Clinical Kidney Journal

ckj

OXFORD

era edta
LEADING EUROPEAN NEPHROLOGY

Clinical Kidney Journal, 2018, vol. 11, no. 5, 681–687

doi: 10.1093/ckj/sfx153
Advance Access Publication Date: 2 February 2018
Original Article

ORIGINAL ARTICLE

Is renal tubular cadmium toxicity clinically relevant?

Kanyarat Boonprasert[1], David A. Vesey[2,3], Glenda C. Gobe[2], Ronnatrai Ruenweerayut[4], David W. Johnson[2,3], Kesara Na-Bangchang[1] and Soisungwan Satarug[2]

[1]Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand, [2]Centre for Kidney Disease Research, UQ Diamantina Institute and Centre for Health Services Research, Translational Research Institute, University of Queensland, Brisbane, Australia, [3]Department of Nephrology, Princess Alexandra Hospital, Brisbane, Australia and [4]Mae Sot General Hospital, Tak Province, Thailand

Correspondence and offprint requests to: David A. Vesey; E-mail: david.vesey@health.qld.gov.au

Abstract

Background: Exposure to cadmium (Cd) has been associated with the development of hypertension, especially in women, but the mechanism of such an association is not understood. We hypothesize that Cd exposure alters renal production of 20-hydroxyeicosatetraenoic acid (20-HETE), which plays an indispensable role in renal salt balance and blood pressure control.

Methods: We examined long-term Cd exposure in relation to urinary 20-HETE excretion levels, tubular dysfunction and blood pressure measures, using data from a population-based, cross-sectional study that included 115 normotensive and 110 hypertensive women, 33–55 years of age, who lived in Cd contamination areas in Thailand.

Results: The mean [standard deviation (SD)] blood Cd level of the study subjects was 3.57 (3.3) μg/L, while the mean (SD) urinary Cd and urinary 20-HETE levels were 0.58 (0.47) μg/g creatinine and 1651 (4793) pg/mL, respectively. Elevated 20-HETE levels were associated with a 90% increase in prevalence odds of hypertension ($P = 0.029$), four times greater odds of having higher urinary Cd levels ($P = 0.030$) and a 53% increase in odds of having higher levels of tubular dysfunction ($P = 0.049$), evident from an increase in urinary excretion of β2-microglobulin. In normotensive subjects, an increase in urinary 20-HETE levels from tertile 1 to tertile 3 was associated with a systolic blood pressure increase of 6 mmHg (95% confidence interval 0.3–12, $P = 0.040$).

Conclusions: This is the first report that links urinary 20-HETE levels to blood pressure increases in Cd-exposed women, thereby providing a plausible mechanism for associated development of hypertension.

Key words: blood pressure, β2-microglobulin, cadmium exposure, estimated glomerular filtration rate, hypertension, 20-hydroxyeicosatetraenoic acid, renal tubular dysfunction

Received: July 27, 2017. **Editorial decision:** November 27, 2017

Introduction

Exposure to cadmium (Cd), a food-chain contaminant, is a common problem worldwide [1–3]. It is estimated that 40–60% of dietary Cd intake, for the average consumer, comes from staple foods such as rice, potatoes and wheat, and that shellfish, crustaceans, molluscs, offal and spinach form the remaining sources of exposure [2]. Women of reproductive age and children are particularly at risk of Cd toxicity due to enhanced Cd uptake [1]. The most frequently reported renal effect of Cd exposure in the general population is injury to the proximal tubular epithelial cells, where Cd selectively accumulates [3]. Renal tubular cells are rich in mitochondria and heavily reliant on autophagy to maintain homeostasis [4], making them highly susceptible to Cd-induced apoptosis [5, 6]. Injury to and death of renal tubular epithelial cells can account for a reduction in tubular reabsorption capacity in Cd-exposed persons. Cd-linked tubular cell injury and functional impairment can be evaluated by urinary biomarkers, such as *N*-acetyl-β-D-glucosaminidase (NAG) enzyme and β2-microglobulin (β2-MG) [7–10], while urinary Cd excretion is an indicator of long-term Cd exposure or body burden [11–13]. However, despite numerous reports, these renal tubular effects associated with Cd exposure have often been considered to be benign and not clinically relevant.

Recent epidemiologic studies have suggested that chronic exposure to Cd, albeit in low levels, may increase the risk of hypertension, especially in non-smoking women [14–16]. The pathogenic mechanisms underlying the pressor effect of chronic Cd exposure remain unexplored. Cytochrome P450 (CYP) enzymes, such as CYP4A11 and CYP4F2, have been linked to the indispensable role of kidneys in blood pressure regulation [17–19]. Previously, we have shown that CYP4A11 and CYP4F2 are differentially expressed in the glomeruli, proximal tubules and distal tubules in human kidney samples [20]. We have also shown that expression levels of CYP4A11 and CYP4F2 protein in human kidneys correlated with donor age and the levels of Cd accumulation [21–23]. We hypothesize that exposure to Cd alters the tubular expression of CYP4A11 and CYP4F2 that catalyze ω-hydroxylation of the fatty acid arachidonic acid [24] to produce the eicosanoid 20-hydroxyeicosatetraenoic acid (20-HETE), which is involved in vascular function, salt balance in the kidney and blood pressure regulation [17–19]. We therefore undertook the present study to evaluate the associations between Cd exposure, urinary 20-HETE levels and development of hypertension in relationship to age, body mass index (BMI), smoking status and levels of tubular injury and tubular dysfunction, assessed by urinary NAG and β2-MG levels, respectively.

