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

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In this study, based on a rat model of human environmental exposure to cadmium (Cd), it has been examined whether low-level lifetime Cd exposure increases the risk of vertebral osteoporosis and vertebrae fractures in the elderly. For this purpose, the lumbar vertebral bodies (L4 or L3) of control and Cd-exposed (1 mg Cd/l in drinking water for 24 months) female Wistar rats were assigned to densitometric, radiographic, biomechanical (compression test), and biochemical studies, as well as to assess their dimensions and chemical composition. The exposure to Cd affected the mineral status of the L4. The decreased mineral content, density (BMD) and bone mineral area of the vertebral body together with the unchanged ratio of non-organic and organic components indicate osteoporotic nature of the Cd-induced changes. The activity of alkaline phosphatase in the L3 decreased. Cd also influenced the mechanical properties of the L4. The yield load and ultimate load decreased indicating a weakness in the vertebral body compression strength. Stiffness of the L4 decreased and the displacement at ultimate increased suggesting its enhanced susceptibility to deformities. Indeed, in the Cd group vertebral deformities (in 30% of females) or even fractures (in 40% of females), including those with disruption of bone continuity were evident. Z-score values for the L4 BMD revealed vertebral osteopenia in 30% and osteoporosis in 70% of the Cd-exposed females. The results allow for the conclusion that low lifetime exposure to Cd may become an important factor increasing the risk of lumbar spine osteoporosis with vertebral deformities and fractures in the elderly.

Key Words: cadmium; vertebral body; bone mineral status; mechanical testing; vertebral fracture; rat.

Cadmium (Cd) is one of the most toxic heavy metals, an environmental and occupational pollutant endangering human and animal health (Järup, 2002; Satarug et al., 2003). It has been suggested that Cd may be an environmental risk factor for osteoporosis (Alfvén et al., 2000; Goyer et al., 1994; Honda et al., 2003; Järup et al., 1998; Wang et al., 2003). The latest epidemiological data (Alfvén et al., 2000; Honda et al., 2003; Järup et al., 1998) and our own experimental findings (Brzóska et al., 2003a, 2004b, unpublished data including those submitted for publication) indicate that chronic, even relatively low, exposure to this metal may affect bone metabolism and decrease bone density. However, the critical level of exposure leading to disorders in bone metabolism and bone fractures are unknown. That is why, at present, it is of great importance to determine whether environmental exposure to Cd occurring in industrialized, but not excessively polluted, countries may be a risk factor increasing the incidence of bone diseases with fractures. Vertebral fractures together with femoral neck and forearm fractures belong to the most frequent postmenopausal and senile osteoporotic fractures (Kanis et al., 2004; Riis, 1996).

Increased incidence of fractures has been noted in human subjects chronically exposed to Cd, especially those patients with Itai-itai disease, which is the most severe form of environmental Cd poisoning characterized by osteoporosis or/and osteomalacia with renal dysfunction (Kasuya et al., 1992; Wang et al., 2003). Cd has also been reported to affect the mineral status of the skeleton and decrease the mechanical strength of bones, including vertebrae, in animal models (Choi et al., 2003; Habeebu et al., 2000; Ogoshi et al., 1989, 1992; Uria et al., 2000; Whelton et al., 1997). However, its influence on the spine at low exposure, except for our findings (Brzóska et al., 2003a, 2004b), is practically unknown. For example, Uria et al. (2000) have noted disturbed mineralization and weakened compression strength of the lumbar vertebral body; however, this effect was observed in ovarioectomized rats after repeated ip administration of a relatively high Cd dose.

Using a rat model of human exposure to Cd, we have observed that the exposure of females since weaning up to the skeletal maturity reduced the lumbar spine (L1–L5) mineralization (Brzóska et al., 2004b). Moreover, the treatment corresponding to human environmental exposure, especially in smokers (5 mg Cd/l in drinking water in our model), weakened the mechanical strength of the lumbar spine vertebral body (the L4 was studied) leading to its deformities and even fractures. At the exposure corresponding to low environmental Cd exposure (1 mg Cd/l), no effect was observed on the vertebral body compression strength (measured based on load at yield and ultimate load).

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and no fractures. However, the stiffness of the L4 was increased suggesting changes in its elastic properties and in some animals the vertebral body was deformed. At higher intoxication with Cd, corresponding to human exposure in heavily contaminated areas and occupational conditions, the L1–L5 demineralization was accompanied by marked weakness in the L4 mechanical properties and its numerous deformities and fractures. Moreover, our results seemed to indicate that the Cd-induced changes in the lumbar spine are osteoporotic in nature (Brzóska et al., 2004b).

