Current Research in Toxicology 6 (2024) 100140

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology

The pathogenesis of albuminuria in cadmium nephropathy

Soisungwan Satarug [a], David A. Vesey [a,b], Glenda C. Gobe [a,c,d], Kenneth R. Phelps [e,*]

[a] Centre for Kidney Disease Research, Translational Research Institute, Brisbane, Australia
[b] Department of Kidney and Transplant Services, Princess Alexandra Hospital, Brisbane, Australia
[c] School of Biomedical Sciences, The University of Queensland, Brisbane, Australia
[d] NHMRC Centre of Research Excellence for CKD QLD, UQ Health Sciences, Royal Brisbane and Women's Hospital, Brisbane, Australia
[e] Stratton Veterans Affairs Medical Center and Albany Medical College, Albany, NY, USA

ARTICLE INFO

Keywords:
Cadmium
Cadmium nephropathy
Albumin
Albuminuria
β_2-microglobulin
β_2-microglobulinuria

ABSTRACT

Background: Urinary cadmium excretion (E_{Cd}) rises with renal tissue content of the metal. Whereas glomerulopathies are sometimes associated with massive albuminuria, tubular accumulation of Cd typically causes modest albuminuria. Since β_2-microglobulinuria ($E_{\beta 2M}$) is an established marker of proximal tubular dysfunction, we hypothesized that a comparison of albuminuria (E_{alb}) to $E_{\beta 2M}$ in Cd-exposed subjects would provide evidence of similar mishandling of both proteins.

Methods: To depict excretion rates per functional nephron, E_{Cd}, E_{alb}, and $E_{\beta 2M}$ were normalized to creatinine clearance (C_{cr}), a surrogate for the glomerular filtration rate (GFR). Estimation of GFR itself (eGFR) was accomplished with CKD-EPI formulas (2009). Linear and logistic regression analyses were performed to relate E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$, and eGFR to several independent variables. Simple linear regressions of eGFR, E_{alb}/C_{cr}, and $E_{\beta 2M}/C_{cr}$ on E_{Cd}/C_{cr} were examined before and after adjustment of dependent variables for age. All regressions were performed after log-transformation of ratios and standardization of all variables. Increments in E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ and decrements in eGFR were quantified through four quartiles of E_{Cd}/C_{cr}.

Results: As age or E_{Cd}/C_{cr} rose, E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ also rose, and eGFR fell. In linear regressions, slopes relating E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ to E_{Cd}/C_{cr} were similar. After adjustment of dependent variables for age, coefficients of determination (R^2) for all regressions rose by a multiple, and slopes approached unity. E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ were similarly associated with each other. Mean E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ rose and mean eGFR fell in stepwise fashion through quartiles of E_{Cd}/C_{cr}. Whereas $E_{\beta 2M}/C_{cr}$ did not vary with blood pressure, E_{alb}/C_{cr} rose in association with hypertension in two of the four quartiles.

Conclusions: Our data indicate that Cd in renal tissue affected tubular reabsorption of albumin and β_2M similarly in a large cohort of exposed subjects. The results suggest that Cd reduced receptor-mediated endocytosis and subsequent lysosomal degradation of each protein by a shared mechanism.

Introduction

Cadmium (Cd), a nonessential and toxic heavy metal, gains access to circulating blood through the lungs and gastrointestinal tract (reviewed in Nordberg and Nordberg, 2022). Thereafter, it binds primarily to hemoglobin in red blood cells but also to albumin and other large proteins in plasma (Carlson and Friberg, 1957; Gibson et al., 2017; Nordberg et al., 1971). The plasma proteins transport Cd to hepatocytes, which take up the metal and store it in complexes with metallothionein (MT), a small, sulfur-rich protein synthesized in the liver and other organs (Sabolic, 2010). As hepatocytes die, they release CdMT to plasma, from which the complex is rapidly cleared by glomerular filtration and tubular reabsorption (Nordberg and Nordberg, 1975; Nomiyama and Foulkes, 1977; Chan et al., 1993). In the proximal tubule (PT),

Abbreviations: β_2M, beta$_2$-microglobulin; $[x]_p$, plasma concentration of *x*, mass/volume; $[x]_u$, urine concentration of *x*, mass/volume; Alb, albumin; C_{cr}, creatinine clearance, volume/time; Cd, cadmium; Cr, creatinine; Dab-2, disabled-2; eGFR, estimated GFR, mL/min/1.73m^2; E_x, excretion rate of *x*, mass/time; E_x/C_{cr}, amount of *x* excreted per volume of filtrate, mass/volume; FcRn, neonatal Fc receptor; FPE, fluid phase endocytosis; GFR, glomerular filtration rate, volume/time; GSC, glomerular sieving coefficient; MT, metallothionein; PCT, proximal convoluted tubule; POR, prevalence odds ratio; PST, proximal straight tubule; PT, proximal tubule; RME, receptor-mediated endocytosis; S1, S2, S3, segments of the proximal tubule.

* Corresponding author at: Stratton Veterans Affairs Medical Center, 113 Holland Avenue, Albany, NY 12208, USA.
E-mail address: kenneth.phelps@va.gov (K.R. Phelps).

https://doi.org/10.1016/j.crtox.2023.100140
Received 10 July 2023; Received in revised form 16 November 2023; Accepted 30 November 2023
Available online 6 December 2023

endosomal reabsorption of CdMT is mediated by the apical receptor megalin (Klassen et al., 2004; Wolff et al., 2006); endosomes transfer CdMT to lysosomes, in which MT dissociates from Cd and undergoes degradation. Cd is then transported to cytoplasm (Abouhamed et al., 2007), where it is stored after binding to MT synthesized in situ. In the distal nephron, CdMT is reabsorbed through the mediation of lipocalin-2 receptor (LCN2-R; also called neutrophil gelatinase-associated lipocalin receptor or 24p3 receptor) (Langelueddecke et al., 2012; Zavala-Guevara et al., 2021). LCN2-R has greater affinity than megalin for CdMT, and distally reabsorbed CdMT may reduce cell viability (Langelueddecke et al., 2012).

As Cd nephropathy progresses, the earliest measureable indicators of toxicity reflect injury to or dysfunction of proximal tubule (PT) cells (Satarug and Phelps, 2021). However, the conventional inference that hepatic CdMT is the principal source of Cd in the PT is undergoing re-examination. Experiments in MT-null mice showed that exogenous Cd can induce PT toxicity in the absence of MT (Liu et al., 1998); moreover, the dissociation constant (K_D) of megalin-CdMT is too high and the plasma concentration of MT is too low for circulating CdMT to be a significant source of proximally reabsorbed Cd (Klassen et al., 2004; Milnerowicz and Bizon, 2010; reviewed in Thevenod and Wolff, 2016). Albumin, beta$_2$-microglobulin (β_2M), and other peptides and proteins appear to be more important carriers of Cd to the PT (Fels et al., 2019).

Albumin has a molecular weight of 66 kD and normally circulates in plasma at a concentration ($[alb]_p$) of 3.5–5.0 g/dL. The fraction of dissolved albumin that passes through the glomerular barrier with plasma is debated (Tojo and Endou, 1992; Russo et al., 2007; Comper et al., 2022), but most filtered albumin is reabsorbed in the proximal tubule and either degraded in situ or recycled to the bloodstream by transcytosis (Park and Maack, 1984; Russo et al., 2007; Sarav et al., 2009; Tenten et al., 2013; Molitoris et al., 2022). A small fraction may be reabsorbed in the distal nephron, especially when the proximal tubule is injured (Galaske et al., 1979; Zavala-Guevara et al., 2021). Excessive albuminuria (E_{alb}) is a reported complication of exposure to cadmium (Cd) (Roels et al., 1982; Elinder et al., 1985; Haswell-Elkins et al., 2008; Liang et al., 2012; Feng et al., 2022), which accumulates predominantly but not exclusively in proximal tubular cells (Torra et al., 1994; Yoshida et al., 1998). Since Cd disrupts multiple functions of these cells (reviewed in Satarug and Phelps, 2021), it may also interfere with proximal tubular reabsorption of albumin.

β_2-microglobulin (β_2M), a small protein with molecular weight 11.8 kD, is an essential component of major histocompability complex I on the surface of nucleated cells. β_2M normally circulates in plasma at a concentration of 1.2–2.7 mg/L and is highly filterable by glomeruli (Argyropoulos et al., 2017). Ordinarily, almost all the filtered protein is reabsorbed and degraded by proximal tubular epithelium (Bernier and Conrad, 1969; Schuh et al., 2018; Polesel and Hall, 2019). The S1 segment of the proximal tubule is uniquely equipped with endolysosomal organelles to carry out these functions (Schuh et al., 2018), and proximal tubulopathies are associated with β_2-microglobulinuria (Peterson et al., 1969; Portman et al., 1986). β_2M may also be taken up from peritubular capillaries through the basolateral membrane (Hall III et al., 1982). Accumulated Cd interferes with reabsorption of filtered β_2M, and advanced Cd tubulopathy is often associated with markedly increased excretion of this protein (Peterson et al., 1969; Satarug et al., 2019). Historically, the excretion rate of β_2M ($E_{\beta 2M}$) has been employed for early detection of renal Cd toxicity (Food and Agriculture Organization of the United Nations (FAO), 2023), but other excretion rates, including that of Cd itself (E_{Cd}), appear to be more sensitive for this purpose (Satarug et al., 2020, 2023b).

Because excessive β_2-microglobulinuria ($E_{\beta 2M}$) is a manifestation of proximal tubular dysfunction, we hypothesized that comparisons of excretion rates of β_2M and albumin ($E_{\beta 2M}$ and E_{alb}) would clarify the role of the proximal tubule in Cd-induced albuminuria. Our results suggest that the hypothesis was correct.

Materials and methods

Participants

We assembled archived data from 519 citizens of Thailand who resided in Cd-contaminated areas of Mae Sot District, Tak Province (Satarug et al., 2013, 2023a). Excessive levels of Cd were documented in soil samples and rice (Suwatvitayakorn et al., 2020). The Institutional Ethical Committees of Chiang Mai University and the Mae Sot Hospital approved the study protocol (Satarug et al., 2013).

All participants gave informed consent prior to participation. They had lived at their current addresses for at least 30 years. Exclusion criteria were pregnancy, breast-feeding, a history of metal work, and a hospital record or physician's diagnosis of an advanced chronic disease. Because occupational exposure was an exclusion criterion, we presumed that all participants had acquired Cd from the environment.

Diabetes was defined as fasting plasma glucose levels $\geq$ 126 mg/dL or a physician's prescription of anti-diabetic medications. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg (Bloch and Basile, 2013), a physician's diagnosis, or prescription of anti-hypertensive medications.

Sample collection, analyses, and assays

Second aliquots of morning urine were collected after an overnight fast, and whole blood samples were obtained within 3 h after urine sampling. Urine, whole blood, and plasma were stored at − 20 °C or − 80 °C for later analysis. The assay for urine and plasma concentrations of creatinine ($[cr]_u$ and $[cr]_p$) was based on the Jaffe reaction.

The urine concentration of Cd ($[Cd]_u$) was determined with atomic absorption spectrophotometry (Shimadzu Model AA-6300, Kyoto, Japan). Urine standard reference material No. 2670 (National Institute of Standards, Washington, DC, USA) was used for quality assurance and control purposes. The limit of detection of Cd quantitation, defined as 3 times the standard deviation of blank measurements, was 0.06 μg/L. None of the urine samples exhibited $[Cd]_u$ below the detection limit.

