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Estimation of the Cadmium Nephrotoxicity Threshold from Loss of GFR and Albuminuria

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Abstract: Cadmium (Cd) is a pervasive, toxic environmental pollutant that preferentially accumulates in tubular epithelium of the kidney. Current evidence suggests that the cumulative burden of Cd here leads to progress loss of the glomerular filtration rate (GFR). In this study, we have quantified changes in estimated GFR (eGFR) and albumin excretion (E_{alb}) in according to levels of blood Cd ($[Cd]_b$) and excretion of Cd (E_{Cd}) after adjustment for confounders. E_{Cd} and E_{alb} were normalized to creatinine clearance (C_{cr}) as E_{Cd}/C_{cr} and E_{alb}/C_{cr}. Among 482 residents of Cd-polluted and non-polluted regions of Thailand, 8.1% had low eGFR and 16.9% had albuminuria, $(E_{alb}/C_{cr})\times100 \geq 20$ mg/L filtrate. In the low Cd burden group, $(E_{Cd}/C_{cr})\times100 < 1.44$ µg/L filtrate, eGFR did not correlate with E_{Cd}/C_{cr} ($\beta = 0.007$), while an inverse association with E_{Cd}/C_{cr} was found in the medium ($\beta = -0.230$) and high-burden groups ($\beta = -0.349$). Prevalence odds ratios (POR) for low eGFR were increased in the medium (POR 8.26) and high Cd burden groups (POR 3.64). Also, eGFR explained a significant proportion of E_{alb}/C_{cr} variation among those with middle (η^2 0.093) and high $[Cd]_b$ tertiles (η^2 0.132), but did not with low tertile (η^2 0.001). With adjustment of eGFR, age and BMI, POR values for albuminuria were increased in the middle (POR 2.36) and high $[Cd]_b$ tertiles (POR 2.74) and those with diabetes (POR 6.02) and hypertension (2.05). These data argue that E_{Cd}/C_{cr} of 1.44 µg/L filtrate (0.01–0.02 µg/g creatinine) could determine a Cd threshold level from which protective exposure guidelines should be formulated.

Keywords: albuminuria; blood pressure; cadmium; chronic kidney disease; estimated GFR

1. Introduction

Chronic kidney disease (CKD) is a progressive disease with high morbidity and mortality, affecting 8% to 16% of the world's population [1–3]. CKD is diagnosed when the estimated glomerular filtration rate (eGFR) falls below 60 mL/min/1.73 m^2, termed low eGFR, or albuminuria, defined as albumin to creatinine ratios (ACR) ≥ 30 mg/g creatinine in women and ≥ 20 mg/g creatinine in men that persists for at least 3 months [1,2]. Environmental exposure to the toxic metal pollutant cadmium (Cd) is linked to increases in risk of both low eGFR and albuminuria. Typically, studies of CKD associated with environmental Cd exposure employed levels of blood Cd ([Cd[$_b$) and excretion of Cd (E_{Cd}), normalized to creatine excretion (E_{Cd}/E_{cr}) as indicators of exposure [3,4]. Notably, however, these Cd exposure indicators have been found to be also associated with increased risks of diabetes [5–10] and hypertension [11–16] both of which are major risk factors for CKD. Hypertension is known to be a cause and the result of CKD [17,18]. Furthermore, in Dutch prospective cohort study, smoking, which is a source of Cd exposure, promoted kidney failure, evident from a reduction of eGFR to below 15 mL/min/1.73 m^2 [19]. It is imperative that these covariates are adequately adjusted for in the estimation of risks of GFR loss and albuminuria due to renal Cd accumulation.

The dose-response relationship between Cd exposure and adverse kidney outcomes measured by a reduction in eGFR and albuminuria are limited. In comparison, numerous dose-effect studies employed tubule damage and tubulopathy reflected by tubular proteinuria have been employed as signs of the nephrotoxicity due to Cd accumulation. However, current evidence suggests that a sustained decrease in eGFR after Cd exposure is more suitable than the tubulopathy endpoint, especially for the purpose of determining protective exposure guidelines.

Also, current evidence suggests that excreted Cd (E_{Cd}) is related to tubular cell injury and death by a cumulative burden of Cd [20]. The excreted Cd emanates from tubular epithelial cells in complexed with metallothionein (MT) as CdMT [21], the storage form of Cd. Consequently, it is logical to normalize E_{Cd} to creatinine clearance (C_{cr}) rather than creatinine excretion (E_{cr}) because C_{cr} is a surrogate for GFR, which is the measurable analog of nephron number [3,20,22].

The present study aims to explore if there is a potential toxicity threshold level of Cd accumulation in kidneys. To achieve this aim, changes in eGFR and albumin excretion (E_{alb}) together with risks of low eGFR and albuminuria were quantified in relation to both E_{Cd} and $[Cd]_b$. The confounding impact of smoking, diabetes, and hypertension were also evaluated. We collected data from women and men who resided in Cd-polluted and non-polluted regions of Thailand to obtain a wide range of exposure level pivotal to dose-response analysis.

We normalized E_{Cd} and E_{alb} to C_{cr} as E_{Cd}/C_{cr} and E_{alb}/C_{cr}, respectively. This normalization method depicts an amount of Cd and albumin excreted per volume of filtrate, which approximates the amount of Cd and albumin excreted per nephron [23]. The E_{Cd}/C_{cr} and E_{alb}/C_{cr} ratios are unaffected by creatinine excretion (E_{cr}), while the differences in urine flow rate (dilution) and the number of functioning nephrons among cohort participants are eliminated [23]. $E_{alb}/C_{cr} \geq 20$ mg/g creatinine indicate albuminuria in both men and women.

2. Materials and Methods

2.1. Cohort Participants

To establish a clear dose–response relationship, a population exposed to a wide range of Cd doses is required. Therefore, we selected subjects from two-population-based cross- sectional studies undertaken in a Cd-contaminated area of the Mae Sot District, Tak Province [24], and a non-contaminated location in Nakhon-Si-Thammarat Province [25] of Thailand. More females (n = 354) were recruited to the present study than males (n = 118), given that an increase in mortality from kidney disease was found, especially in women in a prospective cohort study of residents of a Cd -polluted area of Japan [26].

The data from a nationwide survey of Cd levels in soils and food crops indicated that environmental exposure to Cd in Nakhon Si Thammarat was low [27]. In comparison, the Cd content of the paddy soil samples from the Mae Sot district exceeded the standard of 0.15 mg/kg, and the rice samples collected from household storage contained four times the amount of the permissible Cd level of 0.1 mg/kg [28]. An independent health survey reported the prevalence of low eGFR among those resided in the Cd-contaminated area of Mae Sot District to be 16.1% [29].

The study protocol for the Mae Sot group was approved by the Institutional Ethical Committees of Chiang Mai University and the Mae Sot Hospital. The Office of the Human Research Ethics Committee, Walailak University of Thailand approved the study protocol for the Nakhon Si Thammarat group.

Prior to participation, all participants provided informed consent. 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. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg [30], a physician's diagnosis, or prescription of anti-hypertensive medications. Diabetes was diagnosed,

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when fasting plasma glucose levels ≥ 126 mg/dL (https://www.cdc.gov/diabetes/basics/getting-tested.html) (accessed on 12 August 2023) or a physician's prescription of anti-diabetic medications.

