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Low-Level Environmental Cadmium Exposure Induces Kidney Tubule Damage in the General Population of Korean Adults

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Abstract Cadmium (Cd) is the most potent nephrotoxic heavy metal and may affect bone; it also has a long biological half-life in the human body. This study was designed to assess the effect of environmental low-level Cd exposure on kidney function and bone in the general population. The subjects of this cross-sectional study were 1907 healthy Korean adults who had not been exposed to Cd occupationally. We analyzed the concentrations of Cd in the urine, markers of renal tubule damage, such as β_2-microglobulin (β_2-MG) and N-acetyl-β-D-glucosaminidase (NAG) activity in the urine, calculated the estimated glomerular filtration rate (eGFR) using serum creatinine, and measured bone mineral density (BMD). Also, we analyzed malondialdehyde (MDA) levels in the urine. The geometric mean concentration of Cd in urine was higher in women (1.36 μg/g creatinine) than in men (0.82 μg/g creatinine). Urinary Cd was significantly positively correlated with urinary β_2-MG and NAG activity, whereas it was negatively correlated with eGFR and BMD. The risk of renal tubule damage was significantly associated with urine Cd level, and the association remained significant after controlling for various confounding variables. However, no association was observed between urinary Cd level and glomerular dysfunction or bone damage. The concentration of MDA was increased with urinary Cd level in a dose-dependent manner. These findings suggest that low-level environmental Cd exposure may cause microscopic damage to renal tubules through oxidative stress but might not impair kidney glomeruli or bones.

Cadmium (Cd) is a toxic transition metal that is ubiquitous in the environment and is widely used in various fields, such as the production of stabilizers, pigments, batteries, alloys, and plated products (ATSDR 2012). The environmental Cd concentration continuously increases, because Cd dose not decompose in the environment and is used continuously still in various fields (Moulis and Thévenod 2010). Cd tends to accumulate in crops (such as rice, wheat, root vegetables, tuber vegetables, leafy vegetables, etc.), and the human body may eventually be exposed to high levels of Cd through the food chain; this is especially true for inhabitants of Cd-contaminated areas (Tsuchiya 1969; EFSA 2009; Moulis and Thévenod 2010). Consumption of Cd-contaminated food and cigarette smoking are the major sources of chronic environmental exposure to Cd in the general population (Järup et al. 1998).

Workers in certain industries are mainly exposed to Cd through inhalation, and it has negative effects on the lungs, kidneys, and bones, whereas the general population is mainly exposed via ingestion and the effects are largely seen in kidneys and bones (Järup et al. 1998; Järup and Akesson 2009). Absorption of Cd via the gastrointestinal tract is relatively low compared with absorption via inhalation (ATSDR 2012). Gastrointestinal absorption of

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Cd is affected by an individual's nutritional status, such as body levels of iron and zinc (Ryu et al. 2004; EFSA 2009; ATSDR 2012). However, most of the absorbed Cd accumulates in the kidneys and liver. Because accumulated Cd is very poorly excreted in urine (approximately 0.001% of the body's burden is excreted per day), Cd has a long biological half-life in humans of more than 20 years (Bernard 2004; ATSDR 2012). Cd induces the synthesis of metallothionein (MT), a high-affinity metal binding protein, in the liver (Klaassen et al. 2009). The Cd-MT complex is released from the liver and transported to the kidneys, where it is reabsorbed and degraded into the highly toxic Cd^{2+} ion in proximal tubules (Bernard 2008; Nordberg 2009). Free Cd ions provoke microscopic renal tubule damage, which is asymptomatic condition and subclinical finding of impaired kidney function (Järup et al. 1998; Satarug et al. 2010). Cd-induced renal tubule damage is usually demonstrated by increased urinary excretion of low molecular weight proteins, such as β_2-microglobulin (β_2-MG), α1-microglobulin, and retinol-binding protein, as well as intracellular tubular enzymes, such as N-acetyl-β-D-glucosaminidase (NAG) (Järup and Akesson 2009). However, if renal tubule damage is continuously caused by Cd exposure, the affected individual may display a decreased glomerular filtration rate as a representative of kidney dysfunction (Bernard 2004). The effects of Cd on bone are well recognized, because a historical event involving Cd poisoning in Japan, so-called "itai–itai disease" (Tsuchiya 1969). It is understood that bone damage by Cd may follow renal tubule damage caused by relatively high-level Cd exposure (Kazantzis 2004). Alternatively, it has been shown in both experimental and epidemiologic studies that long-term exposure to low levels of Cd also affect bone, leading to decreased bone mineral density (BMD) (Alfvén et al. 2000; Brzóska and Moniuszko-Jakoniuk 2005; Wallin et al. 2016). Thus, the mechanism of the effects of Cd on bone is still under debate, including whether the effects are secondary or direct.