The assay of β_2M in urine ($[\beta_2M]_u$) was based on the latex immunoagglutination method (LX test, Eiken 2MGII; Eiken and Shionogi Co., Tokyo, Japan). The assay of urinary albumin ($[alb]_u$) was based on an immunoturbidimetric method.

Calculation of derived parameters

Estimated GFR (eGFR) was calculated with equations developed by the Chronic Kidney Disease Epidemiology Collaboration (Levey et al., 2009). For dichotomous comparisons, CKD was defined as eGFR $\leq$ 60 mL/min/1.73 m^2. CKD stages G1, G2, G3a, G3b, G4, and G5 corresponded respectively to eGFR of 90–119, 60–89, 45–59, 30–44, 15–29, and < 15 mL/min/1.73 m^2.

Urinary excretion rates of all substances x (E_x) were expressed as mass excreted per volume of filtrate (E_x/C_{cr}), and calculated as $[x]_u[cr]_p/[cr]_u$, where cr is creatinine, C_{cr} is creatinine clearance, and x is Cd, albumin, or β_2M (Phelps and Gosmanova, 2020; see Supplemental Material (SM), Item I). For purposes of comparison, E_x was also expressed as E_x/E_{cr} (simplified to $[x]_u/[cr]_u$). Results based on this normalization are presented in SM, Item II.

Statistical analysis

Data were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The one-sample Kolmogorov–Smirnov test was used to identify departures of continuous variables from a normal distribution, and logarithmic transformation was applied to variables that showed rightward skewing before they were subjected to parametric statistical analysis. The Kruskal-Wallis test was used to compare mean differences among the three eGFR groups. The Chi-square test was used to analyze

differences in percentage and prevalence data. For all analyses, p-values ≤ 0.05 were assumed to indicate statistical significance.

Multiple linear regression analysis was used to determine standardized β coefficients describing the strength of association of $\log[(E_{alb}/C_{cr}) \times 10^4]$, $\log[(E_{\beta 2M} \times 10^4]$, and eGFR with age, body mass index (BMI), $\log[(E_{Cd}/C_{cr}) \times 10^5]$, gender, smoking, and hypertension. Multivariate (covariance) analysis was used to estimate the size of effects (η^2) of each independent variable on $\log[(E_{alb}/C_{cr}) \times 10^4]$, $[\log(E_{\beta 2M} \times 10^4]$, and eGFR. Multivariable logistic regression analysis was used to determine the Prevalence Odds Ratio (POR) for albuminuria, β_2-microglobulinuria, and CKD in relation to the same independent variables, except that effects of two expressions of Cd excretion (E_{Cd}) were examined. Model 1 incorporated $\log(E_{Cd}/E_{cr})$ or E_{Cd}/E_{cr} quartiles, and model 2 incorporated $\log(E_{Cd}/C_{cr})$ or E_{Cd}/C_{cr} quartiles. Results based on model 1 are presented in SM, Item II; those based on model 2 are reported herein.

Simple linear regressions were used to examine relationships between the independent variable E_{Cd}/C_{cr} and dependent variables E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$, and eGFR. For these examinations, all variables except eGFR were log-transformed, and eGFR and logarithmic values were standardized. Regressions were repeated after adjustment of logs of dependent variables for age. To obtain covariate (age)-adjusted values for eGFR, $\log[(E_{alb}/C_{cr}) \times 10^4]$, and $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$, the generic trivariate equation below was applied:

$$y = a + \beta_1 * age + \beta_2 * x$$

where y = eGFR, $\log[(E_{alb}/C_{cr}) \times 10^4]$ or $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$; a = y-intercept; β_1 = slope for age; β_2 = slope for $\log[(E_{Cd}/C_{cr}) \times 10^5]$; and x = $[\log[(E_{Cd}/C_{cr}) \times 10^5]$. Values of a, β_1, and β_2 when y equaled eGFR, $\log[(E_{alb}/C_{cr}) \times 10^4]$, or $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$ are provided in the table below.

y	x	a	β_1	β_2
eGFR	$\log[(E_{Cd}/C_{cr}) \times 10^5]$	210	−1.10	−18.4
$\log[(E_{alb}/C_{cr}) \times 10^4]$	$\log[(E_{Cd}/C_{cr}) \times 10^5]$	0.195	0.018	0.423
$\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$	$\log[(E_{Cd}/C_{cr}) \times 10^5]$	1.398	0.012	0.672

Univariate analysis of covariance with Bonferroni correction for multiple comparisons was used to obtain mean $\log[(E_{alb}/C_{cr}) \times 10^4]$, mean $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$ and mean eGFR in subjects grouped by E_{Cd}/C_{cr} quartiles and blood pressure status. All means were adjusted for covariates (age, BMI, gender and smoking) plus interactions.

Results

In Table 1, subsets of the study sample are defined by eGFR > 90, 61–90, or ≤ 60 mL/min/1.73 m^2. Mean values of variables pertinent to albuminuria are provided for each subset. The data show the following accompaniments of falling eGFR: a rise in age and in prevalence of male gender and diabetes; increased excretion rates of albumin, β_2M, and Cd, whether rates were normalized to E_{cr} or C_{cr}; rising prevalence of $[(E_{Cd}/C_{cr}) \times 100] = 5$–9.99 or ≥ 10 μg/L of filtrate; and simultaneously falling prevalence of $[(E_{Cd}/C_{cr}) \times 100] < 2$ or = 2–4.99 μg/L of filtrate.

Table 2 summarizes multivariable analyses done to examine relationships of $\log[(E_{alb}/C_{cr}) \times 10^4]$, $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$, and eGFR to several independent variables. β-coefficients indicate that each of the three dependent variables was associated with age and with $\log[(E_{Cd}/C_{cr}) \times 10^5]$. Values of η^2 show that E_{Cd}/C_{cr} explained more variability in E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ than age did, and age explained more variability in eGFR than E_{Cd}/C_{cr} did. With respect to eGFR, values of η^2 were uncommonly high for both age and E_{Cd}/C_{cr}. Associations of $\log[(E_{alb}/C_{cr}) \times 10^4]$, $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$, and eGFR with hypertension were weak in general, and were statistically significant only for $\log[(E_{alb}/C_{cr}) \times 10^4]$ and eGFR. Adjusted R^2 values for regression models analyzing log $[(E_{alb}/C_{cr}) \times 10^4]$, $\log[(E_{\beta 2M}/C_{cr}) \times 10^4]$, and eGFR were 0.147, 0.116, and 0.474, respectively.

Table 3 summarizes prevalence odds ratios (PORs) for abnormal E_{alb}/C_{cr}, abnormal $E_{\beta 2M}/C_{cr}$, and eGFR < 60 mL/min/1.73 m^2 in relation to several independent variables. The POR for abnormal E_{alb}/C_{cr} was increased significantly by age, smoking, hypertension, and E_{Cd}/C_{cr} higher than 5 μg/L of filtrate. Gradations of E_{Cd}/C_{cr} were the most potent of the independent variables. The POR for abnormal $E_{\beta 2M}/C_{cr}$ was also increased by gradations of E_{Cd}/C_{cr}, but not by any other independent variable. The POR for eGFR ≤ 60 mL/min/1.73 m^2 was increased by age, hypertension, and E_{Cd}/C_{cr}, but again, gradations of E_{Cd}/C_{cr} were by far

Table 1
Descriptive characteristics of study subjects grouped by eGFR levels.

Variables	All subjects *N* 519	eGFR [a], mL/min/1.73 m^2 > 90, *n* 234	61–90, *n* 225	≤ 60, *n* 60	*p*
Age (years)	51.2 ± 9.3	46.7 ± 5.78	52.8 ± 8.4	62.4 ± 11.8	<0.001
BMI (kg/m^2)	23.2 ± 3.9	23.9 ± 3.59	22.8 ± 3.94	22.0 ± 4.16	0.026
eGFR, mL/min/ 1.73 m^2	86 ± 21	105 ± 7.53	77.0 ± 8.38	46.6 ± 10.6	<0.001
eGFR range	22–131	91–131	61–90	22–60	
Men (%)	35.3	32.9	34.7	46.7	0.134
Smoking (%)	50.5	58.1	46.2	36.7	0.003
Hypertension (%)	36.6	37.2	35.6	38.3	0.897
Diabetes (%)	2.1	0.0	3.1	6.7	0.002
Albuminuria (%)[b]	14.1	7.7 (18)	13.3 (30)	41.7	<0.001
Serum creatinine, mg/dL	0.90 ± 0.30	0.72 ± 0.13	0.95 ± 0.14	1.46 ± 0.41	<0.001
Urine creatinine, mg/dL	122.3 ± 72.4	117.6 ± 73.1	126.6 ± 72.8	125.0 ± 68.7	0.266
Urine albumin, mg/L	19.5 ± 54.0	10.5 ± 23.4	21.9 ± 66.8	45.8 ± 74.2	<0.001
Urine β_2M, μg/L	4523 ± 20721	772 ± 1378	3181 ± 15398	24188 ± 49051	<0.001
Urine Cd, μg/L	8.33 ± 9.65	6.26 ± 8.45	8.87 ± 8.39	14.40 ± 14.6	<0.001
Normalized to E_{cr} (E_x/E_{cr})[c]					
ACR, mg/g creatinine	18.1 ± 47.5	11.4 ± 35.9	18.7 ± 50.2	42.2 ± 66.2	<0.001
$E_{\beta 2MG}/E_{cr}$, μg/g creatinine	4446 ± 22865	829 ± 1448	2636 ± 11777	25336 ± 59556	<0.001
E_{Cd}/E_{cr}, μg/g creatinine	7.49 ± 6.70	6.08 ± 5.66	7.87 ± 6.57	11.57 ± 8.82	<0.001
Normalized to C_{cr}, (E_x/C_{cr})[d]					
$(E_{alb}/C_{cr}) \times 100$, mg/L	19.83 ± 59.98	8.57 ± 32.04	18.08 ± 48.39	70.27 ± 124.67	<0.001
Abnormal E_{alb}/C_{cr} (%)[e]	16	6.4	16.9	50.0	<0.001
$(E_{\beta 2MG}/C_{cr})$, μg/L	61.56 ± 338.4	5.97 ± 10.65	26.29 ± 124.38	410.66 ± 897.38	<0.001
Abnormal $E_{\beta 2M}/C_{cr}$ (%)[f]	56.1	56.0	48.0	86.7	<0.001
$(E_{Cd}/C_{cr}) \times 100$, μg/L	7.14 ± 7.82	4.34 ± 4.55	7.48 ± 6.27	16.76 ± 13.4	<0.001
% $(E_{Cd}/C_{cr}) \times 100$, μg/L					
< 2	17.3	26.5	11.6	2.3	<0.001
2–4.99	37.8	47.0	34.2	15.0	<0.001
5–9.99	24.5	20.1	30.7	18.3	<0.001
≥ 10	20.4	6.4	23.6	63.9	<0.001

Abbreviations: *n*, number of subjects; BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio; alb, albumin; β_2M, β_2-microglobulin. [a]Estimated GFR (eGFR) is determined with equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD − EPI) [Levey et al., 2009]. [b]Albuminuria is defined as ACR ≥ 20 mg/g for men and ≥ 30 mg/g for women. [c]$E_x/E_{cr} = [x]_u/[cr]_u$, where x = alb, β_2M or Cd. [d]$E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = alb, β_2M, or Cd. [e]Abnormal E_{alb}/C_{cr} is defined as $[(E_{alb}/C_{cr}) \times 100] \geq 20$ mg/L of filtrate. [f]Abnormal $E_{\beta 2M}/C_{cr}$ is defined as $(E_{\beta 2M}/C_{cr}) \geq 2$ μg/L of filtrate. Data for all continuous variables are arithmetic mean ± standard deviation (SD). For all tests, $p \leq 0.05$ identifies statistical significance, determined with the Pearson Chi-Square test for differences between percentages and the Kruskal-Wallis test for differences of means.

