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Cadmium Osteotoxicity in Experimental Animals: Mechanisms and Relationship to Human Exposures

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Abstract

Extensive epidemiological studies have recently demonstrated increased cadmium exposure correlating significantly with decreased bone mineral density and increased fracture incidence in humans at lower exposure levels than ever before evaluated. Studies in experimental animals have addressed whether very low concentrations of dietary cadmium can negatively impact the skeleton. This overview evaluates results in experimental animals regarding mechanisms of action on bone and the application of these results to humans. Results demonstrate that long-term dietary exposures in rats, at levels corresponding to environmental exposures in humans, result in increased skeletal fragility and decreased mineral density. Cadmium-induced demineralization begins soon after exposure, within 24 hours of an oral dose to mice. In bone culture systems, cadmium at low concentrations acts directly on bone cells to cause both decreases in bone formation and increases in bone resorption, independent of its effects on kidney, intestine, or circulating hormone concentrations. Results from gene expression microarray and gene knock-out mouse models provide insight into mechanisms by which cadmium may affect bone. Application of the results to humans is considered with respect to cigarette smoke exposure pathways and direct vs. indirect effects of cadmium. Clearly, understanding the mechanism(s) by which cadmium causes bone loss in experimental animals will provide insight into its diverse effects in humans. Preventing bone loss is critical to maintaining an active, independent lifestyle, particularly among elderly persons. Identifying environmental factors such as cadmium that contribute to increased fractures in humans is an important undertaking and a first step to prevention.

Keywords

cadmium; bone; skeleton; osteoporosis; osteoblast; osteoclast; gene expression; mouse; rat; organ culture; cell culture; environmental exposure; postmenopausal women; pregnancy; lactation

Bone loss in experimental animals

Studies of bone loss in cadmium-exposed experimental animals initially were conducted in the context of proteinuria, renal calcium stones, and bone fractures, which were reported among persons exposed to high levels of cadmium either in industry or via the contaminated rice pathway in Japan that led to skeletal demise in Itai-Itai disease patients. Urine cadmium concentrations among these populations were high, in range of 20–30 µg Cd/g creatinine (Ezaki et al., 2003).

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More recently, extensive epidemiological studies have been conducted that evaluate effects of much lower levels of cadmium exposure via environmental routes, for example among populations in Belgium, Sweden, China, Japan, and the United States (Alfvén et al., 2000, 2002, 2004; Honda et al., 2003; Kazanzis, 2004; Järup and Alfvén, 2005; Åkesson et al., 2006; Qian et al., 2006; Bernard, 2008; Gallagher, 2008; Schutte et al., 2008; Engström et al., 2009; Järup and Åkesson, 2009). These studies provide repeated demonstrations of increased cadmium exposure correlating significantly with decreased bone mineral density (BMD) and increased fracture incidence at lower exposure levels than ever before evaluated. Persons in these populations are exposed to environmental cadmium due to the proximity of their homes to cadmium-related industries, including zinc smelters or nickel-cadmium battery plants. Urine cadmium concentrations are in range of 1–2 µg Cd/g creatinine, about 10-fold below those in the more highly exposed Itai-Itai population mentioned above.

A number of investigators have successfully developed animal models for the extreme bone loss of Itai-Itai disease (e.g., Fujiwara, 1980; Katsuta et al., 1994; Li et al., 1997; Umemura, 2000; Wang et al., 1994). However, more recent studies with experimental animals have also addressed the question of whether very low concentrations of dietary cadmium can negatively impact the skeleton. Ogoshi and colleagues (1989) were among the first to report a clear decrease in mechanical strength of bones in weanling rats after only 4 weeks of exposure to 5 or 10 µg Cd/ml in the drinking water – exposure levels that give urine cadmium concentrations in range of values for persons who smoke cigarettes.