Materials and methods

We undertook a cross-sectional study, including 225 women who participated in a larger population-based study of men and women, drawn from Cd contamination areas in the Mae Sot District, Tak Province, Thailand [25]. The Human Research Ethics Committee of the Thailand's Ministry of Public Health approved this study and participants provided written informed consent prior to participation. We restricted the analysis to women, based on the literature reports of an association of Cd exposure and increased risk of hypertension development in women [14–16]. Inclusion criteria were apparently healthy, 33–55 years of age and resident at the current address for at least 30 years. Exclusion criteria were pregnancy, breast-feeding, history of metal work, a hospital record or diagnosis by physician of chronic kidney disease (CKD), heart disease, diabetes, anemia or hyperlipidemia. Smoking, regular use of medications, education, occupation, family health history and anthropometric data were obtained from questionnaires. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg, physician diagnosis or prescription of anti-hypertensive medications.

Assessment of Cd exposure and renal effects

Samples of whole blood were collected after overnight fasting. Second morning void urine samples were collected on the same day of blood sampling. Aliquots of blood and urine samples were stored at −80°C for later analysis. Assessment of long-term Cd exposure was based on urinary Cd levels, while recent Cd exposure was based on blood Cd levels [11–13]. Urine and blood Cd levels were quantified by atomic absorption spectrophotometry with the Zeeman-effect background correction system (Unicam model 989, Thermo Elemental Corp., Franklin, MA, USA). Calibration, quality control and quality assurance for the Cd quantitation were accomplished by simultaneous analysis of blood and urine control samples (ClinChekTM, Munich, Germany). The limit of quantification (LOQ) was 0.5 μg/L for urinary Cd and 0.3 μg/L for blood Cd. Assessment of tubular dysfunction was based on a reduction in tubular reabsorption activity, reflected by an increase in urinary excretion of β2-MG, the low-molecular-weight protein that can pass through the glomerular membrane and is subsequently reabsorbed by tubular cells [7–10]. Assessment of tubular integrity was based on urinary excretion of the enzyme NAG that originates mostly from tubular epithelial cells [7–10]. The levels of β2-MG in urine samples were measured with a solid phase microparticle enzyme immunoassay (AxSYM β2-MG, Abbott Diagnostics, Abbott Park, IL, USA), while the urinary levels of NAG were determined with a colorimetric assay, using 2-methoxy-4-(2′ nitrovinyl)-phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (MNP-GlcNAc) as a substrate (Diazyme Laboratories, Poway, CA, USA). The urinary albumin assay was based on a turbidimetric method (UniCel® DxC800 Synchron system, Beckman Coulter, Fullerton, CA, USA). The urinary 20-HETE assay was based on a competitive enzyme-linked immunoassay (Detroit R&D, Inc. Detroit, MI, USA). Urinary and serum creatinine assays were based on the Jaffe kinetic method using alkaline picrate (Siemens Healthcare Diagnostics, Newark, Delaware, NJ, USA). Renal function was based on estimated glomerular filtration rate (eGFR), calculated with the CKD Epidemiology Collaboration (CKD-EPI) equation [26].

Statistical analysis

The SPSS statistical package 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyze data. The Mann–Whitney U-test was used to compare two groups of subjects. Distribution of the variables was examined for skewness and those showing right skewing were subjected to logarithmic transformation before analysis, where required. Departure from normal distribution of variables was assessed by one sample Kolmogorov–Smirnov test. A linear regression model analysis was used to evaluate effects of Cd exposure on kidneys, based on urinary biomarkers, namely β2-MG, NAG and albumin. Logistic regression analysis was used to estimate prevalence odds ratio (POR) for the presence of hypertension and adverse kidney effects associated with elevated urinary 20-HETE levels. One-way Analysis of the Variance (ANOVA) was used to evaluate the variation in blood pressure measures

across quartiles of urinary 20-HETE levels. $P \leq 0.05$ for a two-tailed test was considered to indicate statistical significance.

Results

Descriptive characteristics of study subjects

Of the 225 women participating in this study, 115 were normotensive and 110 were hypertensive (99 persons were treated for hypertension and 11 persons were untreated). For the untreated group, mean ± standard deviation (SD) values for SBP and DBP were 150 ± 6.3 and 89.1 ± 11.4 mmHg, respectively. For the treated group, mean ± SD values for SBP and DBP were 127.9 ± 14.1 and 81.8 ± 10.9 mmHg, respectively. Treated subjects received anti-hypertensive medications for a mean period of 4 years (range: 0.8 −14.5 years). The distribution of mean arterial pressure showed a tendency to conform to a normal distribution ($Z = 1.274$, $P = 0.078$). There was a substantial correlation between blood Cd and urinary Cd (the Spearman correlation coefficient (r) of 0.541 ($P < 0.001$). Relative to the normotensive group, the hypertensive group had ∼2-fold higher urinary 20-HETE levels ($P = 0.02$), 9.9% higher SBP ($P < 0.001$), 7.7% higher DBP ($P < 0.001$), 4.2% lower eGFR ($P = 0.04$), 22.5% lower blood Cd levels ($P = 0.05$) and 18.8% lower urinary Cd levels ($P = 0.05$). The percentage of urinary Cd ≥1 μg/g creatinine (a threshold for tubular dysfunction) and urinary levels of albumin, NAG and β2-MG in hypertensive group did not differ from the normotensive group (Table 1).

