposed individuals (≥ 10% at 200 µg/g w.w. and ≥ 1% at 50 µg/g w.w.) sustains 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 were not obviously cadmium induced indicates that the ringed seals are not affected by their high cadmium intake because the food composition affect cadmium uptake and toxicty.

Zinc and calcium

Low concentrations of zinc and calcium will enhance the cadmium absorption over 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 over the GI mucosa (Riget et al. 1997, Dietz et al. 1998b). At the same time zinc is able to induce synthesis of metallothionein (Mt = a cystein rich LMW protein), which probably act to detoxify the Cd^{2+} by forming a Cd-Mt complex (Felley-Bosco and Diezi 1992, Ohta and Cherian 1995).

Selenium

The concentration of selenium in the kidney and liver from the ringed seals as well as in the food are also high (Dietz et al. 1996, 1998b-c). Selenium is known to detoxify cadmium (and methylmercury) in insolouble selenid 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 the mercury and thereby detoxifying the 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 et al. in press).

Zinc and cadmium

Dietz et al. (1998c) have shown that zinc and cadmium are positively correlated in the bile of the ringed seals. Cadmium elimination through the bile in ringed seals is about 200 fold higher than found in terrestrial mammals (Friberg et al. 1986, WHO 1992, Dietz et al. 1998c). As only about 5% of the cadmium is believed to be reabsorbed in the gastrointestinal chanel and 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

Osteopenia

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 deficiency of calcium, vitamin D, protein, phosphorus and cadmium which is known to exacerbate this (Ibid.). 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 (Ibid.).

D-vitamine sources

Cadmium induced damage are known to induce Fanconi's Syndrome which is a state of a pathological 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 seals diet is comprised of fish rich in vitamin D, calcium, phosphorus, zinc and proteins, which counteracts cadmium induced osteopenia (Riget et al. 1997, Saxholt 1998). Another vitamin D contribution is obtained in the spring where ringed seals haul out on the ice in connection with their moulting and thereby stimulated to synthesis of cholecalciferol through the UV-radiation from the Arctic midnight sun (Vibe 1981, Haarløv 1986, Génsbøl 1996).

4 Conclusions

High cadmium levels in the food

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

Results

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 occurence related to age and and to measured cadmium and calcium levels.

Adaptation/regulation

The diagnosis and pathogenesis of cadmium induced diseases are usually done 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 in several years (≥ 10 years) show signs on renal and skeleton damage. It seems that the ringed seals have adapted to the high cadmium levels through their cadmium excretion and their constant mineralisation of the skeleton system facilitated by their food composition.

The sample

If any of the ringed seals in the Qaanaaq area have cadmium related diseases, we have not been able to detect them. This can be explained by that the individuals examined were not old or sensible enough, and that the ringed seals have adapted or regulated to the high cadmium concentrations which together with their food content reduces the possibility of finding affected individuals. The food contains namely 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 as well as the females estrogen hormonal activity throughout life.

5 Perspectives and recommendations

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

It is recommended to analyze cadmium concentrations in the liver and muscle, the blood and the urine content of cadmium, calcium, phosphate, aminoacids, protein, glucose and possibly creatinin and %-TRP. These results should then be compared to zoological measurements, BMD and histopathological findings. Finally, it may be neccessary to examine the lumbar vertebrae histopathologically to determine osteodystrophy.

As The National Environmental Research Institute, Deparment of Arctic Environment in Copenhagen have samples from approximately 500 ringed seals analysed for cadmium in muscle, liver and kidney, it is possible to carry out additional studies on the BMD relative to cadmium levels, age and sex.

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 vitamine 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 treatment of osteoporosis and cadmium induced effects.

6 Aknowledgements

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Cadmium concentrations in kidneys from ringed seals (*Phoca hispida*) from North West Greenland (Qaanaaq) are high. Concentrations range at level known to induce renal toxic effects (mainly tubulopathy) and demineralisation (osteopenia) of the skeletal system (Fanconi's Syndrome) in humans as well as laboratory mammals. We have studied possible cadmium induced histopathological changes in the kidneys as well as a demineralisation of the skeletal system (DXA-scanning of lumbal vertebraes). No obvious cadmium induced toxic changes were found. Food composition and physiological adaptations may explain the absence of toxic effects of cadmium in ringed seal.

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