Brzóska and her colleagues have conducted many studies in rats designed to model human exposures to cadmium (Brzóska et al., 2004, 2005a, 2005b, 2005c, 2007, 2008; Brzóska and Moniuska-Jakoniuk 2004a, 2004b, 2005a, 2005b). For example, after exposure to cadmium at only 1 µg Cd/ml in drinking water from weaning to 24 months of age, the lifetime-exposed rats had lumbar vertebrae that were demineralized and decreased in mechanical strength compared to controls (Fig. 1) (Brzóska and Moniuska-Jakoniuk 2004a, 2004b). As indicated this figure, peak vertebral bone mineral density was lower in the exposed females at 12 months, and cadmium effects grew with time through 24 months. Consequently, lifetime cadmium exposure strikingly increased the skeletal involution that occurred naturally in control rats during 18 to 24 months of age. However, urine calcium excretion was increased about 2-fold even at 3 months, the first time point in the study, and remained significantly above control levels to 24 months. The fractional excretion of calcium (FECa) was also increased, meaning the kidneys were excreting a greater fraction of filtered Ca. Blood cadmium in the exposed rats at 24 months was only 0.79 ng Cd/ml, compared to 0.47 ng Cd/ml in controls, while urine Cd concentrations were 3- to 6-fold higher than in controls. This little bit of extra cadmium resulted in decreased bone mass (total skeleton, L1–L5, femur) and increased frequency of osteopenia and osteoporosis in the elderly females.

Regarding age effects, Ogoshi and colleagues (1989, 1992) showed that cadmium-induced skeletal damage is significantly greater when exposure occurs during the rapid growth phase in weanling rats than during adulthood, and that skeletons were compromised when bone cadmium concentrations reached 100–200 ng Cd/g bone in both age ranges. These bone cadmium concentrations are in range although somewhat higher than those reported by Brzóska in rat bones that were weakened by cadmium exposure (40–120 ng Cd/g bone) (Brzóska et al., 2005a).

When applied to humans, the reports of Brzóska and colleagues have extensive potential implications, in that rats reached somewhat less than their full peak bone mass as adults and experienced more extensive involution of the skeleton late in life (Fig. 1) when they had dietary cadmium exposures that resulted in end-of-life blood cadmium concentrations of 0.79 ng Cd/ml. Many humans world-wide have blood cadmium concentrations in this range. The current

convergence of data in both humans and experimental animals makes it critical that the possibility be more fully addressed that lifetime skeletal development in a large number of humans is potentially being compromised by their lifetime, low-level exposures to cadmium.

Increased Susceptibility in Females

Brzóska and Moniuska-Jakoniuk have demonstrated that young male rats, while susceptible to cadmium-induced bone loss, are less susceptible than young females (Brzóska and Moniuska-Jakoniuk, 2005b). In addition, however, a number of investigators have asked whether the particular experiences of multiparity or ovariectomy increase susceptibility of the female skeleton to cadmium-induced bone changes (Watanabe et al., 1986; Bhattacharyya et al., 1988a, 1988b; Bhattacharyya et al., 1992; Sacco-Gibson et al., 1992; Uriu et al., 2000; Umemura, 2000; Ohto et al., 2002). This line of investigation stems from the fact that Itai-Itai disease in Japan was not observed in the entire population. Instead, ~95% of cases were postmenopausal women (~50 to 70 years of age) who had birthed an average of 6 children (Nogawa, 1981).

Some studies, because of high exposure levels, apply more to the etiology of Itai-Itai disease than to bone changes at lower human exposure levels. However, results of all studies, independent of cadmium level, clearly demonstrate that the female skeleton sustains increased bone loss from cadmium exposure during states of increased bone turnover, either during pregnancy and lactation, when maternal bone is mobilized to build bone in the offspring, or after menopause, when turnover increases under low estrogen conditions. As one example, the femurs of mouse dams exposed to cadmium at 5 µg Cd/g diet lost 20% of their calcium content during a first 40-day period of pregnancy and lactation, while the femurs of non-exposed dams lost none (Bhattacharyya et al., 1988a). Furthermore, there remained a significant dose-dependent reduction in maternal femur calcium content through additional reproductive cycles, and this effect of cadmium was clearly greater in the dams than in the non-pregnant controls. The extensive net loss of bone calcium during the first reproductive cycle occurred over a short period of time and at a cadmium exposure level (5 µg Cd/g in diet) anticipated to produce blood cadmium concentrations not far different from those among women who smoke one pack of cigarettes per day (Brzóska et al., 2005a). In another study, microradiographs of thoracic vertebral cross-sections showed the extreme fragility of vertebrae from cadmium-exposed ovariectomized mice, showing bone loss far greater than due to ovariectomy or cadmium exposure alone. (Fig. 2).