Although the primary target of Cd is the proximal tubule of the kidneys in the general population, several studies also evaluated the association between Cd exposure and glomerular dysfunction, as well as decreased bone mineral density. It remains unclear whether environmental Cd exposure can induce glomerular dysfunction and bone damage (Akesson et al. 2005; Hwangbo et al. 2011; Kim and Lee 2012; Myong et al. 2012; Byber et al. 2016; Wang et al. 2016). It is a concerning issue in the field of public health if environmental exposure to low levels of Cd can damage target organs in the general population. Therefore, it is necessary to research continuously the association between chronic low-level Cd exposure and effects on target organs, investigating the link between the observed effects on target organ and a clinical disease or health effects. The levels of Cd in blood and urine are used as biomarkers of human exposure to Cd. Urinary Cd reflects chronic exposure to Cd and the body's burden, whereas blood Cd levels represent relatively recent exposure to Cd (Godt et al. 2006). In this study, we evaluated long-term Cd exposure by measuring Cd in urine and simultaneously assessed biological indicators of renal tubule damage, alteration of glomerular filtration rate, and bone mineral density to evaluate the effects of low-level Cd exposure on kidney function and bone in the general population.

Materials and Methods

Study Subjects

This study used data from a population-based cross-sectional study conducted in Korea. The study design and sampling method used for study subjects were described in detail in previous studies (Eom et al. 2014; Lim et al. 2015). Study subjects were collected by sex- and age-stratified probability method from the 102 sampling sites, which included metropolitan, urban, and rural areas. The subjects of this study were 1907 healthy Korean adults aged 19 years or older. There were 759 men and 1148 women; of whom none were exposed to Cd occupationally. After written, informed consent was obtained from all subjects, trained interviewers collected information on demographic characteristics, smoking habits, alcohol drinking, dietary habits, occupation, duration of current residence, and past medical history. Whole blood was sampled and centrifuged at 3000 rpm for 10 min to separate serum. Spot urine in the morning and overnight urine (approximately 15 h from dinner on the day before the interview to the next morning) samples were collected from all subjects and stored at −80 °C until analysis. The study protocol was approved by the Chung-Ang University Ethical Committee for Medical Research and Other Studies Involving Human Subjects.

Determination of Cd in Urine

The concentration of Cd in overnight urine was analyzed by a flameless method. Briefly, urine was added to nitric acid and diluted with diammonium hydrogen phosphate, followed by vigorous mixing. Determination of the Cd level in the solution was performed using a flameless atomic absorption spectrophotometer (Model Z-8270, Hitachi) equipped with a Zeeman graphite furnace. The detection limit was 0.03 μg/L for urinary Cd. For samples with concentrations of Cd below the limit of detection, the concentration was substituted with the value of the limit of detection divided by the square root of 2 (Hornung and

Reed 1990). Three samples contained Cd at a level lower than the limit of detection.