Table 2
Multivariate analyses to determine strength of association of log[($E_{alb}/C_{cr} \times 10^4$), log[($E_{\beta 2M}/C_{cr}$) × 10^3], and eGFR with independent variables.

Independent variables/factors	Log [(E_{alb}/C_{cr}) × 10^4]			Log[($E_{\beta 2MG}/C_{cr}$) × 10^4]			eGFR		
	β	η^2	*p*	β	η^2	*p*	β	η^2	*p*
Age	0.219	0.042	<0.001	0.106	0.027	0.010	−0.515	0.284	<0.001
BMI	0.063	0.004	0.177	−0.021	0.000	0.758	−0.068	0.012	0.015
Log[(E_{Cd}/C_{cr}) × 10^5]	0.250	0.053	<0.001	0.291	0.073	<0.001	−0.368	0.173	<0.001
Gender	0.001	0.001	0.975	0.018	0.002	0.277	−0/029	0.002	0.327
Smoking	0.058	0.000	0.227	0.036	0.000	0.991	0.024	0.000	0.623
Hypertension	0.113	0.021	0.008	0.074	0.007	0.061	−0.068	0.012	0.015
Gender × SMK × HTN	–	0.008	0.049	–	–	–	–	–	–
Adjusted R^2	0.142	–	<0.001	0.116	–	<0.001	0.474	–	<0.001

Abbreviations: β, standardized regression coefficient; η^2, eta squared; SMK, smoking status; HTN, hypertension; Gender × SMK × HTN, interaction term; adjusted R^2, coefficient of determination. Coding: female, 1, male, 2; non-smoker, 1, smoker, 2; normotension, 1, hypertension, 2. Positive β for associations of log[(E_{alb}/C_{cr}) × 10^4] and log[($E_{\beta 2MG}/C_{cr}$) × 10^4] indicate that hypertension was predictive of increased E_{alb} and $E_{\beta 2M}$. Negative β for the association of eGFR with hypertension implies that hypertension was predictive of a decline in GFR. β indicates strength of association of dependent variables with independent variables (first column). Adjusted R^2 indicates the fraction of total variation of each dependent variable explained by all independent variables. η^2 indicates the fraction of the variability of each dependent variable explained by a corresponding independent variable. *p*-values ≤ 0.05 indicate a statistically significant contribution of variation of an independent variable to variation of a dependent variable.

Table 3
Prevalence odds ratios for albuminuria, β_2-microglobuminuria, and reduced eGFR in relation to cadmium excretion and other variables.

Independent variables/ factors	Abnormal E_{alb}/C_{cr} [a]		Abnormal $E_{\beta 2M}/C_{cr}$ [b]		eGFR ≤ 60 mL/min/ 1.73 m^2	
	POR (95 % CI)	*p*	POR (95 % CI)	*p*	POR (95 % CI)	*p*
Age	1.050 (1.021, 1.079)	0.001	1.008 (0.986, 1.030)	0.497	1.135 (1.094, 1.178)	<0.001
BMI	1.017 (0.946, 1.093)	0.648	0.989 (0.939, 1.043)	0.689	1.083 (0.984, 1.192)	0.103
Gender	1.196 (0.676, 2.116)	0.539	0.962 (0.627, 1.474)	0.857	1.258 (0.576, 2.744)	0.565
Smoking	2.009 (1.118, 3.619)	0.020	1.011 (0.667, 1.534)	0.957	1.280 (0.589, 2.782)	0.533
Hypertension	1.912 (1.129, 3.237)	0.016	1.328 (0.907, 1.945)	0.145	2.063 (0.992, 4.294)	0.053
(E_{Cd}/C_{cr}) × 100, μg/L of filtrate						
< 2	Referent		Referent		Referent	
2–4.99	1.764 (0.886, 3.514)	0.106	1.914 (1.100, 3.330)	0.022	5.704 (2.414, 13.48)	<0.001
5–9.99	1.950 (1.009, 3.766)	0.047	1.744 (1.030, 2.951)	0.038	10.35 (4.160, 25.76	<0.001
≥ 10	2.849 (1.136, 7.146)	0.026	2.462 (1.320, 4.595)	0.005	18.06 (3.702, 88.15)	<0.001

Definitions: [a]abnormal E_{alb}/C_{cr}, (E_{alb}/C_{cr}) × 100 ≥ 20 mg/L of filtrate; [b]abnormal $E_{\beta 2M}/C_{cr}$, ($E_{\beta 2MG}/C_{cr}$) ≥ 2.0 μg/L of filtrate. Abbreviations: eGFR, estimated glomerular filtration rate; POR, prevalence odds ratio; CI, confidence interval. Coding: abnormal E_{alb}/C_{cr}, 1, normal E_{alb}/C_{cr}, 2; abnormal $E_{\beta 2M}/C_{cr}$, 1, normal $E_{\beta 2M}/C_{cr}$, 2; female, 1, male, 2; non-smoker, 1, smoker, 2; normotension, 1, hypertension, 2. Arithmetic means (SD) of categories of (E_{Cd}/C_{cr}) × 100: <2 μg/L, 1.440 (0.376); 2–4.99 μg/L, 3.315 (0.855); 5–9.99 μg/L, 7.073 (1.411); ≥ 10 μg/L, 19.109 (9.895). Corresponding numbers of subjects in each category were 90, 196, 127, and 106, respectively. For all tests, *p*-values ≤ 0.05 indicate a statistically significant association of POR with a given independent variable.

the most potent independent variables.

Fig. 1 shows linear regressions of log[(E_{alb}/C_{cr}) × 10^4] (A), log[($E_{\beta 2M}/C_{cr}$) × 10^4] (B), and eGFR (C) on log[E_{Cd}/C_{cr}) × 10^5], and the linear regression of log[$E_{alb}/C_{cr} \times 10^4$] on log[($E_{\beta 2M}/C_{cr}$) × 10^4] (D). All x- and y-values are standardized. R^2 values for the four regressions were 0.086, 0.107, 0.252, and 0.127, respectively, and p was < 0.001 for each regression. Table E shows that standardized β-coefficients in graphs A and B were not significantly different from each other, but both were lower than the absolute value of the β-coefficient in graph C.

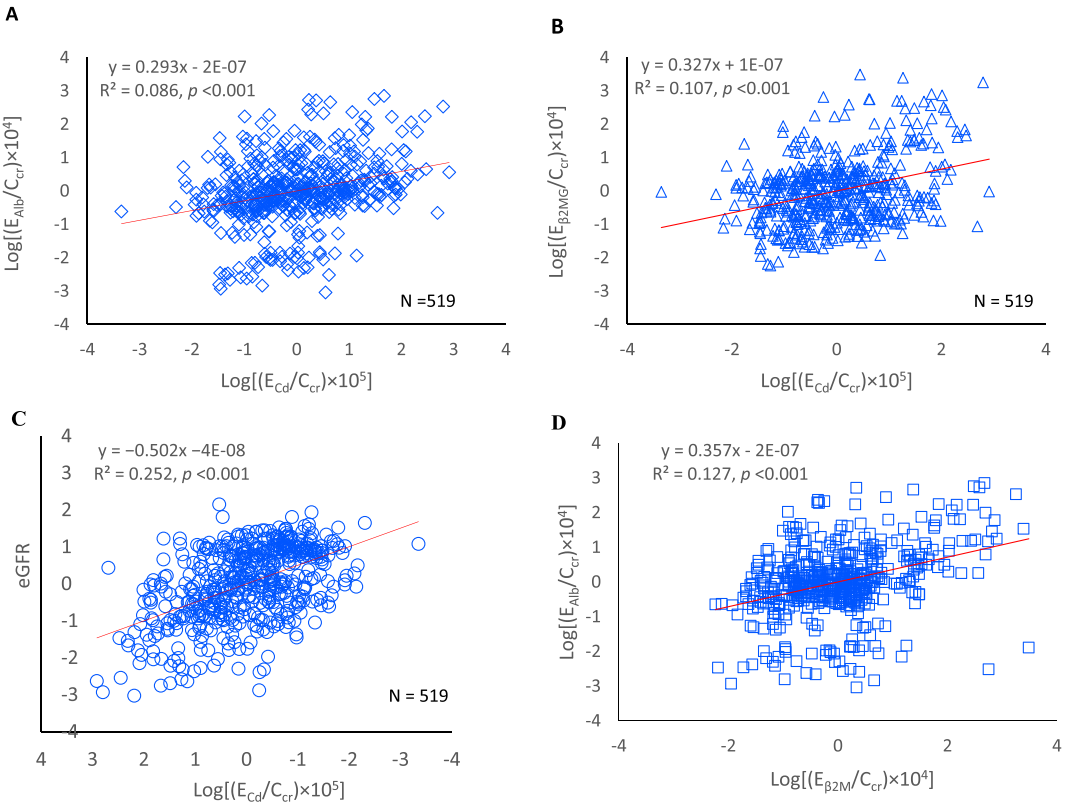
From Table 2, it is apparent that age had effects on E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$, and eGFR that were independent of the effects of Cd on these parameters; moreover, the strength of the effect of age (standardized β) differed among the parameters. Fig. 2A-C depict linear regressions of log[(E_{alb}/C_{cr}) × 10^4] (A), log[($E_{\beta 2M}/C_{cr}$) × 10^4] (B), and eGFR (C) on log[E_{Cd}/C_{cr}) × 10^5] after adjustment of dependent variables for age. All x- and y-values are standardized. In comparison to Fig. 1A-D (before age-adjustment), Fig. 2A-D show much higher R^2 values and substantially reduced dispersion around regression lines. These graphs confirm that age was an important determinant of the relationship of E_{alb}/C_{cr} with $E_{\beta 2M}/C_{cr}$ and relationships of E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$, and eGFR to E_{Cd}/C_{cr}. However, the graphs also reveal strong associations between dependent variables and E_{Cd}/C_{cr} after adjustment for age. The R^2 value in Fig. 2A, 0.660, suggests that adjusted log[(E_{alb}/C_{cr}) × 10^4] may have also been affected by hypertension (Table 2). R^2 values in Fig. 2B and 2D, 0.889 and 0.922, imply that $E_{\beta 2M}/C_{cr}$ varied almost exclusively with E_{Cd}/C_{cr} (B), and E_{alb}/C_{cr} varied almost exclusively with $E_{\beta 2M}/C_{cr}$ (D). Importantly, slopes relating standardized variables in graphs B and D approached unity.

Fig. 3 depicts trajectories of log[(E_{alb}/C_{cr}) × 10^4] (A), log[($E_{\beta 2M}/C_{cr}$) × 10^4] (C), and eGFR (E) through four quartiles of E_{Cd}/C_{cr}. For each quartile, bar graphs show the presence or absence of differences between hypertensive and normotensive subjects with respect to these variables (B, D, and F). Graphs A, C, and E show upward trajectories of log[(E_{alb}/C_{cr}) × 10^4] and log[($E_{\beta 2M}/C_{cr}$) × 10^4] and a downward trajectory of eGFR as E_{Cd}/C_{cr} increased. Hypertension was associated with higher log[(E_{alb}/C_{cr}) × 10^4] in quartiles 1 and 3 (graph B) and lower eGFR in quartile 1 (graph F). Values of log[($E_{\beta 2M}/C_{cr}$) × 10^4] were not affected by hypertension in any quartile (graph D).

