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*Article*

# Gender Differences in the Severity of Cadmium Nephropathy

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**Abstract:** Excretion of β2-microglobulin (β2M) above 300 μg/g creatinine, termed tubulopathy, was regarded as the critical effect of chronic exposure to the metal pollutant cadmium (Cd). However, current evidence suggests that Cd may induce nephron atrophy, resulting in a reduction in the estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m$^2$. Herein, these pathologies were investigated in relation to Cd exposure, smoking, diabetes and hypertension. Data were from 448 residents of Cd-polluted and non-polluted regions of Thailand. The body burden of Cd, indicated by the mean Cd excretion ($E_{Cd}$) normalized to creatinine clearance ($C_{cr}$) as ($E_{Cd}/C_{cr}$) ×100 in women and men did not differ (3.21 vs. 3.12 μg/L filtrate). The prevalence odds ratio (POR) for tubulopathy and a reduced eGFR were increased by 1.9-fold and 3.2-fold for every 10-fold rise in Cd body burden. In women only, a dose-effect relationship was seen between β2M excretion ($E_{β2M}/C_{cr}$) and $E_{Cd}/C_{cr}$ ($F$ = 3.431, $\eta^2$ 0.021). In men, $E_{β2M}/C_{cr}$ was associated with diabetes (β = 0.279). In both genders, eGFR was inversely associated with $E_{β2M}/C_{cr}$. The respective covariate- adjusted mean eGFR values were 16.5 and 12.3 mL/min/1.73 m$^2$ lower in women and men who had severe tubulopathy; ($E_{β2M}/C_{cr}$) ×100 ≥ 1000 μg/L filtrate. These findings indicate that women were particularly susceptible to the nephrotoxicity of Cd, and that the increment of $E_{β2M}/C_{cr}$ could be attributable mostly to Cd-induced impairment in tubular reabsorption of the protein together with Cd-induced nephron loss, evident from an inverse relationship between $E_{β2M}/C_{cr}$ and the eGFR.

**Keywords:** β2-microglobulin; cadmium; diabetes; GFR; hypertension; smoking; tubular proteinuria

## 1. Introduction

Cadmium (Cd) is a toxic metal pollutant that accumulates in the proximal tubule of kidneys, where it causes tubular cell injury, resulting in cell death, tubular and nephron atrophy, and eventually a reduction in the estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m$^2$ [1–4]. Because diet is an inevitable Cd exposure source, Cd has become one of the environmental toxicants of increasing public health concern worldwide. The Japan total diet study undertaken from 2013 to 2018 reported that rice and its products, green vegetables, cereals, and seeds plus potatoes constituted 38%, 17%, and 11% of total dietary exposure, respectively [5].

To safeguard against excessive exposure to Cd in the human diet, exposure guidelines, referred to as a tolerable intake level of Cd, was derived by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) [6]. Of note, the "tolerable" intake level derived was based on the risk assessment model that assumed tubular proteinuria, reflected by an increase in the excretion of the low-molecular-weight protein β2-microglubulin (β2M, $E_{β2M}$) above 300 μg/g creatinine to be an early warning sign of the nephrotoxicity of Cd. Consequently, tubulopathy is the most frequently

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reported as an adverse effect of Cd exposure. Numerous studies, however, have cast considerable doubt on the utility of $E_{\beta 2M}$ for such purposes.

This study aimed to examine if exposure to Cd adversely impacts kidney toxicity differently in men and women. To this end, tubular dysfunction and changes in the eGFR were quantified in residents of Cd-polluted and non-polluted regions of Thailand and analyzed in relation to Cd exposure levels. The confounding impact of smoking, diabetes and hypertension were also evaluated. Exposure to Cd was assessed by measurement of blood Cd concentration ($[Cd]_b$), and urinary Cd excretion ($E_{Cd}$). Tubular dysfunction was assessed by $E_{\beta 2M}$. Equations developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) were used to compute the estimated GFR (eGFR) [7].

## 2. Materials and Methods

### *2.1. Participants*

To obtain a group with a wide range of environmental Cd exposure amenable to dose-effect relationship assessment, we assembled data from 334 women and 114 men who participated in the cross-sectional studies conducted in a high-exposure area of the Mae Sot District, Tak Province [8] and a low-exposure locality in Pakpoon Municipality of Nakhon-Si-Thammarat Province [9]. Based on the data from a nationwide survey of Cd levels in soils and food crops [10], environmental exposure to Cd in Nakhon Si Thammarat were low.

