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ORIGINAL ARTICLE

A longitudinal study on urinary cadmium and renal tubular protein excretion of nickel–cadmium battery workers after cessation of cadmium exposure

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Abstract
Purpose This study aimed to predict the outcome of urinary cadmium (Cd) excretion and renal tubular function by analyzing their evolution through 10 years after Cd exposure ceased.
Methods Forty-one female, non-smoking workers were recruited from the year 2004 to 2009 when being removed from a nickel–cadmium battery factory, and they were asked to provide morning urine samples on three consecutive days at enrollment and in every follow-up year until 2014. Urinary Cd and renal tubular function biomarkers including urinary β_2-microglobulin (β_2-m) and retinol-binding protein (RBP) concentrations were determined with the graphite furnace atomic absorption spectrometry and the enzyme-linked immunosorbent assays, respectively.
Results The medians of baseline Cd, β_2-m and RBP concentrations at enrollment were 6.19, 105.38 and 71.84 μg/g creatinine, respectively. Urinary β_2-m and RBP concentrations were both related to Cd concentrations over the years ($\beta_{\text{absolute-β2-m}} = 9.16$, $P = 0.008$ and $\beta_{\text{absolute-RBP}} = 6.42$, $P < 0.001$, respectively). Cd, β_2-m and RBP concentrations in the follow-up years were all associated with their baseline concentrations ($\beta_{\text{absolute-Cd}} = 0.61$, $P < 0.001$; $\beta_{\text{absolute-β2-m}} = 0.64$, $P < 0.001$; and $\beta_{\text{absolute-RBP}} = 0.60$, $P < 0.001$, respectively), and showed a decreasing tendency with the number of elapsed years relative to their baseline concentrations ($\beta_{\text{relative-Cd}} = -0.20$, $P = 0.010$; $\beta_{\text{relative-β2-m}} = -17.19$, $P = 0.002$; and $\beta_{\text{relative-RBP}} = -10.66$, $P < 0.001$, respectively).
Conclusions Urinary Cd might eventually decrease to the general population level, and Cd-related tubular function would improve under the baseline conditions of this cohort.

Keywords Cadmium · β_2-Microglobulin · Retinol-binding protein · Exposure cessation · Repeated measurement

Introduction

Cadmium (Cd) is an industrial toxicant as well as an environmental contaminant, which causes multiple adverse health effects including renal dysfunction, bone mineral density reduction and lung impairment (Järup and Akesson 2009; Johri et al. 2010; Satarug et al. 2010). International Agency for Research on Cancer classified Cd and its compounds as "carcinogenic to humans (Group 1)" early in 1993 (IARC 1993) and reconfirmed their carcinogenicity on the lung and suggested carcinogenicity on the kidney and the prostate in the recent evaluation (IARC 2012). As a by-product of Zinc and lead smelter and a primary material used in electroplating, pigments and battery production, etc. (Nordberg et al. 2015), Cd in dust could be inhaled by workers without proper occupational protections. Cd in industrial sewage or dust may also pollute the soil or water where crops, rice particularly, and marine animals grow, and then, bioaccumulation of Cd may lead to Cd intake in the local residents who consume the rice or seafood (Satarug et al. 2003; Zhang et al. 2012). Besides, cigarette smoking also gives rise to Cd exposure (Johri et al. 2010). All circumstances could result in accumulation of Cd in the human body.

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Cd mainly accumulates in the kidney and excretes through urine (Godt et al. 2006). Urinary Cd was found correlated well with accumulation of Cd in the kidney and therefore is a good substitute measure of total body burden of Cd (Godt et al. 2006). The renal proximal tubular cells are the primary target site attacked by Cd due to the receptor-mediated endocytosis of freely filtered and metallothionein-bound Cd, leading to impaired reabsorption of renal proximal tubules (Jin et al. 1998; Prozialeck and Edwards 2012). The earliest sign of tubular dysfunction is increased urinary excretion of low molecular weight (LMW) proteins (molecular weight <40 kD) such as β_2-microglobulin (β_2-m) and retinol-binding protein (RBP), which has been previously observed among active workers in the Cd industry and among Cd-contaminated food consumers (Bernard 2004). The LMW proteinuria was also reported as a sensitive index for monitoring renal tubular function of those who had ceased known exposure to Cd (Kawasaki et al. 2004), because Cd has a very long biological half-life of 10–30 years (Järup et al. 1998), and it may continue to cause tubular injury after the exposure ends.