Time-course of Ca release from bone

In 1978, Ando and colleagues, using ⁴⁵Ca isotope administration to rats, first reported that cadmium increases calcium excretion in rats within the first few days of exposure, well before the onset of kidney damage leading to aminoaciduria and proteinuria. Ten years later, a second study using calcium isotope technology reported a similar finding in mice (Bhattacharyya et al., 1988b). The latter report demonstrated that cadmium-induced release of calcium from bone into excreta occurred within 96 hr of the start of dietary cadmium exposure, and that the extent of release was greater in ovariectomized mice than in sham-operated controls. A more detailed examination of the early kinetics of calcium release from bone was conducted in mice on a low calcium diet (Wilson and Bhattacharyya, 1997). In these mice, the cadmium effect could be determined without use of a calcium isotope, because baseline fecal calcium levels were low enough and stable enough to allow measurement of a cadmium-induced rise in stable calcium concentrations in feces over time. Note that a separate study had shown that the calcium excreted in feces after cadmium exposure was bone-derived (Wang and Bhattacharyya, 1993). As shown in Fig. 3, increased release of calcium from bone to feces had not started during the first 8 h, but showed a striking transient rise during 8–48 hours after a single gavage

administration of 200 µg cadmium to CF1 mice. This dose produced a bone response starting at 2–5 ng Cd/ml blood (Wilson and Bhattacharyya, 1997), a concentration range that is below the OSHA standard of 5 ng/ml for industrial exposures (USOSHA92) and is at the upper end of blood cadmium concentrations reported for smokers (Elinder et al., 1983; Pocock et al., 1988; this report Fig. 6).

Direct effects of Cd in bone organ and cell culture systems

Many investigators have reported direct effects of cadmium on bone organ and cell culture systems. For example, at concentrations as low as 10 nM (1 ng Cd/ml) – in range of blood cadmium concentrations in persons who smoke cigarettes, cadmium caused dramatic demineralization of fetal rat limb bones (FRLB) during 60h of culture (Fig. 4) (Bhattacharyya et al., 1988b). Beginning at 300 nM, Miyahara showed a smaller but still significant demineralization response in this FRLB system (Miyahara et al., 1980a, 1980b), while Cd at 1 µM caused no bone loss but instead inhibited parathyroid-hormone stimulated bone loss (Bhattacharyya et al., 1988b). Together these results indicate that the FRLB system has a biphasic response to cadmium, demineralizing more extensively at low nM concentrations. In cultures of the intramembranous calvarial or parietal bones, cadmium at 250–500 nM, but not at 100 nM, caused a 2- to 3-fold increase in the release of ⁴⁵Ca from prelabeled bones (Suzuki et al., 1989, 1990; Miyahara et al., 1992). Taken together, these results indicate that different bone types may differ in their sensitivity to Cd-induced degradation.

Regarding effects on bone formation in organ cultures, cadmium at 2 to 20 µM caused a striking inhibition of bone formation in cultured chick embryonic long bones, expressed by decreases in bone length, hydroxyproline synthesis, and accumulation of collagen, along with a degeneration of osteogenic mesenchymal cells and osteoblasts (Miyahara et al., 1978, 1983; Kaji et al., 1988a, 1988b, 1990). Similarly, in a mouse osteoblast-like cell line, MC3T3-E1, 0.1 to 1 µM Cd decreased cellular alkaline phosphatase activity, a marker of osteoblast differentiation; at 1 to 2 µM, cadmium decreased intracellular ⁴⁵Ca accumulation and decreased calcified nodule formation (Miyahara et al., 1988; Iwami and Miriyama, 1993). At the latter concentrations, Miyahara and colleagues showed an increase in prostaglandin E2 secretion by MC3T3-E1 cells, mediated by induction of cytosolic phospholipase 2 and cyclooxygenase 2, two enzymes central to PGE2 synthesis from phospholipid-derived arachidonic acid (Miyahara et al., 2001, 2004). Osteoblast-mediated secretion of PGE2 can in turn stimulate formation and activation of osteoclasts, leading to osteoclast-mediated bone resorption. Therefore, at low micromolar concentrations, cadmium-exposed osteoblast-like cells decrease their bone forming activity and at the same time secrete a substance that stimulates formation of bone-resorbing cells. These effects, taken together, could clearly cause an uncoupling of the normal balance between bone formation and bone resorption.