As Cd is a common contaminant of food and natural environment (Satarug et al., 2003), humans are exposed to this metal from birth to death. Thus, in our opinion, it was also important to study the effect of low lifetime exposure to Cd on the skeletal damage in the elderly. Recently, we have reported that low exposure to Cd can affect the accumulation of bone mass during skeletal development, resulting in low peak bone mass, and may increase the age-related bone loss in the second half of animal life (Brzóska et al., 2003a, 2004b). Low peak bone mass and enhanced rate of bone loss are the main factors increasing the risk of osteoporosis and fractures in the elderly (Riis, 1996).

Thus, based on our findings in the females exposed to 1 mg Cd/l, we hypothesized that the low lifetime exposure could also reduce the lumbar spine load-bearing capacity and as a consequence increase the incidence of vertebral deformities and fractures in the elderly. The present study was aimed to investigate this hypothesis. According to our knowledge, the effect of low lifetime exposure to Cd on the risk of vertebral osteoporosis with fractures in the elderly has not been investigated until now.

MATERIALS AND METHODS

Animals and experimental design. Twenty young (three-week-old, body weight ~ 50 g) female Wistar rats were used. During the whole experiment (24 months) the animals were maintained at controlled conditions (22 ± 2°C, 12h light/dark cycle, a relative humidity of 50 ± 10%) and had free access to food (the LSM dry diet; Agropol, Motycz, Poland) containing 1.11% calcium (Ca), 0.72% phosphorus, and 1 IU vitamin D3/g, and drinking water. Cd concentration in the diet consumed during the whole experiment was approximately 0.1 μg/g.

Ten randomly selected females were exposed to Cd in the form of an aqueous solution (prepared using redistilled water free of Cd and other contaminants) of cadmium chloride (CdCl2 · 2½H2O; POCh, Gliwice, Poland) at a concentration of 1 mg Cd/l administered as the only drinking fluid (ad libitum), for 24 months. The remaining 10 animals drank redistilled water without Cd and served as controls. Drinking water and food consumption and body weight of rats were monitored during the whole experiment. Every third month, the rats were housed in individual metabolic cages for 24-h urine collection for Cd analysis.

At the end of the experiment, the third and fourth lumbar vertebrae were removed at necropsy (under barbiturate anaesthesia with Vetalbal, 30 mg/kg b. wt., ip.; Biowet, Pulawy, Poland) and immediately cleaned of all the vertebral processes, vertebral arch and of all adherent soft tissues to obtain vertebral bodies (L3 and L4) and weighed (WW, wet weight; OHAUS, Switzerland). The L4 were assigned to densitometric measurements, evaluation of the incidence of deformities or fractures, mechanical testing and to analysis of their chemical composition, including Cd accumulation. The L3 were subjected to the determination of alkaline phosphatase (ALP) activity.

At the last month of this experiment one female of the Cd-exposed group died, but her L4 was immediately isolated and assigned for all studies. Since the results for the L4 were in the range of values noted for other animals they were analyzed statistically together with other results of the Cd group. The ALP activity in the L3 was measured only for surviving females.

The experimental design was approved by the Local Ethics Committee for Animal Experiments in Białystok (Poland).

Vertebral body densitometry. The L4 bone mineral content (BMC), density (BMD), and bone mineral area were measured by DEXA technique (dual-energy X-ray absorptiometry) using Lunar DPX-L (USA) small animal software. To evaluate the in vitro reproducibility of the densitometric measurements, five repeated scans of the two control L4s were made. The coefficient of variation (CV) for the measurements was 0.9% for BMC, 0.8% for BMD, and 0.9% for the bone mineral area of the L4.

Prevalence of osteopenia and osteoporosis of vertebral body. To evaluate the prevalence of osteopenia and osteoporosis of the L4 in Cd-exposed females, age- and gender-standardized Z-score values for the vertebral body BMD were calculated (Alfén et al., 2000; Supplementary Data 1). According to the WHO criteria applied in humans (WHO, 1994), osteopenia was recognized when −1 > Z-score > −2.5, whereas the Z-score values ≤−2.5 indicated osteoporosis.

Estimation of vertebral body deformities and fractures. To evaluate possible deformities or fractures of vertebral bodies, the posterior height (H P) and anterior height (H A) were measured with a precision calliper (<0.02 mm; ARTPOL, Poland) and the percentage of difference between the heights (% HP – H A) and the ratio of HA and HP (HA/HP) were calculated. The two criteria were used to estimate the vertebral body deformity or fracture (Eastell et al., 1991; Brzóska et al., 2004b). For more details see the legend for Table 2 and the Supplementary Data 2.