2.2. Assessment of Cadmium Exposure and Adverse Effects

Assessment of long-term Cd exposure was based on E_{Cd}, while a recent exposure was indicated by $[Cd]_b$. Kidney functional assessment was based on E_{alb} and eGFR. For these measurements of exposure and effects, samples of urine and whole blood were collected after overnight fast. Blood samples were collected within 3 h after urine sampling. Aliquots of blood and urine samples were stored at −80°C for later analysis.

Levels of Cd in urine and blood ($[Cd]_u$ and $[Cd]_b$) were quantified by atomic absorption spectrophotometry. Blood control samples (ClinChek, Munich, Germany), urine standard reference material No. 2670 (National Institute of Standards, Washington, DC, USA), and the reference urine metal control levels 1, 2, and 3 (Lyphocheck, Bio-Rad, Hercules, CA, USA) were used for quality control, analytical accuracy, and precision assurance. The limit of detection (LOD) of Cd, defined as 3 times the standard deviation of blank measurements, was 0.3 μg/L for $[Cd]_b$ and 0.1 μg/L for $[Cd]_u$. When a sample contained Cd below its LOD, the Cd concentration assigned was the LOD value divided by the square root of 2 [31].

Urinary and plasma creatinine ($[cr]_u$ and $[cr]_p$) assays were based on the Jaffe reaction. Urinary albumin assay was based on a turbidimetric method.

2.3. Estimated Glomerular Filtration Rate (eGFR)

In theory GFR is a measure of nephron function which is the product of the functioning nephron number and mean single nephron GFR [32–34]. In practice, GFR is estimated from established chronic kidney disease epidemiology collaboration (CKD-EPI) equations, and is reported as estimated GFR (eGFR) [34]. These CKD-EPI equations have been validated with inulin clearance [35].

Male eGFR = 141 × [plasma creatinine/0.9]^{Y} × 0.993^{age}, where Y = −0.411 if $[cr]_p \le 0.9$ mg/dL, and Y = −1.209 if $[cr]_p > 0.9$ mg/dL. Female eGFR = 144 × [plasma creatinine/0.7]^{Y} × 0.993^{age}, where Y = −0.329 if $[cr]_p \le 0.7$ mg/dL, and Y = −1.209 if $[cr]_p > 0.7$ mg/dL. CKD stages 1, 2, 3a, 3b, 4, and 5 corresponded to eGFRs of 90–119, 60–89, 45–59, 30–44, 15–29, and < 15 mL/min/1.73 m^2, respectively. For dichotomized comparison, CKD (abnormal eGFR) was defined eGFR ≤ 60 mL/min/1.73 m^2.

2.4. Normalization of Excretion Rate

E_x was normalized to E_{cr} as $[x]_u/[cr]_u$, where x = Cd or alb; $[x]_u$ = urine concentration of x (mass/volume); and $[cr]_u$ = urine creatinine concentration (mg/dL). The ratio $[x]_u/[cr]_u$ was expressed in μg/g of creatinine.

E_x was normalized to C_{cr} as $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd or alb; $[x]_u$ = urine concentration of x (mass/volume); $[cr]_p$ = plasma creatinine concentration (mg/dL); and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/C_{cr} was expressed as the excretion of x per volume of filtrate [23].

Results obtained with C_{cr}-normalized data are shown herein. Results of analogous analyses with E_{cr}-normalized data are provide in Supplemental Material (SM).

2.5. Statistical Analysis

Data were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The Mann–Whitney U test was used to compared two groups, and Pearson's chi-squared test was used to assess differences in percentages. 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 multiple linear regression model analysis was used to identify predictors of eGFR and E_{alb}/C_{cr}. The multivariable logistic regression analysis was used to determine the prevalence odds ratio (POR) for low eGFR and albuminuria. Univariate analysis of covariance via Bonferroni correction in multiple comparisons was used to obtain covariate-adjusted mean E_{alb}/C_{cr} and eta square (η^2) values. For all tests, p-values ≤ 0.05 were considered to indicate statistical significance.

3. Results

3.1. Cohort Participants

Table 1 provides descriptive characteristics of this Thai cohort participants, recruited from a low-exposure locality, and a high-exposure region due to environmental Cd pollution.

Table 1. Characteristics of participants according to eGFR levels and gender.

Parameters	All	Normal eGFR [a]		Low eGFR	
	n = 482	Women *n* = 329	Men *n* = 114	Women *n* = 35	Men *n* = 4
Age (years)	51.8 ± 9.2	51.2 ± 8.7	49.8 ± 7.5	63.1 ± 11.3	56.0 ± 6.4
BMI (kg/m^2)	24.8 ± 4.0	25.3 ± 4.0	23.5 ± 3.7 ***	24.0 ± 3.6	26.7 ± 5.2
eGFR [b], mL/min/1.73 m^2	90 ± 19	93 ± 15	94 ± 14	51 ± 9	47 ± 11
Smoking (%)	29.7	17.6	68.4 ***	11.4	75.0 ##
Diabetes (%)	18.3	16.7	13.2	45.7	50.0
Hypertension (%)	48.3	49.8	40.4	54.3	100
SBP (480)	129 ± 17	129 ± 16	127 ± 113	138 ± 18	147 ± 5
DBP (480)	81 ± 10	81 ± 10	81 ± 11	79 ± 8	86 ± 4
Serum creatinine, mg/dL	0.82 ± 0.22	0.74 ± 0.13	0.92 ± 0.14 ***	1.19 ± 0.31	1.68 ± 0.43 ##
Urine creatinine, mg/dL	113 ± 73	110 ± 74	134 ± 72 ***	72 ± 33	73 ± 24
Urine albumin, mg/L	21 ± 57	13 ± 31	29 ± 77	60 ± 118	86 ± 106
Blood Cd, μg/L	2.60 ± 3.13	2.43 ± 2.96	3.13 ± 3.32 *	2.19 ± 3.67	5.03 ± 5.03
Urine Cd, μg/L	4.21 ± 5.66	4.44 ± 6.15	3.84 ± 4.04	3.31 ± 5.71	2.26 ± 2.80
Normalized to E_{cr} (E_x/E_{cr}) [c]					
ACR, mg/g creatinine	24 ± 71	15 ± 41	27 ± 70	93 ± 181	112 ± 139
Albuminuria (%) [d]	15.0	11.4	17.0	34.4	100 #
E_{Cd}/E_{cr}, μg/g creatinine	4.05 ± 4.43	4.27 ± 4.46	3.32 ± 3.71 *	4.63 ± 6.31	2.62 ± 3.04
Normalized to C_{cr}, (E_x/C_{cr}) [e]					
(E_{alb}/C_{cr}) ×100, mg/L	24 ± 85	12 ± 32	26 ± 67	125 ± 252	174 ± 205
Abnormal E_{alb}/C_{cr} (%) [f]	16.9	12.9	17.0	46.9	100 #
(E_{Cd}/C_{cr}) ×100, μg/L	3.20 ± 3.73	3.05 ± 3.25	3.05 ± 3.41	5.14 ± 7.44	5.20 ± 6.80

n, number of subjects; eGFR, estimated glomerular filtration rate; BMI, body mass index; ACR, albumin-to-creatinine ratio; alb, albumin. [a]The cutoff eGFR of 60 mL/min/1.73 m^2 was used to define departure from normalcy. [b] eGFR was determined with equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD−EPI) [35]. [c] $E_x/E_{cr} = [x]_u/[cr]_u$, where x = alb or Cd. [d] Albuminuria is defined as ACR ≥ 20 mg/g for men and ≥ 30 mg/g for women. e $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = alb, or Cd [23]. [f] Abnormal E_{alb}/C_{cr} was defined as $[(E_{alb}/C_{cr})\times100] \geq 20$ mg/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 Mann-Whitney U test for differences of means. For the normal eGFR group, ***$p < 0.001$; * $p = 0.016$–0.021. For the low eGFR group, ## p = 0.002; # p = 0.012 – 0.045.