The study protocol for the Mae Sot group was approved by the Institutional Ethical Committees of Chiang Mai University and the Mae Sot Hospital [8]. The study protocol for the Nakhon Si Thammarat group was approved by the Office of the Human Research Ethics Committee of Walailak University in Thailand [9].

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. Diabetes was defined as fasting plasma glucose levels ≥126 mg/dL (https://www.cdc.gov/diabetes/basics/getting-tested.html (accessed on 25 June 2023) or a physician's prescription of anti-diabetic medications. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg [11], a physician's diagnosis, or prescription of anti-hypertensive medications.

### *2.2. Collection and Analysis of Blood and Urine Samples*

Second morning urine samples were collected after overnight fast, and whole blood samples were obtained within 3 h after the urine sampling. Aliquots of urine, whole blood, and plasma were stored at −20 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 assay of urinary β2M concentration ($[\beta_2M]_u$) was based on the latex immunoagglutination method (LX test, Eiken 2MGII; Eiken and Shionogi Co., Tokyo, Japan) or the ELISA method (Sino Biological Inc., Wayne, PA, USA).

Urinary Cd concentration ($[Cd]_u$) was determined using atomic absorption spectrophotometer. Urine standard reference material No. 2670 (National Institute of Standards, Washington, DC, USA) or 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. was used for quality assurance and control purposes. The limit of detection (LOD) of Cd quantitation was defined as 3 times the standard deviation of blank measurements. When $[Cd]_u$ was below its detection limit (0.1 μg/L), the Cd concentration assigned was the LOD divided by the square root of 2 [12]

### *2.3. Estimated Glomerular Filtration Rates (eGFRs)*

The GFR is the product of nephron number and mean single nephron GFR, and in theory, the GFR is indicative of nephron function [13–15]. In practice, the GFR is estimated from established chronic kidney disease–epidemiology collaboration (CKD-EPI) equations and is reported as eGFR [15].

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

### *2.4. Normalization of Excretion Rate*

$E_x$ was normalized to $E_{cr}$ as $[x]_u/[cr]_u$, where x = Cd or $\beta_2$M; $[x]_u$ = 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 $\beta_2$M; $[x]_u$ = 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 [7].

### *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 assess differences in mean values in women and men, and the Pearson chi-squared test was used to assess differences in percentages. 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 multivariable logistic regression analysis was used to determine the Prevalence Odds Ratio (POR) for categorical outcomes. Reduced eGFR was assigned when eGFR ≤ 60 mL/min/1.73 m$^2$. For $C_{cr}$-normalized data, tubular dysfunction was defined as $(E_{\beta 2M}/C_{cr}) \times 100 \geq 300$ μg/L of filtrate. For $E_{cr}$-normalized data, tubular dysfunction was defined as $E_{\beta 2M}/E_{cr} \geq 300$ μg/g creatinine [6]. Univariate analysis of covariance with Bonferroni correction in multiple comparisons was used to obtain covariate-adjusted mean $E_{Cd}/C_{cr}$ and mean $E_{\beta 2M}/C_{cr}$. For all tests, p-values ≤ 0.05 were considered to indicate statistical significance.

## 3. Results

### *3.1. Descriptive Characteristics of Participants*

This cohort consisted of 334 women (mean age 51.5 years) and 114 men (mean age 49.9 years) (Table 1).