Effects of Cd exposure on kidneys

To statistically evaluate the effect of Cd exposure on kidney function, we used a linear regression model analysis of Cd body burden, assessed by urinary Cd levels. In such model analysis (Table 2), urinary Cd was a continuous dependent variable, while age, BMI and blood pressure status were independent variables as were the urinary levels of biomarkers of kidney effects, namely urinary β-MG, NAG and albumin. Given a known independent effect of smoking on the kidney, results are shown separately for non-smokers ($n = 159$) and smokers ($n = 60$). Only 6.7% of the variation in Cd body burden in non-smokers was associated with the six independent variables ($P = 0.011$), but a larger proportion (34.6%) of the total variation in Cd body burden in smokers was associated with these six independent variables ($P < 0.001$). In non-smokers, urinary Cd levels did not show association with the blood pressure group ($\beta = 0.036$, $P = 0.642$), while higher urinary Cd levels showed moderate associations with lower BMI ($\beta = -0.165$, $P = 0.038$), and higher tubular injury levels, reflected by urinary NAG levels ($\beta = -0.171$, $P = 0.037$). In smokers, however, higher urinary Cd levels were moderately associated with blood pressure group ($\beta = 0.269$, $P = 0.020$), while they were strongly associated with lower BMI ($\beta = -0.472$, $P < 0.001$) and moderately associated with higher urinary NAG levels ($\beta = 0.349$, $P = 0.005$), indicative of tubular injury. In addition, higher urinary Cd levels in smokers also showed a tendency for a moderate association with higher urinary albumin levels ($\beta = 0.227$, $P = 0.055$).

Cd body burden as a predictor of elevated urinary 20-HETE levels

To explore potential involvement of 20-HETE in Cd toxicity, we tested if Cd body burden indicator, assessed by urinary Cd measurement, was a predictor of urinary 20-HETE excretion. Figure 1 shows scatter plots of urinary Cd versus 20-HETE levels, stratified according to blood pressure status and treatment for hypertension. The strength of association (standardized β coefficient) was greatest in the untreated hypertensive group [$\beta = 0.664$, 95% confidence interval (CI) 0.304–3.724, $P = 0.026$], followed by the normotensive group ($\beta = 0.283$, 95% CI 0.258–1.145, $P = 0.002$) and the treated group ($\beta = 0.270$, 95% CI 0.236–1.465, $P = 0.007$). To test for interaction, we used a generalized linear model that included all subjects and incorporated urinary 20-HETE excretion as a continuous dependent variable. Age, BMI, urinary Cd, smoking status and blood pressure groups were incorporated as covariates along with blood pressure versus smoking interaction term. Urinary Cd excretion and BMI

Table 1. Characteristics of study subjects

Descriptors/variables	All subjects	Hypertensive	Normotensive	P-value
Number of subjects	225	110	115	
Smoking prevalence (%)	27.1	20.9	33.0	0.04
Age (years)	47.2 ± 4.6	47.2 ± 4.4	47.2 ± 4.7	0.99
BMI (kg/m^2)	24.8 ± 3.6	25.3 ± 3.6	24.4 ± 3.5	0.07
Anti-hypertensive medication (%)	48.4	90	0	
Systolic pressure (mmHg)	124.2 ± 14.0	130.2 ± 15.0	118.5 ± 10.1	<0.001
Diastolic pressure (mmHg)	79.6 ± 10.4	82.6 ± 11.1	76.7 ± 8.8	<0.001
Mean arterial pressure (mmHg)	94.4 ± 10.6	98.4 ± 10.4	90.6 ± 8.1	<0.001
eGFR (mL/min/1.73 m^2)	95.4 ± 16.5	93.3 ± 16.8	97.4 ± 15.9	0.04
Urinary 20-HETE (pg/mL)	1651 ± 4793	2429 ± 6657	913 ± 1348	0.02
Exposure biomarkers				
Blood Cd (μg/L)	3.6 ± 3.3	3.1 ± 2.6	4.0 ± 3.9	0.05
Urinary Cd (μg/g creatinine)	0.58 ± 0.47	0.52 ± 0.39	0.64 ± 0.54	0.05
Urinary Cd ≥1 μg/g creatinine (%)	14.2	12.7	15.7	0.53
Urinary biomarkers for kidney effects				
Albumin (mg/g creatinine)	1.12 ± 2.13	1.38 ± 2.55	0.88 ± 1.61	0.18
NAG (units/g creatinine)	1.43 ± 1.28	1.41 ± 1.14	1.45 ± 1.41	0.81
β2-MG (μg/g creatinine)	46.2 ± 301.8	21.3 ± 60.5	69.6 ± 415.7	0.12

Numbers are arithmetic mean ± standard deviation (SD).
Mean arterial pressure = diastolic pressure + (pulse pressure)/3, where pulse pressure = systolic − diastolic.
eGFR is determined with the CKD-EPI equation [26].

