Bone Mineral Health relates to Environmental Cadmium Exposure- Experimental and Human data

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Abbreviations: cadmium (Cd), zinc (Zn), calcium (Ca), phosphorus (P), boron (B), copper (Cu), silicon (Si), manganese (Mn), magnesium (Mg), trabecular bone mineral density (tBMD), One-way analysis of variance (ANOVA), Fisher's least significant difference (LSD), critical effect dose (CED), critical effect size (CES), CED lower bound of 95% confidence interval (CED-L), no observed adverse effect level (NOAEL)

Abstract

Exposure to cadmium (Cd) is recognised as one of the risk factors for osteoporosis, although critical exposure levels and exact mechanisms are still unknown.

Here, we first confirmed that in male Wistar rats challenged orally with 7 different levels of Cd (0.3-10 mg/kg b.w.), over 28 days, there was a direct dose relationship to bone Cd concentration. Moreover, bone mineral content was significantly diminished by ~ 15% (p <0.0001) plateauing already at the lowest exposure level. For the other essential bone elements zinc (Zn) loss was most marked. Having established the sensitive metrics (measures of Cd exposure), we then applied them to 20 randomly selected human femoral head bone samples from 16 independent subjects. Bone Cd concentration was inversely proportional to trabecular bone mineral density and mineral (calcium) content and Zn content of bone, but not the donor's age.

Our findings, through direct bone analyses, support the emerging epidemiological view that bone health, adjudged by mineral density, is extremely sensitive to even background levels of environmental Cd. Importantly, however, our data also suggest that Cd may play an even greater role in compromised bone health than prior indirect estimates of exposure could reveal. Environmental Cd may be a substantially determining factor in osteoporosis and large cohort studies with direct bone analyses are now merited.

Ethical approval

All procedures performed in study involving human participants have been carried out in accordance with the Code of Ethics of the World Medical Association and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Both animal and human studies have approvals of appropriate Ethical Committees.

1. INTRODUCTION

It is well recognised that exposure to cadmium (Cd) has a potentially negative impact on human health. Following exposure, this toxic metal is retained in the body and levels accumulate with age, due to its long biological half-life of 10-30 years (Järup and Åkesson, 2009). Cadmium is readily taken up from contaminated soil by various crops, especially vegetables and rice which, together with other foodstuffs such as shellfish and offal, can contribute to excessively high levels of Cd intake (Renieri et al., 2019; Satarug et al., 2017)Furthermore, tobacco smoking increases the body's burden to Cd. Hence, the estimated exposure to Cd in many areas, particularly industrial, is high enough to be hazardous to human health (EFSA, 2009; WHO, 2011). Of additional concern is the fact that exposure to Cd has not decreased over the past decades, except in some highly contaminated areas (Åkesson et al., 2014). On the contrary, trends for an increase in exposure to Cd have been documented (Nordberg et al., 2015).

Cadmium is broadly toxic and can cause damage to the kidneys, liver, testes, cardiovascular and endocrine system (ATSDR, 2012; Andjelkovic et al., 2019; Buha et al., 2018; Deering et al., 2018; Mezynska and Brzóska, 2017; Satarug, 2018). Cadmium and its compounds have been classified as known human carcinogens by the International Agency for Research on Cancer since 1993 (IARC, 1993) based on epidemiological studies showing a causal connection with the development of lung cancer, while new IARC monograph on Cd and Cd compounds stated positive association between Cd exposure and cancer of the kidney and the prostate cancer (IARC, 2012). Studies have also implicated its connection to pancreatic, bladder, and breast cancer (Buha et al., 2017; Djordjevic et al., 2019; Feki-Tounsi and Hamza-Chaffai, 2014; Van Maele-Fabry et al., 2016; Wallace et al., 2019). Furthermore, it has been reported that exposure to Cd affects the skeletal system, potentially increasing the risk of osteoporosis and bone fractures, especially in populations occupationally exposed to Cd or populations residing near areas of Cd contamination (Kazantzis, 2004). However, recent observational studies, reviewed by James and Meliker (James and Meliker, 2013), suggest that it is not only exposure to relatively high-levels of Cd that is osteotoxic, but more typical, low environmental levels of Cd could also have adverse effects (Åkesson et al., 2006; Gallagher et al., 2008; Wu et al., 2010). Similarly, data from the Swedish cohort of the Osteoporotic Fractures in Men, the MrOS study, showed that even relatively low Cd

exposure, through the diet and from smoking, increases the risk of low bone mineral density and osteoporosis-related fractures in the elderly. This was supported by the negative association between urine Cd levels and bone mineral density (Wallin et al., 2016). Within the Swedish Mammography Cohort, urinary Cd (as a marker of lifetime exposure) and bone mineral density were assessed. Low-level dietary exposure to Cd showed modest, but significant association with bone mineral density and fractures (Engstrom et al., 2011). In recent IUPAC technical review document, statistically significant associations were reported between markers of Cd exposure and decreased bone mineral density, risk of osteoporosis, increased urinary deoxypyridinoline, and risk of bone fractures (Nordberg et al., 2018). Furthermore, recent study raised concerns over the possible impact of early-life Cd exposure on bone-related biomarkers and suggested that Cd exposure during childhood might affect bone remodeling and growth at pubertal age (Malin Igra et al., 2019).

It is generally considered that for 'low' (ie typical environmental) background levels of Cd exposure, the association is weak between urinary or blood levels of the element and true organ exposure (Buser et al., 2016; Friberg and Elinder, 1993). In this respect, direct analysis only would provide a true measure of what the organ is exposed to at the time of assessment.

In rats the toxic effects of cadmium on bone can be replicated and antagonism of the beneficial zinc (Zn) effects are anticipated based upon (a) displacement of Zn ions by Cd ions in bone and (b) protection of supplemental Zn against Cd-induced osteotoxicity(Andrulewicz-Botulińska et al., 2018). However, the dose response relationship between oral exposure to Cd and bone levels of Cd or the essential elements is not well established. Hence, we first undertook this work in rats and then applied the most sensitive outcomes to human bone samples.

2. MATERIALS AND METHODS

2.1.   Animal study

2.1.1. Experimental design

The study was conducted on male albino Wistar rats of 6-8 weeks of age and 150-200 g body weight. Rats (n=49) were obtained, free of typical rodent pathogens, from a commercial

breeder (the Military Medical Academy, Belgrade) and acclimatized for one week prior to use in the study. Rats were housed in stainless steel cages under standard laboratory conditions (i.e. room temperature of 22°C, 50-60% humidity and 12 h night:12 h day photoperiod) with free access to standard pelleted diet (Veterinary Institute ''Subotica'', Subotica, Serbia) and tap water. The diet contained 19.2 ng Cd/g diet (determined according to the method described in section 2.1.4.2.). Following acclimatisation, rats were randomly allocated into seven groups, with 7 rats per group: a control (untreated) group and six treatment groups (0.3, 0.6, 1.25, 2.5, 5 and 10 mg Cd/kg b.w. per day, in the form of aqueous solutions of Cd chloride (CdCl$_2$xH$_2$O; Merck, Darmstadt, Germany) for 4 weeks, which corresponds to subacute exposure. Cadmium treatment of all animals was performed by oral gavage. The control group did not receive any treatment. Doses of Cd were selected to represent environmental levels of exposure in the general population (e.g. in smokers and non-smokers) based upon literature data (ATSDR, 2012). Rats were monitored continuously throughout the study period. This study was conducted in accordance with Guidelines for animal studies no. 9667-1/2011 issued by the Ethics Committee of Military Medial Academy in Belgrade, Serbia which is in accordance with EU Directive 2010/63/EU for animal experiments.