**Table 1.** Characteristics of Study Subjects.

| Parameters | All Subjects, *n* = 448 | Women, *n* = 334 | Men, *n* = 114 | *p* |
|---|---|---|---|---|
| Age, years | 51.1 ± 8.6 | 51.5 ± 9.0 | 49.9 ± 7.2 | 0.344 |
| BMI, kg/m$^2$ | 24.8 ± 4.0 | 25.2 ± 4.0 | 23.7 ± 3.6 | <0.001 |
| Smoking, % | 31.3 | 18.6 | 68.4 | <0.001 |
| Hypertension, % | 48.7 | 50.6 | 43.0 | 0.160 |
| Diabetes, % | 15.4 | 16.2 | 0.442 | 0.442 |
| eGFR [a], mL/min/1.73 m$^2$ | 90 ± 18 | 89 ± 19 | 93 ± 16 | 0.145 |
| Reduced eGFR [b], % | 6.9 | 8.1 | 3.5 | 0.097 |
| Plasma creatinine, mg/dL | 0.82 ± 0.22 | 0.78 ± 0.21 | 0.95 ± 0.21 | <0.001 |
| Urine creatinine, mg/dL | 114 ± 74 | 108 ± 74 | 132 ± 72 | <0.001 |
| Blood Cd, μg/L | 2.75 ± 3.19 | 2.58 ± 3.10 | 3.25 ± 3.41 | 0.038 |
| Urine Cd, μg/L | 4.22 ± 5.67 | 4.36 ± 6.14 | 3.82 ± 4.01 | 0.875 |
| Urine $\beta_2$M, μg/L | 3122 ± 18836 | 2596 ± 17238 | 4665 ± 22903 | 0.544 |
| Normalized to $C_{cr}$ ($E_x/C_{cr}$) [c] | | | | |

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| | | | | |
|---|---|---|---|---|
| ($E_{Cd}/C_{cr}$) ×100, µg/L filtrate | 3.19 ± 3.72 | 3.21 ± 3.79 | 3.12 ± 3.55 | 0.639 |
| ($E_{\beta 2M}/C_{cr}$) ×100, µg/L filtrate | 3839 ± 30422 | 3078 ± 26986 | 6072 ± 38837 | 0.212 |
| ($E_{\beta 2M}/C_{cr}$) ×100, µg/L filtrate, % | | | | |
| < 300 | 41.1 | 38.9 | 47.4 | |
| 300–999 | 34.8 | 35.9 | 31.6 | |
| ≥ 1000 | 24.1 | 25.1 # | 21.1 ** | |
| Normalized to $E_{cr}$ ($E_x/E_{cr}$) [d] | | | | |
| $E_{Cd}/E_{cr}$, µg/g creatinine | 4.02 ± 4.41 | 4.26 ± 4.62 | 3.30 ± 3.68 | 0.028 |
| $E_{\beta 2M}/E_{cr}$, µg/g creatinine | 3220 ± 21847 | 3005 ± 22812 | 3850 ± 18815 | 0.017 |
| $E_{\beta 2M}/E_{cr}$, µg/g creatinine, % | | | | |
| < 300 | 35.5 | 32.3 | 45.6 | |
| 300–999 | 34.6 | 34.7 | 34.2 | |
| ≥ 1000 | 29.7 | 32.9 | 20.2 * | |

Notes: *n*, number of subjects; BMI, body mass index; eGFR, estimated glomerular filtration rate; $\beta_2$M, $\beta_2$-microglobulin; $E_x$, excretion of x; cr, creatinine; $C_{cr}$, creatinine clearance; Cd, cadmium; [a] eGFR, was determined by established CKD-EPI equations [15]; [b] reduced eGFR corresponds to eGFR ≤60 mL/min/1.73m$^2$; [c] $E_x/E_{cr} = [x]_u/[cr]_u$; [d] $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd or $\beta_2$M [7]. Data for all continuous variables are arithmetic means ± standard deviation (SD). For all tests, $p \leq 0.05$ identifies statistical significance, determined by Pearson chi-square test for % differences and by the Mann–Whitney U-test for mean differences between women and men. #$p = 0.005$; * $p = 0.004$; **$p = 0.002$.

The respective overall percentages of smoking, hypertension, diabetes and reduced eGFR were 31.3%, 48.7% 15.4% and 6.9%. More than half of men (68.4%) smoked cigarettes, while only 18.6% of women did. The % all other ill health conditions in men and women did not differ nor did their mean age.

With the exception for BMI, mean plasma creatinine, mean urine creatinine, and mean blood Cd all were lower in women than men. Mean eGFR, mean urine Cd and mean $\beta_2$M concentrations in women and men were not statistically different.

For $C_{cr}$-normalized data, the mean $E_{Cd}/C_{cr}$ and mean $E_{\beta 2M}/C_{cr}$ in women and men both did not differ statistically. However, there were statistically significant differences in the % of women and men across three $E_{\beta 2M}/C_{cr}$ groups.