Discussion

Summary of findings

The present study addresses the pathogenesis of albuminuria in Cd nephropathy. Participants inhabited regions of Thailand characterized by heavy exposure to the metal. In a previous report, mean E_{Cd}/E_{cr} was 0.19 μg/g cr in subjects from a low-exposure area (Satarug et al., 2021); in the present study, mean E_{Cd}/E_{cr} was 7.49 μg/g cr. We normalized E_{Cd}, E_{alb}, and $E_{\beta 2M}$ to C_{cr} rather than E_{cr} in order to depict amounts excreted per volume of filtrate, which in theory are proportional to amounts excreted per functioning nephron (Satarug et al., 2021). The resulting



E

Graphs	Standardized β coefficients		
	Mean	95% CI for β	*p*-values
A	0.298 (0.041)	0.218, 0.385	<0.001
B	0.331 (0.047)	0.237, 0.418	<0.001
C	−0.507 (0.039)	−0.578, −0.438	<0.001
D	0.358 (0.049)	0.264, 0.447	<0.001
Δ E_{Alb} vs $E_{β2MG}$	0.033	n/a	0.561
Δ E_{Alb} vs eGFR	0.209	n/a	<0.001
Δ $E_{β2MG}$ vs eGFR	0.176	n/a	0.002

β and 95% CI were obtained by bootstrap.
Differences between β values (Δ) in pairwise comparisons of graphs A-C were assessed with a t-test.
p-values ≤ 0.05 indicate statistically significant associations between x- and y-variables or statistically significant differences between values of β in pairwise comparisons.

Fig. 1. Regression analyses relating albumin excretion rate, β_2-microglobulin excretion rate, and eGFR to cadmium excretion rate. Scatterplots A-C relate $\log[(E_{alb}/C_{cr})\times10^4]$ (A), $\log[(E_{\beta2M}/C_{cr})\times10^4]$ (B), and eGFR (C) to $\log[(E_{Cd}/C_{cr})\times10^5]$. Scatterplot D relates $\log[(E_{alb}/C_{cr})\times10^4]$ to $\log[(E_{\beta2M}/C_{cr})\times10^4]$. Respective units of E_{alb}/C_{cr}, $E_{\beta2M}/C_{cr}$, E_{Cd}/C_{cr}, and eGFR are mg/L of filtrate, m g/L of filtrate, m g/L of filtrate, and mL/min/1.73m^2. Standardization of logarithmic values is accomplished by transformation to z-scores using the equation $z = x - \mu/\sigma$, where x = raw score, μ = mean, and σ = standard deviation. Regression equations, coefficients of determination (R^2), and p-values are provided for all scatterplots. Differences between pairs of standardized β-coefficients (Δ) are provided in Table E.

ratios were log-transformed for analyses, and regressions were performed after standardization of eGFR and logarithmic values.

Tabular data indicate that as age or E_{Cd}/C_{cr} rose, E_{alb}/C_{cr} and $E_{\beta2M}/C_{cr}$ also rose and eGFR fell (Table 2). Prevalence odds ratios (PORs) for abnormal E_{alb}/C_{cr}, $E_{\beta2M}/C_{cr}$, and eGFR (Table 3) were compatible with the results of multivariable regression analyses (Table 2). Simple linear regressions show that positive slopes of lines relating E_{alb}/C_{cr} or $E_{\beta2M}/C_{cr}$ to E_{Cd}/C_{cr} were not significantly different (Fig. 1A,B), but eGFR was inversely related to E_{Cd}/C_{cr} with a steeper slope (Fig. 1C). When eGFR and logs of E_{alb}/C_{cr} and $E_{\beta2M}/C_{cr}$ were adjusted for age, relationships of these variables to $\log(E_{Cd}/C_{cr})$ were strengthened considerably (Fig. 2); R^2 rose by a multiple for all associations, and standardized slopes of lines relating $\log(E_{\beta2M}/C_{cr})$ to $\log(E_{Cd}/C_{cr})$ and $\log(E_{alb}/C_{cr})$ approached unity. Variation in age and E_{Cd}/C_{cr} explained almost all variation in E_{alb}/C_{cr}, $E_{\beta2M}/C_{cr}$, and eGFR; moreover, after adjustment for age, standardized $\log(E_{\beta2M}/C_{cr})$ varied almost exactly as standardized logs of E_{Cd}/C_{cr} and E_{alb}/C_{cr} varied (Fig. 2). In selected quartiles, hypertension affected the relationship of E_{alb}/C_{cr} (but not $E_{\beta2M}/C_{cr}$) to E_{Cd}/C_{cr} (Fig. 3).

Pertinent aspects of albumin and β_2M homeostasis

Albumin is the most abundant protein in plasma. Ordinarily, 20 % of plasma entering the glomerulus is filtered, and a minute fraction of albumin dissolved in plasma accompanies filtrate into the tubular lumen. Published estimates of that fraction, the glomerular sieving coefficient for albumin (GSC_{alb}), range from 6×10^{-4} (Tojo and Endou, 1992) to 7×10^{-2} (Eppel et al., 1999; Comper et al., 2022). Although both extremes of estimated GSC_{alb} indicate minimal filterability in comparison to that of

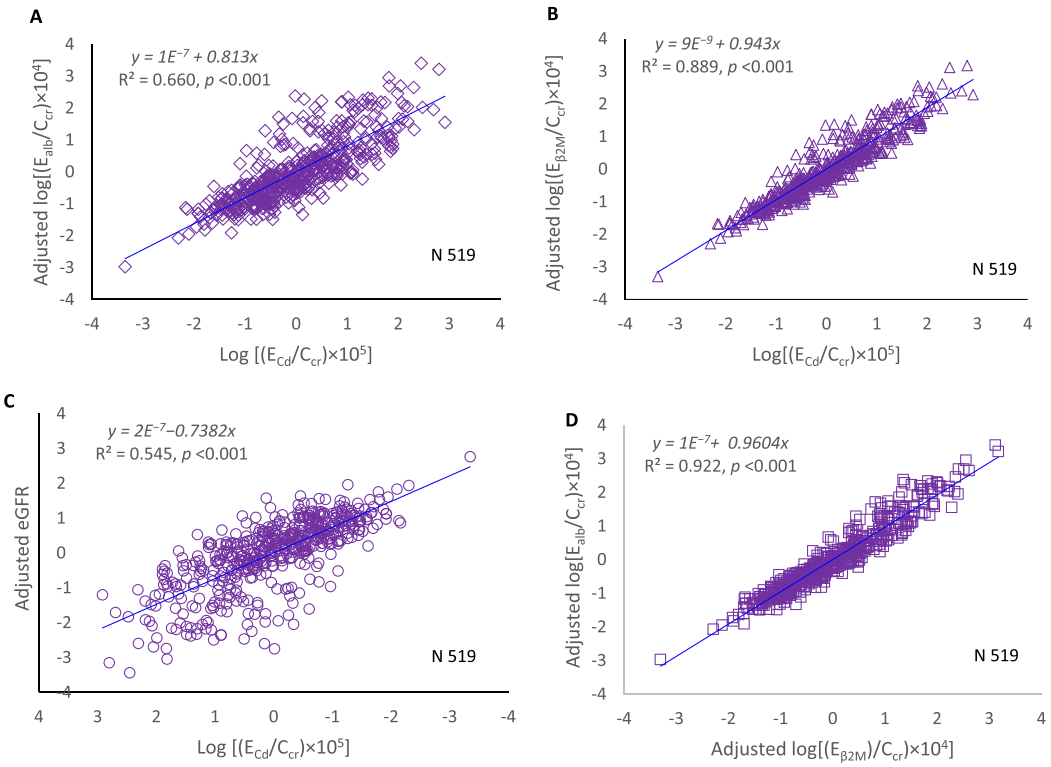


Fig. 2. Regression analyses relating albumin excretion rate, β_2-microglobulin excretion rate, and eGFR to cadmium excretion rate after adjustment of dependent variables for age.

Scatterplots relate z-scores of age-adjusted log[(E_{alb}/C_{cr})×10^4] (A), age-adjusted log[($E_{\beta2M}/C_{cr}$)×10^4] (B), and age-adjusted eGFR (C) to log[(E_{Cd}/C_{cr})×10^5]. An additional scatterplot relates z-scores of age-adjusted log[(E_{alb}/C_{cr})×10^4] to age-adjusted log[($E_{\beta2M}/C_{cr}$)×10^4] (D). Respective units of E_{alb}/C_{cr}, $E_{\beta2M}/C_{cr}$, E_{Cd}/C_{cr}, and eGFR are mg/L of filtrate, m g/L of filtrate, m g/L of filtrate, and mL/min/1.73m^2. Transformation of age-adjusted log[(E_{alb} /C_{cr})×10^4], age-adjusted log[($E_{\beta2M}$ /C_{cr})×10^4], and age-adjusted eGFR to z-scores is accomplished with the equation z = x – μ/σ, where x = raw score, μ = mean, and σ = standard deviation. Regression equations, coefficients of determination (R^2), and *p* -values are provided for all scatterplots. The generic age-adjusted equation is $y = \alpha + \beta_1{*}age + \beta_2{*}x$, where x = log [(E_{Cd} /C_{cr})×10^5]; y = age-adjusted log[(E_{alb} /C_{cr})×10^4], age-adjusted log[($E_{\beta2M}$ /C_{cr})×10^4], or age-adjusted eGFR; α = y-intercept; β_1 = slope for age; and β_2 = slope for log[(E_{Cd} /C_{cr})×10^5]. The equation to describe age-adjusted E_{alb} /C_{cr} is log[(E_{alb} /C_{cr})×10^4] = 0.195 + 0.018* age + 0.423* log[(E_{Cd} /C_{cr})×10^5]. The equation to describe age-adjusted $E_{\beta2M}$ is log[($E_{\beta2M}$ /C_{cr})×10^4] = 1.398 + 0.012* age + 0.678* log[(E_{Cd} /C_{cr})×10^5]. The equation to describe age-adjusted eGFR is eGFR = 210 − 1.10* age − 18.4* log[(E_{Cd} /C_{cr})×10^5].

many plasma solutes, values within the reported range of GSC_{alb} imply markedly different absolute rates of albumin filtration. For example, at a GFR of 100 mL/min (1440 dL/d) and $[alb]_p$ of 4.0 g/dL, the filtration rate of albumin if GSC_{alb} equals 10^{-3} is (1 0 −3) (4.0 gm/dL) (1440 dL/d), or 5.76 gm/d; at the same GFR and $[alb]_p$, the filtration rate of albumin if GSC_{alb} equals 10^{-2} is 57.6 gm/d. Since the higher value greatly exceeds the maximal hepatic synthetic rate of albumin (Ballmer et al., 1992; de Sain-van der Velden et al., 1998), a normal $[alb]_p$ at GSC_{alb} of 10^{-2} necessarily implies recycling of filtered albumin to the circulation. A large body of evidence suggests that GSC_{alb} is in fact 10^{-2} or higher (Eppel et al., 1999; Russo et al., 2007; Wagner et al., 2023; reviewed in Comper et al., 2022); if this conclusion is correct, the principal fate of filtered albumin is reabsorption and transcytosis by proximal tubular epithelium, extrusion into the interstitium, and diffusion into peritubular capillaries. These phenomena have been amply documented (Park and Maack, 1984; Eppel et al., 1999; Russo et al., 2007; Sarav et al., 2009; Tenten et al., 2013).