Regarding effects on osteoclasts in culture, cadmium at 10 to 100 nM has been shown to stimulate the formation of osteoclast-like multinuclear cells from progenitors in bone marrow cultures (Konz et al., 1989; Bhattacharyya, 1991; Miyahara et al., 1991; Wilson et al., 1996) and increase the numbers and bone-degrading activity of multinuclear osteoclasts formed by the fusion of mononuclear precursors in primary rat osteoclast cultures (Wilson et al., 1996).

In overview, the lower cadmium concentrations that affect osteoclast pathways (10–500 nM) vs. osteoblast pathways (0.1–20 µM) in bone organ and cell culture systems indicate that osteoclast formation, activation, and bone-degrading activities may be more sensitive to stimulation by cadmium than are the osteoblast processes leading to decreased bone formation. Ultimately, however, net bone loss will only occur when cadmium stimulates bone resorption or decreases bone formation in such a way that the normal balance between these two processes become uncoupled.

Cd pathways in bone based on gene expression microarray

Many investigators have evaluated gene expression pathways involved in the normal processes of osteoclast-mediated bone resorption and osteoblast-mediated bone formation, unaffected by toxins. A number have also applied gene expression microarray techniques to evaluate cadmium-induced changes in non-bone cell systems (Koizumi and Yamada, 2003; Martinez-Campa et al., 2006; Badisa et al., 2008; Dakeshita et al., 2009). However, very few have applied these techniques to study cadmium effects in bone cells. This approach does, however, provide a way to identify mechanisms that are not necessarily related to known effects of cadmium.

For example, Ohba and colleagues (Ohba et al., 2007) used an Affymetrix protocol and a rat genome array to analyze RNA extracted from the marrow-free femurs of cadmium-exposed rats. Four weeks after a 6-week protocol of daily oral cadmium (5 mg/kg/d), the RNA from femurs of exposed rats showed 13 genes upregulated (3- to 5-fold) and 10 genes down-regulated (3- to 10-fold) compared to controls, out of 28,734 genes evaluated. Among the upregulated genes was the gene for Ssp1, a non-collagenous bone matrix protein with several functions, including bone remodeling, angiogenesis, cell adhesion, and tumor migration (Merry et al., 1993). Further studies would be required to identify the role of Ssp1 in bone changes brought about by continuous oral exposure to cadmium. Until now, however, this protein has not been connected to known effects of cadmium on bone.

In another study, changes in bone cell gene expression in the mouse model described in Figure 3 were determined by cDNA microarray analysis (Regunathan et al., 2003). RNA was prepared from marrow-free femurs and tibiae at 2h and 4h after a single oral cadmium gavage (200 µg/mouse). These time points are well before the start of calcium release from bone, which starts at ~8h in this model (Wilson and Bhattacharyya, 1997); blood cadmium concentrations were ~2 ng/ml, relevant to cadmium concentrations in persons who smoke cigarettes. Mouse strains were CF1, SvJ-Metallothionein-Normal (MTN), and SvJ-Metallothionein1,2-deficient (MT1,2KO). The bone responses by mouse strain, detected by a rise in fecal calcium excretion (as in Fig. 3), were in the order expected, i.e., the MT1,2KO mice, which lack the cellular protection provided by the cadmium-induced metal-binding proteins MT1 and MT2, showed a 2-fold increase in the release of bone calcium compared to the MTN controls (Bhattacharyya et al., 1998).