A total of 482 persons (354 women and 118 men), mean age of 51.8 years, were included in this investigation. The respective percentages of smoking and diabetes were 29.7% and 18.3%, while about half (48.5%) had hypertension.

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The overall % low eGFR and albuminuria (ACR criterion) were 8.1% and 15%. For C_{cr}-normalized data, the % (E_{alb}/C_{cr}) ×100 ≥ 20 mg/L filtrate was 16.9%. Of note the % abnormal E_{alb} was higher in the low eGFR group than normal eGFR group for both ACR and E_{alb}/C_{cr} criteria in both genders. Similarly, the % hypertension and diabetes were higher in the low eGFR than the normal eGFR group.

The mean $[Cd]_b$ (range) was 2.60 (0.03–20) μg/L. The mean (E_{Cd}/C_{cr}) ×100 (range) was 3.20 (0.02–25) μg/L filtrate. Corresponding mean E_{Cd}/E_{cr} (range) was 4.05 (0.03–31) μg/g creatinine.

3.2. Predictors of eGFR

Table 2 provides results of multiple linear regression analysis to define the independent variables that contributed to differences in eGFR.

Table 2. Predictors of eGFR in women and men.

Independent Variables/Factors	eGFR, mL/min/1.73 m²					
	All, *n* = 444		**Women, *n* = 332**		**Men, *n* = 113**	
	β	*p*	β	*p*	β	*p*
Age, years	−0.503	<0.001	−0.511	<0.001	−0.472	<0.001
BMI, kg/m²	−0.066	0.129	−0.050	0.310	−0.134	0.149
Log[(E_{Cd}/C_{cr}) ×10⁵], μg/L filtrate	−0.121	0.022	−0.126	0.043	−0.097	0.367
Systolic pressure, mmHg	−0.087	0.134	−0.067	0.320	−0.147	0.230
Diastolic pressure, mmHg	−0.011	0.836	−0.020	0.745	0.022	0.856
Gender	−0.006	0.898	−	−	−	−
Smoking	0.033	0.492	0.031	0.533	0.034	0.710
Diabetes	−0.101	0.038	−0.119	0.036	−0.033	0.741
Adjusted R^2	0.278	<0.001	0.279	<0.001	0.216	<0.001

eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted R^2, coefficient of determination. Coding: male, 1, female, 2; non-smoker, 1, smoker, 2; non-diabetic, 1, diabetic, 2. β indicates strength of association of eGFR with eight independent variables (first column). Adjusted R^2 indicates the proportion of eGFR variance explained by all independent variables. For all tests, p-values ≤ 0.05 indicate a statistically significant effect of individual independent variables on eGFR.

In an inclusive model, all eight independent variables explained 27.8% of the total variation in eGFR. Age, E_{Cd}/C_{cr} and diabetes were the three main influential independent variables. All other five independent variables did not show a significant association with eGFR. In subgroup analysis, female eGFR was inversely associated with age (β = −0.511), E_{Cd}/C_{cr} (β = −0.126) and diabetes (β = −0.119). In comparison, male eGFR was associated with age only (β = −0.472).

In an analogous regression of eGFR with E_{Cd} normalized to creatinine excretion (E_{Cd}/E_{cr}) (Table S1), eGFR was inversely associated with age only (β = −0.442). No significant association was seen between eGFR and all other six variables, including E_{Cd}/C_{cr}.

To investigate dose-response relationship of Cd exposure and changes in eGFR, we designated the low, middle and high E_{Cd}/C_{cr} tertiles to represent low, medium and high Cd burdens, respectively. In women, the cut-off values of (E_{Cd}/C_{cr}) ×100 for the low, medium and high Cd burdens were ≤ 1.44, 1.45–3.26, > 3.26 μg/L filtrate, respectively. Corresponding cut-off values of (E_{Cd}/C_{cr}) ×100 in men were ≤ 1.25, 1.26-3.25, > 3.25 μg/L filtrate.

Figure 1 provides scatterplots relating eGFR to Cd excretion rate in women and men, and their subgroups according to Cd burden levels, described above.

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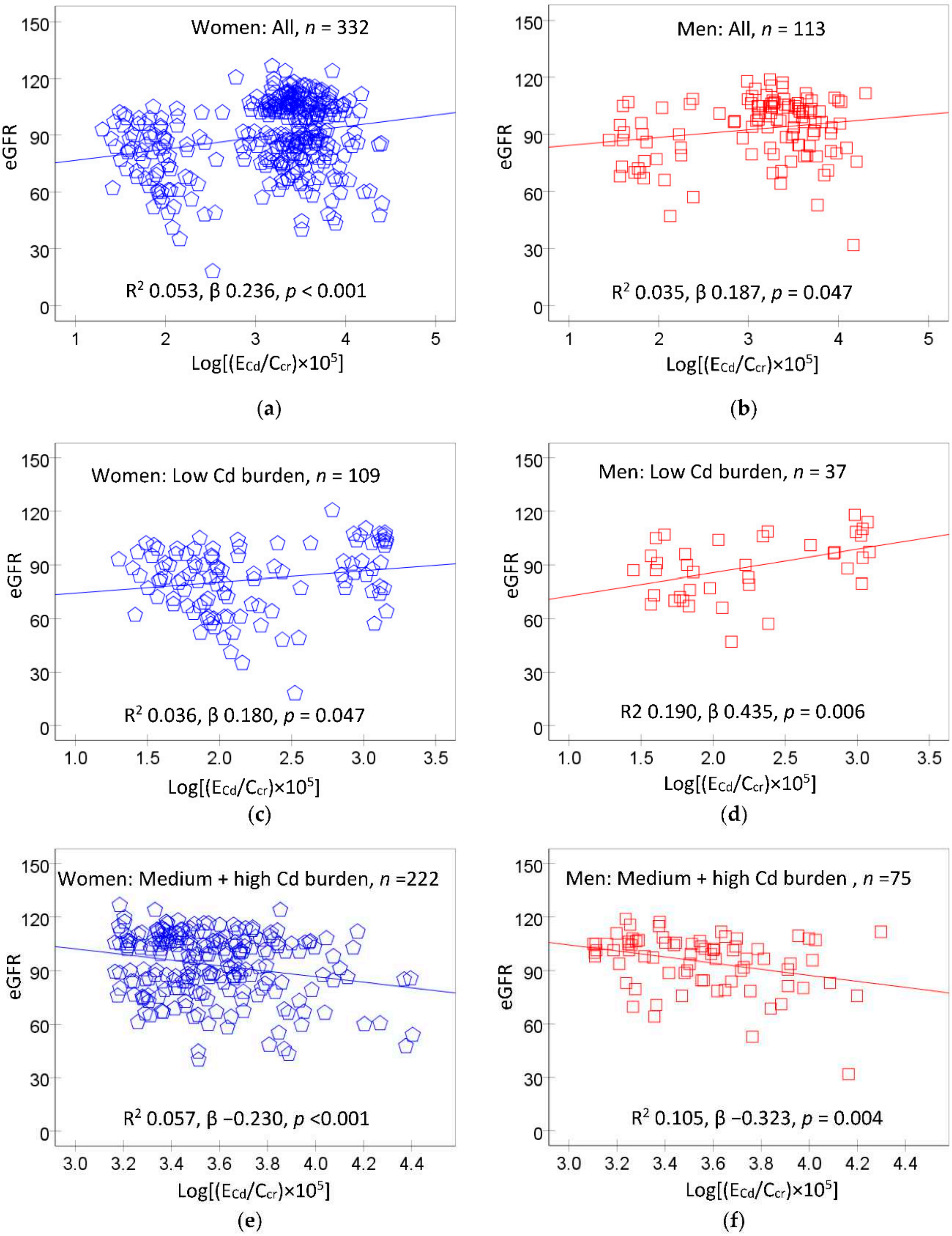