The proximal tubule is divisible by ultrastructural morphology into segments S1, S2, and S3 (Clapp et al., 1988). S1 is confined to the proximal convoluted tubule (PCT) and S3 to the proximal straight tubule (PST). S2 bridges the PCT and the PST and exhibits features of both S1 and S3. Fluid phase endocytosis (FPE), which is assessed by reabsorption of dextran, occurs in S1, but in contrast to receptor-mediated endocytosis (RME), FPE is also evident in S2 and S3 (Schuh et al., 2018; Wagner et al., 2023) and is believed to initiate most transcytosis of albumin. (Molitoris et al., 2022). Like FPE, albumin reabsorption occurs in S1, S2, and S3 (Clapp et al., 1988; Tojo and Endou, 1992), and megalin, which is distributed evenly throughout these segments (Schuh et al., 2018), is required for optimal FPE (Ren et al., 2020; Wagner et al., 2023). Endosomes created by FPE are trafficked through the mediation of neonatal Fc receptor (FcRn) to the basolateral membrane, where intact albumin is extruded (Russo et al., 2007; Sarav et al., 2009; Tenten et al., 2013).

In S1, albumin is also subjected to RME by the megalin-cubilin system and subsequently to lysosomal degradation (Schuh et al., 2018). Albumin altered by glycation or binding of other substances is diverted to this pathway (reviewed in Molitoris et al., 2022); since binding of Cd to albumin alters the conformational structure of the protein (Chen et al., 2014; Liu et al., 2014), we speculate that Cd-albumin complexes are also subjected to receptor-mediated endocytosis (RME) and subsequent degradation (Fels et al., 2019). Because the excreted form of Cd is primarily Cd-metallothionein (Cd-MT) (Wolf et al., 2009), *i.e.*, the storage form of the metal in proximal tubular cells (reviewed in Sabolic et al., 2010), we infer that Cd released from catabolized albumin in those cells is retained as Cd-MT while the breakdown products of albumin are excreted in urine or delivered to the bloodstream.

β_2M is normally released to the circulation from the surface of nucleated cells (Argyropoulos et al., 2017). Binding to a carrier protein in plasma was observed in rats after a bolus injection of β_2M (Nguyen-Simmonet et al., 1982), but to the best of our knowledge, the bound fraction at normal or chronically elevated plasma concentrations has not been quantified, and it may be negligible. In an isolated perfused kidney

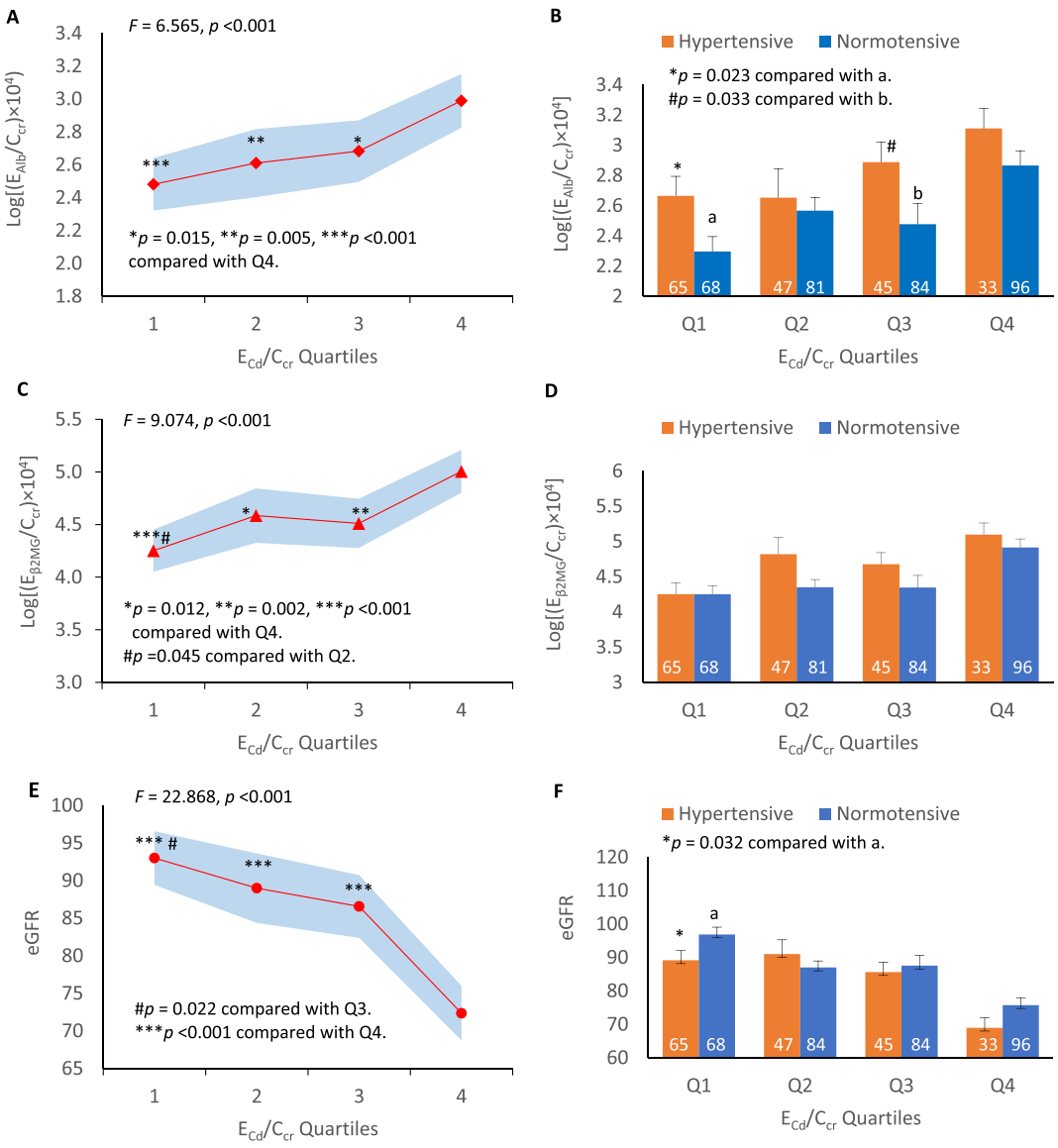


Fig. 3. Covariance analysis of effects of cadmium on excretion of albumin, excretion of β_2-microglobulin, and eGFR.
The color-coded area graphs depict means of $\log[E_{alb}/C_{cr})\times10^4]$ (A), $\log[(E_{\beta2MG}/C_{cr})\times10^4$ (C), and eGFR (E) across E_{Cd}/C_{cr} quartiles. Shaded areas indicate variability of means. The bar graphs compare means of $\log[E_{alb}/C_{cr})\times10^4]$ (B), $\log[(E_{\beta2MG}/C_{cr})\times10^4$ (D), and eGFR (F) in subgroups of subjects with and without hypertension in each of four E_{Cd}/C_{cr} quartiles. The respective numbers of subjects in quartiles 1, 2, 3 and 4 are 133, 128, 129 and 129. Means for subgroups were obtained by analysis of covariance with adjustment for age and BMI. Bonferroni correction was applied to multiple comparisons. Respective arithmetic means and standard deviations (SD) for $(E_{Cd}/C_{cr})\times100$ quartiles 1, 2, 3 and 4 are 1.71 (0.50), 3.38 (0.57), 6.23 (1.17) and 17.36 (9.72) μg/L of filtrate. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

model, $GSC_{\beta2M}$ was 0.94 even though the perfusate contained albumin, a potential carrier of β_2M (Sumpio and Maack, 1982); in five patients with Dent's disease, a tubulopathy presumed to disable β_2M reabsorption, $GSC_{\beta2M}$ (calculated as fractional excretion of β_2M) was 0.91 (Norden et al., 2001). Significant binding of β_2M to a carrier in plasma would have lowered this estimate. In Cd nephropathy, reabsorption of β_2M commonly falls and its excretion rate ($E_{\beta2M}$) rises accordingly (Peterson et al., 1969; Satarug et al., 2019).

Multiple lines of evidence suggest that all β_2M taken up by the proximal tubule is either degraded or incorporated into another molecule. In normal rats, $[\beta_2M]_p$ was 20 % lower in the renal vein than in the renal artery even though the protein was not found in urine (Bernier and Conway, 1969). In dogs, a comparison of renal arterial delivery to venous outflow of β_2M showed that extraction by the kidney exceeded glomerular filtration of β_2M even though a glomerular sieving coefficient ($GSC_{\beta2M}$) of 1 was assumed and $E_{\beta2M}$ was negligible (Hall III et al., 1982). This result suggested that β_2M entered proximal tubular cells through both apical and basolateral membranes, and implied that transcytosis of filtered β_2M did not occur or was quantitatively insignificant. Basolateral uptake of the protein was observed in cultured LLC-PK_1 cells (Cohen et al., 1995) but not in Sprague-Dawley rat proximal tubules (Sundin et al., 1994). The current consensus is that virtually all β_2M entering proximal tubular cells is reabsorbed and degraded in S1 and to a lesser extent in S2 (Polesel et al., 2022), but β_2M is also a constituent of FcRn, which mediates transcytosis of albumin reabsorbed from glomerular filtrate (Sarav et al., 2009; Tenten et al., 2013; reviewed in Argyropoulos et al., 2017).

Inferences from excretion rates of albumin and β2M in the present study

The data reported herein compare renal handling of albumin to that of β_2M in subjects exposed to Cd. After adjustments of $\log(E_{alb}/C_{cr})$ and

$\log(E_{\beta 2M}/C_{cr})$ for age (Fig. 2), standardized slopes (β) of regressions relating $\log(E_{alb}/C_{cr})$ and $\log(E_{\beta 2M}/C_{cr})$ to $\log(E_{Cd}/C_{cr})$ were 0.813 and 0.943, respectively; similarly, standardized β for the regression of $\log(E_{alb}/C_{cr})$ on $\log(E_{\beta 2M}/C_{cr})$ was 0.960. In each of these regressions, slopes approaching unity indicate that on average, x- and y-coordinates for any single point differed from mean values of the coordinates by a similar standard deviation. Most significantly the standardized β for the regression of $\log(E_{alb}/C_{cr})$ on $\log(E_{\beta 2M}/C_{cr})$ implies that Cd disrupted a single, shared mechanism for RME and degradation of albumin and β_2M. Since these phenomena occur primarily in S1 (Schuh et al., 2018), it is likely that the impairment of reabsorption documented in the present study took place in this segment.

Clinical observations are compatible with this proposition. In contrast to values of $E_{alb} \geq 3.5$ g/d in nephrotic syndrome, albuminuria associated with Cd tubulopathy is rarely more than 500 mg/d, even when exposure to the metal has been extreme (Peterson et al., 1969; Roels et al., 1982; Elinder et al., 1985; Haswell-Elkins et al., 2008; Liang et al., 2012). This observation might be expected if only a few grams of albumin entered glomerular filtrate each day, but moderate albuminuria is also compatible with the concept that many grams of albumin traverse the glomerular filtration barrier and most are returned to the circulation by the proximal tubule. If the majority of albumin in filtrate undergoes transcytosis and a minority is subjected to RME and lysosomal degradation (Comper et al., 2022), then impediments to processing in S1 can be expected to yield modest albuminuria and massive beta-2-microglobulinuria simultaneously.