2.1.2. Tissue sampling

After 28 days, rats were fasted overnight and euthanized by decapitation and the following tissues were immediately collected: whole blood was collected from the carotid arteries in tubes containing 6% K$_2$EDTA and frozen at −20 C until Cd and calcium (Ca) analysis. Another aliquot of blood was collected for serum separation at 3,000 g for 30 minutes in order to analyse for creatinine, urea and albumin. The collected sample of whole blood was trunk blood and hence, may have been subjected to non-blood contamination. However, Cd is mainly transported in the blood via erythrocytes (bound with proteins in the membrane) and plasma proteins (Świergosz-Kowalewska, 2001). Moreover, since all samples were collected in the same manner, possible dissolution of the blood with this fluid would be the same in all groups. The right femur was collected from each animal, freed of soft tissue and stored frozen at -20°C until analysis for Cd and other bone-associated elements Ca, phosphorus (P), Zn, boron (B), cooper (Cu), silicium (Si), manganese (Mn) and magnesium (Mg).

2.1.3. Determination of creatinine, urea and albumin in serum

All biochemical analyses were performed using an automated biochemical analyzer (Cobas® 6000 Anlyzer Series, Roche Diagnostics, USA). These analyses followed good laboratory practice in laboratories accredited by the Accreditation Body of Serbia according to SRPS ISO 15189:2008 standards.

2.1.4. Elemental analyses

2.1.4.1. Microwave-assisted acid digestion

Prior to elemental analysis, the blood and bones samples were digested in acid. Blood samples (1 mL) were digested in a mixture of 7 mL of p.a. nitric acid (65% w/v; Merck; Darmstadt, Germany) and 1 ml of p.a. hydrogen peroxide (30% w/v; Sigma-Aldrich, St.Louis, USA) in acid-cleaned TFM vessels in an Ethos One Microwave System (Milestone; Sorisole, Italy). Microwave conditions were: 15 min ramp to 200 °C and maintained at this temperature for 15 min. Blanks containing just the acid mixture without bloodsample were prepared and digested in parallel. The cooled digested samples and blanks were transferred into clean 50 ml bottles and diluted with UHP water (Elga Purelab Prima; Bucks, United Kingdom). Similar procedures were performed on the whole bone samples: namely, 0.2-0.4 g of femoral bone (containing both trabeculae and cortical bone) were digested in 10 mL of a 1+3 mixture of p.a. nitric acid (65% w/v; Sigma-Aldrich, St.Louis, USA) and UHP water in acid-cleaned 15 mL PTFE vessels in an UltraWave Single Reaction Chamber Microwave Digestion System (Milestone Srl; Sorisole, Italy) under the following conditions: 5 min ramp to 120°C, then 10 min ramp from 120°C to 230°C and maintained at 230°C for 15 min. Blanks, containing just the acid mixture without any samples, were digested under the same conditions for each batch to determine the background contribution for the analyzed elements. The digested samples and blanks were transferred to pre-cleaned pre-weighted polypropylene 13 ml tubes (Sarstedt Ltd) for ICP-OES analysis.

2.1.4.2. Determination of Cd and Ca levels in blood

Total Cd and Ca analysis in whole blood was performed by inductively coupled plasma mass spectrometry (ICP-MS; iCap Q, Thermo Scientific, Bremen, Germany) equipped with a collision cell and operating in kinetic energy discrimination (KED) mode. The ^{44}Ca and ^{111}Cd isotopes were assessed.

Torch position, ion optics, and detector settings were adjusted using a tuning solution (Thermo Scientific Tune B), to optimize measurements (signals) and minimize possible interferences. An additional line of the peristaltic pump was used for online introduction of a multielement internal standard (^{6}Li and ^{45}Sc at 10 ng/mL; ^{71}Ga, ^{89}Y and ^{209}Bi at 2 ng/mL) covering a wide mass range. The concentration of each monitored isotope was corrected for response factors of both higher and lower mass internal standards using the interpolation method.

Accuracy and precision of the analytical method was assessed with SeronormTM Trace Elements Whole Blood standard reference material (Sero AS, Billingstad, Norway). The reference material was prepared in the same manner as the blood samples by microwave digestion and was prepared with each batch of digested samples. The reference material was run at the beginning, middle and end of each sample batch run. Measured concentrations were within the range of the certified values for both isotopes.

2.1.4.3.   Determination of Cd and bioelements levels in femur

The bone digests were analyzed for Cd, Ca, P, Zn, Mg, Mn, Si, B, and Cu by inductively coupled plasma optical emission spectrometry (ICP-OES: Jobin Yvon Horiba Ultima 2C; Instrument SA, Longjumeau, France), equipped with a concentric nebulizer and cyclonic spray chamber. A sample introduction pump speed of 10 rates/min, nebulizer flow rate of 0.74 ml/min and a plasma gas flow rate of 10 L/min was used. Analytical lines used were: 228.802 nm (Cd), 317.933 nm (Ca), 213.618 nm (P), 251.611 nm (Si), 208.959 nm (B), 279.079 nm (Mg), 206.191 nm (Zn), 257.610 nm (Mn), and 324.754 nm (Cu). Both acid and pooled sample-based multi-element standards were used (0-20 ppm). The latter were prepared by pooling 1 ml from each of the digested bone samples and then spiking aliquots with a multi-element standard (100 or 1000 mg/L). All samples, including sample blanks, were analyzed in a single batch.

2.2.   Human study

Twenty randomly selected samples of human femoral heads (10 from female osteoporotic patients aged between 75 and 98 years and the remainder from five male and one female subjects without osteoporosis and aged between 58 and 90 years) were obtained from the Stanmore Musculoskeletal Biobank at the Royal National Orthopaedic Hospital in London, UK (ethics approval no. 08/H0304/78). The osteoporotic bone samples were from female subjects who had undergone hip replacement surgery. Osteoporosis mainly effects females after the menopause and hip replacement surgery is generally undertaken between 60 and 80 years of age. The non-osteoporotic (healthy) bone samples were, where possible, matched for the same age range as the osteoporotic samples. A sub-sample (0.4-1 g) of each bone, containing both trabecular and cortical bone, underwent microwave assisted acid digestion as described in section 2.1.4.1. Samples blanks were similarly prepared. The digested samples were then analysed for Ca and Zn by ICP-OES and [111]Cd by ICP-MS using similar methods described in section 2.1.4.3. Trabecular bone mineral density (tBMD) measures, obtained by peripheral quantitative computed tomography, were supplied by the Biobank for each bone sample.

2.3.   Statistical analysis

Bone element levels range from mg/g for the major (structural) elements (Ca, P and Mg), ug/g for the trace elements (Zn, Cu, Mn and Si) and ng/g for the ultra-trace elements such as Cd. Thus it would be difficult to use the same units for all the elements reported here. However, the same units were used for the elements within the same category (major, trace and ultra-trace), to enable easy visualisation and comparison of the data.