Measurements of β_2-MG, NAG Activity and MDA in Urine and BMD

Urinary NAG activity and β_2-MG level were measured as indicators of renal tubule damage. The concentration of β_2-MG in urine was determined using an ELISA kit (β_2-MG ELISA Kit, Immundiagnostik, Germany) and NAG activity in urine was measured with a commercial kit (NAG quantitative kit, Shionogi, Osaka, Japan) according to the manufacturer's instructions. Serum creatinine level was determined by the Jaffe method to estimate the glomerular filtration rate (GFR). The estimated GFR (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula [$\text{eGFR} = 175 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female)] (Levey et al. 2007). The concentration of malondialdehyde (MDA) in urine as a marker of oxidative stress was determined using a high-performance liquid chromatographic system with a fluorescence detector (Agarwal and Chase 2002). BMD was measured using an ultrasound bone densitometer (Osteopro, BMtech, Seongnam, Korea) in the right calcaneus bone.

Statistical Analyses

The concentrations of Cd in urine were log-transformed for statistical analyses because log-transformed values are a better fit with a normal distribution than are measured values. The distribution of study subjects by demographic factors was analyzed by the Chi square test. Comparisons of means was performed with the two-tailed Student's *t* test or analysis of variance (ANOVA) following multiple comparison tests using Duncan's method. The correlation between Cd levels and various biomarkers were evaluated using Spearman's correlation coefficients. The Cd level in urine was categorized into tertiles (lowest, middle, and highest groups). "High NAG" and "high β_2-MG" were defined as greater than 11.5 U/g creatinine and 300 μg/g creatinine, respectively, and "renal tubule damage" was defined as having at least one "high" marker. A low eGFR was defined as lower than 60 ml/min/1.73 m^2 and was considered to indicate glomerular dysfunction (Levey et al. 2007). Bone damage was defined as a *t* score of less than −2.5 (NIH 2001). The associations of urinary Cd levels with renal tubule damage, glomerular dysfunction, and bone damage were evaluated by logistic regression analyses. Multivariate analyses were adjusted for various confounding variables, including sex, age, body mass index, household income, smoking history, alcohol drinking, hypertension, and diabetes and also included blood lead level, because lead exposure could affect the kidney function and bone mineral density (Campbell and Auinger 2007; Kim and Lee 2012). The level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY).

Results

The general characteristics of the 1907 study subjects (759 men and 1148 women) are presented in Table 1. The mean age was not different between men (45.5 ± 15.5 years) and women (45.6 ± 14.2 years). The proportions of current or ex-smokers and alcohol drinkers were higher in men than in women. Education level and household income also were higher in men than in women. There was a significant difference in the prevalence of hypertension and diabetes between men and women. The geometric mean concentration of Cd in urine was higher in women (1.36 μg/g creatinine) than in men (0.82 μg/g creatinine). The 95th percentile value of urinary Cd corresponded to 2.34 μg/g creatinine and 3.39 μg/g creatinine in men and women, respectively. No significant differences in NAG, β_2-MG, or MDA levels in urine were observed between men and women. However, the eGFR was significantly lower in men than in women. BMD was not different between men and women.

Urinary Cd levels were positively correlated with urinary NAG activity, β_2-MG, and MDA in both genders. eGFR and BMD were negatively correlated with urinary Cd in both men and women ($p < 0.05$; Table 2). Furthermore, mean NAG activity and β_2-MG levels increased according to the tertile level of urinary Cd in a dose-dependent manner. These dose–response relationships remained after controlling for various confounding variables, such as body mass index, household income, smoking history, alcohol drinking, hypertension, diabetes, and blood lead level, as well as sex and age. eGFR and BMD also were decreased according to urinary Cd levels; however, the decreases were not observed after adjustment for covariates. The concentrations of MDA increased dose-dependently with urinary Cd levels (Table 3).