Table 2. Cd body burden and urinary biomarkers of adverse kidney effects

	Non-smokers (n=159)		Smokers (n=60)	
Independent variables	β (95% CI)	P-value	β (95% CI)	P-value
Age (years)	−0.028 (−0.012–0.008)	0.719	0.032 (−0.015–0.020)	0.774
BMI (kg/m^2)	−0.165 (−0.029–0.000)	0.038	−0.472 (−0.053 to−0.018)	<0.001
Blood pressure status	0.036 (−0.071–0.114)	0.642	0.269 (0.027–0.313)	0.020
Log urine β2-MG (μg/g creatinine)	0.120 (−0.019–0.114)	0.160	0.185 (−0.018–0.160)	0.117
Log urine NAG (units/g creatinine)	0.171 (0.013–0.404)	0.037	0.349 (0.119–0.619)	0.005
Log urine albumin (mg/g creatinine)	−0.016 (−0.075–0.060)	0.834	0.227 (−0.002–0.180)	0.055
Adjusted R^2	0.067	0.011	0.346	<0.001

Cd body burden, as logarithmically transformed urinary Cd (μg/g creatinine) was a continuous dependent variable. Creatinine-adjusted urinary β-MG, NAG and albumin levels were continuous independent variables, while blood pressure status was categorized independent variable (normotensive = 1, hypertensive = 2).
Adjusted R^2 indicates the total variation in Cd body burden, explained by all six independent variables.
$P \leq 0.05$ is considered to indicate statistical significant levels.

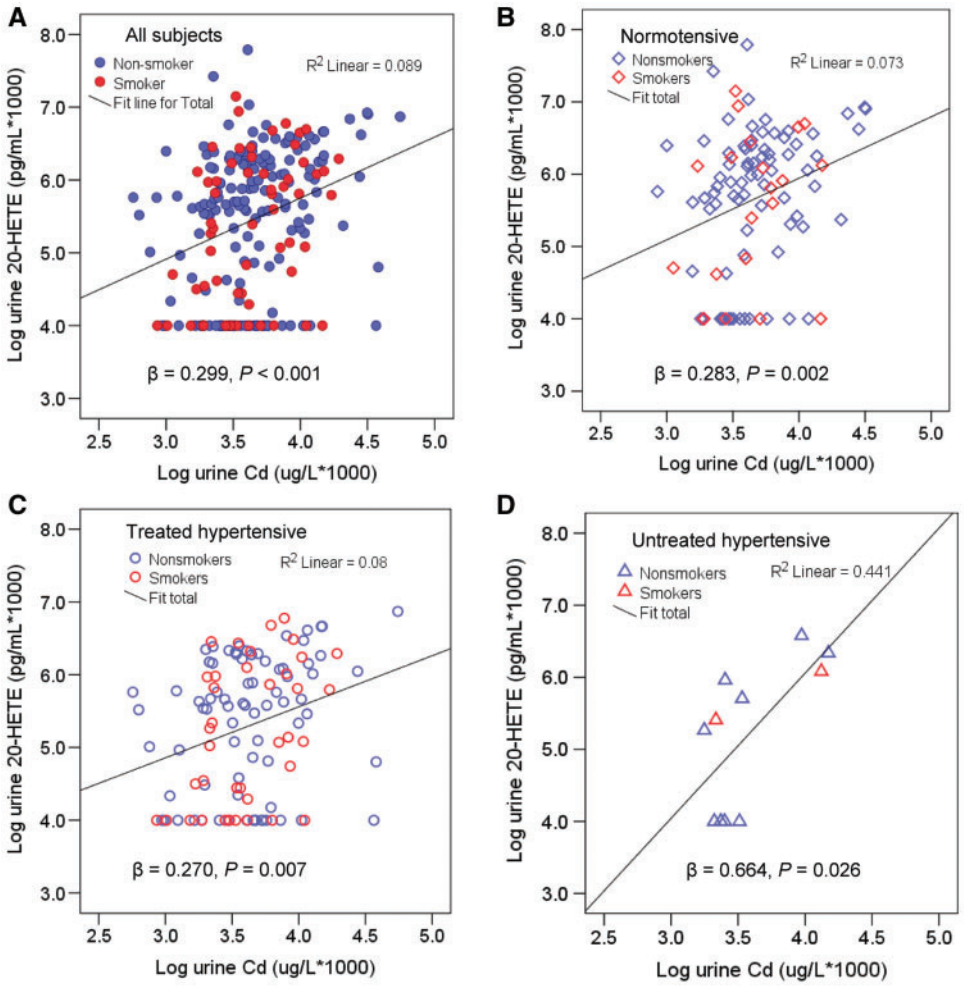

Fig. 1. Scatter plots of urinary 20-HETE excretion versus Cd body burden. The regression lines of logarithmically transformed urinary 20-HETE concentrations versus urinary Cd concentrations are shown separately for (**A**) all subjects, (**B**) normotensive, (**C**) treated hypertensive and (**D**) untreated hypertensive subjects. The R^2 for linear regression values are indicated together with the standardized β coefficients for (A), (B), (C) and (D) as 0.299 ($P < 0.001$), 0.283 ($P = 0.002$), 0.270 ($P = 0.007$) and 0.664 ($P = 0.026$), respectively.