Although previous studies have reported that urinary Cd decreased after Cd exposure reduced or ceased (Iwata et al. 1993; Kawasaki et al. 2004; Kobayashi et al. 2008; Wu et al. 2008), whether it could decrease to the level of the general population is less documented. More importantly, the outcome of Cd-induced tubular damage after Cd exposure is still debatable and has elicited contradictory reports in the scientific literature. Some authors concluded that tubular proteinuria of the exposed population was irreversible or progressive in a long period after the exposure (Cai et al. 2001; Iwata et al. 1993; Kobayashi et al. 2008; Sato et al. 2010; Swaddiwudhipong et al. 2012; Zhang et al. 2014), whereas others such as Hotz et al. (1999) and Liang et al. (2012) believed that renal impairment in mild and early forms or induced in relatively low exposure levels might be reversible and that the kidney function could recover to some extent. A thorough examination of urinary Cd excretion and renal tubular function after the exposure, specifically their yearly evolution in a relatively long term, may help to clarify these issues. In addition, it is still to be determined whether the evolution of urinary Cd and tubular protein excretion is related to their baseline conditions when the exposure ends. Such relations can support and help to identify specific cutoff values for predicting the outcomes.

In the present study, we recruited a cohort of 41 female, non-smoking workers and measured Cd, β_2-m and RBP concentrations in their morning urine samples collected on three consecutive days at enrollment and in each of the follow-up years after their exposure to Cd had ceased. We analyzed the 10-year evolution of urinary Cd excretion and of renal tubular function to predict their outcomes.

Methods

Participants

We recruited 41 female workers from September 2004 to December 2009 when they were removed from their previous Cd-exposed positions in a nickel–cadmium battery factory in Shenzhen, China. After written informed consents were obtained, we collected detailed information on age, sex, and life styles such as smoking, drinking and food consumption, medical conditions and occupational history with structured questionnaires. The 41 female workers had been followed up as a cohort from their enrollment till December 2014. At enrollment and in the same period of each follow-up year, they were asked to provide morning urine samples on three consecutive days for repeated measurement of Cd, β_2-m and RBP concentrations. Ethical approval of the study was granted by the Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases.

Urinary Cd concentrations

The collected urine samples were immediately sent to the laboratory for determination of the biological indices. Urinary Cd was measured by means of the graphite furnace atomic absorption spectrometry with a contrAA 700 instrument (Analytik Jena AG, Jena, Germany) according to the Industrial Hygiene Standard of the People's Republic of China (WS/T 32-1996). Briefly, 1.0 mL of urine specimens was diluted quantitatively with 1.0 mL of matrix modifier (1.0 g of diammonium phosphate dissolved in 100 mL of 1 % (*V/V*) HNO_3) and 8.0 mL of 1 % (*V/V*) HNO_3 and measured under the following conditions: a wavelength of 228.8 nm, a slit of 0.3 nm, a lamp current of 6 mA and an injection volume of 10 μL. The detection limit of this method was 0.28 μg/L. All the Cd concentrations were subsequently adjusted with the urinary creatinine concentrations that were determined by a modified Jaffé reaction using a Beckman Synchron LX 20 analyzer (Beckman Coulter, Krefeld, Germany) (Peake and Whiting 2006).

Urinary β_2-m and RBP concentrations

Urinary β_2-m and RBP concentrations were measured using commercial β_2-MG and RBP ELISA Kits (SUNBIO, Shanghai, China) following the manufacturer's instructions, respectively. Each urine specimen was fivefold diluted and measured in duplicate. The means of the duplicate measures of urinary β_2-m and RBP concentrations were finally adjusted with urinary creatinine concentrations as well.