The associations of urinary Cd level with the risk of renal tubule damage, glomerular dysfunction, and bone damage were evaluated (Table 4). The prevalence of high NAG and high β_2-MG were significantly increased according to the levels of urinary Cd; however, the significant positive association was observed with high β_2-MG only in the highest urinary Cd group after adjustment for covariates. In the present study, the prevalence of renal tubule damage, which was defined as either high NAG or high β_2-MG levels in urine, increased with the tertile level

Table 1 General characteristics of the study subjects

Variables	Men	Women	*p* value[a]
Total, n (%)	759 (39.8)	1148 (60.2)	–
Age, yr, mean ± SD	45.5 ± 15.5	45.6 ± 14.2	0.933
Body mass index, kg/m^2, Mean ± SD	24.5 ± 3.1	23.7 ± 3.5	<0.01
Current or ex-smokers, n (%)	528 (70.0)	94 (8.2)	<0.01
Alcoholic drinkers, n (%)	660 (87.0)	750 (65.3)	<0.01
Low education[b], n (%)	97 (12.8)	243 (21.2)	<0.01
Low household income[c], n (%)	182 (24.0)	333 (29.1)	0.032
Hypertension, n (%)	260 (34.3)	241 (21.0)	<0.01
Diabetes, n (%)	89 (11.7)	68 (5.9)	<0.01
Blood Pb, μg/dL, GM (95% CI)	2.56 (2.49, 2.63)	1.96 (1.92, 2.00)	<0.01
Urinary Cd, μg/g creatinine, GM (95% CI)	0.82 (0.79, 0.86)	1.36 (1.31, 1.41)	<0.01
95th percentile	2.34	3.39	–
Nonsmokers, GM (95% CI)	0.68 (0.63, 0.75)	1.37 (1.32, 1.42)	–
Current or ex-smokers, GM (95% CI)	0.89 (0.85, 0.94)**	1.21 (1.06, 1.38)	–
Urinary NAG, unit/g creatinine, AM (95% CI)	4.17 (3.74, 4.59)	4.14 (3.74, 4.54)	0.941
Urinary β_2-MG, μg/g creatinine, AM (95% CI)	80.47 (72.22, 88.72)	79.86 (74.29, 85.43)	0.904
Estimated eGFR[d], mL/min/1.73 m^2, AM (95% CI)	91.88 (90.63, 93.13)	97.89 (96.79, 98.99)	<0.01
Malondialdehyde, μmol/g creatinine, AM (95% CI)	1.71 (1.61, 1.80)	1.68 (1.61, 1.76)	0.729
Bone mineral density, AM (95% CI)	−1.38 (−1.48, −1.27)	−1.26 (−1.33, −1.18)	0.061

SD standard deviation; *Pb* lead; *Cd* cadmium; *GM* geometric mean; *CI* confidence interval; *NAG* N-acetyl-**β**-D-glucosaminidase; *AM* arithmetic mean; **β_2**-*MG* **β_2**-microglobulin; *eGFR* estimated glomerular filtration rate

** Statistically significant between nonsmokers and current or ex-smokers, $p < 0.01$

[a] *p* values were determined by *t* tests or Chi squared tests for the difference between men and women

[b] Less than a high school education

[c] Total household annual income less than USD 16,000

[d] Estimated GFR = 175 × serum creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 (if female)

Table 2 Spearman's correlation coefficients between urinary cadmium and biomarkers of renal tubule damage, glomerular dysfunction, and bone damage

	Urine Cd, μg/g creatinine		
	Men	Women	Total
Urinary NAG, unit/g creatinine	0.328**	0.244**	0.260**
Urinary β_2-MG, μg/g creatinine	0.194**	0.156**	0.189**
Estimated eGFR, mL/min/1.73 m^2	−0.096**	−0.173**	−0.071**
Bone mineral density (BMD)	−0.218**	−0.240**	−0.196**
Malondialdehyde (MDA), μmol/g creatinine	0.155**	0.152**	0.135**

Cd cadmium; *NAG* N-acetyl-β-D-glucosaminidase; β_2-*MG* β_2-microglobulin; *eGFR* estimated glomerular filtration rate