For $E_{cr}$-normalized data, the mean $E_{Cd}/E_{cr}$ and mean $E_{\beta 2M}/E_{cr}$ were higher in women than men. The % of men across three $E_{\beta 2M}/E_{cr}$ groups differed, but there was no difference in the % of women across $E_{\beta 2M}/E_{cr}$ groups.

### *3.2. Cadmium Exposure Characterization*

Figure 1 provides scatterplots relating two Cd exposure indicators, namely blood Cd concentration and excretion rate of Cd as $E_{Cd}/C_{cr}$.

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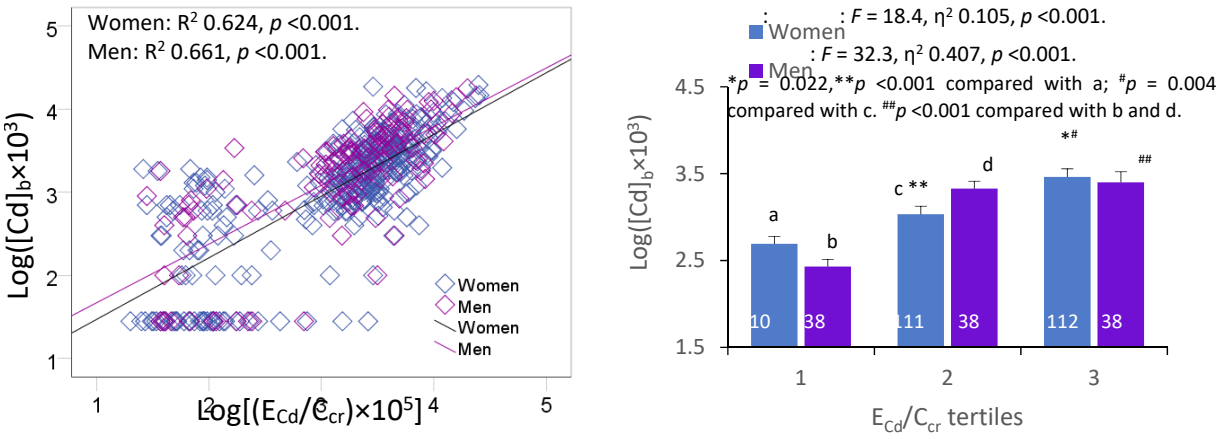


(**a**) (**b**)

**Figure 1.** Blood cadmium and urinary cadmium excretion relationship. Scatterplots relate log([Cd]$_b$ × $10^3$] to log[($E_{Cd}/C_{cr}$) × $10^5$] in women and men (**a**). Coefficients of determination ($R^2$) and *p*-values are provided for all scatterplots. Bar graph (**b**) depicts means log([Cd]$_b$ × $10^3$] values in women and men across $E_{Cd}/C_{cr}$ tertiles. All means were obtained by univariate analysis with adjustment for covariates and interactions. For women, respective arithmetic means and standard deviations (SD) for ($E_{Cd}/C_{cr}$)×100 tertiles 1, 2 and 3 are 0.37 (0.47), 2.34 (0.52) and 6.84 (4.46) µg/L of filtrate. For men, respective arithmetic means and standard deviations (SD) for ($E_{Cd}/C_{cr}$)×100 tertiles quartiles 1, 2 and 3 are 0.36 (0.42), 2.14 (0.63) and 6.81 (3.78) µg/L of filtrate. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

A strong positive association between log([Cd]$_b$×$10^3$) and [log[($E_{Cd}/C_{cr}$)×$10^5$] was evident in both women and men (Figure 1a). After adjustment for covariates and interactions, Cd body burden ([log[($E_{Cd}/C_{cr}$) ×$10^5$] explained a larger proportion of the variation in blood Cd concentrations (log([Cd]$_b$×$10^3$) in men ($\eta^2$ = 0.407) than in women ($\eta^2$ = 0.105) (Figure 1b).

To further address the variables/factors that may influence blood Cd levels, we conducted multiple regression and univariate analyses of variance that incorporated age, BMI, log[($E_{Cd}/C_{cr}$) ×$10^5$], smoking, diabetes and hypertension as independent variables. Table 2 provides results of these analyses.