Microarray results demonstrated that only ~15 genes were increased significantly by cadmium in all 4 microarrays or in 3 of the 4 arrays (Table 1); no genes showed analogous decreases. Changes in 16 genes tested by Northern analyses all verified the microarray results (Table 1). Study of the functions of the genes in Table 1 support the hypothesis that cadmium begins by stimulating osteoclast-mediated bone resorption and protecting the cells by inducing MT1 and MT2. For example, the vacuolar proton pump subunit, integrin α V, transferrin receptor, aquaporin, and p38 MAPK are proteins known to be integral to osteoclast formation and activity, while induction of acidic chitinase, an acidic glycoprotein hydrolase not currently identified as being associated with osteoclasts, is consistent with the need to break down prominent bone matrix glycoproteins, such as osteopontin, in the acidic environment of osteoclast-mediated bone resorption.

The most highly induced message is that of Cyr61, also called Ccn1. Ccn1 is an extracellular matrix protein that specifically binds integrin heterodimers, including integrin α V β 3. It is secreted by osteoblasts in response to estrogen and 1,25(OH)₂ vitamin D. Researchers have shown that adherent conditions, such as those provided by matrix proteins that bind osteoclast precursors via the α V β 3 integrin monomers, are required for formation of multinucleated osteoclasts (Miyamoto et al., 2000). Results of this gene expression microarray study suggest

that CCN1 may stimulate osteoclast-mediated bone resorption by promoting adhesion of osteoclasts or their integrin $\alpha V\beta 3$ -rich precursors to the bone matrix.

Role of *fos* and *src* in Cd-induced bone loss

The bones of mice that lack the *fos* gene contain very few osteoclasts, because the Fos protein is required for formation of osteoclasts from their monocyte precursors (Johnson et al., 1992; Wang et al., 1992; Gigoriadis et al., 1994). Bones of *src*-deficient mice contain osteoclasts, but they fail to resorb bone, because the Src protein is required for osteoclast activation (Soriano et al., 1991; Boyce et al., 1992). Both *fos*^{-/-} and *src*^{-/-} mice have dense osteopetrotic bones that cannot remodel normally due to a lack of active osteoclasts; they also are toothless because active osteoclasts are required for tooth eruption.

An experiment designed to ask whether cadmium can cause bone loss in *fos*^{-/-} and/or *src*^{-/-} mice demonstrated that cadmium stimulates bone loss in mice via a pathway independent of cFos but requires cSrc for its action on bone (Regunathan et al., 2002). In fact, bone loss *in vivo* was the same in wild type and *fos*^{-/-} mice, with several of the *fos*^{-/-} mice erupting teeth, and showing osteoclast-like cells (Fig. 5) and signs of bone remodeling in the densely mineralized femur shaft. These results imply that cadmium may stimulate osteoclast formation via a Fos-independent pathway. However, the bone loss response to cadmium requires active osteoclasts, which the *src*^{-/-} mice do not have.

Application of results from experimental animals to humans

Cigarettes as a source of cadmium exposure

Many investigators have demonstrated that cigarettes are a major source of cadmium exposure (Elinder et al., 1983; Pocock et al., 1988). Although people who smoke ingest much more cadmium from the grains and leafy vegetables in their diet than they inhale via cigarette smoke, the intestines are a much better barrier against cadmium transfer to blood than are the lungs. For example, in a recent study of postmenopausal women who smoke cigarettes, blood cadmium concentrations for the majority of the population were directly proportional to the cadmium that they inhaled from their cigarettes (Fig 6). Note that inhaled cadmium was determined with a smoking machine and varied with the type of cigarette and fraction of cigarette length typically smoked by each individual, in addition to the number of cigarettes smoked per day. Interesting was the finding that about one-third of the women who smoked had much higher blood cadmium concentrations than predicted from the relationship of blood cadmium to inhaled cadmium defined by the others. The possibility exists that their lungs or GI tracts had become more permeable to cadmium than in the other women. In any case, the cause appears to be related to smoking, as none of the non-smokers showed excursions in blood cadmium similar to those in the women who smoke.

Key to these results is the fact that blood cadmium concentrations ranged from 0.6 ng/ml to 2.7 ng/ml, in range of those that resulted in striking skeletal involution late in the lives of female rats that were exposed to low levels of cadmium in the studies of Brzóska and colleagues reviewed at the start of this article. The possibility needs to be considered that the cadmium component of cigarettes is sufficient to increase bone loss in postmenopausal women who smoke, indicating that if cadmium were decreased in tobacco leaves via restrictions on cadmium in fertilizers, one environmental cause of osteoporosis might be removed. Additional research is required, however, to determine whether the cadmium component in cigarettes is sufficient to cause bone loss in persons who smoke.