Statistical analyses

The means of repeated measures of the biomarkers on 3 days were calculated as final values for each worker at enrollment and in the follow-up years. Urinary Cd, β_2-m and RBP concentrations of the workers were presented as medians with interquartile ranges (IQRs), while age and length of employment were presented as means with standard deviations (SDs). We used random-effects linear regression models to analyze the effects of baseline concentrations, age, length of employment, and time lapse on Cd, β_2-m and RBP concentrations after the exposure and also the association of Cd concentrations with β_2-m and RBP concentrations. To minimize the potential influence of selection bias caused by loss of follow-up, we calculated relative concentrations by subtracting baseline Cd, β_2-m and RBP concentrations from the Cd, β_2-m and RBP concentrations in each of the follow-up years, respectively. The random-effects linear regression models were also used to analyze linear trends of relative Cd, β_2-m and RBP concentrations with the number of years elapsed after Cd exposure. Subsequently, we fitted a quadratic curve of relative Cd concentrations and cubic curves of relative β_2-m and RBP concentrations through the follow-up years. All statistical tests were two-tailed with a significance level of $P < 0.05$ and performed using the Stata statistical software (Stata statistical software, release 12.0; Stata Corp, College Station, TX, USA).

Results

General characteristics and baseline Cd, β_2-m and RBP concentrations

The worker cohort was followed up for a median of 8 years, with a maximum of 10 years and a minimum of 1 year (Table 1). It is notable that most of the workers were not followed up consecutively, because they left the city when they were removed from the battery factory and might refuse in a particular follow-up year or forever to participate in the study due to tight work schedule, inconvenience of traffic, or other reasons as they stated by phone. The detailed information on participation of each worker at enrollment and in the follow-up years was illustrated in Online Resource 1.

The 41 female workers had an average age of 30.8 years at enrollment, and they reported no smoking or drinking habit and had an average employment time of 7.47 years in the nickel–cadmium battery factory. None of the workers reported having been consuming food from Cd-polluted areas, such as Hunan, Northeast Guangxi and Northwest Guangdong. None of them had hypertension, diabetes, urinary stones or other diseases that could impair renal function according to their medical history. At the enrollment, the median of baseline urinary Cd concentrations for the cohort was 6.19 μg/g creatinine with a maximum of 15.43 μg/g creatinine and a minimum of 2.18 μg/g creatinine, and the medians of baseline urinary β_2-m and RBP concentrations were 105.38 and 71.84 μg/g creatinine, respectively (Table 1).

Cd, β_2-m and RBP concentrations and the influencing factors

Using the random-effects linear regression model which is one of the appropriate statistical models for analyzing panel data from a follow-up study, we found that Cd concentrations were significantly associated with β_2-m and RBP concentrations over the years ($\beta_{\text{absolute-β2-m}} = 9.16$, $P = 0.008$ and $\beta_{\text{absolute-RBP}} = 6.42$, $P < 0.001$, respectively), suggesting a potential relationship between Cd burden and renal tubular function. According to the model, 1.0 μg/g creatinine decrease in urinary Cd was related to 9.16 μg/g creatinine decrease in urinary β_2-m and 6.42 μg/g creatinine decrease in urinary RBP in 10 years. Cd, β_2-m and RBP

Table 1 Characteristics and baseline urinary Cd, β_2-m and RBP concentrations of the nickel–cadmium battery worker cohort at enrollment ($n = 41$)

Variable	Value
Age (year, mean ± SD)	30.78 ± 6.70
Sex [female, *n* (%)]	41 (100.0)
Non-smoker [yes, *n* (%)]	41 (100.0)
Nondrinker (yes, *n* (%))	41 (100.0)
Length of employment (year, mean ± SD)	7.47 ± 4.19
Length of follow-up years [year, median (min, max)]	8 (1, 10)
Urinary Cd concentration [μg/g creatinine, median (IQR)]	6.19 (5.17–7.45)
Urinary β_2-m concentration [μg/g creatinine, median (IQR)]	105.38 (76.16–190.23)
Urinary RBP concentration [μg/g creatinine, median (IQR)]	71.84 (44.98–115.57)

Cd cadmium, *β₂-m* β_2-microglobulin, *RBP* retinol-binding protein, *SD* standard deviation, and *IQR* interquartile range

Table 2 Regressions of the potential influencing factors on urinary Cd, β_2-m and RBP concentrations in the worker cohort