**Table 2.** Determinants of blood cadmium concentration with cadmium excretion and other variables.

| Independent Variables/Factors | Log([Cd]$_b$ ×$10^3$), µg/L | | | | | |
|---|---|---|---|---|---|---|
| | Women, *n* = 334 | | | Men, *n* = 114 | | |
| | β | $\eta^2$ | *p* | *p* | $\eta^2$ | *p* |
| Age, years | −0.170 | 0.057 | <0.001 | −0.004 | 1×$10^{-6}$ | 0.946 |
| BMI, kg/m² | −0.019 | 0.001 | 0.586 | −0.079 | 0.022 | 0.180 |
| Log[($E_{Cd}/C_{cr}$)×$10^5$], µg/L filtrate | 0.619 | 0.367 | <0.001 | 0.581 | 0.420 | <0.001 |
| Smoking | 0.123 | 0.028 | 0.001 | 0.184 | 0.055 | 0.002 |
| Diabetes | -0.053 | 0.010 | 0.182 | −0.246 | 0.095 | <0.001 |
| Hypertension | 0.048 | 0.007 | 0.162 | −0.061 | 0.004 | 0.287 |
| DM× HTN | n/a | 0.016 | 0.023 | n/a | n/a | n/a |
| SMK × DM × HTN | n/a | n/a | n/a | n/a | 0.042 | 0.036 |
| Adjusted $R^2$ | 0.624 | n/a | <0.001 | 0.661 | n/a | <0.001 |

β, standardized regression coefficient; adjusted $R^2$, coefficient of determination; DM, diabetes; HTN, hypertension; SMK, smoking; n/a, not applicable. β indicates strength of association of log([Cd]$_b$×$10^3$) with independent variables (first column). Adjusted $R^2$ indicates a fractional variation

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of log($[Cd]_b \times 10^3$) explained by all independent variables. Eta square ($\eta^2$) 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.

In women, higher $[Cd]_b$ values were associated strongly with higher $E_{Cd}/C_{cr}$ (β = 0.619), and moderately associated with smoking (β = 0.123), and younger age (β = −0.170). $E_{Cd}/C_{cr}$, age, and smoking explained respectively 36.7%, 5.7% and 2.8% of the variation of $[Cd]_b$ in women, while the interaction between diabetes and hypertension contributed to 1.6% of $[Cd]_b$ variability. In men, higher $[Cd]_b$ values were associated strongly with higher $E_{Cd}/C_{cr}$ (β = 0.581), moderately with smoking (β = 0.184) and not having diabetes (β = −0.246). $E_{Cd}/C_{cr}$, smoking and diabetes accounted, respectively for 42%%, 5.5% and 9.5% of the variation of $[Cd]_b$ in men, while the interaction between smoking, diabetes and hypertension contributed to 4.2 % of $[Cd]_b$ variability.

### *3.3. Effects of Cadmium Exposure on β2M excretion*

We assessed the effects of Cd exposure on $E_{\beta 2M}$ with multiple linear regression and univariate/covariance analysis, where the indicators of Cd exposure ($[Cd]_b$ and $E_{Cd}$) were incorporated as the independent variables together with age, BMI, smoking, diabetes, and hypertension (Table 3).

**Table 3.** Associations of β2-mcirogloubulin excretion with cadmium exposure measures.

| Independent Variables/Factors | Log[($E_{\beta 2M}/C_{cr}$) ×10³], μg/ L filtrate | | | | | |
|---|---|---|---|---|---|---|
| | All subjects | | Women | | Men | |
| | β | *p* | β | *p* | β | *p* |
| Age, years | 0.137 | 0.013 | 0.131 | 0.041 | 0.128 | 0.238 |
| BMI, kg/m² | −0.089 | 0.065 | −0.102 | 0.062 | −0.043 | 0.664 |
| Log($[Cd]_b \times 10^3$), μg/L filtrate | −0.016 | 0.824 | −0.083 | 0.328 | 0.217 | 0.180 |
| Log[($E_{Cd}/C_{cr}$)×10⁵], μg/L filtrate | 0.283 | <0.001 | 0.306 | <0.001 | 0.175 | 0.247 |
| Gender (male) | 0.052 | 0.318 | – | – | – | – |
| Smoking | 0.063 | 0.255 | 0.093 | 0.094 | −0.065 | 0.526 |
| Diabetes | 0.323 | <0.001 | 0.349 | <0.001 | 0.279 | 0.017 |
| Hypertension | 0.015 | 0.745 | −0.023 | 0.660 | 0.142 | 0.139 |
| Adjusted $R^2$ | 0.105 | <0.001 | 0.125 | <0.001 | 0.059 | 0.060 |