Figure 1. The relationship between eGFR and cadmium excretion rate. Scatterplots relate eGFR to log[(E_{Cd}/C_{cr}) ×10^5) in women (**a**) and men (**b**), women (**c**) and men (**d**) with low Cd burden and women (**e**) and men (**f**) with medium plus high Cd burden. Coefficients of determination (R^2) and *p*-values are provided for all scatterplots together with numbers of participants in all subgroups. The cut-off values of (E_{Cd}/C_{cr}) ×100 for low, medium, and high Cd burden in women were ≤ 1.44, 1.45–3.26, > 3.26 µg/L filtrate, respectively. Corresponding cut-off values of (E_{Cd}/C_{cr}) ×100 in men were ≤ 1.25, 1.26-3.25, > 3.25 µg/L filtrate.

In scatterplots including all women (Figure 1a) and all men (Figure 1b), a direct relationship between eGFR and E_{Cd}/C_{cr} was suggested. In subgroup analysis, a direct relationship of eGFR and E_{Cd}/C_{cr} was seen in women and men who had of the low Cd burden (Figure 1c,d). In contrast, eGFR was inversely related to E_{Cd}/C_{cr} in women and men of the medium plus high Cd burden groups (Figure 1e,f). These data indicate that E_{Cd}/C_{cr} of 1.44 μg/L filtrate may be a threshold level of Cd accumulation, above which eGFR began to drop.

To confirm or dispute the contrasting eGFR responses below or above E_{Cd}/C_{cr} of 1.44 μg/L filtrate, multiple regression analyses of eGFR across Cd burden groups were undertaken. Results are provided in Table 3.

Table 3. Comparing strength of association of eGFR with cadmium excretion rate across cadmium burden groups.

Independent Variables/Factors	eGFR, mL/min/1.73 m²					
	Low Cd burden		Medium burden		High burden	
	β	*p*	β	*p*	β	*p*
Age, years	−0.455	<0.001	−0.505	<0.001	−0.410	<0.001
BMI, kg/m²	−0.022	0.777	−0.136	0.064	−0.094	0.221
Log[(E_{Cd}/C_{cr}) ×10⁵], μg/L filtrate	0.007	0.934	−0.230	0.002	−0.349	<0.001
Systolic pressure, mmHg	−0.038	0.722	−0.284	0.004	−0.050	0.611
Diastolic pressure, mmHg	0.087	0.372	0.113	0.226	−0.170	0.078
Gender	−0.024	0.798	−0.064	0.434	0.106	0.194
Smoking	0.094	0.324	−0.081	0.301	0.132	0.108
Diabetes	−0.128	0.104	0.089	0.260	0.022	0.775
Adjusted R^2	0.248	<0.001	0.321	<0.001	0.255	<0.001

eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted R^2, coefficient of determination. Coding: male, 1, female, 2; non-smoker, 1, smoker, 2; non-diabetic, 1, diabetic, 2. β indicates strength of association of eGFR with eight independent variables (first column). Adjusted R^2 indicates the proportion of eGFR variance explained by all independent variables. The cut-off values of (E_{Cd}/C_{cr}) ×100 for the low, medium, and high Cd burdens in women were ≤ 1.44, 1.45–3.26, > 3.26 μg/L filtrate, respectively. Corresponding cut-off values of (E_{Cd}/C_{cr}) ×100 in men were ≤ 1.25, 1.26–3.25, > 3.25 μg/L filtrate. The numbers of subjects in low, moderate and high Cd burdens were 147, 148 and 147, respectively. For all tests, p-values ≤ 0.05 indicate a statistically significant effect of individual independent variables on eGFR.

In distinction from a bivariate analysis (Figure 1c,d), the association between eGFR and E_{Cd}/C_{cr} was statistically insignificant in the low Cd burden group after adjustment for confounding variables (Table 3). In this low Cd burden group, eGFR was inversely associated with age only (β = −0.455), while its association of with E_{Cd}/C_{cr} became statistically insignificant (Table 3).

Of interest, there was an inverse relationship between eGFR and E_{Cd}/C_{cr} in the medium and high Cd burden groups following similar adjustment for confounders. All eight independent variables explained, respectively 32.1% and 25.5% of total eGFR variation in the medium and high Cd burden groups. In the high Cd burden group, eGFR was more closely associated with E_{Cd}/C_{cr} (β = −0.349), compared with the medium Cd burden group (β = −0.284). Other predictors of eGFR in the medium Cd burden group, were age (β = −0.505) and systolic blood pressure (β = −0.230). Age was the other predictor of eGFR (β=−0.410) in the high Cd burden group.

3.3. Logistic Regression Analysis of Low eGFR

Table 4 provides results of logistic regression analysis that evaluated effects of Cd body burden and other six independent variables, on the prevalence odds ratios (POR) for low eGFR.

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Table 4. Prevalence odds ratios for low eGFR in relation to cadmium body burden and other independent variables.

Independent variables/factors	Low eGFR					
	All, n = 446		Women, n = 332		Men, n = 114	
	POR (95% CI)	p	POR (95% CI)	p	POR (95% CI)	p
Age, years	1.118 (1.062, 1.176)	<0.001	1.114 (1.057, 1.175)	<0.001	1.291 (0.935, 1.783)	0.120
BMI, kg/m^2	1.002 (0.908, 1.106)	0.967	1.029 (0.923, 1.147)	0.604	1.351 (0.876, 2.084)	0.174
Gender	0.482 (0.133, 1.742)	0.265	−	−	−	−
Smoking	1.388 (0.433, 4.450)	0.582	1.123 (0.300, 4.206)	0.863	0.495 (0.018, 13.92)	0.679
Diabetes	3.042 (1.126, 8.213)	0.028	2.709 (0.932, 7.878)	0.067	3.713 (0.110, 125.6)	0.465
Hypertension	1.175 (0.516, 2.679)	0.701	1.228 (0.505, 2.990)	0.651	2.478 (1.983, 3.098)	0.030 [a]
Cd body burden						
Low	Referent		Referent		Referent	
Medium	8.265 (1.711, 39.92)	0.009	7.204 (1.438, 36.10)	0.016	n/a	n/a
High	3.643 (1.150, 11.54)	0.028	3.218 (0.934, 11.09)	0.064	n/a	n/a

POR, prevalence odds ratio; CI, confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate. Coding, male 1, female 2; non-smoker 1, smoker 2; non-diabetic 1, diabetic 2; code 1 is referent. Data were generated from logistic regression relating POR for low eGFR to seven independent variables (first column). [a] Effect of hypertension was assessed by Fisher's exact test using data from 118 men. The cut-off values of (E_{Cd}/C_{cr}) ×100 for the low, medium, and high Cd burdens in women were ≤ 1.44, 1.45−3.26, > 3.26 μg/L filtrate, respectively. Corresponding cut-off values of (ECd/Ccr) ×100 in men were ≤ 1.25, 1.26−3.25, > 3.25 μg/L filtrate. For all tests, p-values ≤ 0.05 indicate a statistically significant effect of individual independent variables to the POR for low eGFR.