Dependent variable	Independent variable	$\beta_{absolute}$ (95 % CI)	*P*
Urinary Cd (μg/g creatinine)	Baseline urinary Cd (μg/g creatinine)	0.61 (0.45–0.77)	<0.001
	Age (year)	0.03 (−0.05 to 0.10)	0.445
	Length of employment (year)	−0.02 (−0.13 to 0.10)	0.791
	Time elapsed after Cd exposure (year)	−0.30 (−0.41 to −0.18)	<0.001
Urinary β_2-m (μg/g creatinine)	Baseline urinary β_2-m (μg/g creatinine)	0.64 (0.51–0.77)	<0.001
	Urinary Cd (μg/g creatinine)	9.16 (2.39–15.94)	0.008
	Age (year)	0.28 (−5.20 to 5.77)	0.919
	Length of employment (year)	−0.83 (−9.13 to 7.46)	0.844
	Time elapsed after Cd exposure (year)	−5.40 (−13.46 to 2.66)	0.189
Urinary RBP (μg/g creatinine)	Baseline urinary RBP (μg/g creatinine)	0.60 (0.48–0.73)	<0.001
	Urinary Cd (μg/g creatinine)	6.42 (3.10–9.74)	<0.001
	Age (year)	0.13 (−1.98 to 2.25)	0.903
	Length of employment (year)	0.30 (−2.95 to 3.55)	0.857
	Time elapsed after Cd exposure (year)	−4.83 (−8.50 to −1.16)	0.010

Random-effects linear regression models

Cd cadmium, *β_2-m* β_2-microglobulin, *RBP* retinol-binding protein, and *CI* confidence interval

Table 3 Linear trends of relative Cd, β_2-m and RBP concentrations with the time elapsed

Dependent variable	Independent variable	$\beta_{relative}$ (95 % CI)	*P*
Relative Cd concentration (μg/g creatinine)	Age (year)	−0.05 (−0.17 to 0.08)	0.467
	Length of employment (year)	0.01 (−0.19 to 0.20)	0.926
	Time elapsed after Cd exposure (year)	−0.20 (−0.35 to −0.05)	0.010
Relative β_2-m concentration (μg/g creatinine)	Age (year)	7.27 (−0.81 to 15.34)	0.078
	Length of employment (year)	−4.82 (−17.72 to 8.08)	0.464
	Time elapsed after Cd exposure (year)	−17.19 (−27.97 to −6.41)	0.002
Relative RBP concentration (μg/g creatinine)	Age (year)	2.85 (−0.42 to 6.11)	0.087
	Length of employment (year)	0.40 (−4.82 to 5.62)	0.880
	Time elapsed after Cd exposure (year)	−10.66 (−15.83 to −5.48)	<0.001

Random-effects linear regression models

Cd cadmium, *β_2-m* β_2-microglobulin, *RBP* retinol-binding protein, and *CI* confidence interval

concentrations after the exposure were all associated with their respective baseline concentrations ($\beta_{absolute\text{-}Cd} = 0.61$, $P < 0.001$; $\beta_{absolute\text{-}\beta2\text{-}m} = 0.64$, $P < 0.001$; and $\beta_{absolute\text{-}RBP} = 0.60$, $P < 0.001$, respectively), suggesting that workers with higher baseline Cd concentrations had significantly more urinary Cd excretion and those with less tubular protein excretion at enrollment had better kidney function in the following years. Cd concentrations showed a decreasing trend from enrollment through the follow-up years ($\beta_{absolute\text{-}Cd} = -0.30$, $P < 0.001$), whereas the significant trend of β_2-m concentrations with time lapse disappeared when Cd concentrations were added into the model, suggesting that the decrement of β_2-m concentrations was attributed to the decreasing of urinary Cd with the number of years elapsed. Unlike β_2-m concentrations, RBP concentrations still showed a decreasing tendency through the follow-up years after the effect of urinary Cd was adjusted ($\beta_{absolute\text{-}RBP} = -4.83$, $P = 0.010$). Neither age nor length of employment had a significant influence on Cd, β_2-m or RBP concentrations (Table 2).

Relative Cd, β_2-m and RBP concentrations through the follow-up years

Linear trends of relative Cd, β_2-m and RBP concentrations with the number of years elapsed were also analyzed to minimize the potential influence of selection bias caused by loss of follow-up. We observed that relative Cd, β_2-m

and RBP concentrations all decreased through the 10 years ($\beta_{\text{relative-Cd}} = -0.20$, $P = 0.010$; $\beta_{\text{relative-β2-m}} = -17.19$, $P = 0.002$; and $\beta_{\text{relative-RBP}} = -10.66$, $P < 0.001$, respectively) (Table 3), which confirmed results in Table 2. In order to better understand the dynamic change of Cd, β_2-m and RBP concentrations, we further fitted a quadratic curve of relative Cd concentrations and cubic curves of relative β_2-m and RBP concentrations through the follow-up years, respectively. It is interesting that the decreasing of Cd concentrations was slightly accelerated through the years, while β_2-m and RBP concentrations first shortly increased and then gradually decreased and then lightly increased, in other words, fluctuated relative to their respective baseline concentrations (Fig. 1).