All calculations were performed in Microsoft EXCEL (version 2016 for Windows) while statistical tests were performed using IBM SPSS Statistics (version 18.0 for Windows) and Statistica 7 software. Artwork was created using GraphPad Prism 5 software (GraphPad software, Inc., La Jolla, CA, USA). The one-sample Kolmogorov-Smirnov test was used to determine whether the investigated parameters followed a normal distribution. For normally distributed data One-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc test was used, otherwise the Kruskal–Wallis test

followed by the Median test was used. Post hoc comparisons were made to identify which pairs of means are statistically different. Pearson's and Spearman's correlation analysis was used to determine the relationship between Cd and investigated bioelements levels. The level of significance for all tests was set at $p<0.05$.

Bone element levels were analyzed by the Benchmark dose method based on dose-response modeling of the full data set (Slob, 2002). Benchmark dose is defined as the exposure level that produces change in a response known as a Benchmark response. These analyses were performed using software PROAST (the Dutch National Institute for Public Health and the Environment). When obtained data are continous (as in present study), instead of terms Benchmark dose and Benchmark response, terms critical effect doses (CED) and critical effect size (CES) should be used. CED and its lower bound of 95% confidence interval (CED-L) were calculated at critical effect sizes (CES) of 5% and 10%. This was done according to the EPA guidelines (2012) that recommends the use of CES of one control SD (that were around 5% of the determined values for the majority of investigated parameters) while using CES of 10% appears to minimize the adversity and animal variations.

3. RESULTS

3.1. Rat study samples

Rats appeared healthy throughout the study period, however body weight gain was significantly lower in rats treated with ≥ 1.25 mg Cd/kg b.w./day, when compared to control (untreated) rats (Buha et al., 2013). Rats treated with the highest Cd dose (10 mg Cd/kg b.w.) displayed a significant increase in serum creatinine when compared with the control group and rats treated with lower doses of Cd ($p = 0.02$). Rats treated with 5 and 10 mg Cd/kg b.w/day also displayed an increase in serum urea levels compared to the controls ($p < 0.001$). Serum albumin was significantly lower in the Cd-treated groups, showing a dose response relationship, i.e. a progressive decrease in serum levels with increasing Cd doses. Data is graphically displayed in Figure 1.

Total fasting whole blood Cd concentration was significantly increased following oral dosing with Cd, showing a marked positive correlation (r=0.928, $p<0.0001$) between the administered Cd dose and the concentration of Cd in blood after 28 days of dosing (Figure 2). Whole blood Ca concentration was unaffected at the lower doses of Cd (0.3 and 0.6 mg Cd/kg b.w/day), but at the higher doses (≥ 1.25 mg Cd/kg b.w/day) there was a dose-response decrease ($p<0.05$). Levels of Cd and Ca measured in blood are graphically presented in Figure

3.

3.1.1. Bone elements

As with whole blood Cd levels, there was a marked correlation (Spearman's rank) between the administered Cd doses and the levels of Cd in the femurs (ρ=0.912, p<0.0001). Graphical presentation is given in Figure 4. Femur Cd levels also correlated strongly with blood Cd levels ($\rho = 0.94$, $p< 0.0001$).

Calcium and P levels were both significantly decreased in the femur of Cd-exposed rats when compared to the control rats. Femur Ca was decreased by 9 to 15% (mean 12%), while femur P was decreased by 5 to 15% (mean 9%) compared to control rats. These decreases in femur Ca and P levels occured at the lowest dose of Cd administered and remained low, by the same magnitude, at the higher Cd doses, suggesting that a dose response, if present, would occur at doses < 0.3 mg/kg b.w. (Figure 5a, Figure 5b). Femur Mg levels showed a slight decrease with Cd exposure and again, this decrease occured at the lowest Cd dose investigated and remained low by the same magnitude at the other (higher) Cd doses (Figure 5c).

Zinc showed a dose response decrease in the femur with Cd dosing (Figure 6; r = -0.73, $p<$ 0.001) and less so with blood Cd levels (r = -0.44, $p < 0.008$). At the highest dose of Cd, the decrease in femur Zn was around 30% compared to the control rats. Correlation between femur Zn and Cd levels in femur showed a strong significant negative association ($\rho = -0.74$; $p<0.001$).

In contrast, Cu levels of the femur showed a slight increase with Cd dosing, namely with the 2.5 and 5 mg Cd/kg b.w/day doses. However, at the highest Cd dose a decrease in femur Cu, compared to the control group, was observed, just as for Zn (Table 1).

Femur Si was largely unaffected with Cd dosing, except at the 1.25 and 5.0 mg Cd/kg b.w./day doses, where significant increases in femur Si levels were observed compared to the untreated group. Femur B and Mn levels, on the other hand, showed significant decreases at all Cd doses investigated (Table 1). Indeed, strong significant negative correlations with femur Cd were obtained for femur B ($\rho= -0.43$, $p<0.001$) and femur Mn ($\rho= -0.49$, $p<0.001$).

Table 1. The effects of different doses of Cd, for 28 days, on femur Si, B and Mn concentrations of male Wistar rats.

Cd doses	Cu	Si	B	Mn

(mg/kg b.w.)		(µg/g femur)	(µg/g femur)	(µg/g femur)	(µg/g femur)
0	Median	1.34	1.67	1.94	0.52
	Range	1.10-1.34	1.14-2.57	1.61-2.85	0.36-0.91
0.3	Median	0.93	1.14	1.73*	0.40*
	Range	0.85-1.25	0.77-2.62	1.42-2.20	0.39-0.54
0.6	Median	1.45	1.26	1.76*	0.45*
	Range	1.27-2.11	1.03-2.51	1.63-1.92	0.39-0.60
1.25	Median	1.31	2.59*	1.70*	0.45*
	Range	0.95-2.10	0.85-8.64	1.29-2.03	0.43-0.51
2.5	Median	1.48*	1.42*	1.87*	0.44*
	Range	1.11-2.56	0.60-5.08	1.56-1.97	0.34-0.49
5	Median	2.10*	2.11*	1.47†	0.39*
	Range	1.61-3.25	1.55-2.86	1.13-1.71	0.33-0.48
10	Median	1.29*	1.49*	1.50†	0.37*
	Range	0.66-2.27	0.27-4.34	1.07-2.01	0.32-0.43

Results are presented as the medians and ranges.
Each group contained 7 animals.
Medians marked by * are significantly different from controls (Kruskal–Wallis test, Median test, $p<0.05$).
Medians marked by †. are significantly different from controls (Kruskal–Wallis test, Median test, $p<0.01$)

3.1.2. Benchmark doses for Cd effects on essential bone elements

The dose dependence relationships of the femur elements to Cd exposure are presented in Table 2. Dose–response relationship was characterized using the PROAST software and was confirmed for femur P, Zn, B and Mn. Values of CED10 and CED5 and their lower confidence levels were calculated from the best-fit curves. CED/CEDL ratio was used as a measure of statistical uncertainty and was under 10 for all investigated effects indicating the statistical relevance of these data.