were associated with variability in urinary 20-HETE excretion among subjects in the entire group ($P < 0.001$ for urinary Cd, $P = 0.006$ for BMI). Age, smoking status and blood pressure groups were not associated with urinary 20-HETE variability ($P = 0.257$ for age, $P = 0.557$ for smoking status and $P = 0.310$ for blood pressure).

Elevated 20-HETE levels and prevalence odds of hypertension and tubular dysfunction

To further test if the eicosanoid 20-HETE could be linked to hypertension development, a logistic regression analysis was used (Table 3). In this analysis, urinary 20-HETE level was a categorical dependent variable, based on a median value of

Table 3. Elevated urinary 20-HETE excretion and prevalence odds of hypertension

Independent variables	Exp (β) or POR	95% CI for Exp (β)		P-value
		Lower	Upper	
Age (years)	0.976	0.913	1.044	0.480
BMI (kg/m^2)	0.886	0.809	0.969	0.008
Smoking	0.944	0.468	1.903	0.872
Hypertension	1.905	1.068	3.397	0.029
Log blood Cd (μg/L)	0.213	0.065	0.701	0.011
Log urine Cd (μg/g creatinine)	4.357	1.152	16.481	0.030
Log urine β2-MG (mg/g creatinine)	1.530	1.001	2.338	0.049
Log urine NAG (units/g creatinine)	0.737	0.217	2.497	0.624
Log urine albumin (mg/g creatinine)	1.074	0.702	1.643	0.741

Exp (β) = Explanatory β.
Urinary 20-HETE was a categorical dependent variable, based on median value of 469 pg/mL. Blood Cd levels and creatinine adjusted urinary Cd, β-MG, NAG and albumin levels were continuous independent variables. Smoking status was categorized independent variable (non-smoker = 1, smoker = 2) as was blood pressure status (normotensive = 1, hypertensive = 2).
$P \leq 0.05$ is considered to indicate statistical significant levels.

469 pg/mL. The independent variables incorporated in a model were age, BMI, smoking and blood pressure groups, along with the measures of Cd exposure (blood and urinary Cd) and toxicity (urinary β2-MG, NAG and albumin). Both blood and urinary Cd were incorporated in a model, given the potential for an independent effect of circulating Cd along with the effects of Cd accumulation in kidneys. Urinary 20-HETE levels ≥469 pg/mL were found to be associated with an increase in the prevalence odds of hypertension (POR 1.905, 95% CI 1.068–3.397, P = 0.029), greater odds of having higher urinary Cd levels (POR 4.357, 95% CI 1.152–16.481, P = 0.030) and higher urinary excretion of β2-MG, a measure of renal tubular dysfunction (POR 1.530, 95% CI 1.001–2.338, P = 0.049). Urinary 20-HETE levels ≥469 pg/mL did not show a significant association with higher urinary excretion of NAG (P = 0.624) or albumin (P = 0.741). In the model that omitted blood Cd levels, an association between urinary 20-HETE and prevalence odds of hypertension persisted as did the association between 20-HETE and tubular dysfunction, while the association between 20-HETE and urinary Cd levels was attenuated (POR 1.424, 95% CI 0.522–3.882, P = 0.490).

Urinary 20-HETE levels and blood pressure increases

To evaluate an effect of 20-HETE on blood pressure measures, SBP and DBP and mean arterial blood pressure were stratified according to the tertile of urinary 20-HETE levels (Figure 2). We found that the variability in blood pressure measures among subjects in the treated and untreated hypertensive groups were not associated with the tertile levels of urinary 20-HETE. However, SBP levels in the normotensive subjects increased linearly with increasing tertiles of 20-HETE levels (P = 0.046 for variability across tertile groups, P = 0.037 for linearity), as did mean arterial blood pressures (P = 0.048 for variability across quartile groups, P = 0.023 for linearity). DBP levels also exhibited a linear association with tertile levels of urinary 20-HETE (P = 0.051 for linearity), but such increases in DBP levels across tertile groups did not reach statistical significance (P = 0.131). In a univariate analysis with adjustment for age and BMI, an increase in urinary 20-HETE in normotensive subjects from the lowest tertile to the highest tertile was associated with increases in SBP of 6 mmHg (95% CI 0.3–12, P = 0.040) and mean arterial pressure of 4.7 mmHg (95% CI −0.1–9.5, P = 0.057).