Discussion

The outcome of urinary Cd excretion and of Cd-affected renal function after Cd exposure has been one of concerns for studies on Cd-related health effects and aroused a long-standing debate in the literature because of its uncertainty. To try to settle this dispute, we conducted a follow-up study examining the dynamic change of urinary Cd and of β_2-m and RBP concentrations as biomarkers of tubular function in 10 years in a cohort of 41 female, non-smoking workers after their exposure to Cd had ceased. We found that Cd concentrations showed a gradual or, according to the fitted curve, a slightly accelerated decline through the follow-up years, suggesting that Cd burden in the workers continuously reduced after the exposure ended. Moreover, urinary β_2-m and RBP concentrations were both associated with Cd concentrations over the 10 years and generally decreased, which suggested that Cd-related tubular function improved to some extent. We also noticed that the baseline conditions were predictive of urinary Cd excretion and renal function in the following years.

It is well known that Cd accumulates mostly in the kidney and is difficult to be removed (Johri et al. 2010). To date, the evolution of urinary Cd excretion after Cd exposure ceases has not been thoroughly examined, specifically the every-year condition in as long as 10 years. As demonstrated in our study, urinary Cd concentrations as well as relative Cd concentrations kept decreasing from enrollment through the follow-up years, which is biologically plausible because of the ongoing excretion of Cd from the kidney. Most of relevant studies reported similar results about urinary Cd. For instance, Liang et al. (2012) reported that urinary Cd concentrations decreased from a geometric mean of 11.6 to 9.0 μg/g creatinine over 8 years after reduction in environmental Cd exposure. Kobayashi et al. (2008) even calculated a reduction rate of 5.0 % per year in women. The reduction rate in our cohort was comparable