* $p < 0.05$; ** $p < 0.01$

of urinary Cd; the prevalence of renal tubule damage was 3.2, 5.9, and 11.2% in the lowest, middle, and highest urinary Cd groups, respectively. Thus, the risk of renal tubule damage was significantly (1.89 times) higher in the middle urinary Cd group and 3.79 times higher in the highest urinary Cd group compared with the lowest urinary Cd group. Statistical significance was remained in the highest urinary Cd group versus the lowest group; the risk was 2.76 times higher after controlling for sex and age (Model 1) and 2.50 times higher after controlling for various potential confounders, such as body mass index, household income, smoking history, alcohol drinking, hypertension, diabetes, and blood lead levels, as well as sex and age (Model 2), but disappeared after adjustment for various covariates in the middle urinary Cd group (Model 1 and Model 2, both). No association was observed between

Table 3 Mean concentrations of β_2-MG, NAG, eGFR, BMD, and MDA in the urine according to urinary Cd level

Level of Cd	NAG (unit/g creatinine)				β_2-MG (μg/g creatinine)				eGFR (mL/min/1.73 m^2)				BMD				MDA (μmol/g creatinine)			
	N	Crude	M1	M2	N	Crude	M1	M2	N	Crude	M1	M2	N	Crude	M1	M2	N	Crude	M1	M2
T1	631	$3.1^{c} \pm 2.9$	3.5	3.5	629	$57.7^{c} \pm 51.2$	66.8	67.7	636	$97.3^{a} \pm 19.0$	92.8	92.8	635	$-1.0^{a} \pm 1.4$	−1.4	−1.4	627	$1.53^{b} \pm 1.18$	1.58	1.59
T2	630	$4.3^{b} \pm 7.9$	4.2	4.2	632	$78.0^{b} \pm 86.7$	75.5	75.9	635	$94.7^{b} \pm 18.3$	95.9	95.9	633	$-1.3^{b} \pm 1.3$	−1.2	−1.2	628	$1.62^{b} \pm 1.21$	1.60	1.60
T3	630	$5.0^{a} \pm 7.5$	4.8	4.7	634	$104.4^{a} \pm 145.7$	97.8	96.5	636	$94.5^{b} \pm 18.4$	97.7	97.6	636	$-1.6^{c} \pm 1.2$	−1.3	−1.3	630	$1.93^{a} \pm 1.51$	1.90	1.87
p for trend		<0.001	0.011	0.021		<0.001	<0.001	<0.001		0.011	<0.001	<0.001		<0.001	0.072	0.109		<0.001	<0.001	<0.001

M1 (Model 1): Adjusted for age and sex

M2 (Model 2): Adjusted for age, sex, body mass index, household income, smoking history, alcohol drinking, hypertension, diabetes, and blood lead level

Urinary Cd criteria: T1 (<0.87), T2 (0.87–1.55), T3 (≥ 1.55)

a,b,c Duncan grouping

urinary Cd level and the prevalence of glomerular dysfunction. The prevalence of bone damage increased according to urinary Cd levels; it was 11.3, 16.7, and 22.6% in the lowest, middle, and highest urinary Cd groups, respectively. The statistical significance of the associations disappeared after controlling for various confounding variables as well as sex and age.

Discussion

The present study evaluated whether environmental Cd exposure affects microscopic renal tubule damage, glomerular dysfunction, and bone damage by measuring urinary Cd levels (as an indicator of chronic exposure and body burden) in the Korean general population. The primary results of this study show that the exposure to low-level Cd in the environment was not associated with decreased glomerular filtration rate or bone damage but was significantly associated with microscopic damage to the proximal tubules, which can be described as a precursor of kidney dysfunction.