If the higher reported values of GSC_{alb} are accepted, we estimate that in our most severely affected subjects, *i.e.*, those with eGFR ≤ 60 mL/min/1.73 m^2 (Table 1), mean fractional excretions of filtered albumin and β_2M were 0.18 % and 21 %, respectively (SM, Item III). Since reabsorption of both proteins in S1 is essentially complete under normal circumstances, these percentages also approximate the fractions by which tubular reabsorption of the proteins was reduced.

What aspect of protein reabsorption and degradation did Cd disrupt? Megalin and cubilin are large receptor proteins found in proximity to each other on villous apical membranes of proximal tubular cells. Cubilin is found primarily in S1, but megalin is distributed evenly among S1, S2, and S3 (Schuh, 2018). Some filtered ligands bind specifically to one of the receptors, and others bind to both (Nielsen et al., 2016). Albumin binds to cubilin and does not undergo RME in the absence of this receptor (Amsellem et al., 2010); however, because megalin promotes endocytosis of albumin-cubilin complexes, both receptor proteins are required for maximal RME of albumin (Amsellem et al., 2010), and hereditary deficiency of either protein causes excessive albuminuria (Storm et al., 2011; 2013). Megalin also appears to be required for optimal FPE of albumin (Ren et al., 2020; Wagner et al., 2023). In contrast to albumin, β_2M binds specifically to megalin and not cubilin; consequently, megalin deficiency increases excretion of β_2M (Storm et al., 2013), but cubilin deficiency does not (Bedin et al., 2020; Gan et al., 2022). Taken together, these observations suggest that in our subjects, the close association of albuminuria with beta-2-microglobulinuria was a consequence of reduced availabity, abnormal function, or impaired recycling of megalin. Since the effects of simultaneous deficiencies of megalin and cubilin on albumin uptake were not additive in mice (Amsellem et al., 2010), it is possible that Cd also disrupted the function of cubilin in the present study (Santoyo-Sanchez et al., 2013), but this consideration is not required to explain our findings.

Additional observations are compatible with the concept that Cd affects some aspect of megalin homeostasis. In S1 cells, exposure to sublethal Cd doses impeded uptake of β_2M much more than that of albumin (Fujishiro et al., 2020). This observation is expected if a small fraction of filtered albumin and most or all filtered β_2M enter tubular cells by a shared mechanism. In cultures of pig proximal tubular cells, exposure to Cd suppressed production of megalin RNA, promoted lysosomal degradation of megalin, and reduced uptake of fluorescein-labeled albumin (Gena et al., 2010). In megalin-deficient mice, binding of exogenous megalin to proteins in urine required ionized calcium (Ca^{++}) (Leheste et al., 1999); this observation raises the possibility that Cd^{++} undermines binding of ligands to megalin by displacing Ca^{++}. In Wistar rats, chronic sublethal dosing of Cd led to disruption of microtubules, which facilitate recycling of brush-border membrane proteins (Sabolic et al., 2001).

Our inference that Cd compromised some aspect of megalin function leaves numerous questions unanswered. If megalin is present in S2 and S3, why is processing of filtered β_2M essentially limited to S1 (Polesel et al., 2022)? Does a relative deficiency of the clathrin adaptor disabled-2 (dab-2) limit RME by megalin in S2 and S3 (Weisz, 2021)? If FPE can retrieve dozens of grams of albumin in S2 and S3, why doesn't it prevent tubular proteinuria originating in S1? In the distal tubule, the lipocalin-2 receptor can mediate reabsorption of albumin and β_2M (Zavala-Guevara et al., 2019), but did this activity occur in our subjects? If it did, given the tight association of $\log(E_{alb}/C_{cr})$ and $\log(E_{\beta 2M}/C_{cr})$, we must infer that reabsorption rates of distally displaced albumin and β_2M preserved the close relationship between those rates in the proximal tubule. This scenario seems implausible.

Fig. 4 summarizes our view of the pathogenesis of albuminuria in Cd nephropathy.

Cd, albuminuria, eGFR, and hypertension

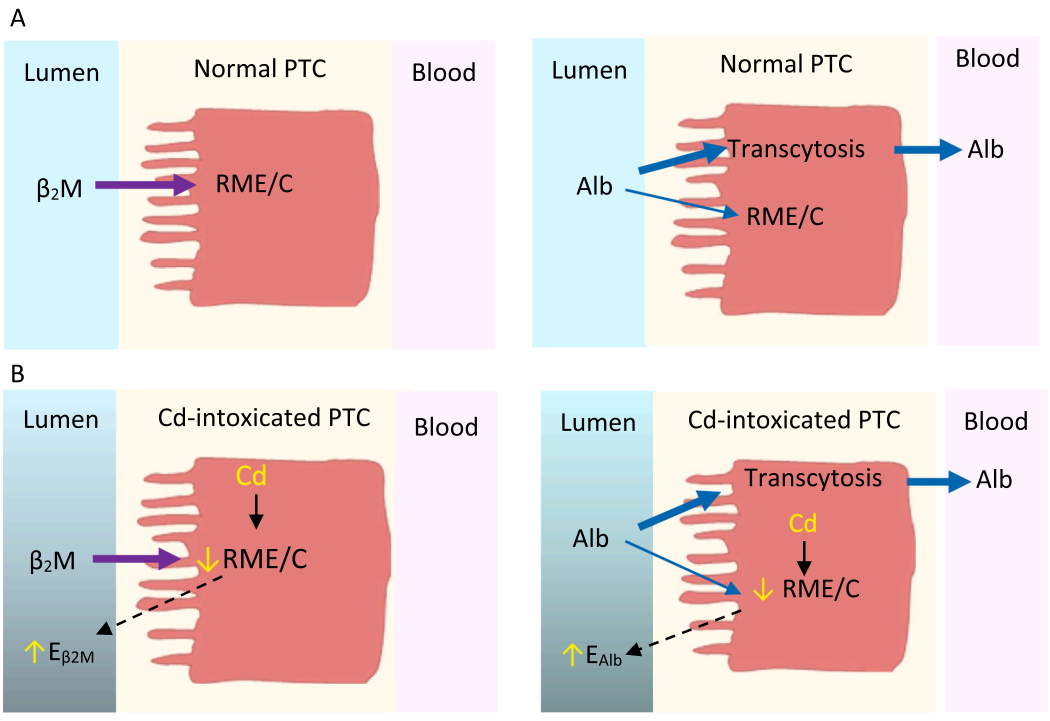
All the tabular and graphic data reported herein show that E_{alb}/C_{cr} rises and eGFR falls as E_{Cd}/C_{cr} increases. Other investigators have documented either direct relationships between E_{alb} and E_{Cd} [Roels et al., 1982; Elinder et al., 1985; Haswell-Elkins et al., 2008; Liang et al., 2012; Feng et al., 2022) or inverse relationships between eGFR and E_{Cd} (Win-Thu et al., 2021; Butler-Dawson et al., 2022). Madrigal and colleagues demonstrated that E_{alb}/E_{cr} varied directly and eGFR varied inversely with blood Cd (Madrigal et al., 2019). In the present study, we found that relationships of E_{Cd} to E_{alb} and eGFR were sharpened considerably by normalization of excretion rates to C_{cr} rather than E_{cr} (Tables 1–3, S1–S2).

Associations of E_{Cd}/C_{cr} with E_{alb}/C_{cr} and eGFR raise questions of cause and effect among the three variables. In glomerular disease, increased concentrations in filtrate apparently augment both tubular reabsorption and excretion of protein, and the reabsorbed component injures tubular epithelium (Ruggenenti and Remuzzi, 2000). The magnitude of proteinuria correlates with the rate of nephron loss, presumably because excretion and reabsorption of protein are directly related, and interventions that reduce proteinuria mitigate the decline in GFR (GISEN group, 1997; Hou et al., 2007). In Cd nephropathy, albuminuria results from reduced tubular reabsorption rather than increased filtrate content of albumin, and excessive reabsorption of protein presumably makes no contribution to tubular injury. Accumulated Cd destroys nephrons and impedes albumin reabsorption simultaneously.

Whereas hypertension did not affect $E_{\beta 2M}/C_{cr}$ significantly in any of the four quartiles of E_{Cd}/C_{cr} (Fig. 3), it appears to have increased E_{alb}/C_{cr} in two of the quartiles. Since $GSC_{\beta 2M}$ approaches 1 (Sumpio and Maack, 1982), hypertension cannot be expected to promote filtration of this protein. In contrast, even if the more recent, higher estimates of GSC_{alb} are accepted (Comper et al., 2022), they provide ample opportunity for enhancement of albumin filtration by hypertension. Published evidence suggests that hypertension promotes albuminuria in type 2 diabetes (Retnakaran et al., 2006; Schrier et al., 2002; UK Prospective Diabetes Study Group, 1998), and it apparently has the same effect in Cd nephropathy. Once again, however, we must ask why S2, S3, and the distal tubule do not prevent excretion of protein that is filtered excessively.

Conclusions

Herein, we attempt to elucidate the mechanism underlying excessive albuminuria in Cd nephropathy. In Thai subjects heavily exposed to



C

PTC status	Protein	Filtration rate	Excretion rate	Catabolic rate	Transcytosis rate
Normal	Albumin	60 gm/d	20 mg/d	2.980 gm/d	57 gm/d
	β_2M	300 mg/d	100 μg/d	2.999 mg/d	0
Cd intoxicated	Albumin	60 gm/d	0.5 gm/d	2.5 gm/d	57 gm/d
	β_2M	300 mg/d	1000 μg/d	2.997 mg/d	0

Assumptions: plasma albumin is 40 gm/L; plasma β_2M is 2.0 mg/L; GFR is 150 L/d; the glomerular sieving coefficient for albumin (GSC_{alb}) is 0.02; and $GSC_{\beta 2M}$ is 1.

Fig. 4. Proposed pathogenesis of reduced proximal tubular reabsorption of albumin and β_2-microglobulin in Cd nephropathy.
Data reported herein show close associations of excretion rates of albumin and β_2-microglobulin (E_{alb} and $E_{b\ 2M}$) in Cd-exposed subjects. Normally, proximal tubular cells (PTCs) reabsorb virtually all filtered b_2M by receptor-mediated endocytosis (RME) involving the apical protein megalin. b_2M is then catabolized (C) in lysosomes (row A). PTCs return most filtered albumin to the circulation by transcytosis, but a small fraction is subjected to RME by the apical proteins megalin and cubilin and subsequently catabolized in lysosomes (row A). Our results suggest that Cd impairs a single mechanism for RME and degradation of both b_2M and albumin, and we propose that disruption of the function of megalin explains the results. This disruption increases excretion rates of both proteins (row B). The table summarizes representative quantitative effects of Cd on handling of albumin and b_2M by PTCs. Note that fractional reductions in reabsorption of both proteins are miniscule even though absolute excretion rates of the proteins are distinctly increased.

environmental Cd, E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ rose unequivocally as eGFR fell. After log transformation of E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$, and E_{Cd}/C_{cr}, adjustment of log(E_{alb}/C_{cr}), log($E_{\beta 2M}/C_{cr}$), and eGFR for age, and standardization of all resulting values, our data show that variation in age and E_{Cd}/C_{cr} explained most variation in E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$ in the cohort. Moreover, standardized slopes of regressions of log(E_{alb}/C_{cr}) on log(E_{Cd}/C_{cr}), log($E_{\beta 2M}/C_{cr}$) on log(E_{Cd}/C_{cr}), and log(E_{alb}/C_{cr}) on log($E_{\beta 2M}/C_{cr}$) approached unity. These results suggest that accumulated Cd impaired a single mechanism for proximal tubular reabsorption and degradation of filtered albumin and β_2M. From other published information, we infer that the mechanism probably involved the availability or function of the apical receptor megalin in S1 of the proximal tubule.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Soisungwan Satarug: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **David A. Vesey:** Resources, Writing – review & editing. **Glenda C. Gobe:** Resources, Writing – review & editing. **Kenneth R. Phelps:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported with resources of the Centre for Kidney Disease Research, and the Department of Kidney and Transplant Services, Princess Alexandra Hospital. It was also supported with resources of the Stratton Veterans Affairs Medical Center, Albany, NY, USA. Opinions expressed herein are those of the authors and do not represent the official position of the United States Department of Veterans Affairs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2023.100140.