Discussion

Cumulative lifetime Cd exposure levels in the study subjects, reflected by the mean (range) urinary Cd of 0.58 (0.09−3.12) μg/g creatinine, are considered to be low to moderate. These exposure levels are in line with those found in the majority of non-occupationally exposed populations [2, 7–10]. Consistent with numerous studies, Cd exposure levels in the subjects in the present study were associated with tubular injury, based on elevated urinary NAG levels [1–3, 7–10]. In a previous Thai study [7], a 3-fold increase in urinary Cd level was associated with a 32% increase in the prevalence odds of having tubular injury. In addition, those with elevated urinary NAG levels had a 20% increase in the probability of developing hypertension. In the US National Health and Nutritional Examination Survey (NHANES) 1999–2006 data, blood Cd levels ≥0.6 μg/L were associated with kidney damage and CKD, defined as eGFR <60 mL/min/1.73 m^2 and [27], while blood Cd levels ≥0.4 μg/L were associated with an increased risk of hypertension in Caucasian women and Mexican–American women [14]. In the US NHANES 2011–2012 data, blood Cd levels >0.53 μg/L were associated with increases in the risks of albuminuria and low GFR [28]. Blood and urinary Cd levels >1 μg/L have been associated with CKD in the adult participants in the US NHANES 1999–2006 study [29]. In a Korean population study [30], blood Cd levels in the highest tertile were associated with 1.85 mL/min/1.73 m^2 (95% CI −3.55 to −0.16) lower eGFR values, compared with the lowest tertile. An inverse association between blood Cd and eGFR was observed also in the US NHANES 2007–2012 data [31].

To the best of our knowledge, there are no previous studies that report chronic Cd exposure as a predictor of urinary 20-HETE levels nor are there reports of an association between urinary 20-HETE levels and tubular dysfunction together with hypertension prevalence. Thus, we are the first to observe an association between Cd exposure and urinary 20-HETE levels. Further, we have observed an association between urinary 20-HETE levels ≥469 pg/mL and increased odds of hypertension development together with tubular dysfunction, assessed by urinary β2-MG levels. It is noteworthy that 90% of hypertensive subjects in the present study received anti-hypertensive medication, raising the possibility for an effect of the medication on urinary Cd excretion levels. However, in an interaction analysis, there was little evidence for such effect. Therefore, it is conceivable that anti-hypertensive medication may have caused a

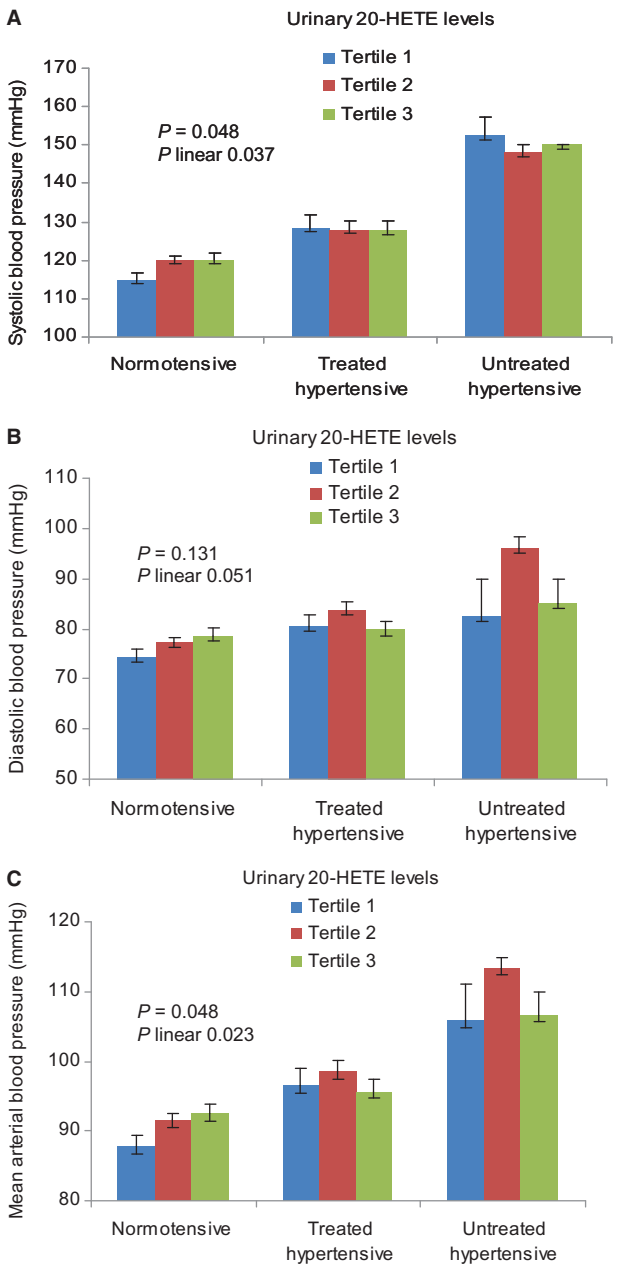

Fig. 2. Blood pressure measures stratified by quartile of urinary 20-HETE excretion levels. Bar graphs representing (**A**) SBP, (**B**) DBP and (**C**) mean arterial pressure, stratified by tertile of urinary 20-HETE excretion levels, are shown separately for the normotensive, treated hypertensive and untreated hypertensive groups. The mean (SE) values for urinary 20-HETE excretion levels in tertiles 1, 2 and 3 are 11 (4), 620 (493) and 5351 (8590) pg/mL, respectively. $P \leq 0.05$, obtained by one-way ANOVA, indicate statistical differences in blood pressure levels across the urinary 20-HETE excretion groups.

reduction in 20-HETE levels concurrently with lowering of blood pressure levels.