Based on the very low CEDL5 and CEDL10 values, the effect of Cd on femur Zn levels can be regarded as the most critical effect of Cd on the investigated femur elements.

Table 2. Dose dependency and calculated CED doses for Cd effects on femur elements of male Wistar rats treated with Cd for 28 days.

Bioelements in femur	Dose dependence	CED_5 (mg Cd/kg b.w.)	$CEDL_5$ (mg Cd/kg b.w.)	CED_{10} (mg Cd/kg b.w.)	$CEDL_{10}$ (mg Cd/kg b.w.)
Calcium	/				
Phosphorus	$y=a*[c-(c-1)exp(-bx)]$	0.35	0.21	0.81	0.49
Zinc	$y=a*exp(bx^d)$	0.10	0.01	0.58	0.15
Boron	$y=a*exp(bx)$	1.92	1.13	3.95	2.31
Magnesium	/				
Silicon	/				
Copper	/				
Manganese	$y=a*exp(bx)$	1.96	1.30	4.02	2.67

Data are fitted and calculated with PROAST software.
Dose dependence is calculated and represented with corresponding equation.
Critical effect dose (CED) match the critical effects size (CES) for endpoints set at 5% (CED_5) or at 10% (CED_{10}). CEDL represents the lower 95% confidence level.
Mark / means that there were no dose dependence for investigated parameter.

3.2. Human bone samples

We finally considered whether very low levels of Cd in bone (i.e. through 'natural' long term exposure) might also associate with lower levels of mineral (Ca content and tBMD) or Zn in human bone, by correlating these data from the 20 samples analysed. We confirmed that age and bone Cd content were unrelated (r = 0.32; p= 0.16). In contrast, significant negative correlations were obtained between bone Cd and Ca contents (r = -0.45, Figure 7a) and between bone Cd content and tBMD (r= -0.44, Figure 7b). Moreover, bone Zn content was negatively associated with bone Cd content (r = 0.54, Figure 7c) and positively with tBMD (r

= 0.48, Figure 7d).

4. DISCUSSION

In terms of bone Cd content, it has not been clear how this relates to oral dosing. We show here that, just as for blood, there is a dose response relationship with oral Cd ingestion, but the variance for any dose group is high and overall data were non-normally distributed. Given these findings, under carefully controlled laboratory conditions, it is likely that for humans variance would be even more marked. Indeed, on a number of occasions estimates of human Cd exposure, notably from the diet or via urinary analysis, have been called into question (Bernard, 2016; Needham et al., 2007; Quraishi et al., 2016). Taken together, it seems that quantifying the impact of current 'normal' Cd exposure levels to human bone will be best delivered through human bone analyses rather than relying on biomarkers. In this respect, we report that bone Zn levels were especially sensitive, as was macro mineral content (Ca, P, Mg), to Cd exposure in rats. Indeed, the latter had already bottomed out at the lowest dose used. When these measures were applied to randomly selected human bone samples, the associations held up. Higher Cd content was associated with lower macro mineral (Ca or tBMD) and Zn levels. It begs the question as to whether, for bone: Are there any safe Cd levels? Are the typical surrogate biomarkers that are used to determine Cd effects on bone revealing the true extent of the problem? Further large scale analytical human bone studies that consider both Cd and Zn content, are now merited. In the following discussion we place our findings in the context of the known literature.

4.1. Cadmium effects on bone

It has been suggested that Cd could affect bone directly or indirectly, hence two different hypothesis have been proposed. The first proposes that Cd has a direct effect on bone cells i.e. by stimulating osteoclast differentiation and activity (Chen et al., 2012; Coonse et al., 2007). The second hypothesis proposes that Cd indirectly impacts bone through its effects on other organ systems such as the gastrointestinal tract, thyroid, parathyroid glands and especially the kidneys (Bhattacharyya, 2009; Brzóska and Moniuszko-Jakoniuk, 2005a; M.M. Brzóska and Moniuszko-Jakoniuk, 2004). Such effects can result in disturbances in the metabolism of vitamin D and bone-associated elements. We discuss these two possible mechanisms of action

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below and how our findings provide evidence for the former, i.e. for a direct effect of Cd on bone.

Although no data on bone mineral density or bone quality were collected for the rat bones, our findings indicate Cd-induced disturbances in bone mineralization and points clearly to the fact that even low environmental Cd doses can influence Ca and P homeostasis in male rats bone. Possible mechanisms of how Cd could interfere with Ca levels in bones is its ability to compromise Ca absorption and excretion and/or replace Ca in the hydroxyapatite crystals promoting bone resorption, osteomalacia or osteoporosis (Hamilton and Smith, 1978). The results of our study are in agreement with the study performed by Choi et al. (Choi et al., 2003) in which they reported that male Sprague-Dawley rats had lower bone Ca content and lower bone mineral density after treatment with 50 mg/L Cd in their drinking water for 20 weeks. Although the observed changes were relatively small (ranging from 5-15% overall) this could certainly elicit larger effects on bone function over a long period of time (i.e. life-time exposure). The ability of Cd to time-dependently influenced bone mineral density and chemical composition has been already established with special importance given to exposure during skeletal development (Brzóska et al., 2005).

We also observed a similar, but smaller effect of Cd on femur Mg levels, although the decrease in Mg levels did not show a dose response with Cd doses, suggesting this could be an indirect or secondary effect of Cd. Previous work have not reported any significant effects on bone Mg content from Cd exposure (Brzóska et al., 2005; Bulat et al., 2012). Thus, the role of Mg in Cd osteotoxicity remains rather unclear.

Decreases in bone Zn levels following Cd exposure have previously been reported. Decreased concentrations of Zn was reported in the bones of rats treated with 50 mg Cd/L for 8-12 weeks (Brzóska et al., 2001) and in rats treated with a much lower dose of 1 mg Cd/L (in their drinking water) for 12 months (Małgorzata M. Brzóska and Moniuszko-Jakoniuk, 2004). Exposure of female rats to Cd (50 mg/L Cd as $CdCl_2$) in their drinking water induced maternal hypozincemia and Zn depletion in the femur of the fetuses suggesting that the observed toxic effects of Cd on prenatal bone formation are, at least in part, mediated by the disruption of maternal Zn metabolism by Cd during pregnancy (Boughammoura et al., 2017). In a recent study we reported decreased levels of Zn in the bones of rabbits treated with 10 mg Cd/kg b.w. for four weeks (Bulat et al., 2012) and here, we similarly report lower Zn levels in the femur of rats exposed to much lower doses of Cd and a significant negative association

between bone Cd and Zn levels in the human bone samples with much lower total Cd levels.

Another investigated trace element in this study, Cu was influenced in a non-monotonic manner; moderate Cd doses were associated with an increase in femur Cu, the highest Cd dose caused a decrease in femur Cu, while no effect was observed with low Cd doses. Similar inconclusive results on Cd and Cu interactions have previously been reported (Bulat et al., 2012; Ishitobi and Watanabe, 2005). Cadmium ability to produce non-monotonic effects has been already shown for various parameters in zebra fish linking it to hormesis phenomena (Renieri et al., 2017).