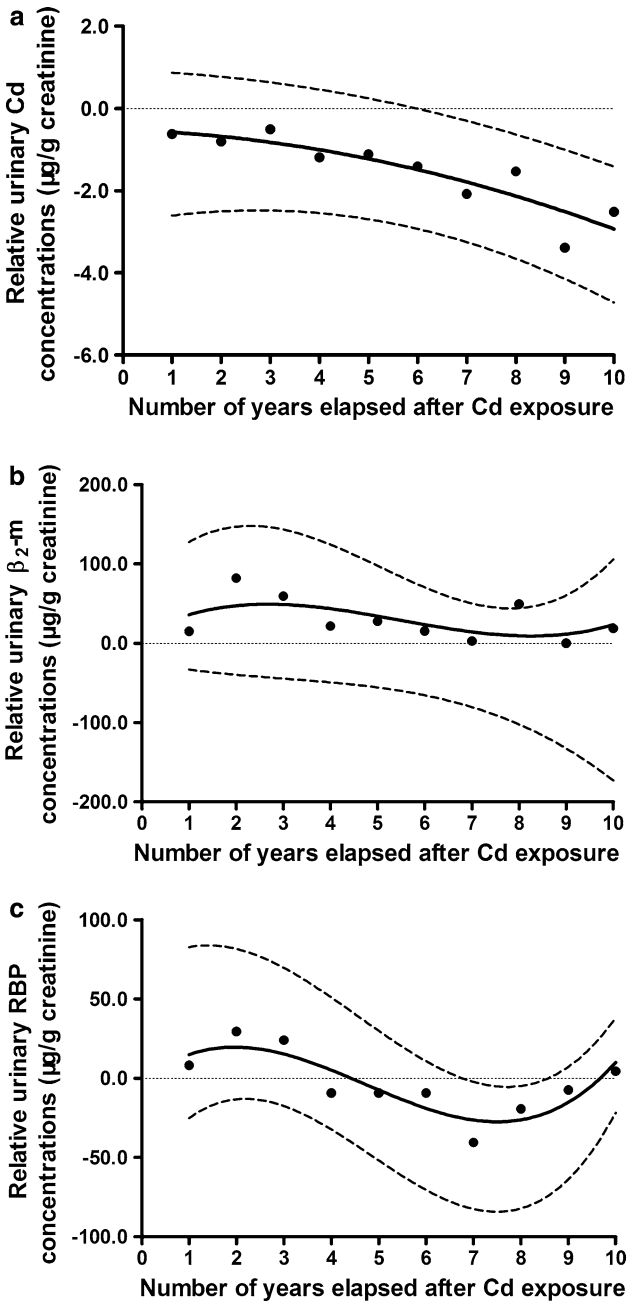

Fig. 1 Relative urinary cadmium (Cd), β_2-microglobulin (β_2-m) and retinol-binding protein (RBP) concentrations of the workers through 10 years after cessation of Cd exposure. The concentrations were all relative to their respective baseline concentrations at enrollment. The *black dots* represent medians of the relative concentrations in each year, the *solid curves* represent the quadratic trend of relative Cd concentrations and the cubic trends of relative β_2-m and RBP concentrations through the 10 years, respectively, and the *dash curves* represent the interquartile ranges of the *solid curves*

(4.8 %) according to the random-effects linear regression model. However, in a 3-year follow-up study conducted by Wu et al. (2008), the geometric mean of urinary Cd increased from 3.7 to 5.5 μg/g creatinine, probably because

Cd exposure of the cohort was only reduced on some level instead of completely ceased as in our study. Based on the continuously decreasing trend of urinary Cd in 10 years, it seems quite reasonable and possible that urinary Cd of the workers could eventually decrease to the level of the general population. Calculated from the regression model of urinary Cd, the decrement of urinary Cd was 3.0 μg/g creatinine in 10 years and decreasing of urinary Cd to the local general population level [2.03 μg/g creatinine (Zhang et al. 2015a)] would take about 14 years on average.

The Cd burden before exposure ends does have a significant influence on the evolution of Cd excretion afterward. We observed in our cohort that higher baseline Cd concentrations led to relatively high urinary Cd excretion after the exposure. This phenomenon may be related to the toxicokinetics of Cd in the kidney, and it indicates that the kidney functions well enough to handle the amount of Cd burden within the studied range. Therefore, the outcome of urinary Cd decreasing to the general population level might be also fit for other people with baseline urinary Cd under the level of this cohort. The above findings were first reported in a human population as far as we know, and they help to evaluate renal function on excreting Cd.

The causality of associations between urinary Cd indicating Cd exposure and biomarkers of tubular function has been questioned recently in populations with low-level Cd exposure, because of possible confounding by smoking or physiological factors (Bernard 2008; Chaumont et al. 2011, 2012; Haddam et al. 2011). In order to clarify this question, Akerstrom et al. (2013) conducted a study by repeated short-term sampling within individual non-smoking participants. They concluded that the associations between short-term changes in urinary Cd and kidney function biomarkers more likely resulted from normal variation in renal function than reflecting effects of Cd toxicity, and that the adverse effects of low-level Cd exposure on kidney function might be overestimated. Nevertheless, Wallin et al. (2014) confirmed the effects of low-level Cd exposure later and attributed false associations to insensitivity of biomarkers. Although the baseline Cd exposure in our cohort was high relative to the Cd level in the local general population (2.03 μg/g creatinine, Zhang et al. 2015a), and all the participants were non-smokers, there was still possibility that the observed associations might be confounded by physiological factors such as diuresis or glomerular filtration rate. However, we collected urine samples from the same individual on three consecutive days on each sampling occasion, and the average values of biomarkers were relatively stable with respect to physiological sources of variability (Akerstrom et al. 2013). Also, according to Akerstrom et al. (2013), urinary creatinine-adjusted biomarkers as used in our study were less affected by normal physiological variations. Moreover, the associations of urinary Cd with the effect biomarkers were obtained based on measures over 10 years, reflecting long-term instead of short-term effects of Cd on renal function, and thus were more accurate and reliable. The variations of urinary proteins over the years were not coincident with that of urinary Cd according to the curves of relative concentrations we fitted; therefore, it is actually impossible that their associations were confounded by physiological variation or other factors in the same direction. All above support that the tubular function indicated by urinary β_2-m and RBP was related to Cd exposure in this cohort.