Moreover, to evaluate vertebral deformities or fractures, X-ray film (RTG technique; TUR800; NRD) of the L4 was taken. The RTG picture may be also useful in estimating of markedly advanced demineralization of the L4. To eliminate differences in radiographic technique (variability in exposure and development of the films), all L4s were radiographed on the same X-ray film using one exposure.

Mechanical testing of vertebral body. To estimate the effect of chronic exposure to Cd on the mechanical properties of the lumbar spine, the compression test of the L4 was performed as described previously (Brzóska et al., 2004b). The L4 was broken in compression along its longitudinal axis (Figs. 1A and 1B) and a load-displacement curve (Fig. 1C) was recorded on line and analysed for yield load (the force causing the first damage of the L4 visible in the load-displacement curve), ultimate load (the force causing total disintegration of the L4 structure), displacement at yield (deformation of the L4 under yield load), displacement at failure (deformation of the L4 before its failure under ultimate load), stiffness (the slope of the linear, elastic part of the load-displacement curve) and work to failure (total energy absorbed by the L4 during the compression test). The ultimate load and yield load describe the L4 compression strength.

Analysis of chemical composition of vertebral body. After mechanical testing, the totally disintegrated L4 were quantitatively transferred (using small forceps) for the analysis of their chemical composition, including Cd accumulation. For this purpose, the specimens were dried (110°C) to water elimination and after weighing (Wd, dry weight) they were defatted, dried again and weighed to obtain dry defatted weight (DW), ashed (525°C) and weighed to obtain ash weight (AW), as reported in details previously (Brzóska et al., 2004b). The difference between the WW and Wd is a weight of water, whereas the difference between the DW and AW is a weight of organic component (OW). The percentage of water content (%) and organic (%) comp. and non-organic (%) non-org. comp.) components content was calculated based on the obtained weights (Supplementary Data 3). The ash residue of the L4 was wet-digested with trace-pure 69% nitric acid (HNO3; Merck Darmstadt, Germany) and dissolved with ultra-pure water (received from two-way water purification MAXIMA system; ELGA, Bucks, UK). In such preparations, concentrations of Ca, magnesium (Mg), zinc (Zn),
copper (Cu), and Cd were determined by atomic absorption spectrometry method (AAS) using an atomic absorption spectrophotometer model Z-5000 (Hitachi, Tokyo, Japan) according to the manufacturer’s recommendation with our own modification (Brzóśka et al., 2004b; Supplementary Data 3). In the analyses stocks of standard solutions of particular elements assigned for AAS (Sigma, St. Louis, MO) were used. Total phosphate was determined in the acid digests of the L4 by molybdenum blue colorimetric analysis (Allen et al., 1974) using a Hitachi U-3010 spectrophotometer (Tokyo, Japan). Total content of particular metals and phosphates in the L4 was given. Internal quality control was employed to keep the measurement processes reliable. The CV for metals analysis was <4% and <5% for phosphate.

**Determination of alkaline phosphatase activity in vertebral body.** The L3, after crumbling and washing out (to eliminate of the remove-available bone marrow) with physiological saline (0.9% NaCl), was homogenized in glycine buffer (pH = 10.4) using a high-performance homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany) equipped with stainless-steel dispersing element (S25N-8G, IKA) to receive an appearing homogenous liquid and centrifuged (Supplementary Data 4). The activity of ALP in the supernatant was determined colorimetrically (Supplementary Data 4) using a commercially available diagnostic laboratory test (POCh, Gliwice, Poland). To express the activity as IU/g protein, the concentration of total protein in the supernatant was determined according to the Lowry method modified by Peterson (1977) with bovine albumin (Sigma-Aldrich Chemie GmbH, Stainheim, Germany) as a standard. A Hitachi U-3010 spectrophotometer was used in these measurements. The CV for ALP measurements was <8%.