In all subjects, the POR values for low eGFR increased with age (POR 1.118) and in those with diabetes (POR 3.024), medium Cd burden (POR 8.265), and high Cd burden (POR 3.643). All other four independent variables did not significantly affect the POR for low eGFR.

Among women, the POR values for low eGFR increased with age (POR 1.114) and in the medium Cd burden group (POR 7.204). An increase in the POR for low eGFR in the high Cd burden group did not reach a statistically significant level (POR 3.218, p = 0.064).

An effects of Cd exposure on the POR for low eGFR in men could not be evaluated due a small number of men with low eGFR (n = 4). However, the POR for low eGFR among men was associated with hypertension (POR 2.478).

3.4. Multiple Regression Analysis of Albumin Excretion Rate

We used multiple regression analysis to identify if E_{Cd}/C_{cr} and other independent variables predicted rising E_{alb}/C_{cr}. We included eGFR in regression models to evaluate if functioning nephrons, indicated by eGFR values, had an independent effect on E_{alb}/C_{cr}. Results are provided in Table 5.

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Table 5. Comparing strength of association of albumin excretion rate with cadmium excretion rate across cadmium burden groups.

Independent Variables/Factors	Log [(E_{alb}/C_{cr}) ×10^4], µg/L filtrate					
	Low Cd burden		Medium burden		High burden	
	β	*p*	β	*p*	β	*p*
Age, years	0.049	0.609	−0.019	0.846	−0.082	0.381
BMI, kg/m²	−0.007	0.932	0.248	0.004	−0.021	0.803
Log[(E_{Cd}/C_{cr}) × 10^5], µg/L filtrate	0.177	0.050	0.144	0.098	0.173	0.044
eGFR, mL/min/1.73 m²	−0.110	0.216	−0.147	0.130	−0.214	0.021
Gender	−0.150	0.122	−0.111	0.233	0.139	0.120
Smoking	−0.183	0.064	0.024	0.788	0.129	0.153
Diabetes	0.263	0.001	0.093	0.280	0.115	0.176
Hypertension	0.278	<0.001	0.121	0.148	0.104	0.204
Adjusted R^2	0.174	<0.001	0.103	0.003	0.082	0.009

eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted R^2, coefficient of determination. Coding: male, 1, female, 2; non-smoker, 1, smoker, 2; non-diabetic, 1, diabetic, 2. β indicates strength of association of eGFR with eight independent variables (first column). Adjusted R^2 indicates the proportion of eGFR variance explained by all independent variables. The cut-off values of (E_{Cd}/C_{cr}) ×100 for the low, medium, and high Cd burdens in women were ≤ 1.44, 1.45–3.26, > 3.26 µg/L filtrate, respectively. Corresponding cut-off values of (E_{Cd}/C_{cr}) ×100 in men were ≤ 1.25, 1.26-3.25, > 3.25 µg/L filtrate. The numbers of subjects in low, medium and high Cd burdens were 147, 148 and 149, respectively. For all tests, p-values ≤ 0.05 indicate a statistically significant effect of individual independent variables on eGFR.

In the low and medium Cd burden groups, neither E_{Cd}/C_{cr} nor eGFR showed a significant association with E_{alb}/C_{cr}. However, diabetes and hypertension predicted E_{alb}/C_{cr} in the low Cd burden group, while BMI was a sole predictor of E_{alb}/C_{cr} in the medium Cd burden group.

In the high Cd burden group, E_{Cd}/C_{cr} and eGFR both were associated with E_{alb}/C_{cr}. There was an inverse association between eGFR and E_{alb}/C_{cr} (β =−0.214), while E_{Cd}/C_{cr} showed a direct relationship with E_{alb}/C_{cr} (β = 0.173). All other six independent variables did not have a significant effect on E_{alb}/C_{cr}.

Of note, the relationship between albumin excretion and eGFR was obscure, when E_{alb} was normalized to E_{cr} (E_{alb}/E_{cr}). As data in Table S2 indicate, E_{alb}/E_{cr} did not show a significant inverse association with eGFR (β = −0.092, p = 0.078).

In subsequent analysis, scatterplots were used together with univariate analysis to quantify independent effects of E_{Cd}/C_{cr}, eGFR, diabetes and hypertension on E_{alb}/C_{cr}. Covariate adjusted mean E_{alb}/C_{cr} values were obtained for various subgroups as shown in Figure 2.

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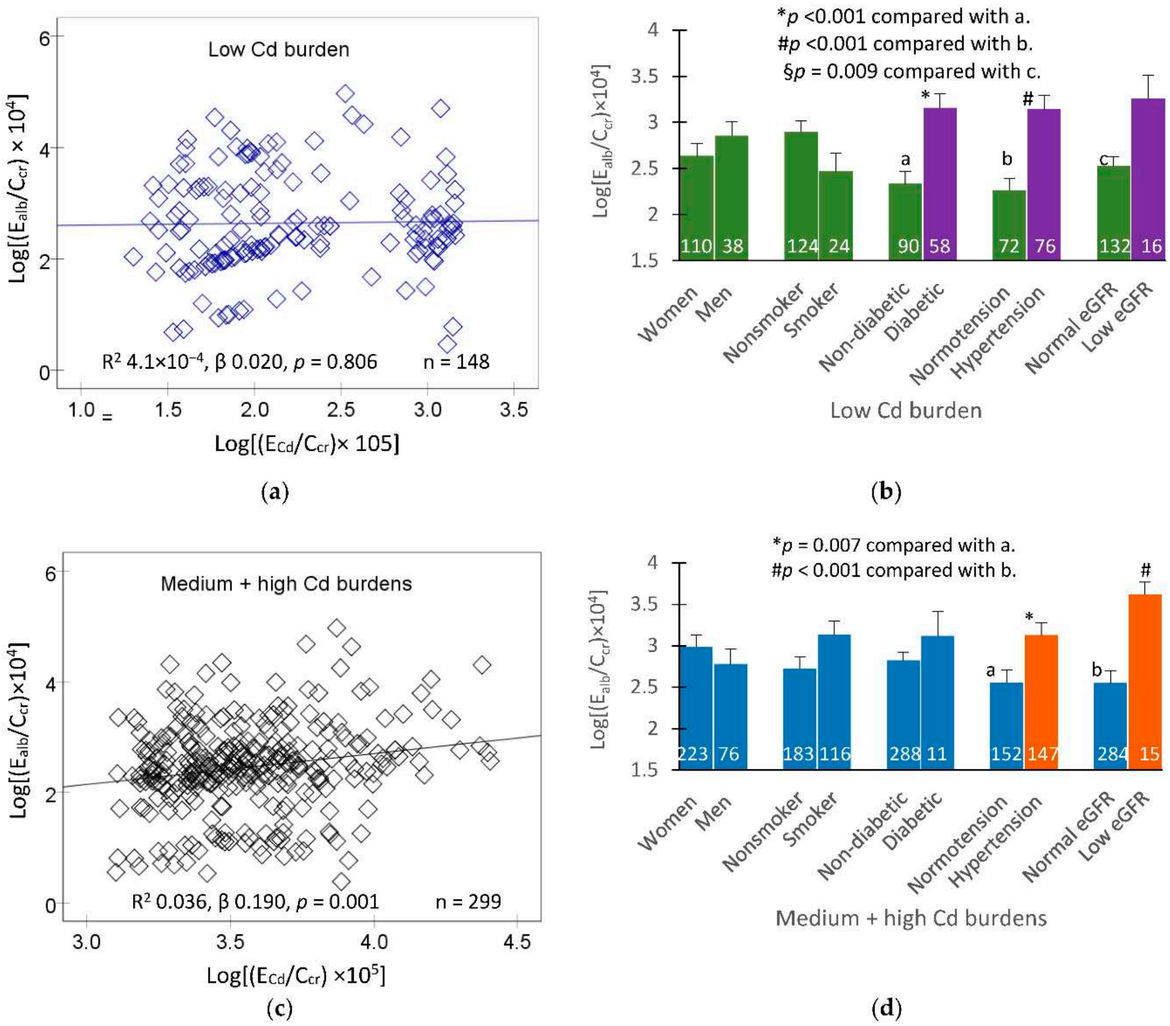