Direct vs. indirect effects of cadmium on bone

Results reviewed in this article demonstrate unequivocally that cadmium can act directly on bone cells in bone organ and cell culture systems to decrease bone formation and increase bone resorption. The low concentration of cadmium that causes fetal rat limb bones to demineralize in culture (10 nM, i.e., 1 ng Cd/ml) (Fig. 5) is directly in range of blood cadmium concentrations among persons environmentally exposed to cadmium (e.g. Figure 6). These results strongly support the hypothesis that cadmium, at low environmental levels of exposure in humans, can act directly on bone cells, in the absence of other effects, to cause loss of skeletal integrity in the form of decreased mineral density and increased risk of fracture.

As many toxins do, however, cadmium affects many organ systems, and a number of them can indirectly affect bone. The question therefore is being asked whether or not bone loss responses to cadmium that are documented in humans are solely the result of cadmium having a direct effect on bone.

Regarding cadmium-induced renal damage, the severe osteoporosis and osteomalacia with multiple fractures among persons with Itai-Itai disease in Japan was most certainly a result of both direct effects of cadmium on bone and indirect effects via the kidney; both organs were severely damaged. However, at lower levels of exposure, investigators report bone effects independent of kidney effects in experimental animals and humans (Bhattacharyya et al., 1988c; Honda et al., 2003; Schutte et al., 2008), while others report that skeletal effects in humans do not occur in the absence of kidney damage (Horiguchi et al., 2005).

On the basis of human data, including benchmark dose modeling, investigators have concluded that, at low environmental exposures that include urine cadmium concentrations of 1–2 µg Cd/g creatinine, an increase occurs in the risk of persons showing increased urinary excretion of low-molecular weight proteins indicative of cadmium-induced renal tubular dysfunction, including increased excretion of β 2-microglobulin, α 1-microglobulin, retinol binding protein, and N-acetyl β -D glucosaminidase (Buchet et al., 1990; Jarup et al., 2000; Uno et al., 2005; Aoshima et al., 2006). These cadmium effects themselves, however, are of little clinical significance except as potential indicators of greater kidney damage in the future, and no mechanistic link has been made between early tubular low-molecular-weight proteinuria and pathways leading to bone loss. Consequently, current results support the interpretation that decreases in mineral density and mechanical strength from long-term, low-level cadmium exposures most likely result from bone effects of cadmium independent of effects on the kidney.

Regarding cadmium-induced hormone disturbances, for example those affecting vitamin D metabolic pathways, Engström and colleagues recently demonstrated that the cadmium-related bone changes observed among postmenopausal women in Sweden occur with no change in circulating concentrations of 1,25(OH)₂ vitamin D (Engström et al., 2009). These results are supported by those of Bhattacharyya and colleagues in dogs, where a cadmium-induced release of calcium from bone occurred in the absence of changes in circulating concentrations of 1,25(OH)₂ vitamin D, parathyroid hormone, and calcitonin (Sacco-Gibson et al., 1992). Regarding estrogen hormone pathways, cadmium at low micromolar concentrations has been shown to activate the estrogen receptor (ER) in cultured MCF7 breast cancer cells (Martínez-Campa et al., 2006). Furthermore, cadmium binds with a very high affinity to the isolated ER, with an equilibrium dissociation constant of 0.4–0.5 nM Cd for purified human ER- α ; cadmium also blocks the binding of estrogen to its receptor *in vitro* with a K_i of 0.3 nM Cd (Stoica et al., 2000). Because of the high affinity of cadmium for the hormone binding domain of the ER, exposure levels at which cadmium as a metallo-estrogen might indirectly affect the skeleton by altering estrogen pathways need to be explored.

Clearly, understanding the mechanism(s) by which cadmium causes bone loss in experimental animals will provide a better understanding of the diverse effects of cadmium in humans and provide a basis for countering its adverse effects. Preventing bone loss is critical to maintaining an active, independent lifestyle, particularly among elderly persons. Identifying environmental factors such as cadmium that contribute to bone loss and increased fractures in humans is an important undertaking and a first step to prevention.