Our findings on the ultra-trace elements suggest the involvement of B and Mn in Cd osteotoxicity, as the decreases in femur Mn and B were significantly correlated with Cd exposure. Even the lowest dose of Cd (0.3 mg/kg b.w.) significantly decreased the level of femur B. Olgun and Bahtiyana (Olgun and Bahtiyarca, 2015) similarly, reported that the addition of Cd to the diet decreased tibia B content in chickens. There is growing evidence that Si may be essential for the normal health of bone and other connective tissues as its deficiency in young growing animals can cause stunted growth and abnormal development of bone and other connective tissue (Jugdaohsingh et al., 2015b). Recently two of the co-authors reported that Si is the major element associated with collagen fraction of bone, followed by Mn and Cu at much lower levels, whilst B, Zn and Ca were negligibly associated with the collagen fraction (Jugdaohsingh et al., 2015a). Our study here showed that Cd exposure had minimal effect on the levels of Si in the femur, implying that Si is not significantly affected by Cd induced osteotoxicity.

The above reported findings suggest that the primary detrimental effect of Cd on bone is on bone mineralisation and the mineral fraction of bone, hence the significant associations between Cd exposure and femur Ca, P, Zn and B (i.e. the mineral associated elements).

The second hypothesis on Cd osteotoxicity proposes that Cd indirectly impacts bone through its effects on other organ systems such as the gastrointestinal tract, kidneys, thyroid and parathyroid glands (Bhattacharyya, 2009; Brzóska and Moniuszko-Jakoniuk, 2005a; M.M. Brzóska and Moniuszko-Jakoniuk, 2004). However, there is a lack of understanding of the relationship between the concentration of Cd required for renal dysfunction and the concentration required for bone disturbances. In the present study, disturbances in serum urea and serum creatinine levels were only observed at the higher doses of Cd (i.e $\geq$ 5 mg Cd/kg b.w./day). The decrease in serum albumin concentration observed in this study for all

investigated doses of Cd may be the consequence of reduced liver function rather than the effect of early albuminuria (unpublished data). It could be hypothesized that Cd-induced disturbances in bone mineral status occurred before renal dysfunction suggesting that Cd may disrupt bone metabolism by directly reaching the bone compartment at levels that do not produce impaired tubular reabsorption, i.e. increased Ca excretion. This is in accordance with the results obtained in several human studies. In a longitudinal study that included 936 Swedish men, aged 70-81 years, in which urinary Cd showed a positive correlation with bone mineral density while suggesting that this demineralization could also be independent of Cd kidney toxicity (Burm et al., 2015). Similarly, recent cross sectional study on women aged 50 years and higher revealed that decreased bone mineral density and the risk of osteoporosis in postmenopausal women might be the consequence of environmental Cd exposure with these effects being independent of Cd-induced renal toxicity (Callan et al., 2015). However, with higher Cd doses the effects on the kidneys, which were shown here by statistically higher serum levels of urea and creatinine, as well as oxidative status disturbances in the kidneys (unpublished data) certainly may have a role in the impairment of bone mineral content. This could be mainly through increased Ca excretion as confirmed by marked decrease in Ca blood levels after treatment with 5 and 10 mg Cd/kg b.w./day (45% and 65% decrease, respectively). A recent study in adult male Sprague/Dawley rats that received daily subcutaneous injections of $CdCl_2$ at a dose of 0.6 mg /kg for up to 12 weeks, revealed that the change in periodontal bone levels became statistically significant after the time point when overt renal toxicity was apparent (i.e., after the ninth week), as revealed by the increase in kidney injury molecule-1, a sensitive urinary biomarker of renal injury (Browar et al., 2018). This points to the possibility that some of the Cd effects on the periodontium may be the consequence of indirect effects on whole-body calcium levels as a consequence of Cd-induced renal dysfunction.

Another possible mechanism of Cd osteotoxicity that should not be overlooked is the mechanism connected to Cd endocrine disruptive properties. Cadmium's role as an aetiological factor in oestrogen-dependent disease in humans was critically evaluated in a review by Silve at al (2012). Persuasive in vitro and in vivo evidence pointed to Cd having oestrogenic properties, although reasonable knowledge gaps exist on the potential oestrogenic effect of cadmium in humans.A recent Chinese cohort study of 429 women revealed hat cadmium exposure was associated with earlier menarche and pointing to Cd as a potent

nonsteroidal estrogen (Chen et al., 2017). Taking into consideration that oestrogen affects the skeleton in all species via both osteoblast and osteoclast function (Krassas and Papadopoulou, 2001), Cd role as a metalloestrogen can certainly be one of the possible mechanisms of Cd effects on bones. Furthermore, studies have shown that FSH negatively regulates osteoblast number (Zhu et al., 2012). Another possible endocrine disruptive mechanism of Cd osteotoxicity, is that Cd can have thyroid disruptive effects, as reported in many previous studies (Buha et al., 2018, 2013). Namely, administrated recombinant human TSH was shown to prevent ovariectomy-induced bone loss (Sun et al., 2008). Moreover, studies have indentified the kidneys as an important target of thyroid hormone action (Den Hollander et al., 2005).

4.2.   Dose dependance and reference points of Cd effects on bone

An important aim of this study was to identify the dose-response relationship and CEDs for the bioelements affected in bone following Cd dosing. Relationships between Cd dose and femur P, Zn, B and Mn levels were observed, with the lowest CED levels calculated for Zn. The BMD method, used in the present study, has been increasingly used in health risk assessment of environmental contaminants with CEDL more and more replacing the use of no observed adverse effect level (NOAEL) in the risk assessment (EPA 2012). One of the major advantages of this method, as compared to NOAEL, is the ability to utilize the information from the entire dose-response curve.

In the present study, the selected doses of Cd were relatively low if we consider the already set reference doses. At thepresent point, provisional tolerable monthly intake-PTMI is set to 25 µg Cd per kg b.w. per month ie dietary Cd exposure of 0.8 µg/kg body weight per day (JECFA, 2010). When transferred into daily animal doses (multiplied by the safety factor of 100 and the additional factor of 3 or 10 for tolerable intake derivation on an incomplete database), the following animal doses were calculated: 0.27 or 0.8 mg Cd/kg b.w./day. Recently, EFSA (2009) conducted a meta-analysis applying the BMD approach with reference point set for renal tubular effects with 5% BMR. Derived tolerable weekly intake-TWI of 2.5 µg Cd/kg b.w was much lower than the one established by FAO/WHO Expert Committee on Food Additives. When transferred into doses for animal study this would be either 0.11 mg Cd/kg b.w./day or 0.36 mg Cd/kg b.w./day. In the present study, Ca and P