References

Abouhamed, M., Wolff, N.A., Lee, W.-K., Smith, C.P., Thevenod, F., 2007. Knockdown of endosomal/lysosomal divalent metal transporter 1 by RNA interference prevents cadmium-metallothionein-1 toxicity in renal proximal tubule cells. Am. J. Physiol. Renal Physiol. 293, F705–F712.

Amsellem, S., Gburek, J., Harnard, G., Nielsen, R., Willnow, T.E., Devuyst, O., Nexo, E., Verroust, P.J., Christensen, E.I., Kozyraki, R., 2010. Cubilin is essential for albumin reabsorption in the renal proximal tubule. J. Am. Soc. Nephrol. 21, 1859–1867.

Argyropoulos, C.P., Chen, S.S., Ng, Y.-H., Roumelioti, M.-E., Shaffi, K., Singh, P.P., Tzamaloukas, A.H., 2017. Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. Front. Med. 4, 73.

Ballmer, P.E., Weber, B.K., Roy-Chaudhury, P., McNurlan, M.A., Watson, H., Power, D. A., Garlick, P.J., 1992. Elevation of albumin synthesis rates in nephrotic patients measured with [1-13C] leucine. Kidney Int. 41, 132–138.

Bedin, M., Boyer, O., Servais, A., Li, Y., Villoing-Gaudé, L., Tête, M.J., Cambier, A., Hogan, J., Baudouin, V., Krid, S., Bensman, A., Lammens, F., Louillet, F., Ranchin, B., Vigneau, C., Bouteau, I., Isnard-Bagnis, C., Mache, C.J., Schäfer, T., Pape, L., Gödel, M., Huber, T.B., Benz, M., Klaus, G., Hansen, M., Latta, K., Gribouval, O., Morinière, V., Tournant, C., Grohmann, M., Kuhn, E., Wagner, T., Bole-Feysot, C., Jabot-Hanin, F., Nitschké, P., Ahluwalia, T.S., Köttgen, A., Andersen, C.B.F., Bergmann, C., Antignac, C., Simons, M., 2020. Human C-terminal CUBN variants associate with chronic proteinuria and normal renal function. J. Clin. Invest. 130, 335–344.

Bernier, G.M., Conrad, M.E., 1969. Catabolism of human β_2-microglobulin by the rat kidney. Am. J. Physiol. 217, 1359–1362.

Bloch, M.J., Basile, J.N., 2013. Review of recent literature in hypertension: updated clinical practice guidelines for chronic kidney disease now include albuminuria in the classification system. J. Clin. Hypertens. (Greenwich) 15, 865–867.

Butler-Dawson, J., James, K.A., Krisher, L., Jaramillo, D., Dally, M., Neumann, N., Pilloni, D., Cruz, A., Asensio, C., Johnson, R.J., Adgate, J., Newman, L.S., 2022. Environmental metal exposures and kidney function of Guatemalan sugarcane workers. J. Expo. Sci. Environ. Epidemiol. 32, 461–471.

Carlson, L.A., Friberg, L., 1957. The distribution of cadmium in blood after repeated exposure. Scand. J. Clin. Lab. Invest. 9, 67–70.

Chan, H.M., Zhu, L.-F., Zhong, R., Grant, D., Goyer, R.A., Cherian, M.G., 1993. Nephrotoxicity in rats following liver transplantation from cadmium-exposed rats. Toxicol. Appl. Pharmacol. 123, 89–96.

Chen, M., Guo, H., Liu, Y., Zhang, Q., 2014. Structural changes of human serum albumin induced by calcium acetate. J. Biochem. Mol. Toxicol. 28, 281–287.

Clapp, W.L., Park, C.H., Madsen, K.M., Tisher, C.C., 1988. Axial heterogeneity in the handling of albumin by the rabbit proximal tubule. Lab. Invest. 58, 549–558.

Cohen, M., Sundin, D.P., Dahl, R., Molitoris, B.A., 1995. Convergence of apical and basolateral endocytic pathways for β2-microglobulin in LLC-PK1 cells. Am. J. Physiol. 268, F829–F838.

Comper, W.D., Vuchkova, J., McCarthy, K.J., 2022. New insights in proteinuria/albuminuria. Front. Physiol. 13, 991756.

de Sain-van der Velden, M.G.M., Kaysen, G.A., de Meer, K., Stellaard, F., Voorbij, H.A.M., Reijngoud, D.-J., Rabelink, T.J., Koomans, H.A., 1998. Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: measurements with stable isotopes. Kidney Int. 53, 181-188.

Elinder, C.G., Edling, C., Lindberg, E., Agedal, B.K., Vesterberg, A., 1985. Assessment of renal function in workers previously exposed to cadmium. Br. J. Ind. Med. 42, 754–760.

Eppel, G.A., Osicka, T.M., Pratt, L.M., Jablonski, P., Howden, B.O., Glasgow, E.F., Comper, W.D., 1999. The return of glomerular-filtered albumin to the rat renal vein. Kidney Int. 55, 1861–1870.

Fels, J., Scharner, B., Zarbock, R., Zavala Guevara, I.P., Lee, W.K., Barbier, O.C., Thévenod, F., 2019. Cadmium complexed with β2-microglobulin, albumin and lipocalin-2 rather than metallothionein cause megalin:cubilin dependent toxicity of the renal proximal tubule. Int. J. Mol. Sci. 20, 2379.

Feng, X., Zhou, R., Jiang, Q., Wang, Y., Yu, C., 2022. Analysis of cadmium accumulation in community adults and its correlation with low-grade albuminuria. Sci. Total Environ. 834, 155210.

Food and Agriculture Organization of the United Nations (FAO): Available online: Safety evaluation of certain food additives and contaminants: prepared by the Seventy-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA). Accessed June 14, 2023. https://apps.who.int/iris/handle/10665/44521.

Fujishiro, H., Yamamoto, H., Otera, N., Oka, N., Jinno, M., Himeno, S., 2020. In vitro evaluation of effects of cadmium on endocytic uptakes of proteins into cultured proximal tubule epithelial cells. Toxics 8, 24.

Galaske, R.G., van Liew, J.B., Feld, L.G., 1979. Filtration and reabsorption of endogenous low-molecular-weight protein in the rat kidney. Kidney Int. 16, 394–403.

Gan, C., Zhou, X., Chen, D., Chi, H., Qiu, J., Chen, Y., Wang, M., Yang, H., Jiang, W., Li, Q., 2022. Novel pathogenic variants in *CUBN* uncouple proteinuria from renal function. J. Transl. Med. 20, 480.

Gena, P., Calamita, G., Guggino, W.B., 2010. Cadmium impairs albumin reabsorption by down-regulating megalin and ClC5 channels in renal proximal tubule cells. Environ. Health Perspect. 118, 1551–1556.

Gibson, M.A., Sarpong-Kumankomah, S., Nehzati, S., George, G.N., Gailer, J., 2017. Remarkable differences in the biochemical fate of Cd2+, Hg2+, CH3Hg+ and thimerosal in red blood cell lysate. Metallomics 9, 1060.

GISEN group,, 1997. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 349, 1857–11186.

Hall III, P.W., Chung Park, M., Vacca, C.V., London, M., Crowley, A.Q., 1982. The renal handling of beta2-microglobulin in the dog. Kidney Int. 22, 156–161.

Haswell-Elkins, M., Satarug, S., O'Rourke, P., Moore, M., Ng, J., McGrath, V., Walmby, M., 2008. Striking association between urinary cadmium level and albuminuria among Torres Strait Islander people with diabetes. Environ. Res. 106, 379–383.

Hou, F.F., Xie, D., Zhang, X., Chen, P.Y., Zhang, W.R., Liang, M., Guo, Z.J., Jiang, J.P., Hou, F.F., Xie, D., Zhang, X., Chen, P.Y., Zhang, W.R., Liang, M., Guo, Z.J., Jiang, J. P., 2007. Renoprotection of optimal antiproteinuric doses (ROAD) study: a randomized controlled study of benazepril and losartan in chronic renal insufficiency. J. Am. Soc. Nephrol. 18, 1889–1898.

Klassen, R.B., Crenshaw, K., Kozyraki, R., Verroust, P.J., Tio, L., Atrian, S., Allen, P.L., Hammond, T.G., 2004. Megalin mediates renal uptake of heavy metal metallothionein complexes. Am. J. Physiol. Renal Physiol. 287, F393–F403.

Langelueddecke, C., Roussa, E., Fenton, R.A., Wolff, N.A., Lee, W.-K., Thevenod, F., 2012. Lipocalin-2 (24p3/Neutrophil Gelatinase-associated Lipocalin (NGAL)) receptor is expressed in distal nephron and mediates protein endocytosis. J. Biol. Chem. 287, 159–169.

Leheste, J.-R., Rolinski, B., Vorum, H., Hilpert, J., Nykjaer, A., Jacobsen, C., Aucouturier, P., Moskaug, J.O., Otto, A., Christensen, E.I., Willnow, T.E., 1999. Megalin knockout mice as an animal model of low molecular weight proteinuria. Am. J. Pathol. 155, 1361–1370.

Levey, A.S., Stevens, L.A., Schmid, C.H., Zhang, Y.L., Castro III, A.F., Feldman, H.I., Kusek, J.W., Eggers, P., Van Lente, F., Greene, T., Coresh, J., 2009. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration): A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150, 604–612.

Liang, Y., Lei, L., Nilsson, J., Li, H., Nordberg, M., Bernard, A., Nordberg, G.F., Bergdahl, I.A., Jin, T., 2012. Renal function after reduction in cadmium exposure: an 8-year follow-up of residents in cadmium-polluted areas. Environ. Health Perspect. 120, 223–228.

Liu, Y., Chen, M., Jiang, L., Song, L., 2014. New insight into molecular interaction of heavy metal pollutant–cadmium (II) with human serum albumin. Environ. Sci. Pollut. Res. 21, 6994–7005.

Liu, J., Liu, Y., Habeebu, S.S., Klaassen, C.D., 1998. Susceptibility of MT-null mice to chronic $CdCl_2$-induced nephrotoxicity indicates that renal injury is not mediated by the CdMT complex. Toxicol. Sci. 46, 197–203.

Madrigal, J.M., Ricardo, A.C., Persky, V., Turyk, M., 2019. Associations between blood cadmium concentration and kidney function in the U.S. population: Impact of sex, diabetes and hypertension. Environ. Res. 169, 180–188.

Milnerowicz, H., Bizon, A., 2010. Determination of metallothionein in biological fluids using enzyme-linked immunoassay with commercial antibody. Acta Biochim. Polonica 57, 99–104.

Molitoris, B.A., Sandoval, R.M., Yadav, S.P.S., Wagner, M.C., 2022. Albumin uptake and processing by the proximal tubule: physiologoical, pathological, and therapeutic implications. Physiol. Rev. 102, 1625–1667.