Figure 2. Albumin excretion rates among participants grouped by cadmium burden and other risk factors. Scatterplots relate log[(E_{alb}/C_{cr}) ×10⁴] to log[E_{Cd}/C_{cr}) ×10⁵] in participants with low Cd burden (**a**) and middle plus high Cd burdens (**c**). Coefficients of determination (R^2) and p-values and numbers of participants are provided for all scatterplots. Bar graphs depict mean log[(E_{alb}/C_{cr}) ×104] in participants with low Cd burden (b) and middle plus high Cd burdens (d), grouped by gender, smoking status, blood pressure status, diabetes diagnosis and eGFR. Normal eGFR and low eGFR were defined, respectively, as eGFR > 60 and eGFR ≤ 60 mL/min/1.73 m². Numbers of subjects are provided for all subgroups. All means were obtained via univariate covariance analysis with adjustment for covariates and interactions. The cut-off values of (E_{Cd}/C_{cr}) ×100 for the low, middle and high tertiles of Cd burden in women were ≤ 1.44, 1.45–3.26, > 3.26 µg/L filtrate, respectively. Corresponding cut-off values of (E_{Cd}/C_{cr}) ×100 in men were ≤ 1.25, 1.26-3.25, > 3.25 µg/L filtrate.

In the low Cd burden group, a scatterplot indicated that the correlation of E_{alb}/C_{cr} and E_{Cd}/C_{cr} was statistically insignificant (Figure 2a). After adjustment for covariates and interactions, those with diabetes, hypertension and low eGFR had higher E_{alb}/C_{cr} than non-diabetics, normotension and normal eGFR (Figure 2b).

In the medium plus high Cd burden group, a scatterplot indicated a statistically significant relationship between E_{alb}/C_{cr} and E_{Cd}/C_{cr} (Figure 2c). After adjustment for covariates and interactions, those with hypertension and low eGFR had higher E_{alb}/C_{cr} than those with normotension and normal eGFR (Figure 2d).

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3.5. Blood Cadmium and eGFR as Predictos of Albuminuria

Table 6 provides results of logistic regression analysis that evaluated if $[Cd]_b$, like E_{Cd}, predicted an increase in risk of abnormally high E_{alb} defined as $(E_{alb}/Ccr)\times100 \geq 20$ µg/L filtrate.

Table 6. Prevalence odds ratios for albuminuria in relation to blood cadmium levels and other independent variables.

Independent Variables/factors	Albuminuria, $(E_{alb}/C_{cr})\times100 \geq 20$ µg/L filtrate					
	All, *n* = 473		Women, *n* = 357		Men, *n* = 116	
	POR (95% CI)	*p*	POR (95% CI)	*p*	POR (95% CI)	*p*
Age, years	0.990 (0.955, 1.027)	0.609	1.007 (0.965, 1.051)	0.733	0.988 (0.904, 1.079)	0.781
BMI, kg/m²	1.028 (0.957, 1.104)	0.448	1.033 (0.950, 1.123)	0.450	1.011 (0.877, 1.166)	0.881
eGFR, mL/min/1.73 m²	1.043 (1.026, 1.061)	<0.001	1.045 (1.025, 1.065)	<0.001	1.045 (1.008, 1.084)	0.017
Gender	0.559 (0.273, 1.146)	0.112	−	−	−	−
Smoking	1.037 (0.494, 2.177)	0.924	1.258 (0.496, 3.192)	0.629	0.809 (0.254, 2.572)	0.719
Diabetes	6.021 (2.813, 12.89)	<0.001	5.996 (2.446, 14.69)	<0.001	8.324 (1.642, 42.21)	0.011
Hypertension	2.053 (1.167, 3.609)	0.013	2.785 (1.397, 5.552)	0.004	1.133 (0.371, 3.463)	0.827
Tertile of $[Cd]_b$, µg/L						
Low: < 0.82	Referent		Referent		Referent	
Middle: 0.83−2.63	2.360 (1.097, 5.076)	0.028	3.402 (1.324, 8.745)	0.011	0.925 (0.237, 3.604)	0.911
High: ≥ 2.64	2.740 (1.174, 6.394)	0.020	3.783 (1.369, 10.46)	0.010	1.425 (0.263, 7.732)	0.681

POR, prevalence odds ratio; CI, confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; [Cd]b, blood Cd concentration. Coding, male 1, female 2; non-smoker 1, smoker 2; non-diabetic 1, diabetic 2; code 1 is referent. Data were generated from logistic regression relating POR for albuminuria to seven independent variables (first column). Arithmetic means (SD) of blood Cd in low, middle and high tertiles were 0.31 (0.29), 1.86 (1.98), 5.58 (3.28), respectively. Corresponding numbers of subjects were 157, 164, and 161, respectively. For all tests, *p*-values ≤ 0.05 indicate a statistically significant effect of individual independent variables to the POR for albuminuria.

In all subjects, eGFR, diabetes, hypertension, and $[Cd]_b$ were independent variables showing associations with the POR for albuminuria. Comparing those with $[Cd]_b < 0.82$ µg/L, POR values for albuminuria were increased by 2.4-fold and 2.7-fold in those with $[Cd]_b$ of 0.83−2.63 and ≥ 2.64 µg/L, respectively. For every 1 mL/min/1.73 m² loss of eGFR, the POR for albuminuria rose by 4.3%. There was no effect of Cd on albuminuria in an analysis with Ecr-normalized data (Table 3S).

Among women, there was $[Cd]_b$-related increment of the POR for albuminuria. The POR values for high albuminuria were increased by 3.4-fold and 3.8-fold in those with middle and high $[Cd]_b$ tertiles, respectively. In addition, eGFR, diabetes, and hypertension were independent variables showing associations with the POR for albumin in women. For every 1 mL/min/1.73 m² loss of eGFR, the POR for albuminuria rose by 4.5%.

Among men, $[Cd]_b$ did not show an association with POR for albuminuria, eGFR did. For every 1 mL/min/1.73 m² loss of eGFR, the POR for albuminuria rose by 4.5%, similar to the effect size found in women.

In a following analysis, covariate adjusted mean E_{alb}/C_{cr} values were obtained for groups of participants with different eGFR levels (≤ 60, 61−90 and > 90 mL/min/1.73 m²) to reveal an effect of GFR loss, reflected by a reduction in eGFR. Results are shown in Figure 3.