**Determination of Cd in urine.** Cd concentration in the urine samples, after appropriate dilution with 0.5% HNO₃, was determined by flameless AAS (Brzóśka et al., 2003b; Supplementary Data 3). The measurements of Cd were corrected by creatinine concentration (determined colorimetrically based on Jaffe reaction using a diagnostic laboratory test by POCh). Statistical analysis. To evaluate statistically significant differences between control and Cd-exposed groups, a one-way ANOVA followed by the Kruskal-Wallis rank test was conducted. Spearman rank correlation analysis was performed to investigate the relationship between some of the variables measured. Moreover, a linear Pearson’s correlation analysis was used to evaluate the relationship between the BMD Z-score for the L4 and %H₄ – H₅ stiffness, ultimate load, and yield load reflecting the effect of the Cd-induced demineralization of the L4 on the risk of its deformities and fractures. Differences and correlations were considered statistically significant at \( p < 0.05 \). All statistical calculations were made using Statistica 5.0 package (StatSoft, Tulsa, OK).

**RESULTS**

**Drinking Water and Cd Intake, Food Consumption, and Body Weight Gain**

The addition of CdCl₂ into drinking water had no effect on its consumption during the whole experiment (data not shown). Average Cd intake, calculated on the basis of fluid consumption, ranged from 17.1 to 38.0 \( \mu g/24 \) h and depended on animal age (drinking water consumption and thus Cd intake in young females in the first month of the experiment was lower than thereafter). Mean daily Cd intake during the experiment was 24.84 ± 0.23 \( \mu g/24 \) h (mean ± SE).

The administration of Cd had no influence on food consumption (and thus the nutritional status of the animals) and body weight of females during the whole experimental period (data not shown). The body weight gain of Cd-exposed females during the 24-month study period was similar to that in the control group (339.0 ± 15.5 g; mean ± SE).

**BMC, BMD, and Bone Mineral Area of the L4**

In the females exposed to Cd for 24 months, the BMC and BMD at the L4 were decreased by 57 and 15%, respectively, compared to control. Moreover, the L4 bone mineral area in the Cd-exposed rats was lower by 30% in comparison to the control (Table 1). Significant positive correlation was noted between the L4 BMC and BMD (\( r = 0.9284 \), \( p < 0.001 \)).

Radiographs of the L4 also revealed lower mineralization of the vertebral body in the Cd group (Fig. 2). The RTG
picture seems to indicate changes in the trabecular bone microarchitecture due to Cd (Fig. 2).

**Prevalence of Osteopenia and Osteoporosis of the L4**

The Cd-induced decrease in the L4 BMD resulted in Z-score values < −1 (−3.33 ± 0.422; mean ± SE). The analysis of the Z-score BMD revealed vertebral osteopenia in three females (in 30% of females) and osteoporosis in seven cases (in 70% of females) as a result of exposure to Cd. There were no females having proper the L4 BMD values (−1 < Z-score < 1) after 24 months of exposure to 1 mg Cd/l.

**Incidence of Deformities and Fractures of the L4**

The HA and the H_P/H_P of the L4 of the Cd-exposed females were lower (by 4 and 1.5%, respectively) and the %H_P – H_A was higher (by 78%) compared to control, whereas the H_P was similar in both groups (Table 2).

![FIG. 2. Radiographs of the fourth lumbar spine vertebral body (L4) of control and exposed to cadmium (1 mg Cd/l in drinking water for 24 months) female rats. (A) Proper picture of the L4 of control rat. (B, C, D) Fractured L4 of Cd-exposed rats: Biconcave fracture (B) and fracture with a clearly evident disruption of bone continuity (C, D). Demineralization of the L4 in B is clearly evident. To make the Cd-induced changes more evident, the picture of the L4 has been somewhat magnified.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Bone Mineral Content (BMC), Density (BMD), and Area of the Fourth Lumbar Vertebral Body (L4) of Control and Cadmium-Exposed (1 mg Cd/l in Drinking Water for 24 Months) Female Rats</th>
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<tr>
<td>Variable</td>
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<tr>
<td>BMC (mg)</td>
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<tr>
<td>BMD (mg/cm²)</td>
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<tr>
<td>Bone mineral area (cm²)</td>
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*Note.* Values are means ± SE. ***p < 0.001 vs. control group (ANOVA + Kruskal-Wallis ranks test).

According to the first criterion used, the L4 was deformed when its %H_P – H_A was more than 2.54% and less than 4.02%; when the %H_P – H_A was higher than 4.02% it was fractured, whereas if the %H_P – H_A was less than 2.54% the L4 was recognized as intact. Assuming the second criterion, the L4 was classified as intact for H_A/H_P higher than 0.975; when the ratio ranged from 0.975 to 0.961 it was deformed, whereas in the case of values lower than 0.961 the vertebral body was fractured. The two criteria used gave consistent results regarding the status of the L4 in all of the 10 evaluated cases of exposure to Cd. The L4 was intact in three females, deformed in three and fractured in four cases (Table 2).