β, standardized regression coefficient; adjusted $R^2$, coefficient of determination; β indicates strength of association of log[($E_{\beta 2M}/C_{cr}$) ×10³] with independent variables (first column). Adjusted $R^2$ indicates a fractional variation of log[($E_{\beta 2M}/C_{cr}$)×10³] explained by all independent variables. For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of an independent variable to log[($E_{\beta 2M}/C_{cr}$)×10³] variability.

In all subjects, $E_{\beta 2M}/C_{cr}$ was associated age (β = 0.137), $E_{Cd}/C_{cr}$ (β = 0.283) and diabetes (β = 0.323). In women, the associations of $E_{\beta 2M}/C_{cr}$ with these three independent variables were evident. In men, $E_{\beta 2M}/C_{cr}$ showed a significant association only with diabetes (β = 0.279).

We next examined the association between the $E_{Cd}/C_{cr}$ and $E_{\beta 2M}/C_{cr}$ with scatterplots and the covariate-adjusted mean $E_{\beta 2M}$ in subjects grouped by $E_{Cd}/C_{cr}$ tertiles (Figure 2).

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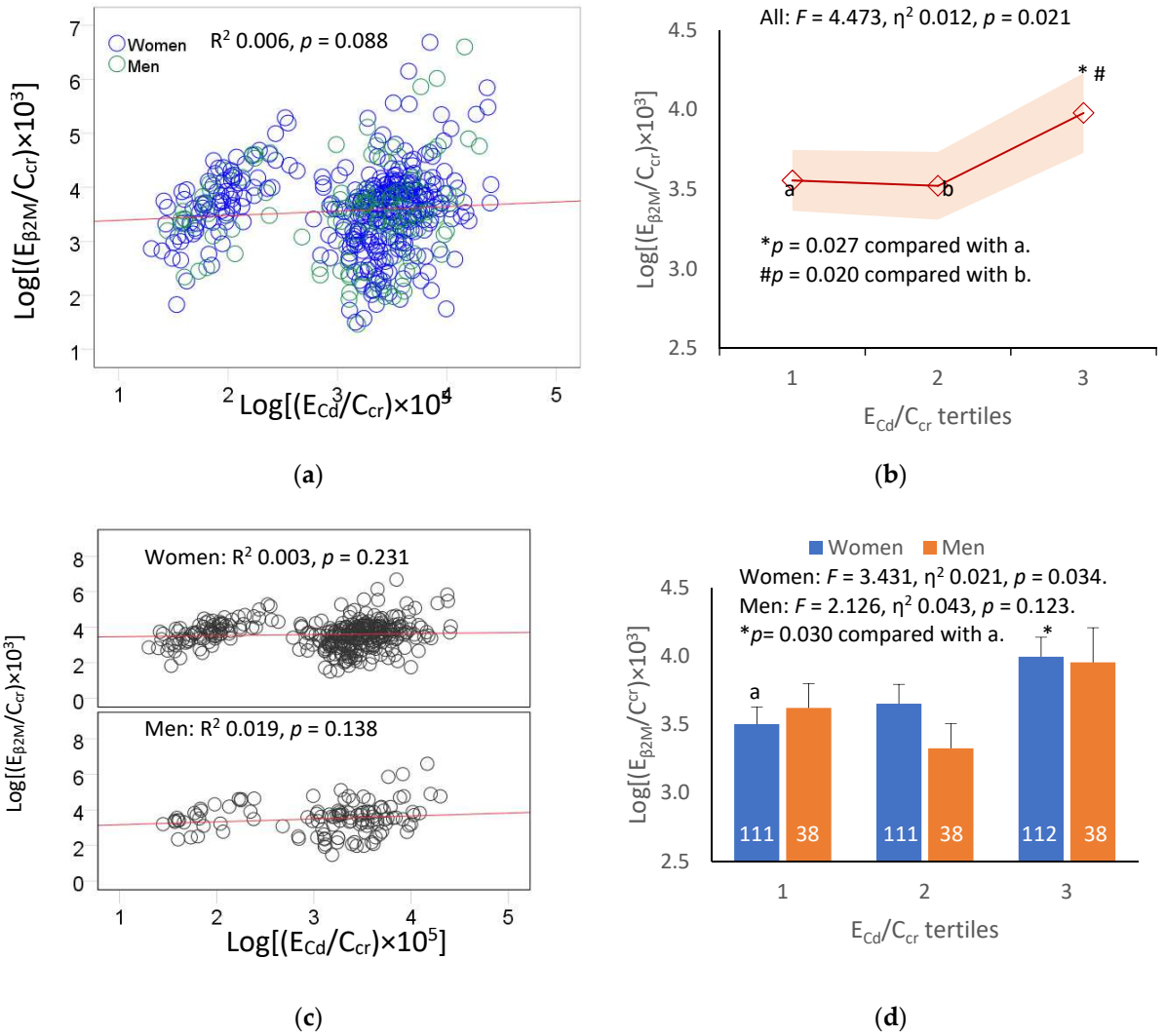