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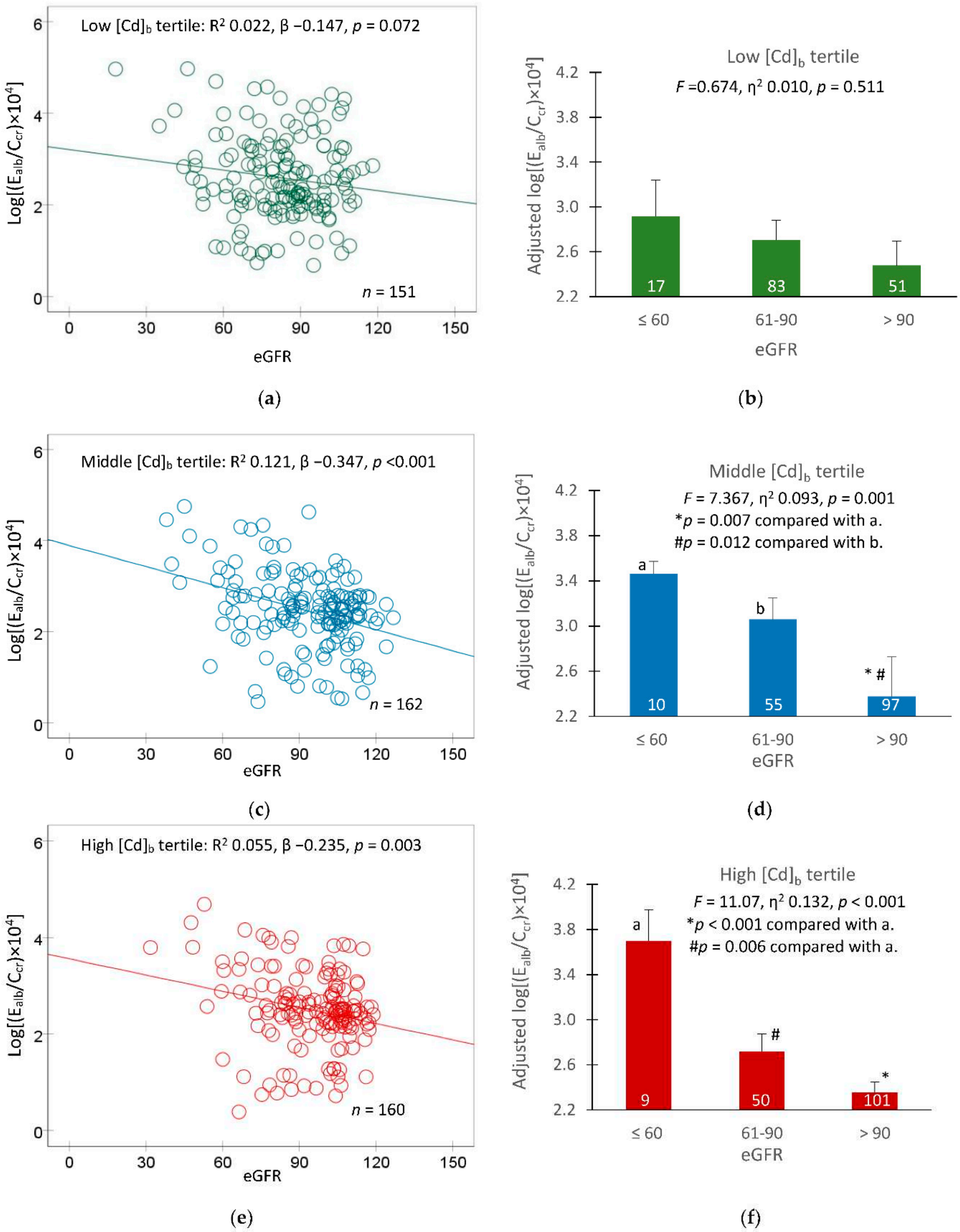

Figure 3. Albumin excretion rates in participants grouped by blood cadmium and eGFR levels. Scatterplots relate log[(E_{alb}/C_{cr}) ×10^4] to eGFR in participants with low (**a**), middle (**c**) and high [Cd]$_b$ tertiles (**e**). Coefficients of determination (R^2), p-values and numbers of subjects are provided for all scatterplots. Bar graphs depict mean log[(E_{alb}/C_{cr}) ×10^4] in participants of low (**b**), middle (d) and high [Cd]$_b$ tertiles (**f**) who had eGFR ≤ 60, 61−90 and > 90 mL/min/1.73 m^2. All means were obtained via univariate covariance analysis with adjustment for covariates and interactions. The cut-off values of [Cd]$_b$ for low, middle, and high tertiles were < 0.82, 0.83−2.63, and ≥2.64 µg/L, respectively. Arithmetic means (SD) of [Cd]b in low, middle, and high tertiles were 0.31 (0.29), 1.86 (1.98), and 5.58 (3.28), respectively. For all tests, *p*-values ≤ 0.05 indicate statistically significant levels.

In the low $[Cd]_b$ tertile group, the relationship between E_{alb}/C_{cr} and eGFR was statistically insignificant (Figure 3a). Similarly, the covariate-adjusted mean E_{alb}/C_{cr} values did not show a significant variation across three eGFR subgroups.

In the middle and high $[Cd]_b$ tertile groups, an inverse relationship was seen between E_{alb}/C_{cr} and eGFR (Figure 3c,e), and an effect of GFR loss on E_{alb}/C_{cr} was apparent (Figure 3d,f). In these two $[Cd]_b$ tertile groups, mean E_{alb}/C_{cr} value was highest, middle and lowest in those with eGFR ≤ 60, 60–90, and > 90 mL/min/1.73 m^2, respectively.

4. Discussion

Herein, we used eGFR decline as a primary endpoint in our attempt to define a toxic accumulation level of Cd. We focused on this eGFR because attenuation of eGFR decline is employed in clinical trials to evaluate effects of treatment of CKD [1,2]. Albuminuria was concurrently examined, because it has also been associated with Cd exposure along with low eGFR [4]. Previous studies show that the burden of Cd as µg/ g kidney tissue weight increases with age [36–38]. They show also that sufficient tubule damage from the Cd accumulation disables GFR, leading to nephron atrophy, and a fall of GFR follows [20,22,39,40]. We hypothesized that this eGFR effect of Cd occurs at a very low kidney burden, producing E_{Cd}/E_{cr} below 5.24 µg/g creatinine. It is noteworthy that E_{Cd} itself is indicative of tubular cell injury and death [3,20]

We analyzed data were from 482 Thai nationals of which 8.1% had low eGFR and 15% had albuminuria (ACR criterion). Nearly half (48.3%) had hypertension, while 29.7% reported to be smokers, and 18.3% had diabetes. There was a 1250-fold difference in E_{Cd}/C_{cr} values, ranging from 0.0002 to 0.25 µg/L filtrate (mean 0.0032 µg/L filtrate). There was a 667-fold difference in $[Cd]_b$, ranging between 0.03 and 20 µg/L (mean 2.60 µg/L). The wide ranges of these Cd burden and exposure matrices together with sufficiently high numbers of smokers and those with diabetes and hypertension provide an ideal scenario to define with certainty a toxic threshold level of Cd should it exist.

Like E_{Cd}/C_{cr}, E_{Cd}/E_{cr} showed a large variation (1033-fold), mean E_{Cd}/E_{cr} (range) was 4.05 (0.03–31) µg/g creatinine (Table 1). However, in distinction from E_{Cd}/C_{cr}, there was no significant association between eGFR and E_{Cd}/E_{cr} (Table S1). Consequently, a dose-response analysis was not possibly determined. Also, when E_{alb} was normalized to E_{cr} (E_{alb}/E_{cr}), an inverse relationship of albuminuria (ACR criterion) and eGFR was obscure (Table S2) and there was no effect of Cd on POR for albuminuria (Table S3).