femur levels were decreased at doses of 0.3 mg Cd/kg b.w./day, while calculated CED values in this study (when CES was set at 5%) were even lower for the effects on femur P and Zn levels. Additionly, according to the CED values, Zn levels in bone was the most sensitive to Cd exposure, which raises some interesting points. The current critical Cd concentrations obtained for renal effects and used for TWI development might be underestimated and disturbances in bone metabolism that later can induce low bone mineral density and subsequently lead to osteopenia, osteoporosis or osteomalacia could occur at lower Cd exposure levels than estimated so far. The results from our human bone analyses strongly support this statement, as even though much lower Cd levels were found in these samples, disturbances (decreases) in Ca and Zn levels were still detected. Moreover the trend of lower Zn and Ca levels in the osteoporotic samples with higher Cd levels was observed. Our findings are in accordance with Åkesson et al. (2014) and Satarug et al. (2017). Åkesson et al. (2014) challenged the basis of existing health risk assessment for Cd exposure after performing a literature review and proposed that non-renal effects and effects on bone in particular, should be considered in future risk assessment of Cd. Meanwhile, Satarug et al. (2017) conducted review of current health risk assessment practice for dietary Cd suggesting a more restrictive dietary Cd intake guideline and re-evaluation of Cd toxicity threshold levels. Moreover, literature data suggest that women are more susceptible to the toxic effects of Cd, including bone effects at high Cd exposure (Chen et al., 2009a, 2009b; Nishijo et al., 2017). Calculated Benchmark dose of urinary Cd was lower in women than in men in a recently performed population-based study (Lv et al., 2017). Brzoska and associates (Brzóska et al., 2010, 2005; Brzóska and Moniuszko-Jakoniuk, 2005b) concluded that male rats are less susceptible to bone disorders than female rats probably as a consequence of already explained Cd effects as a metalloestrogen. Hence, it could be hypothesized that even lower critical concentrations of Cd would have been obtained if we had performed our study with female rats.

Our findings point to an unfavorable action of Cd at low and moderate levels of exposure, which is concerning given that Cd is a universal toxicant that plagues most human populations. The findings reported here suggest that the current 'safe intake level' for Cd based on renal effects may need further scrutiny and the findings from this research should be considered in the determination of critical effects and risk assessment on Cd. Furthermore, our data also suggest that Cd may play an even greater role in poor bone health than prior,

insensitive proxies for exposure could reveal and, hence, this toxic element might be a substantially determining factor in osteoporosis. Large scale human studies with direct bone analyses are merited.

References

Åkesson, A., Barregard, L., Bergdahl, I.A., Nordberg, G.F., Nordberg, M., Skerfving, S., 2014. Non-Renal Effects and the Risk Assessment of Environmental Cadmium Exposure. Environ. Health Perspect. https://doi.org/10.1289/ehp.1307110

Åkesson, A., Bjellerup, P., Lundh, T., Lidfeldt, J., Nerbrand, C., Samsioe, G., Skerfving, S., Vahter, M., 2006. Cadmium-Induced Effects on Bone in a Population-Based Study of Women. Environ. Health Perspect. 114, 830–834. https://doi.org/10.1289/ehp.8763

Andjelkovic, M., Djordjevic, A.B., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D., Bulat, Z., 2019. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int. J. Environ. Res. Public Health. https://doi.org/10.3390/ijerph16020274

Andrulewicz-Botulińska, E., Wiśniewska, R., Brzóska, M.M., Rogalska, J., Galicka, A., 2018. Beneficial impact of zinc supplementation on the collagen in the bone tissue of cadmium-exposed rats. J. Appl. Toxicol. 38, 996–1007. https://doi.org/10.1002/jat.3608

ATSDR, 2012. Toxicological Profile for Cadmium. Agency Toxic Subst. Dis. Regist. Public Heal. Serv. U.S. Dep. Heal. Hum. Serv. 1–487.

Bernard, A., 2016. Confusion about cadmium risks: The unrecognized limitations of an extrapolated paradigm. Environ. Health Perspect. 124. https://doi.org/10.1289/ehp.1509691

Bhattacharyya, M.H., 2009. Cadmium osteotoxicity in experimental animals: Mechanisms and relationship to human exposures. Toxicol. Appl. Pharmacol. 238, 258–265. https://doi.org/10.1016/j.taap.2009.05.015

Boughammoura, S., Chemek, M., Mimouna, S. Ben, Banni, M., Messaoudi, I., 2017. Involvement of Zn Depletion in Cd-Induced Toxicity on Prenatal Bone Formation in Rat. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-017-0981-7

Browar, A., Koufos, E., Wei, Y., Leavitt, L., Prozialeck, W., Edwards, J., 2018. Cadmium Exposure Disrupts Periodontal Bone in Experimental Animals: Implications for Periodontal Disease in Humans. Toxics. https://doi.org/10.3390/toxics6020032

Brzóska, M.M., Majewska, K., Kupraszewicz, E., 2010. Effects of low, moderate and relatively high chronic exposure to cadmium on long bones susceptibility to fractures in male rats. Environ. Toxicol. Pharmacol. 29, 235–245. https://doi.org/10.1016/j.etap.2010.01.005

Brzóska, M.M., Majewska, K., Moniuszko-Jakoniuk, J., 2005. Bone mineral density, chemical composition and biomechanical properties of the tibia of female rats exposed to cadmium since weaning up to skeletal maturity. Food Chem. Toxicol. 43, 1507–1519. https://doi.org/10.1016/j.fct.2005.04.008

Brzóska, M.M., Moniuszko-Jakoniuk, J., 2005a. Bone metabolism of male rats chronically exposed to cadmium. Toxicol. Appl. Pharmacol. 207, 195–211. https://doi.org/10.1016/j.taap.2005.01.003

Brzóska, M.M., Moniuszko-Jakoniuk, J., 2005b. Effect of low-level lifetime exposure to cadmium on calciotropic hormones in aged female rats. Arch. Toxicol. 79, 636–646. https://doi.org/10.1007/s00204-005-0680-6

Brzóska, M.M., Moniuszko-Jakoniuk, J., 2004. Low-level lifetime exposure to cadmium decreases skeletal mineralization and enhances bone loss in aged rats. Bone 35, 1180–1191. https://doi.org/10.1016/j.bone.2004.07.010

Brzóska, M.M., Moniuszko-Jakoniuk, J., 2004. Low-Level Exposure to Cadmium during the

Lifetime Increases the Risk of Osteoporosis and Fractures of the Lumbar Spine in the Elderly: Studies on a Rat Model of Human Environmental Exposure. Toxicol. Sci. 82, 468–477. https://doi.org/10.1093/toxsci/kfh275

Brzóska, M.M., Moniuszko-Jakoniuk, J., Jurczuk, M., Gałażyn-Sidorczuk, M., Rogalska, J., 2001. The effect of zinc supply on cadmium-induced changes in the tibia of rats. Food Chem. Toxicol. 39, 729–737. https://doi.org/10.1016/S0278-6915(01)00007-2

Buha, A., Antonijević, B., Bulat, Z., Jaćević, V., Milovanović, V., Matović, V., 2013. The impact of prolonged cadmium exposure and co-exposure with polychlorinated biphenyls on thyroid function in rats. Toxicol. Lett. 221, 83–90. https://doi.org/10.1016/j.toxlet.2013.06.216

Buha, A., Matovic, V., Antonijevic, B., Bulat, Z., Curcic, M., Renieri, E.A., Tsatsakis, A.M., Schweitzer, A., Wallace, D., 2018. Overview of cadmium thyroid disrupting effects and mechanisms. Int. J. Mol. Sci. https://doi.org/10.3390/ijms19051501

Buha, A., Wallace, D., Matovic, V., Schweitzer, A., Oluic, B., Micic, D., Djordjevic, V., 2017. Cadmium Exposure as a Putative Risk Factor for the Development of Pancreatic Cancer: Three Different Lines of Evidence. Biomed Res. Int. 2017, 1–8. https://doi.org/10.1155/2017/1981837