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Figure 1.

Effects of lifetime exposure to low level Cd in drinking water (1 mg/L) on appendicular skeleton of female rats. ABOVE: Changes in BMD of lumbar vertebrae (L1–L5) with age. Note that Cd differences from controls increase with age, especially during 18–24 months (bdata from Brzóška and Moniuska-Jakoniuk 2004b). BELOW: Radiographs of fourth lumbar vertebral body (L4) from female rats represented in above graph. Note the biconcave fracture in B and fractures with a clearly evident disruption of bone continuity in C and D (a data from Brzóška and Moniuska-Jakoniuk, 2004a).

Figure 2.

Microradiographs of cross sections of thoracic vertebrae from cadmium-exposed mice 6 months after ovariectomy (OV). White portions show calcified parts of vertebral cross sections. Mice were exposed to 50 ppm Cd in diet, relevant to Itai-Itai exposures. Each sectioned bone came from a mouse whose lumbar vertebrae had a calcium content identical to the mean for the group. The low calcium content of bones from the OV+Cd group is clearly visible (data from Bhattacharyya et al., 1988b)

Figure 3. Effects of cadmium on fecal calcium excretion after a single gavage dose of cadmium (200 µg Cd) to adult mice on a low-calcium diet

The length of each horizontal line depicts the time span of the fecal collection. Its vertical position shows the mean fecal calcium excretion for that collection; the vertical bar gives the standard error ($n=6-18$). The four dashed horizontal lines after the Cd gavage provide results for the Cd-exposed mice; the four solid lines show results for no-Cd controls. In clear contrast to the controls, Cd-gavaged mice experience a sharp transient rise in fecal calcium excretion, which starts after the first 8h collection and peaks between 8 and 48 hours after Cd. An earlier publication (Wang and Bhattacharyya, 1993) demonstrates that this fecal calcium comes from bone. (Figure from Wilson and Bhattacharyya, 1997).

Figure 4. Photomicrograph of fetal rat limb bones cultured with or without 10 nM Cd
On day 19 of gestation in rat dams, midshafts of the forelimb bones were dissected from fetal rats and incubated in modified BGJ medium containing 0.1% bovine serum albumin under 5% CO₂/95% air, either without Cd (left) or with 10 nM Cd as CdCl₂ (right). After 60h of incubation, photomicrographs were obtained to document changes in the limb bones. At left, control bone shows intact midshaft with beginning stages of mineralization. At right, cadmium at 10 nM stimulated extensive degradation of the midshaft, leaving only the cartilaginous ends. This extent of degradation was the same as in cultures with 10 nM parathyroid hormone. Note that actual limb bone length is ~1 mm. (unpublished photomicrographs from experiments published in Bhattacharyya et al., 1988b)

Figure 5. Multinucleated osteoclast-like cell associated with osteopetrotic bone of a 45-d-old *fos*^{-/-} mouse

Sections (5 μ m thick) were stained with von Kossa with tetrachrome. Black areas are calcified bone. Mouse was continuously exposed to cadmium beginning at age 17 days (10 then 20 μ g Cd/ml drinking water). Note multiple nuclei present in circled cell. (from Regunathan et al., 2003).

Figure 6. Relationship between blood cadmium concentration and amount of cadmium inhaled via cigarette smoke in 20 postmenopausal women

Inhaled cadmium was determined for each woman's cigarettes using a smoking machine. Total inhaled cadmium varied with type of cigarette and smoking habits (number of cigarettes per day, fraction of cigarette length typically smoked). The line intersects the ordinate at the blood cadmium value for non-smokers in this study. Although data from 2/3 of the women define this line, 1/3 of the women who smoke had blood cadmium concentrations much higher than predicted by the line. (from Bhattacharyya MH and Ebert-McNeill A, unpublished results).