The above analysis of the L4 status conducted based on %H_P – H_A and H_A/H_P revealed that only 30% of females of the Cd group had intact L4 and in 70% of animals it was deformed or fractured. All the osteopenic L4 (−1 > BMD Z-score > −2.5) were intact. Fractured L4 were noted only in females with the BMD Z-score < −2.5 and they occurred in 57% of the females with osteoporosis. In the other cases, the osteoporotic vertebral bodies were deformed (43%). Radiographic examination of the L4 has revealed the vertebral body fracture in three of the Cd-exposed females having the BMD Z-score < −2.5 (Fig. 2). In one case the picture of the

**TABLE 2**

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<tr>
<th>Dimensions and Incidence of Deformities and Fractures of the Fourth Lumbar Vertebral Body (L4) of Control and Cadmium-Exposed (1 mg Cd/l in Drinking Water for 24 Months) Female Rats</th>
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<tr>
<td>Variable</td>
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<tr>
<td>H_P (mm)</td>
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<tr>
<td>H_A (mm)</td>
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<tr>
<td>%H_P – H_A</td>
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<td>H_A/H_P</td>
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<tr>
<td>L4 deformities and fractures**</td>
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<tr>
<td>Intact</td>
</tr>
<tr>
<td>Deformed</td>
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<tr>
<td>Fractured</td>
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</table>

*Note.* Values are means ± SD. 
**Percentage of difference between posterior (H_P) and anterior (H_A) heights of the L4.
*The ratio of H_A and H_P.
**Two criteria based on %H_P – H_A and H_A/H_P were used to recognize the L4 as deformed or fractured. The vertebral body having the %H_P – H_A more than 1 SD but less than 3 SD higher of the arithmetical mean of the control group or the H_A/H_P less than 3 SD but more than 1 SD lower of the control value was recognized as deformed. The fracture threshold was calculated as 3 SD above the %H_P – H_A or 3 SD below the H_A/H_P of the arithmetical mean of the control. For each L4 of the Cd group the same finding was received using both the criteria. 
*Percent and number of animals of the Cd group in which the change was observed, respectively. 
*p < 0.05 vs. control group (ANOVA + Kruskal-Wallis ranks test).
L4 seems to indicate a biconcave fracture (Fig. 2B). The other two cases show a fracture with a clearly evident disruption of continuity of bone trabecules (Figs. 2C and 2D) and in one case a solution of cortical bone continuity is very clearly evident (Fig. 2D).

**Mechanical Properties of the L4**

In the females exposed to Cd, all the variables describing the mechanical properties of the L4 in the compression test, except for the displacement at yield, differed significantly when compared to control (Table 3). Load at yield and ultimate load were lower by 32 and 37%, respectively, indicating decreased compression strength of the vertebral body. The displacement at the L4 failure was increased in the Cd-exposed rats by 69%, whereas the stiffness of the L4 decreased by 42% and work to its failure by 38%.

**Chemical Composition of the L4**

The WW, DW, and AW of the L4 of the Cd-exposed females were lower by 17, 18, and 20%, respectively, when compared to control. The OW of the animals clearly tended to decrease reaching values lower by 15% than that of the control (Table 4). Percent non-org. comp. was decreased (by 6%) and % water increased (by 9%), whereas the content of org. comp. (Fig. 3) as well as the ratio of non-org. comp./org. comp. (1.951 ± 0.019 in the Cd group and 2.062 ± 0.038 in control) were unchanged under Cd treatment. The decrease in the L4 weight (WW, DW, and AW) and % non-org. comp. was accompanied by a reduction in the content of main non-organic constituents such as Ca (by 19%) and phosphate (by 21%) as well as Mg (by 27%), Zn (by 20%), and Cu (by 21%) (Table 4). Cd accumulation in the L4 in the Cd group was higher by 51% of that in the unexposed females (Table 4).

**ALP Activity in the L3**

The activity of ALP in the L3 of the Cd-exposed females (148.81 ± 5.99 IU/g protein) was lower by 30% (p < 0.001) compared to control (212.26 ± 7.86 IU/g protein). The ALP activity in the L3 positively correlated with the L4 BMC (r = 0.6901, p < 0.001), BMD (r = 0.7504, p < 0.001), and Zn content (r = 0.4441, p < 0.05).