Contradiction has long existed on reversibility of renal function after cessation or reduction in Cd exposure. We showed in this study that urinary β_2-m and RBP concentrations were generally decreased across the follow-up years, suggesting an improvement of tubular function in the workers. Similar result was reported by Kawasaki et al. (2004) who observed decrement of urinary LMW protein in the workers over 4-year improvement of exposure condition in a Cd pigment factory in Japan. Wu et al. (2008) and Liang et al. (2012) also reported improving of tubular function in their follow-up studies after the reduction in Cd exposure from rice, and they concluded that the evolution of renal function was determined by the baseline Cd level. For those with baseline urinary Cd less than 10 μg/g creatinine, renal function was found to be improved, otherwise worsened (Wu et al. 2008; Liang et al. 2012). On the contrary, Iwata et al. (1993) and Swaddiwudhipong et al. (2012) reported that renal function after the exposure was worsened over time, but the average baseline Cd concentrations in their cohorts were under 10 μg/g creatinine. Although this cutoff value of baseline Cd level is applicable to our study, its predictive value cannot be verified in our cohort because of the limited sample size and narrow range of urinary Cd concentration. However, by analyzing studies supporting and objecting to the reversibility, which were summarized in Online Resource 2, we do not consider baseline Cd exposure condition as a primary influencing factor. Instead, baseline condition of tubular function is more likely to determine its further evolution. This point of view is strongly supported by our finding that β_2-m and RBP concentrations over the years were positively associated with their baseline concentrations, and also by the result from Liang et al.'s study (2012). Nonetheless, better cutoff values of baseline effect biomarkers are yet to be determined, since the 1500 μg/g creatinine of urinary β_2-m previously proposed by Roels et al. (1997) is also controversial (Online Resource 2).

Fitting curves of β_2-m and RBP concentrations demonstrated variation of tubular function better than linear trends. By doing so, we observed that there was also decline of renal function at some stage after Cd exposure relative to the baseline conditions, although the decline was not the

main trend. According to the patterns of relative β_2-m and RBP concentrations with time lapse, renal function of the workers first worsened shortly and then gradually improved to some extent and then slightly worsened again relative to their baseline conditions. It is well known and supported by the relation between Cd concentrations and β_2-m and RBP concentrations in this study that effects on the kidney are caused by the accumulation of Cd in the proximal tubular cells (Godt et al. 2006); therefore, the increment of β_2-m and RBP concentrations till the second year after cessation of exposure seemed unreasonable, considering the continuously decreasing of urinary Cd. One possible explanation is that development of potential renal damage lagged behind Cd burden in the body. As for the drop of kidney function since the seventh year after Cd exposure, aging could be a possible contributor, because we observed a positive association between aging and relative β_2-m and RBP concentrations (linear regression of age on relative β_2-m and RBP concentrations with time lapse unadjusted, data not shown). There may also be other explanations that beyond our comprehension based on hitherto available resources.

Taken together, both from the perspective of linear and curvilinear trends and from that of cutoff values, Cd-related tubular function in this worker cohort was likely to improve in the long run. The decrements of β_2-m and RBP concentrations in 10 years were calculated to be 85.9 and 70.0 μg/g creatinine, respectively, according to the regression models of urinary β_2-m and RBP. Besides, β_2-m and RBP concentrations of the workers, although higher than the local general population (0.05 and 0.00 mg/g creatinine, respectively, Zhang et al. 2015a), were both within the reference scopes (<1001.6 and <946.9 μg/g creatinine, respectively). Therefore, clinically speaking, the renal function of the workers should not be a matter of concern. Instead, in the most recent health examination of the cohort in 2015, 80 % workers complained about different degrees of backache and/or joint pain, and 53 % were newly found to have kidney stones, which imply a more serious impairment of bones. The long-term relationship of Cd burden and bone damage after cessation of exposure is to be further explored.