Supporting further the connection between 20-HETE and blood pressure increase is a stratification analysis that suggested a linear relationship between tertiles of urinary 20-HETE excretion levels and blood pressure increases in normotensive subjects, where urinary 20-HETE levels in the highest tertile were found to have be on average 6 mmHg higher SBP, compared with the lowest tertile. The findings in the present study thus provide strong evidence linking Cd exposure to hypertension development through 20-HETE. Other mechanistic studies suggest that 20-HETE has anti-hypertensive effects in the proximal tubules, where it activates protein kinase C, which in turn phosphorylates Na-K-ATPase, leading to a reduction in sodium transport activity [17]. In the thick ascending limb of the loop of Henle, 20-HETE had a natriuretic effect as a result of inhibition of Na-K-2Cl− and 70pS K+ transport, thereby causing a reduction in reabsorption of sodium [17]. In vascular smooth muscle cells, 20-HETE was a local vasoconstrictor and thus was pro-hypertensive [17–19].

In an Australian study, a positive correlation between urinary 20-HETE and blood pressure was seen in hypertensive women who had higher urinary 20-HETE levels than normotensive subjects [32]. In the US studies, an expected increase in urinary 20-HETE excretion after administration of furosemide (a diuretic drug) was found in normotensive subjects, but not in salt-sensitive hypertensive subjects, leading to the suggestion that impaired synthesis or actions of renal 20-HETE might contribute to the salt-sensitive hypertension [33, 34]. In a double-blind, placebo-controlled intervention [35], a reduction in blood pressure in Australian CKD patients was observed after 8 weeks of supplementation with n-3 fatty acid that appeared to lower plasma 20-HETE levels.

In conclusion, the present study has demonstrated for the first time that Cd exposure is a predictor of urinary 20-HETE levels, and that 20-HETE is in turn associated with an increase in odds of having hypertension and tubular dysfunction in Thai women who had been chronically exposed to Cd. This study thus provides the first evidence linking 20-HETE to blood pressure increases and hypertension development after chronic Cd exposure.

Strengths and limitations

The strengths of this study include an analysis of apparently healthy individuals, who were relatively young (age 33–55 years, mean age 47.2 years) together with a focus on a susceptible sub-population (women). The high prevalence of hypertension in an area with Cd contamination allowed recruitment of sufficient numbers of hypertensive subjects from villages. The community-based recruitment strategy minimized bias toward certain sub-population groups, frequently encountered in health center-based studies. The limitations of this study were its cross-sectional design, which limited an assessment of temporal relationships between variables or causal inference of Cd exposure on blood pressure, the small sample size and the fact that the results may not be generalizable to men.

Funding

This study was supported in part by the Royal Jubilee Ph.D. scholarship (K.B.) and by the Reverse Brain Drain Award (S.S.) from the Commission for Higher Education, Thailand's Ministry of Education.

Conflict of interest statement

None declared.

References

1. Satarug S, Vesey DA, Gobe GC. Health risk assessment of dietary cadmium intake: do current guidelines indicate how much is safe? *Environ Health Perspect* 2017; 125: 284–288