**TABLE 3**

| Mechanical Properties (Compression Test) of the Fourth Lumbar Vertebral Body (L4) of Control and Cadmium-Exposed (1 mg Cd/l in Drinking Water for 24 Months) Female Rats |
|-----------------|-----------------|-----------------|
| Variable        | Control         | 1 mg Cd/l       |
| Yield load (N)  | 154.72 ± 13.17  | 104.91 ± 7.90** |
| Ultimate load (N) | 224.79 ± 14.35  | 141.46 ± 13.57** |
| Displacement at yield (mm) | 0.679 ± 0.051 | 0.797 ± 0.022 |
| Displacement at ultimate (mm) | 1.280 ± 0.092 | 2.164 ± 0.255* |
| Stiffness (N/mm) | 229.42 ± 12.05  | 132.59 ± 10.82*** |
| Work to failure (J) | 0.379 ± 0.025 | 0.233 ± 0.034** |

*Note. Values are means ± SE.

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control group (ANOVA + Kruskal-Wallis ranks test)."
Correlations between Variables

Numerous statistically significant correlations (positive or negative) were noted between the L4 mechanical properties evaluated in the compression test and the variables describing its geometry, density, and chemical composition including mineral content (Table 5 and 6). The most important of them involve positive relationship between the variables describing the L4 mineral status (BMC, BMD, AW, % non-org. comp., Ca, and phosphate content) and the yield load and ultimate load describing the L4 load-bearing capacity (Tables 5 and 6) as well as negative correlations between the %H_p – H_A and BMC and BMD (r = – 0.6497 and r = – 0.6205, respectively; p < 0.01) and positive relationship between H_A/H_p and BMC and BMD (r = 0.6253 and r = 0.6150, respectively; p < 0.01). Moreover statistically significant correlations were noted between the BMD Z-score for the L4, indicating the degree of Cd-induced demineralization, and %H_p – H_A, stiffness, yield load, and ultimate load, reflecting its susceptibility to deformities and fractures (Fig. 4).

Cd Urinary Excretion

Cd concentration in the urine of the Cd-exposed rats ranged from 0.665 to 2.498 μg/g creatinine depending on the exposure duration. It gradually increased during the first months of the treatment and thereafter remained at a relatively stable level. In the unexposed animals, the urinary Cd excretion remained at similar level during the whole experiment (ranged from 0.197 to 0.466 μg/g creatinine). The mean Cd concentration in the urine of the females treated with this toxic metal was from three to six-fold higher compared to control.

DISCUSSION

In this study, we investigated the hypothesis that low-level exposure to Cd throughout the lifetime can increase the risk of vertebral osteoporosis and fractures in the elderly. For this purpose, we used our own rat model of human environmental exposure in non-Cd-polluted areas. Young female rats were treated with 1 mg Cd/l in drinking water for two years, i.e., almost throughout their lifetime. Cd intake in the females and its urinary concentration during the whole experiment were in the range of values noted in the general population inhabiting non-Cd-polluted areas (Honda et al., 2003; Satarug et al., 2003; Wang et al., 2003). Thus, the experimental model corresponded well to exposure to Cd occurring in the course of human life, not only in terms of the level of exposure but also all the phases of the skeletal development, i.e., its rapid growth and mineralization in a young organism (childhood and adolescence), skeletal maturity in adulthood, and age-related bone loss in the second half of life, analogous with humans (Riis, 1996; Sampson, 2002).

Both osteoporosis and osteomalacia with pathological fractures have been reported as a result of exposure to Cd (Brzo´ska et al., 2001, 2004b; Kasuya et al., 1992; Kjellström, 1992; Ohta et al., 2000; Wang et al., 2003). However, until now it has not been definitely recognized whether the Cd-induced bone damage is osteoporotic or osteomalacian in character. The observations made in this study seem to confirm our indication of osteoporotic character of changes in the lumbar spine due to chronic exposure to Cd since weaning (Brzo´ska et al., 2004b). The reduced content of minerals (reflected in BMC, AW, % non-org. comp. and content of Ca and phosphate), BMD and bone mineral area of the L4 together with the X-ray picture of the rats exposed to Cd indicate a low ‘bone within a bone’ and microarchitectural abnormalities in trabecular bone which are characteristic features of osteoporosis (Riis, 1996). In osteoporosis, due to an inadequate bone matrix formation and its mineralization, the ratio of mineral to organic matter is proper, as it was observed in case of the Cd-exposed females, whereas in osteomalacia the ratio is low because of the disturbed mineralization of bone matrix (Brzo´ska et al., 2001; Riis, 1996).