The subjects of this study were from the general population and did not have occupational exposure to Cd. The geometric mean concentration of urinary Cd was 0.82 μg/g creatinine for men and 1.36 μg/g creatinine for women, which are similar to or less than those reported in previous studies on Koreans [0.95 μg/g creatinine (Huang et al. 2013)] and other Asian countries, such as Japan [1.14 μg/L (Ikeda et al. 2013)] and China [1.38 μg/g creatinine (Jin et al. 2004)] but was higher than those reported in Sweden [0.67 μg/g creatinine (Akesson et al. 2005)], the United States [0.22 μg/L (Buser et al. 2016)], and Germany [0.18 μg/g creatinine (Becker et al. 2003)]. The general population is exposed to Cd primarily through food and smoking (Järup and Akesson 2009). The reason Koreans have high Cd levels compared with western countries may be partially that they frequently consume foods that are relatively high in Cd, such as grains, potatoes, seaweed, and seafood (Moon et al. 2012). In this study, the urinary Cd was significantly higher in current or ex-smokers (0.89 μg/g creatinine) than in nonsmokers (0.68 μg/g creatinine) in males only. No significant difference of urinary Cd was observed in females, which could be ascribed to relatively small number of smokers (8.2%) and younger than nonsmokers (mean age: 36.3 vs. 43.7 years) in females of Korea. In addition, the impact of differences in genetic background among ethnic groups cannot be excluded; in a recent genome-wide association study, a genetic polymorphism involved in Cd absorption and metabolism was confirmed to be associated with Cd levels in the erythrocytes of nonsmokers (Borné et al. 2016).

Table 4 Associations of urinary Cd levels with renal tubule damage, glomerular dysfunction, and bone damage

Level of Cd	High NAG, OR (95% CI)				High β_2-MG, OR (95% CI)				Renal tubular damage[a], OR (95% CI)				Glomerular dysfunction[b], OR (95% CI)				Bone damage[c], OR (95% CI)			
	N (%)	Crude	M1	M2	N (%)	Crude	M1	M2	N (%)	Crude	M1	M2	N (%)	Crude	M1	M2	N (%)	Crude	M1	M2
T1	18	1.00	1.00	1.00	4	1.00	1.00	1.00	20	1.00	1.00	1.00	9	1.00	1.00	1.00	72	1.00	1.00	1.00
	(2.9)	(Ref.)	(Ref.)	(Ref.)	(0.6)	(Ref.)	(Ref.)	(Ref.)	(3.2)	(Ref.)	(Ref.)	(Ref.)	(1.4)	(Ref.)	(Ref.)	(Ref.)	(11.3)	(Ref.)	(Ref.)	(Ref.)
T2	26	1.47	1.19	1.13	16	4.07	2.82	2.70	37	1.89	1.47	1.36	13	1.46	0.70	0.69	106	1.57	0.86	0.90
	(4.1)	(0.80, 2.70)	(0.62, 2.29)	(0.58, 2.18)	(2.5)	(1.35, 12.24)	(0.92, 8.69)	(0.87, 8.39)	(5.9)	(1.08, 3.29)	(0.82, 2.63)	(0.76, 2.46)	(2.1)	(0.62, 3.43)	(0.28, 1.74)	(0.27, 1.76)	(16.7)	(1.14, 2.17)	(0.60, 1.24)	(0.62, 1.29)
T3	35	2.01	1.52	1.40	38	9.98	6.90	6.92	70	3.79	2.76	2.50	16	1.80	0.61	0.63	144	2.29	1.00	1.06
	(5.6)	(1.12, 3.58)	(0.76, 3.05)	(0.69, 2.84)	(6.0)	(3.54, 28.12)	(2.25, 21.15)	(2.24, 21.44)	(11.2)	(2.28, 6.31)	(1.52, 5.00)	(1.37, 4.56)	(2.5)	(0.79, 4.10)	(0.23, 1.61)	(0.23, 1.71)	(22.6)	(1.69, 3.12)	(0.68, 1.48)	(0.71, 1.57)
Total	79 (4.2)				58 (3.1)				127 (6.8)				38 (2.0)				322 (16.9)			