Bulat, Z., Đukić-Ćosić, D., Antonijević, B., Bulat, P., Vujanović, D., Buha, A., Matović, V., 2012. Effect of Magnesium Supplementation on the Distribution Patterns of Zinc, Copper, and Magnesium in Rabbits Exposed to Prolonged Cadmium Intoxication. Sci. World J. 2012, 1–9. https://doi.org/10.1100/2012/572514

Burm, E., Ha, M., Kwon, H.J., 2015. Association between blood cadmium level and bone mineral density reduction modified by renal function in young and middle-aged men. J. Trace Elem. Med. Biol. 32, 60–65. https://doi.org/10.1016/j.jtemb.2015.06.002

Buser, M.C., Ingber, S.Z., Raines, N., Fowler, D.A., Scinicariello, F., 2016. Urinary and blood cadmium and lead and kidney function: NHANES 2007-2012. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2016.01.005

Callan, A.C., Devine, A., Qi, L., Ng, J.C., Hinwood, A.L., 2015. Investigation of the relationship between low environmental exposure to metals and bone mineral density, bone resorption and renal function. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2015.03.010

Chen, X., Zhu, G., Jin, T., 2017. Effects of Cadmium Exposure on Age of Menarche and Menopause. Toxics. https://doi.org/10.3390/toxics6010006

Chen, X., Zhu, G., Jin, T., Åkesson, A., Bergdahl, I.A., Lei, L., Weng, S., Liang, Y., 2009a. Changes in bone mineral density 10 years after marked reduction of cadmium exposure in a Chinese population. Environ. Res. 109, 874–879. https://doi.org/10.1016/j.envres.2009.06.003

Chen, X., Zhu, G., Jin, T., Gu, S., 2009b. Effects of cadmium on forearm bone density after reduction of exposure for 10 years in a Chinese population. Environ. Int. 35, 1164–1168. https://doi.org/10.1016/j.envint.2009.07.014

Chen, X., Zhu, G., Jin, T., Zhou, Z., Gu, S., Qiu, J., Xiao, H., 2012. Cadmium Stimulates the Osteoclastic Differentiation of RAW264.7 Cells in Presence of Osteoblasts. Biol. Trace Elem. Res. 146, 349–353. https://doi.org/10.1007/s12011-011-9256-x

Choi, J.-H., Rhee, I.-K., Park, K.-Y., Park, K.-Y., Kim, J.-K., Rhee, S.-J., 2003. Action of green tea catechin on bone metabolic disorder in chronic cadmium-poisoned rats. Life Sci. 73, 1479–1489. https://doi.org/10.1016/S0024-3205(03)00433-8

Coonse, K.G., Coonts, A.J., Morrison, E. V., Heggland, S.J., 2007. Cadmium Induces Apoptosis in the Human Osteoblast-like Cell Line Saos-2. J. Toxicol. Environ. Heal. Part

A 70, 575–581. https://doi.org/10.1080/15287390600882663

Deering, K.E., Callan, A.C., Prince, R.L., Lim, W.H., Thompson, P.L., Lewis, J.R., Hinwood, A.L., Devine, A., 2018. Low-level cadmium exposure and cardiovascular outcomes in elderly Australian women: A cohort study. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2017.12.007

Den Hollander, J.G., Wulkan, R.W., Mantel, M.J., Berghout, A., 2005. Correlation between severity of thyroid dysfunction and renal function. Clin. Endocrinol. (Oxf). https://doi.org/10.1111/j.1365-2265.2005.02236.x

Djordjevic, V.R., Wallace, D.R., Schweitzer, A., Boricic, N., Knezevic, D., Matic, S., Grubor, N., Kerkez, M., Radenkovic, D., Bulat, Z., Antonijevic, B., Matovic, V., Buha, A., 2019. Environmental cadmium exposure and pancreatic cancer: Evidence from case control, animal and in vitro studies. Environ. Int. 128, 353–361. https://doi.org/10.1016/j.envint.2019.04.048

EFSA, 2009. Cadmium in food - Scientific opinion of the Panel on Contaminants in the Food Chain. EFSA J. 7, 980. https://doi.org/10.2903/j.efsa.2009.980

Engstrom, a, Michaelsson, K., Suwazono, Y., Wolk, a, Vahter, M., Akesson, a, 2011. Long-term cadmium exposure and the association with bone mineral density and fractures in a population-based study among women. J Bone Min. Res 26, 486–495. https://doi.org/10.1002/jbmr.224

Environmental Protection Agency (EPA), 2012. Benchmark Dose Technical Guidance. Washington DC, USA.

Feki-Tounsi, M., Hamza-Chaffai, A., 2014. Cadmium as a possible cause of bladder cancer: a review of accumulated evidence. Environ. Sci. Pollut. Res. 21, 10561–10573. https://doi.org/10.1007/s11356-014-2970-0

Friberg, L., Elinder, C.G., 1993. Biological monitoring of toxic metals, in: Scandinavian Journal of Work, Environment and Health. https://doi.org/10.1007/978-1-4613-0961-1_1

Gallagher, C.M., Kovach, J.S., Meliker, J.R., 2008. Urinary Cadmium and Osteoporosis in U.S. Women ≥50 Years of Age: NHANES 1988–1994 and 1999–2004. Environ. Health Perspect. 116, 1338–1343. https://doi.org/10.1289/ehp.11452

Hamilton, D.L., Smith, M.W., 1978. Inhibition of intestinal calcium uptake by cadmium and the effect of a low calcium diet on cadmium retention. Environ. Res. 15, 175–184. https://doi.org/10.1016/0013-9351(78)90094-4

IARC, 1993. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man., IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans.

Iarc, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. International Agency for Research on Cancer IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Personal habits and indoor combustions. Iarc Monogr. Eval. Carcinog. Risks To Humans 100.

Ishitobi, H., Watanabe, C., 2005. Effects of low-dose perinatal cadmium exposure on tissue zinc and copper concentrations in neonatal mice and on the reproductive development of female offspring. Toxicol. Lett. 159, 38–46. https://doi.org/10.1016/j.toxlet.2005.04.006

James, K.A., Meliker, J.R., 2013. Environmental cadmium exposure and osteoporosis: a review. Int. J. Public Health 58, 737–745. https://doi.org/10.1007/s00038-013-0488-8

Järup, L., Åkesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol. 238, 201–208. https://doi.org/10.1016/j.taap.2009.04.020

Joint FAO/WHO Expert Committee, 2010. Food Additives. Who Food Addit. Ser. 64 1–17.

Jugdaohsingh, R., Pedro, L.D., Watson, A., Powell, J.J., 2015a. Silicon and boron differ in their localization and loading in bone. Bone Reports 1, 9–15. https://doi.org/10.1016/j.bonr.2014.10.002

Jugdaohsingh, R., Watson, A.I.E., Pedro, L.D., Powell, J.J., 2015b. The decrease in silicon concentration of the connective tissues with age in rats is a marker of connective tissue turnover. Bone 75, 40–48. https://doi.org/10.1016/j.bone.2015.02.004

Kazantzis, G., 2004. Cadmium, osteoporosis and calcium metabolism. BioMetals 17, 493–498. https://doi.org/10.1023/B:BIOM.0000045727.76054.f3

Krassas, G.E., Papadopoulou, P., 2001. Oestrogen action on bone cells. J. Musculoskelet. Neuronal Interact.