It is important to note that the densitometrically evaluated effect of Cd on the L4 mineral content is more marked than

| Table 5: Correlation Coefficients between the Bone Mineral Content (BMC) and Density (BMD) at the Lumbar Spine Vertebral Body (L4) and Its Mechanical Properties Evaluated in the Compression Test |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Yield load      | Ultimate load   | Displacement at yield | Displacement at ultimate | Stiffness | Work to failure (J) |
| BMC (mg)                        | 0.6483*         | 0.8147*         | -0.3101*             | -0.6157*             | 0.8142*   | 0.6770*               |
| BMD (mg/cm²)                    | 0.6021*         | 0.7174*         | -0.4750*             | -0.7279*             | 0.8720*   | 0.5667*               |
| Yield load (N)                  |                | 0.7549*         | 0.2677*              | -0.6872*             | 0.7499*   | 0.7234*               |
| Ultimate load (N)               |                |                | -0.0406*             | 0.8296*              | 0.8920*   |
| Displacement at yield (mm)      |                |                | 0.3504*              | -0.3558*             | 0.0625*   |
| Displacement at ultimate (mm)   |                |                | -0.8071*             | -0.5879*             |          |
| Stiffness (N/mm)                |                |                |                      |                    | 0.6935*   |

Note. Sperman rank correlation analysis was conducted to investigate the relationship between variables. Data are presented as correlation coefficient (r) and the level of statistical significance (p).

* p < 0.001, † p < 0.01, ‡ p < 0.05, § p > 0.05.
the effect estimated on the basis of the AW and Ca content. It results from the fact that the BMC is a measure of mineral content of the scanned bone area of the vertebral body, whereas the AW is a measure of all minerals in the vertebral body by chemical analysis. These should be taken into account to avoid an interpretative error. Since the effect of Cd on the BMC and AW is estimated in comparison with the respective control values, both the variables are useful to study the influence of Cd; however, to estimate the quantitative effect on the minerals content in bone, the AW is a better parameter.

The decrease in all the variables measured describing the L4 mineral status in the Cd-exposed rats indicates its impaired mineralization resulting in osteopenia or osteoporosis. The Z-score BMD revealed vertebral osteoporosis due to exposure to Cd in as many as 70% of females and in some of them (in 57%) vertebral fracture occurred indicating advanced osteoporosis. In other Cd-exposed females being osteopenic, vertebral deformities were evident but without fractures.

Decreased ALP activity in the L4 in the Cd group as shown in this study, indicating decreased bone formation rate (Swaminathan, 2001), together with increased bone resorption in these females (data submitted for publication) explain the net loss of vertebral bone due to Cd. The reduced activity of ALP can at least partly result from the reduced vertebral content of Zn being a cofactor of this enzyme (Bonner et al., 1980). It is confirmed by positive correlation between the ALP activity in the trabecular bone and Zn content, BMD, and BMC of the L4. The effect of the lifetime exposure to 1 mg Cd/l on bone metabolism and mineral status of the lumbar spine, including mechanisms of the action, was studied and discussed by us in detail elsewhere (Brzózska et al., 2004b, data submitted for publication). Briefly, it consists in the inhibiting influence on the accumulation of the peak bone mass during growth, the unfavorable effect on the maintenance of bone mass at skeletal maturity and the enhancement of the age-related bone loss. Several possible mechanisms of the Cd-induced bone damage have been proposed. They involve a direct action of Cd on the processes of bone formation and resorption via influencing bone cells and an indirect action, being a secondary effect of kidney and gastrointestinal tract damage (Brzózska et al., 1997, 2003c; Iwami and Moriyama, 1993; Kjellström, 1992; Ohta et al., 2000; Uriu et al., 2000; Wang and Bhattacharyya, 1993). The mechanisms are further under investigation by us. Recently we have reported (Brzózska et al., 2003b, 2004a) structural and functional damage to the renal proximal tubules at relatively low exposure to Cd and its relatively low accumulation in the kidney. Moreover, an important factor that might be, at least partly, responsible for such a strong effect of the low exposure to Cd on the lumbar spine mineral status and mechanical properties was estrogen deficiency in the second year of female life (postmenopausal state; Sampson, 2002). It has been reported that the lack of estrogens makes the bone more susceptible to Cd action (Bhattacharyya et al., 1992).