Nguyen-Simmonet, H., Vincent, C., Gauthier, C., Revillard, J.P., Pellet, M.V., 1982. Turnover studies of human β2-microglobulin in the rat: evidence for a β2-microglobulin-binding protein. Clin. Sci. 62, 403–410.

Nielsen, R., Christensen, E.I., Birn, H., 2016. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. Kidney Int. 89, 58–67.

Nomiyama, K., Foulkes, E.C., 1977. Reabsorption of filtered cadmium-methionein in the rabbit kidney. Proc. Soc. Exp. Biol. Med. 156, 97–99.

Nordberg, M., Nordberg, G.F., 1975. Distribution of metallothionein-bound cadmium and cadmium chloride in mice: preliminary studies. Environ. Health Perspect. 12, 103–108.
Nordberg, M., Nordberg, G.F., 2022. Metallothionein and cadmium toxicology – historical review and commentary. Biomolecules 12, 360.
Nordberg, G.F., Piscator, M., Nordberg, M., 1971. On the distribution of cadmium in blood. Acta Pharmacol. Toxicol. 30, 289–295.
Norden, A.G.W., Lapsley, M., Lee, P.J., Pusey, C.D., Scheinman, S.J., Tam, F.W.K., Thakker, R.V., Unwin, R.J., Wrong, O., 2001. Glomerular protein sieving and implications for renal failure in Fanconi syndrome. Kidney Int. 60, 1885–1892.
Park, C.H., Maack, T., 1984. Albumin absorption and catabolism by isolated perfused proximal convoluted tubules of the rabbit. J. Clin. Invest. 73, 767–777.
Peterson, P.A., Evrin, P.-E., Berggard, I., 1969. Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of β2-microglobulin, albumin, and total protein. J. Clin. Invest. 48, 1189–1198.
Phelps, K.R., Gosmanova, E.O., 2020. A generic method for analysis of plasma concentrations. Clin. Nephrol. 94, 43–49.
Polesel, M., Hall, A.M., 2019. Axial differences in endocytosis along the kidney proximal tubule. Am. J. Physiol. Renal Physiol. 317, F1526–F1530.
Polesel, M., Kaminska, M., Haenni, D., Bugarski, M., Schuh, C., Jankovic, N., Kaech, A., Mateos, J.M., Berquez, M., Hall, A.M., 2022. Spatiotemporal organization of protein processing in the kidney. Nat. Commun. 13, 5732.
Portman, R.J., Kissane, J.M., Robson, A.M., 1986. Use of β2 microglobulin to diagnose tubulo-interstitial renal lesions in children. Kidney Int. 30, 91–98.
Ren, Q., Weyer, K., Rbaibi, Y., Long, K.R., Tan, R.J., Nielsen, R., Christensen, E.I., Baty, C. J., Kashlan, O.B., Weisz, O.A., 2020. Distinct functions of megalin and cubilin receptors in recovery of normal and nephrotic levels of filtered albumin. Am. J. Physiol. Renal Physiol. 318, F1284–F1294.
Retnakaran, R., Cull, C.A., Thorne, K.I., Adler, A.I., Holman, R.R., for the UKPDS Study Group., 2006. Risk factors for renal dysfunction in type 2 diabetes. U.K. prospective diabetes study 74. Diabetes 55, 1832-1839.
Roels, H., Djubgang, J., Buchet, J.P., Bernard, A., Lauwerys, R., 1982. Evolution of cadmium-induced renal dysfunction in workers removed from exposure. Scand. J. Work Environ. Health 8, 191–200.
Ruggenenti, P., Remuzzi, G., 2000. The role of protein traffic in the progression of renal diseases. Annu. Rev. Med. 51, 315–327.
Russo, L.M., Sandoval, R.M., McKee, M., Osicka, T.M., Collins, A.B., Brown, D., Molitoris, B.A., Comper, W.D., 2007. The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells. Retrieval is disrupted in nephrotic states. Kidney Int. 71, 504–513.
Sabolic, I., Herak-Kramberger, C.M., Brown, D., 2001. Subchronic cadmium treatment affects the abundance and arrangement of cytoskeletal proteins in rat renal proximal tubule cells. Toxicol. 165, 205–216.
Sabolic, I., Breljak, D., Skarica, M., Herak-Kramberger, C.M., 2010. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. Biometals 23, 897–926.
Santoyo-Sanchez, M.P., Pedraza-Chaverri, J., Molina-Jijon, E., Arreola-Mendoza, L., Rodriguez-Munoz, R., Barbier, O.C., 2013. Impaired endocytosis in proximal tubule from subchronic exposure to cadmium involves angiotensin II type 1 and cubilin receptors. BMC Nephrol. 14, 211.
Sarav, M., Wang, Y., Hack, B.K., Chang, A., Jensen, M., Bao, L., Quigg, R.J., 2009. Renal FcRn reclaims albumin but facilitates elimination of IgG. J. Am. Soc. Nephrol. 20, 1941–1952.
Satarug, S., Gobe, G.C., Vesey, D.A., Phelps, K.R., 2020. Cadmium and lead exposure, nephrotoxicity, and mortality. Toxics 8, 86.
Satarug, S., Phelps, K.R., 2021. Cadmium exposure and toxicity. In: Bagchi, D., Bagchi, M. (Eds.), Metal Toxicology Handbook. CRC Press, pp. 219–272.
Satarug, S., Swaddiwudhipong, W., Ruangyuttikarn, W., Nishijo, M., Ruiz, P., 2013. Modeling cadmium exposures in low- and high-exposure areas in Thailand. Environ. Health Perspect. 121, 531–536.
Satarug, S., Vesey, D.A., Nishijo, M., Ruangyuttikarn, W., Gobe, G.C., 2019. The inverse association of glomerular function and urinary β2-MG excretion and its implications for cadmium health risk assessment. Environ. Res. 173, 40–47.
Satarug, S., Vesey, D.A., Nishijo, M., Ruangyuttikarn, W., Gobe, G.C., Phelps, K.R., 2021. The effect of cadmium on GFR is clarified by normalization of excretion rates to creatinine clearance. Int. J. Mol. Sci. 22, 762.
Satarug, S., Vesey, D.A., Gobe, G.C., Yimthiang, S., Buha Đorđević, A., 2023a. Health risk in a geographic area of Thailand with endemic cadmium contamination: Focus on albuminuria. Toxics 11, 68.
Satarug, S., Vesey, D.A., Gobe, G.C., Phelps, K.R., 2023b. Estimation of health risks associated with dietary cadmium exposure. Arch. Toxicol. 97, 329–358.
Schrier, R.W., Estacio, R.O., Esler, A., Mehler, P., 2002. Effects of aggressive blood pressure control in normotensive type 2 diabetic patients on albuminuria, retinopathy, and strokes. Kidney Int. 61, 1086–1097.
Schuh, C.D., Polesel, M., Platonova, E., Haenni, D., Gassama, A., Tokonami, N., Ghazi, S., Bugarski, M., Devuyst, O., Ziegler, U., Hall, A.M., 2018. Combined structural and functional imaging of the kidney reveals major axial differences in proximal tubule endocytosis. J. Am. Soc. Nephrol. 29, 2696–2712.
Storm, T., Emma, F., Verroust, P.J., Hertz, J.M., Nielsen, R., Christensen, E.I., 2011. A patient with cubilin deficiency. N. Engl. J. Med. 364, 89–91.
Storm, T., Tranebjaerg, L., Frykholm, C., Birn, H., Verroust, P.J., Neveus, T., Sundelin, B., Hertz, J.M., Holmstrom, G., Eriicson, K., Christensen, E.I., Nielsen, R., 2013. Renal phenotypic investigations of megalin-deficient patients: novel insights into tubular proteinuria and albumin filtration. Nephrol. Dial. Transplant 28, 585–591.
Sumpio, B.E., Maack, T., 1982. Kinetics, competition, and selectivity of tubular absorption of proteins. Am. J. Physiol. 243, F379–F392.
Sundin, D.P., Cohen, M., Dahl, R., Falk, S., Molitoris, B.A., 1994. Characterization of the β_2-microglobulin endocytic pathway in rat proximal tubule cells. Am. J. Physiol. 267, F380–F389.
Suwatvitayakorn, P., Ko, M.S., Kim, K.W., Chanpiwat, P., 2020. Human health risk assessment of cadmium exposure through rice consumption in cadmium-contaminated areas of the Mae Tao sub-district, Tak. Thailand. Environ. Geochem. Health 42, 2331–2344.
Tenten, V., Menzel, S., Kunter, U., Sicking, E.-M., van Roeyen, C.R.C., Sanden, S.K., Kaldenbach, M., Boor, P., Fuss, A., Uhlig, S., Lanzmich, R., Willemsen, B., Dijkman, H., Grepl, M., Wild, K., Kriz, W., Smeets, B., Floege, J., Moeller, M.J., 2013. Albumin is recycled from the primary urine by tubular transcytosis. J. Am. Soc. Nephrol. 24, 1966–1980.
Thevenod, F., Wolff, N.A., 2016. Iron transport in the kidney: implications for physiology and cadmium nephrotoxicity. Metallomics 8, 17–42.
Tojo, A., Endou, H., 1992. Intrarenal handling of proteins in rats using fractional micropuncture technique. Am. J. Physiol. 263, F601–F606.
Torra, M., To-Figueras, J., Brunet, M., Rodamilans, M., Corbella, J., 1994. Total and methionein-bound cadmium in the liver and the kidney of a population in Barcelona (Spain). Bull. Environ. Contam. Toxicol. 53, 509–515.
U.K. Prospective Diabetes Study Group., 1998. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. BMJ 317, 703–713.
Wagner, M.C., Sandoval, R.M., Yadav, S.P.S., Campos, S.B., Rhodes, G.J., Phillips, C.L., Molitoris, B.A., 2023. Lrpap1 (RAP) inhibits proximal tubule clathrin mediated and clathrin independent endocytosis, ameliorating renal aminoglycoside nephrotoxicity. Kidney 360, 591–605.
Weisz, O.A., 2021. Endocytic adaptation to functional demand by the kidney proximal tubule. J. Physiol. 599, 3437–3446.
Win-Thu, M., Myint-Thein, O., Win-Shwe, T.T., Mar, O., 2021. Environmental cadmium exposure induces kidney tubular and glomerular dysfunction in the Myanmar adults. J. Toxicol. Sci. 46, 319–328.
Wolf, C., Strenziok, R., Kyriakopoulous, A., 2009. Elevated metallothionein-bound cadmium concentrations in urine from bladder carcinoma patients, investigated by size exclusion chromatography-inductively coupled plasma mass spectroscopy. Anal. Chim. Acta 631, 218–222.
Wolff, N.A., Marouan, A., Verroust, P.J., Thevenod, F., 2006. Megalin-dependent internalization of cadmium-metallothionein and cytotoxicity in cultured proximal tubule cells. J. Pharmacol. Exp. Ther. 318, 782–791.
Yoshida, M., Ohta, H., Yamauchi, Y., Seki, Y., Sagi, M., Yamazaki, K., Sumi, Y., 1998. Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol. Trace Elem. Res. 63, 167–175.
Zavala-Guevara, I.P., Ortega-Romero, M.S., Narvaez-Morales, J., Jacobo-Estrada, T.L., Lee, W.-K., Arreola-Mendoza, L., Thevenod, F., 2021. Increased endocytosis of cadmium-metallothionein through the 24p3 receptor in an in vivo model with reduced proximal tubular activity. Int. J. Mol. Sci. 22, 7762.