Lv, Y., Wang, P., Huang, R., Liang, X., Wang, P., Tan, J., Chen, Z., Dun, Z., Wang, J., Jiang, Q., Wu, S., Ling, H., Li, Z., Yang, X., 2017. Cadmium Exposure and Osteoporosis: A Population-Based Study and Benchmark Dose Estimation in Southern China. J. Bone Miner. Res. 32, 1990–2000. https://doi.org/10.1002/jbmr.3151

Malin Igra, A., Vahter, M., Raqib, R., Kippler, M., 2019. Early-Life Cadmium Exposure and Bone-Related Biomarkers: A Longitudinal Study in Children. Environ. Health Perspect. https://doi.org/10.1289/EHP3655

Mezynska, M., Brzóska, M.M., 2017. Environmental exposure to cadmium—a risk for health of the general population in industrialized countries and preventive strategies. Environ. Sci. Pollut. Res. 1–22. https://doi.org/10.1007/s11356-017-0827-z

Needham, L.L., Calafat, A.M., Barr, D.B., 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. https://doi.org/10.1016/j.ijheh.2006.11.002

Nishijo, M., Nambunmee, K., Suvagandha, D., Swaddiwudhipong, W., Ruangyuttikarn, W., Nishino, Y., 2017. Gender-Specific Impact of Cadmium Exposure on Bone Metabolism in Older People Living in a Cadmium-Polluted Area in Thailand. Int. J. Environ. Res. Public Health 14, 401. https://doi.org/10.3390/ijerph14040401

Nordberg, G.F., Bernard, A., Diamond, G.L., Duffus, J.H., Illing, P., Nordberg, M., Bergdahl, I.A., Jin, T., Skerfving, S., 2018. Risk assessment of effects of cadmium on human health (IUPAC Technical Report), Pure and Applied Chemistry. https://doi.org/10.1515/pac-2016-0910

Nordberg, G.F., Nogawa, K., Nordberg, M., 2015. Cadmium, in: Handbook on the Toxicology of Metals. Elsevier, pp. 667–716.

Olgun, O., Bahtiyarca, Y., 2015. Effects of Dietary Cadmium and Boron Supplementation on Performance, Eggshell Quality and Mineral Concentrations of Bone in Laying Hens. Biol. Trace Elem. Res. 167, 56–62. https://doi.org/10.1007/s12011-015-0291-x

Quraishi, S.M., Adams, S. V., Shafer, M., Meliker, J.R., Li, W., Luo, J., Neuhouser, M.L., Newcomb, P.A., 2016. Urinary cadmium and estimated dietary cadmium in the Women's Health Initiative. J. Expo. Sci. Environ. Epidemiol. 26, 303–308. https://doi.org/10.1038/jes.2015.40

Renieri, E.A., Safenkova, I. V., Alegakis, A., Slutskaya, E.S., Kokaraki, V., Kentouri, M., Dzantiev, B.B., Tsatsakis, A.M., 2019. Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2018.12.020

Renieri, E.A., Sfakianakis, D.G., Alegakis, A.A., Safenkova, I. V., Buha, A., Matović, V., Tzardi, M., Dzantiev, B.B., Divanach, P., Kentouri, M., Tsatsakis, A.M., 2017. Nonlinear responses to waterborne cadmium exposure in zebrafish. An in vivo study. Environ. Res. 157, 173–181. https://doi.org/10.1016/j.envres.2017.05.021

Satarug, S., 2018. Dietary Cadmium Intake and Its Effects on Kidneys. Toxics 6, 15. https://doi.org/10.3390/toxics6010015

Satarug, S., Vesey, D.A., Gobe, G.C., 2017. Current health risk assessment practice for dietary cadmium: Data from different countries. Food Chem. Toxicol. 106. https://doi.org/10.1016/j.fct.2017.06.013

Satarug, S., Vesey, D.A., Gobe, G.C., 2017. Current health risk assessment practice for dietary cadmium: Data from different countries. Food Chem. Toxicol. 106. https://doi.org/10.1016/j.fct.2017.06.013

Silva, N., Peiris-John, R., Wickremasinghe, R., Senanayake, H., Sathiakumar, N., 2012. Cadmium a metalloestrogen: Are we convinced? J. Appl. Toxicol. https://doi.org/10.1002/jat.1771

Slob, W., 2002. Dose-Response Modeling of Continuous Endpoints. Toxicol. Sci. 66, 298–312. https://doi.org/10.1093/toxsci/66.2.298

Sun, L., Vukicevic, S., Baliram, R., Yang, G., Sendak, R., McPherson, J., Zhu, L.-L., Iqbal, J., Latif, R., Natrajan, A., Arabi, A., Yamoah, K., Moonga, B.S., Gabet, Y., Davies, T.F., Bab, I., Abe, E., Sampath, K., Zaidi, M., 2008. Intermittent recombinant TSH injections prevent ovariectomy-induced bone loss. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.0712395105

Świergosz-Kowalewska, R., 2001. Cadmium distribution and toxicity in tissues of small rodents. Microsc. Res. Tech. 55, 208–222. https://doi.org/10.1002/jemt.1171

Van Maele-Fabry, G., Lombaert, N., Lison, D., 2016. Dietary exposure to cadmium and risk of breast cancer in postmenopausal women: A systematic review and meta-analysis. Environ. Int. https://doi.org/10.1016/j.envint.2015.10.003

Wallace, D., Spandidos, D., Tsatsakis, A., Schweitzer, A., Djordjevic, V., Djordjevic Buha, A., 2019. Potential interaction of cadmium chloride with pancreatic mitochondria: Implications for pancreatic cancer. Int. J. Mol. Med. 1–12. https://doi.org/10.3892/ijmm.2019.4204

Wallin, M., Barregard, L., Sallsten, G., Lundh, T., Karlsson, M.K., Lorentzon, M., Ohlsson, C., Mellström, D., 2016. Low-Level Cadmium Exposure Is Associated With Decreased Bone Mineral Density and Increased Risk of Incident Fractures in Elderly Men: The MrOS Sweden Study: LOW-LEVEL CADMIUM EXPOSURE AND FRACTURES. J. Bone Miner. Res. 31, 732–741. https://doi.org/10.1002/jbmr.2743

World Health Organisation (WHO), 2011. Cadmium in drinking water. Geneva, WHO.

Wu, Q., Magnus, J.H., Hentz, J.G., 2010. Urinary cadmium, osteopenia, and osteoporosis in the US population. Osteoporos. Int. 21, 1449–1454. https://doi.org/10.1007/s00198-009-1111-y

Zhu, L.-L., Blair, H., Cao, J., Yuen, T., Latif, R., Guo, L., Tourkova, I.L., Li, J., Davies, T.F., Sun, L., Bian, Z., Rosen, C., Zallone, A., New, M.I., Zaidi, M., 2012. Blocking antibody to the -subunit of FSH prevents bone loss by inhibiting bone resorption and stimulating bone synthesis. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.1212806109