The vertebral body in approximately 60–70% is composed of the trabecular (cancellous) bone (Riggs et al., 1981). The
mineral status of the vertebral body and the microarchitecture of the trabecular bone are the main determinants of its mechanical competence and at the same time of susceptibility to damage, including fracture (Jiang et al., 1997; Keaveny et al., 2001; Rehman et al., 2003). It is confirmed by statistically significant correlations between variables describing the L4 mineral status and those characterizing its mechanical properties. The strength of the vertebral body also depends on its geometry (size and shape) (Jiang et al., 1997; Rehman et al., 2003) and this is in agreement with strong correlations between the L4 dimensions (expressed as the \%HP – HA and HA/HP) and variables describing its mechanical properties. The Cd-induced changes in the L4 dimensions seem to be small compared to the changes in the mineral status of the vertebral body. It may be caused by the fact that the rat does not hold its spine vertically.

The decrease in the yield load and ultimate load noted in the females treated with Cd indicate a weakness in the vertebral body compression strength and the lumbar spine load-bearing capacity. The analysis of the load-displacement curves recorded during mechanical testing indicated that the Cd-induced decrease in the vertebral body strength resulted from its influence on both elastic (linear, reversible) and plastic (nonlinear, irreversible) deformation components (separated by the yield point). The same degree of the L4 deformation at the point of its first damage under lower loading in the Cd group than in the control and decreased stiffness indicate that the L4 of the females treated with Cd becomes more elastic. An increase in the bone elasticity is connected with its increased susceptibility to deformities. Indeed, in most of the Cd-exposed rats the L4 was deformed. However, in some cases the Cd-induced vertebral fractures were not only typical compression fractures in nature but some of them being accompanied by disruption of bone continuity. Radiographs of the L4 showed changes in the trabecular microarchitecture in the Cd-exposed females. The Cd-induced loss of bone mass led to thinning of bone trabecules decreasing their load-bearing capacity. Mechanical testing of the L4 revealed that the changes in the vertebral body mineralization and microarchitecture were an important determinant of its decreased strength. The link between the L4 demineralization caused by Cd and its increased susceptibility to deformities...
and fractures is confirmed by strong correlations between the BMD Z-score and \( \% H_p - H_A \), stiffness, yield load, and ultimate load.

The bone mechanical strength is also determined by the organic matrix composed mainly of collagen (Burr, 2002; Oxlund et al., 1996). The proper amount and structure of collagen fibers play an important role in bone formation and strength. Collagen fibers constitute an organic matrix into which minerals are incorporated while creating the bone tissue. Moreover, collagen fibers are responsible for bone elasticity and thus influence its biomechanical properties and a risk of fractures. Thus disturbances in collagen metabolism may result in formation of low quality bone tissue susceptible to deformities and fractures (Burr, 2002; Wang et al., 2001). Cd has been reported to inhibit the cross linking of collagen (Iguchi and Sano, 1982) and increase its solubility (Galicka et al., 2004). Cd can influence collagen maturation via inhibiting Cu-dependent enzyme lysyl oxidase that catalyzes the collagen cross linking (Iguchi and Sano, 1982). Decreased Cu content in the L4 and, which was previously noted in those females, the increased serum and urinary concentration of fragments of type I collagen (data submitted for publication) together with the decreasing tendency of the L4 OW, as observed in this study, confirm the possibility of Cd influence on the vertebral body organic matrix. Enhanced, due to exposure to Cd, degradation of bone matrix has also been reported by others (Aoshima et al., 2003; Choi et al., 2000; Uriu et al., 2000). The positive correlations between the \% org. comp. and the yield load and displacement at yield confirm the connection between the Cd-induced changes in the organic matrix and mechanical properties of the L4.

The results of this study together with our previous findings in the Cd-exposed females (Brzońska et al., 2003a, 2004b) give clear evidence that the low lifetime exposure to Cd, which practically did not affect the lumbar spine load-bearing capacity at the skeletal maturity, is very effective in this respect when it takes place up to the elderly.

In summary, the results of the present study show that low lifetime Cd exposure decreases the lumbar spine vertebral body (trabecular bone) mineralization and weakens its compression strength leading to vertebral osteoporosis with deformities or even fractures. Since the effect of Cd was observed at Cd intake and its urinary concentration, being within the range of values noted in the general population, our results seem to confirm the hypothesis that even low environmental exposure to Cd throughout life may be an important risk factor for vertebral osteoporosis with fractures in the elderly. As our experimental model used females, being more vulnerable to disorders in bone tissue metabolism then males, our conclusion refers mainly to environmentally exposed women. In further studies we will investigate the effect of low exposure to Cd on the skeleton in a male rat model to elucidate the gender-related differences in the susceptibility to skeletal damage due to exposure to Cd.

SUPPLEMENTARY DATA

Supplementary data, available online at www.toxsci.oupjournals.org, contains additional information regarding the materials and methods used to carry out this research.

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