2. Satarug S, Vesey DA, Gobe GC. Current health risk assessment practice for dietary cadmium: data from different countries. *Food Chem Toxicol* 2017; 106: 430–445
3. Satarug S, Vesey DA, Gobe GC. Kidney cadmium toxicity, diabetes and high blood pressure: the perfect storm. *Tohoku J Exp Med* 2017; 241: 65–87
4. Havasi A, Dong Z. Autophagy and tubular cell death in the kidney. *Semin Nephrol* 2016; 36: 174–188
5. Gobe G, Crane D. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. *Toxicol Lett* 2010; 198: 49–55
6. Fujiwara Y, Lee JY, Tokumoto M *et al*. Cadmium renal toxicity via apoptotic pathways. *Biol Pharm Bull* 2012; 35: 1892–1897
7. Satarug S, Nishijo M, Ujjin P *et al*. Cadmium-induced nephropathy in the development of high blood pressure. *Toxicol Lett* 2005; 157: 57–68
8. Honda R, Swaddiwudhipong W, Nishijo M *et al*. Cadmium induced renal dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. *Toxicol Lett* 2010; 198: 26–32
9. Callan AC, Devine A, Qi L *et al*. Investigation of the relationship between low environmental exposure to metals and bone mineral density, bone resorption and renal function. *Int J Hyg Environ Health* 2015; 218: 444–451
10. Wallin M, Sallsten G, Lundh T *et al*. Low-level cadmium exposure and effects on kidney function. *Occup Environ Med* 2014; 71: 848–854
11. Satarug S, Baker JR, Reilly PE *et al*. Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. *Arch Environ Health* 2002; 57: 69–77
12. Akerstrom M, Barregard L, Lundh T *et al*. The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. *Toxicol Appl Pharmacol* 2013; 268: 286–293
13. Lech T, Sadlik JK. Cadmium concentration in human autopsy tissues. *Biol Trace Elem Res* 2017; 179: 172–177
14. Scinicariello F, Abadin HG, Murray HE. Association of low-level blood lead and blood pressure in NHANES 1999–2006. *Environ Res* 2011; 111: 1249–1257
15. Lee BK, Kim Y. Association of blood cadmium with hypertension in the Korean general population: analysis of the 2008-2010 Korean National Health and Nutrition Examination Survey data. *Am J Ind Med* 2012; 55: 1060–1067
16. Garner RE, Levallois P. Associations between cadmium levels in blood and urine, blood pressure and hypertension among Canadian adults. *Environ Res* 2017; 155: 64–72
17. Fan F, Muroya Y, Roman RJ. Cytochrome P450 eicosanoids in hypertension and renal disease. *Curr Opin Nephrol Hypertens* 2015; 24: 37–46
18. Zoccali C, Mallamaci F, Grassi G. 20-Hydroxyeicosatetraenoic acid, a far-reaching autacoid in chronic kidney disease: hypertension and beyond. *J Hypertens* 2015; 33: 1764–1766
19. Elshenawy OH, Shoieb SM, Mohamed A *et al*. Clinical implications of 20-hydroxyeicosatetraenoic acid in the kidney, liver, lung and brain: an emerging therapeutic target. *Pharmaceutics* 2017; 9: 9
20. Boonprasert K, Satarug S, Morais C *et al*. The stress response of human proximal tubule cells to cadmium involves upregulation of haemoxygenase 1 and metallothionein but not cytochrome P450 enzymes. *Toxicol Lett* 2016; 249: 5–14
21. Baker JR, Satarug S, Urbenjapol S *et al*. Associations between human liver and kidney cadmium content and immunochemically detected CYP4A11 apoprotein. *Biochem Pharmacol* 2002; 63: 693–696
22. Baker JR, Satarug S, Edwards RJ *et al*. Potential for early involvement of CYP isoforms in aspects of human cadmium toxicity. *Toxicol Lett* 2003; 137: 85–93
23. Baker JR, Edwards RJ, Lasker JM *et al*. Renal and hepatic accumulation of cadmium and lead in the expression of CYP4F2 and CYP2E1. *Toxicol Lett* 2005; 159: 182–191
24. Lasker JM, Chen WB, Wolf I *et al*. Formation of 20-hydroxye, icosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of Cyp4F2 and Cyp4A11. *J Biol Chem* 2000; 275: 4118–4126
25. Boonprasert K, Ruengweerayut R, Satarug S *et al*. Study on the association between environmental cadmium exposure, cytochrome P450-mediated 20-HETE, heme-oxygenase-1 polymorphism and hypertension in Thai population residing in a malaria endemic areas with cadmium pollution. *Environ Toxicol Pharmacol* 2011; 31: 416–426
26. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S1–S266
27. Navas-Acien A, Tellez-Plaza M, Guallar E *et al*. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol* 2009; 170: 1156–1164
28. Lin YS, Ho WC, Caffrey JL *et al*. Low serum zinc is associated with elevated risk of cadmium nephrotoxicity. *Environ Res* 2014; 134: 133–138
29. Ferraro PM, Costanzi S, Naticchia A *et al*. Low level exposure to cadmium increases the risk of chronic kidney disease: analysis of the NHANES 1999-2006. *BMC Public Health* 2010; 10: 304
30. Hwangbo Y, Weaver VM, Tellez-Plaza M *et al*. Blood cadmium and estimated glomerular filtration rate in Korean adults. *Environ Health Perspect* 2011; 119: 1800–1805
31. Buser MC, Ingber SZ, Raines N *et al*. Scinicariello F. Urinary and blood cadmium and lead and kidney function: NHANES 2007-2012. *Int J Hyg Environ Health* 2016; 219: 261–267
32. Ward NC, Rivera J, Hodgson J *et al*. Urinary 20-hydroxyeicosatetraenoic acid is associated with endothelial dysfunction in humans. *Circulation* 2004; 110: 438–443
33. Laffer CL, Laniado-Schwartzman M, Wang MH *et al*. Differential regulation of natriuresis by 20-hydroxyeicosatetraenoic acid in human salt-sensitive versus salt-resistant hypertension. *Circulation* 2003; 107: 574–578
34. Laffer CL, Laniado-Schwartzman M, Wang MH *et al*. 20-HETE and furosemide-induced natriuresis in salt-sensitive essential hypertension. *Hypertension* 2003; 41: 703–708
35. Barden AE, Burke V, Mas E *et al*. n-3 fatty acids reduce plasma 20-hydroxyeicosatetraenoic acid and blood pressure in patients with chronic kidney disease. *J Hypertens* 2015; 33: 1947–1953

Clinical Kidney Journal
ckj