Table 1

Fifteen Genes Most Highly and Consistently Increased in Expression in Bone Cells by Cadmium^a

Rank	Gen Bank ID	Role in Hypothesis?	Gene Name	Cd+/Cd- Expression Ratio				Sum of Four Cd+/Cd- Expression Ratios	Northern Blots ^c CF1, 4h			M Ratio	
				CF 1 2h	CF 1 4h	MT1,2K O 4h	MTN 4h		-Cd	N Ratio			
											≥1.3		≥1.4
Genes with Cd+/Cd- expression ratios ≥ cut-off value in all four groups													
1	AA466852	yes	Cysteine rich protein 61	1.7	2.0	2.8	2.6	9.1		4.1	2.0		
2	W36474	yes	Metallothionein 2 ^b	1.6	1.7	2.7	2.8	8.8		1.8	1.7		
3	AA413490	yes	Transferrin receptor	1.7	2.1	1.4	3.5	8.7		3.2	2.1		
4	AI019469	--	Glutamine synthetase pseudogene 1	1.4	1.7	2.1	3.0	8.2		1.7	1.7		
5	AA638765	yes	Metallothionein 1 ^b	1.4	1.6	2.6	2.2	7.8		1.5	1.6		
6	AA612413	yes	Chitinase, acidic	1.3	1.8	1.9	2.7	7.7					
7	AI390138	--	RIKEN cDNA 3930401B19 gene	1.4	2.0	1.9	2.2	7.5					
8	AA866683	--	Period homolog (Drosophila)	1.4	1.5	2.2	2.1	7.2					

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Rank	Gen Bank ID	Role in Hypothesis?	Gene Name	Cd+/Cd− Expression Ratio				Sum of Four Cd+/Cd− Expression Ratios	Northern Blots ^c CF1, 4h			
				CF 1 2h	CF 1 4h	MT1,2K O 4h	MTN 4h		−Cd	N Ratio	M Ratio	
				≥1.3	≥1.4	≥1.4	≥1.6					
9	AA867150	yes	Src-like adapter protein	1.5	1.4	1.9	2.2	7.0			2.2	1.4
Genes with Cd+/Cd− expression ratios ≥ cut-off value in three of four groups (except for 12)												
10	AA068612	yes	Vacuolar proton pump ATPase, H+ transporting, lysosomal, alpha 70 kDa, isoform 2	1.5	2.2	1.2	2.0	6.9			2.3	2.2
11	AA821980	--	Serine protease inhibitor 2-2	1.2	1.3	2.3	2.1	6.9				
12	AA413794	--	RIKEN cDNA 4930569K13 gene	1.7	2.1	1.3	1.7	6.8				
13	AA547343	yes	Integrin αV	1.6	2.2	1.0	1.6	2.2			3.5	2.2
14	AA241281	yes	Aquaporin 1	1.6	1.5	1.3	2.3	6.7				
15	AA549577	yes	p38 MAP kinase (MAPK 14)	1.5	1.6	1.3	2.2	6.6			2.1	1.6

⁴Data from Regunathan et al., 2003. Genes are listed from high to low according to the sum of four the Cd+/Cd− expression ratios (last column). This approach identifies genes that responded to Cd to the greatest extent and most consistently across the mouse groups. An independent cut-off value was also calculated for each microarray. The cut-off value is the (mean + 2xSD) of the ratios of all genes that have Cd+/Cd− expression ratios ≥ 1.0. These cut-off values, shown in bold at the top of each expression ratio column, provide a measure of whether a given gene is significantly increased in expression by cadmium. The values account for individual variability among ratios for a given microarray. Cd+/Cd− expression ratios that were equal to or greater than the cut-off value are shown in bold font in Columns 5–8.

⁵It should be noted that, contrary to what might be expected, mRNA expression signals for MT1 and MT2 were present in bone cells of the Cd− MT1,2KO mice. These results are the same as those reported by the creators of this KO mouse strain and totally consistent with the nature of the knockout mouse, which has multiple in-frame stop codons in the MT1 and MT2 genes and shows normal levels of MT mRNA but no MT protein. Confirmation via microarray results of this characteristic of MT mRNA expression levels in the MTN vs. MT1,2KO mice gives added validity to the microarray findings.

⁶N Ratio = ratio from Northern blot analysis. M Ratio = ratio from Microarray analysis