4.1. Effects of Cadmium on eGFR

A direct relationship between eGFR and E_{Cd}/C_{cr} was seen in women and men with low-Cd burden (E_{Cd}/C_{cr} ≤ 0.014 in women and ≤ 0.012 µg/L in men) (Figure 1c,d). However, such eGFR/E_{Cd}/C_{cr} relationship was weakened and became statistically insignificant, when covariates were adjusted. In comparison, eGFR showed an inverse association with E_{Cd}/C_{cr}, when E_{Cd}/C_{cr} values rose to levels > 0.014 µg/L filtrate in women and > 0.012 µg/L filtrate in men (Figure 1e,f and Table 3). In reflecting a dose response relationship, eGFR was more closely associated with in E_{Cd}/C_{cr} in the high burden group ($\beta = -0.349$), compared to medium Cd burden group ($\beta = -0.284$). An association of eGFR and E_{Cd}/C_{cr} was statistically insignificant.

Cd burden, diabetes and age were three of seven variables that affected the POR for low eGFR (Table 4). In those with medium, high Cd burden, and diabetes, POR for low eGFR rose by 8.3-fold, 3.6-fold, and 3.0-fold, respectively. Per every 1-year rise in age, there was an 11.8% (95% CI, 6.2, 17.6) increase in the POR for low eGFR.

In summary, no effect of Cd on eGFR was found in those with E_{Cd}/C_{cr} values < 0.012 µg/L filtrate. This E_{Cd}/C_{cr} value of 0.012 µg/L filtrate is in ranges with the no-observed-adverse-effect level (NOAEL) equivalent of E_{Cd}/C_{cr} at 0.010-0.024 µg/L filtrate, obtained by benchmark dose method [41]

4.2. Effects of Cadmium on Prevalence of Albuminuria

The bulk of albumin (~80%) in the glomerular ultrafiltrate is reabsorbed in the S1 sub-segment of the proximal tubule, where the receptor-mediated endocytosis involving the megalin/cubillin system is located [41–44]. Reabsorption of albumin also occurs in the distal tubule and collecting duct, where the process is mediated by the NGAL/lipocalin-2 receptor system [45–47]. Experimental studies show that albuminuria was resulted from Cd selectively disabled albumin endocytosis which is mediated by the cubilin/megalin receptor system [48] and that Cd may diminish expression of megalin and ClC5 channels [49]. However, data in Table 1 indicate that albuminuria, was more prevalent in the low eGFR, compared to normal eGFR groups., thereby suggesting that low eGFR could be a risk factor for albuminuria. We examined this phenomenon further, as described below.

In multiple regressions (Table 5), both eGFR and ECd/Ccr appeared to have an effect on E_{alb}/C_{cr}. In logistic regression (Table 6), POR for albuminuria rose by 4.3% (95% CI: 2.6, 6.1) for every 1 mL/min/1.73 m^2 loss of eGFR, and it was increased by 2.4-fold to 2.7-fold in those who had $[Cd]_b \geq$ 0.83 µg/L. A similar effect size of Cd on eGFR was seen in women and men although the causes of albuminuria differed (Table 6). Additional evidence that Cd may affect eGFR and albumin reuptake simultaneously comes from a covariance analysis, where 9.3% to 13.2% of E_{alb}/C_{cr} variation could be attributable to eGFR levels in those with [Cd]b > 0.83 µg/L (Figure 3d,f). eGFR levels did not explain the variability of E_{alb}/C_{cr} in the low $[Cd]_b$ tertile group ($F = 0.674$, η^2 0.010, $p = 0.511$) (Figure 3b).

Results described above could be interpreted to suggest that albuminuria could be a consequence of Cd-induced destruction of nephrons together with a direct effect on tubular reabsorption of albumin possibly through the cubilin/megalin receptor system of endocytosis. These Cd effects were not found in those who had $[Cd]_b < 0.83$ µg/L.

4.3. Threshold-Based Exposure Guidelines

For most people, exposure to Cd is inevitable because the metal is present in nearly all food types. In Japan, for example, rice and its products, green vegetables, and cereals and seeds plus potatoes constituted, respectively to 38%, 17%, and 11% of total dietary Cd exposure [50]. In response, health guidance such as a tolerable intake level of Cd and a reference dose (RfD) were determined [51–53]. The Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) suggested a tolerable monthly intake (TMI) of Cd to be 25 µg per kg body weight per month, equivalent to 0.83 µg per kg body weight per day (58 µg/day for a 70-kg person), and E_{Cd}/E_{cr} of 5.24 µg/g creatinine was adopted as a nephrotoxicity threshold value [46]. Both figures were based on a risk assessment model that based solely on β_2-microglobulin excretion rate ($E_{\beta 2M}/E_{cr}$) ≥ 300 µg/g creatinine, termed tubular proteinuria, as a toxic endpoint.

The European Food Safety Authority (EFSA) used also β_2M endpoint, but with inclusion of an uncertainty factor (safety margin). The EFSA suggested E_{Cd}/E_{cr} of 1 µg/g creatinine to be the toxicity threshold, and 0.36 µg/kg body weight per day (25 µg/day for a 70-kg person) as the RfD [53]. The higher JECFA guidelines are adopted by most countries. These health guidelines values exceed the nephrotoxicity threshold identified in the present study. In theory, exposure guideline that provides sufficiently healthy protection should only be determined from the most sensitive endpoint with consideration given to subpopulations with increased susceptibility [54] and the most recent scientific knowledge should be considered.

Of concern, eGFR decline due to Cd nephropathy has increasingly been reported in both children and adult populations. Lower eGFR values were associated with higher Cd excretion rates were observed in studies from Guatemala [55] and Myanmar [56]. In a prospective cohort study of Bangladeshi preschool children, an inverse relationship between E_{Cd} and kidney volume was seen in children at 5 years of age. This was in addition to a decrease in eGFR [57]. E_{Cd} was inversely associated with eGFR, especially in girls. In another prospective cohort study, the reported mean for Cd intake among Mexican children was 4.4 µg/d at the baseline rose to 8.1 µg/d after nine years, when such Cd intake levels showed a marginally inverse association with eGFR [58].

5. Conclusions

Environmental exposure to Cd was confirmed to be closely associated with a declining GFR and albuminuria. The current threshold for Cd exposure of 5.24 µg/g creatinine (E_{Cd}/Ecr), which is solely based on an increment of excretion of β_2-microglobulin above 300 µg/g creatinine underestimates the level at which Cd induces kidney damage. Our results show that when a declining GFR is considered along with albuminuria the threshold equivalent is 0.01−0.02 µg/g creatinine. Now is the time to acknowledge there is no safe level of Cd exposure.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Predictors of eGFR based on cadmium excretion rate normalized to creatinine excretion (E_{Cd}/E_{cr}); Table S2: Predictors of albumin excretion rate normalized to creatinine excretion (E_{alb}/E_{cr}); Table S3: Prevalence odds ratios for low eGFR and albuminuria in relation to cadmium excretion rate normalized to creatine excretion (E_{Cd}/E_{cr}).

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Informed Consent Statement: Informed consent was obtained from all participants in the study prior to their participation.

Data Availability Statement: All data are contained within this article.

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