[a] Renal tubule damage was defined as either high NAG (>11.5) or high β_2-MG (>300.0)

[b] Glomerular dysfunction was defined as lower than the cutoff low eGFR value (<60.0)

[c] Bone damage was defined as lower than the cutoff low *T* score (<−2.5)

M1 (Model 1): Adjusted for age and sex

M2 (Model 2): Adjusted for age, sex, body mass index, household income, smoking history, alcohol drinking, hypertension, diabetes, and blood lead level

Urinary Cd criteria: T1 (<0.87), T2 (0.87–1.55), T3 (≥1.55)

Absorbed Cd in the body is transported to the kidney through the liver and then is reabsorbed and accumulates in the renal tubules after being combined with MT; thus, it primarily causes toxicity in the renal tubules (Järup et al. 1998; Bernard 2008; Järup and Akesson 2009). In this study, β_2-MG and NAG in urine, which are the most widely used indicators of renal tubule damage, were measured to assess microscopic renal tubule damage caused by Cd. Although β_2-MG is a low molecular weight protein that is present in blood plasma, it is reabsorbed in the renal tubules and is not excreted under normal conditions; however, it is excreted in the urine when the renal tubules are damaged. NAG is a lysosomal enzyme that is present in the proximal tubule epithelial cells and is excreted in the urine when cells are damaged by Cd (Prozialeck and Edwards 2010). Both indicators reflect early kidney damage caused by Cd, and the present study has identified a significant correlation between urinary Cd and both indicators of microscopic renal tubule damage. Furthermore, increases of NAG and β_2-MG were observed according to urinary Cd levels in a dose-dependent manner. Previous studies, both in vitro and in vivo, indicated that Cd induces the production of oxygen radicals, which may play a role in acute and chronic Cd toxicity by oxidative stress (Shaikh et al. 1999; Thévenod and Friedmann 1999; Thijssen et al. 2007). In this study, MDA in urine was positively correlated with urinary Cd levels, and the concentration of MDA increased according to urinary Cd level in a dose-dependent manner.

Although both urinary NAG and β_2-MG are indicators of renal tubule damage, each reflects the effects of Cd in a different manner, namely damage to tubular epithelial cells and deterioration of reabsorption function, respectively. At present study, mean NAG activity and β_2-MG levels increased according to the tertile level of urinary Cd in a dose-dependent manner. The correlation coefficient between the urinary NAG activity and urinary Cd was higher than the relationship with urinary β_2-MG, whereas the association with urinary Cd level was significant with the high β_2-MG rather than the high NAG. Further study is necessary to clarify those ambiguous findings, which might be related to sensitive or specific response of each indicator, NAG or β_2-MG, to the Cd exposure. Therefore, in this study, we defined microscopic renal tubule damage as being present when either of the two indicators, β_2-MG or NAG, was higher than the corresponding reference level (Bernard 2008). Similar to our findings, many studies have shown a correlation between environmental Cd exposure and microscopic renal damage in the general population (Järup and Akesson 2009; Akesson et al. 2005; Huang et al. 2013; Wang et al. 2016). Although Järup et al. (1998) estimated that renal tubule damage occurred when the renal cortical Cd level reached approximately 150–200 μg/g kidney wet weight (urinary Cd level, 2–10 μg/g creatinine), several recent studies have reported that tubular impairment may occur even at tissue Cd concentrations below 20–50 μg/g kidney wet weight (Akesson et al. 2005; Satarug et al. 2010). In the general population, the threshold value of microscopic renal damage by environmental exposure to Cd has been reported to be gradually decreasing (Järup and Akesson 2009; Satarug et al. 2010; Wang et al. 2016). On the contrary, Ezaki et al. (2003) reported that no relationship was observed between environmental Cd exposure and renal tubule damage in an analysis by age group in a general population of middle-aged women. Recently, several authors have posited that the correlation between exposure to a low level of Cd and indicators of renal tubule damage may be the result of the co-excretion effects of Cd and renal damage indicators in urine, rather than the toxic effects of Cd alone (Chaumont et al. 2012; Akerstrom et al. 2013; Weaver et al. 2016). However, because the urinary Cd levels in those studies (0.09–0.29 μg/g creatinine) were lower than the urinary Cd levels of our study subjects, it is difficult to interpret the apparent correlation in our study in the same manner. Moreover, in our study, we used values corrected to urinary creatinine for both urinary Cd and indicators of renal tubule damage to control for co-excretion effects.