Normally found on the surface of nearly all nucleated cells and in small amounts in the blood and urine, β_2-m is readily filtered through the glomerular membrane and almost completely resorbed by the renal proximal tubules (Wu et al. 2008). RBP is produced in the liver as a transport protein of vitamin A, and free RBP, like β_2-m, mostly absorbed and catabolized in the proximal tubules (Goodman 1980; Marinó et al. 2001). According to Bernard et al. (1987), β_2-m is slightly more sensitive in detecting very early tubular injury, while RBP is relatively stable in acid urines. On condition that glomerular filtration rates of the workers were normal, we examined both indices to find that urinary β_2-m and RBP concentrations basically showed concordant results in this study, except that β_2-m concentrations were more specifically related to Cd concentrations. The independent association of RBP concentrations with the number of years elapsed might reflect change of liver function over time. Age has been considered as a potential confounding factor on kidney function markers in previous studies (Kobayashi et al. 2008; Liang et al. 2012), but we did not find a significant influence of it on urinary Cd, β_2-m and RBP concentrations, as well as on their relative concentrations. A possible reason is that our worker cohort has a relative narrow range of age. Another factor that was taken into account in our study is length of employment, which was expected to estimate cumulative Cd exposure for the workers. Unexpectedly, the employment time was not associated with urinary Cd concentrations, not to mention β_2-m and RBP concentrations. We surmise that length of employment is merely a coarse indicator and it could not differentiate the exposure levels between workplaces. Before being removed from the factory, the workers might be also exposed to nickel which was possibly another threat to their kidney function (Liu et al. 2015), but nickel is rapidly metabolized in the body; thus, it should have no lasting effect in the follow-up years. Because none of the workers had any known disease that could impair renal function, took medicine to accelerate Cd excretion or to improve renal function, had habit of smoking or drinking or regularly consumed food potentially leading to Cd intake, the influence of these factors was all excluded from this study.

Urinary creatinine had been typically used to adjust analyte concentrations in spot urine samples for urine concentration/dilution, but this method was challenged lately because urinary creatinine concentrations vary by age, sex, race/ethnicity, time of day, etc. (Barr et al. 2005), apart from changing water content in urine. New methods of adjustment were subsequently proposed, such as using creatinine as a predictor variable in linear regressions of the analyte (Barr et al. 2005; Zhang et al. 2015b), or even the better and more complicated method incorporating both covariate-adjusted standardization and the inclusion of creatinine as a covariate in the regression model (O'Brien et al. 2016). Admittedly, creatinine adjustment is not a perfect method or even confounding for people group with much demographic diversity; however, it is practicable to this cohort of workers because the recruited workers had a relatively narrow range of age and were all female and Han Chinese, and their urine samples were all first-morning voids, showing high homogeneity in most influencing factors of urinary creatinine. The reason why other better methods were not applied in this study is that urinary concentrations of the biomarkers before the year 2010 were traditionally reported as creatinine-adjusted concentrations

from the laboratory instead of analyte per liter urine; therefore, the creatinine adjustment is genuinely a compromised solution.

That 37 % of the participations were lost to follow-up inevitably leads to the concern for selection bias in this study. To minimize the potential influence of selection bias, we introduced relative Cd, β_2-m and RBP concentrations in the analyses which eliminated the inter-individual difference of baseline conditions. We also evaluated the potential selection bias by comparing baseline Cd, β_2-m and RBP concentrations between participants and those lost to follow-up in every follow-up year (Online Resource 3). The results did not suggest that selection bias could explain the main findings in the study, except that the acceleration of urinary Cd decrement might be caused by the loss of participants with lower baseline Cd concentrations. The relatively small sample size raises another concern for the statistical power of the study. However, the general characteristics of the workers in the cohort showed a high homogeneity, providing enough statistical power. Nevertheless, results from this study need validation in further studies with larger sample size. On the other hand, the high homogeneity of the cohort limited our effort to analyze the evolution of Cd excretion and of renal function by age and sex. The relatively narrow range of baseline Cd, β_2-m and RBP concentrations may prevent us from identifying specific cutoff values for different outcomes. As for the strength of the present study, it lies mainly in the repeated measurement of the exposure and effect biomarkers both on three consecutive days each year and in each of the follow-up years. The former largely eliminated potential confounding by physiological variation, and the later, together with the quadratic and cubic curves we fitted, enabled us to examine the yearly dynamic change of the biomarkers in a relative long period for the first time.

In this work, we thoroughly examined and analyzed the evolution of urinary Cd excretion and of renal tubular function through 10 years after cessation of Cd exposure, providing valuable information for prognostic evaluation on nephrotoxicity of Cd. We concluded that urinary Cd would likely decrease to the general population level through continuous urinary excretion, and the Cd-related renal function would also improve under the baseline levels of urinary Cd and tubular proteins in the cohort.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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