Microscopic renal tubule damage in the general population may be reversible and individuals can recover when Cd exposure ceases; it also is a preclinical stage that does not indicate the presence of disease. However, when renal tubule damage caused by Cd is maintained continuously, it may proceed to kidney dysfunction and decreases in glomerular filtration rate (Järup et al. 1998; Bernard 2008; Prozialeck and Edwards 2010). In this study, the prevalence of renal tubule damage increased according to urinary Cd level, namely, 3.2, 5.9, and 11.2% in the lowest, middle, and highest urinary Cd groups, respectively. Also, the risk of renal tubule damage was significantly associated with urinary Cd levels such that it was 1.89 times higher in the middle and 3.79 times higher in the highest urinary Cd group compared with the lowest group. A significant association between the risk of renal tubule damage and urinary Cd was observed; the risk was 2.50 times higher (95% confidence interval, 1.37–4.56) in the highest Cd group versus the lowest Cd group after controlling for various potential confounding variables, including sex and age. A statistical significance of the tubular damage risk disappeared after adjustment for covariates in the middle urinary Cd group, which could be understood that Cd exposure dose (0.87–1.54 μg/g creatinine) was relatively low to induce tubular damage. So, the crude risk of tubular damage in the middle urinary Cd group might be ascertained by the effects of potential confounding variables, including sex and age rather than the Cd only.

eGFR was negatively correlated with urinary Cd in our study subjects, but the prevalence of eGFR abnormality was not associated with urinary Cd levels. Although the prevalence of bone damage increased according to urinary Cd levels, the association was not significant after controlling for covariates, including sex and age. Accordingly, the present study does not provide evidence of a relationship between long-term exposure to low levels Cd and decreased glomerular filtration rate or effects on bone health, which is consistent with the findings of several previous studies (Horiguchi et al. 2005; Trzcinka-Ochocka et al. 2010; Kim and Lee 2012; Wang et al. 2016). On the other hand, significant correlations between Cd exposure and decreased glomerular filtration rate or decreased BMD in the general population have been reported in several other studies (Alfvén et al. 2000; Akesson et al. 2005, 2006; Hwangbo et al. 2011; Myong et al. 2012). Given studies on the general population, it is still controversial whether or not environmental Cd exposure is associated with decreased glomerular filtration rate. It is understood that kidney dysfunction is not an indicator of early adverse effects of Cd exposure, such as low molecular weight proteinuria, but progresses relatively slowly and appears at a later stage of chronic Cd exposure (Järup et al. 1998). Our data also suggest that bone damage caused by low level Cd exposure would be induced indirectly rather than by direct effects on the bone. Namely, BMD reduction in the general population exposed to low levels of Cd may be caused via loss of calcium or impaired vitamin D metabolism secondary to kidney dysfunction (Kjellstrom 1992; Järup et al. 1998; Alfvén et al. 2000). However, a systematic prospective cohort epidemiological study rather than a cross-sectional study would be necessary to evaluate the association between Cd exposure and target organic diseases, such as kidney dysfunction and bone damage, in the general population.

In conclusion, the level of urinary Cd in the general Korean population is higher than that in western countries, such as Sweden, the United States, and Germany, but similar to or less than that in Asian countries, such as Japan and China. Our findings support the view that environmental Cd exposure may induce early effects on renal tubules but may not result in glomerular dysfunction or bone damage. Also, oxidative stress may play a role in the early effects of Cd on renal tubules.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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