**Figure 2.** Dose-effect relationship of β2-microgloubulin and cadmium excretion. Scatterplots relate log[($E_{\beta M}/C_{cr}$) × $10^3$] to log[($E_{Cd}/C_{cr}$) × $10^5$] in all subjects (**a**), and women and men (**c**). Coefficients of determination ($R^2$) and *p*-values are provided for all scatterplots. The color-coded area graph (**b**) depicts means of log[($E_{\beta 2M}/C_{cr}$)×$10^3$] across $E_{Cd}/C_{cr}$ tertiles. Shaded areas indicate variability of means. Bar graph (**d**) depicts mean log[($E_{\beta 2M}/C_{cr}$) × $10^3$] in women and men in each $E_{Cd}/C_{cr}$ tertile. The respective numbers of subjects in $E_{Cd}/C_{cr}$ tertiles 1, 2 and 3 are 149, 149 and 150. All means were obtained by univariate analysis with adjustment for covariates and interaction. For women, respective arithmetic means and standard deviations (SD) for ($E_{Cd}/C_{cr}$)×100 tertiles 1, 2 and 3 are 0.37 (0.47), 2.34 (0.52) and 6.84 (4.46) µg/L of filtrate. For men, respective arithmetic means and standard deviations (SD) for ($E_{Cd}/C_{cr}$)×100 tertiles quartiles 1, 2 and 3 are 0.36 (0.42), 2.14 (0.63) and 6.81 (3.78) µg/L of filtrate. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

The relationship between $E_{\beta 2M}/C_{cr}$ and $E_{Cd}/C_{cr}$ was weak and statistically insignificant in all subjects (Figure 1a), as were in women and men (Figure 2c). However, with adjustment for covariates that included age and BMI, diabetes, hypertension, and smoking (Figure 2b), a significant contribution of Cd body burden to the variability of $E_{\beta 2M}$ became was evident when all subjects were included in an analysis ($F$ = 4.473, $\eta^2$ 0.012, $p$ = 0.021). The $E_{\beta 2M}$ in subjects of the high $E_{Cd}/C_{cr}$ tertile was higher, compared with those of middle and low $E_{Cd}/C_{cr}$ tertiles (Figure 1b). In subgroup analysis (Figure 1d), a dose-effect relationship of $E_{Cd}$ and $E_{\beta 2M}$ was seen in women only ($F$ = 3.431, $\eta^2$ 0.021, $p$ = 0.034).

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*3.4. Effects of Cadmium Exposure on the Prevalenec Odds of Tubulopathy*

Table 4 provides the results of the logistic regression analysis of abnormal $E_{\beta 2M}$ that incorporated age, BMI, log[($E_{Cd}/C_{cr}$)×$10^5$], gender, smoking, diabetes, and hypertension as independent variables.

**Table 4.** Prevalence odds for excessive excretion of $\beta_2$M in relation to cadmium excretion and other variables.

| Independent variables/factors | Number of Subjects | ($E_{\beta 2M}/C_{cr}$) ×100 ≥ 300 μg/L | | ($E_{\beta 2M}/C_{cr}$) × 100 ≥ 1000 μg/L | |
|---|---|---|---|---|---|
| | | POR (95% CI) | *p* | POR (95% CI) | *p* |
| Age, years | 448 | 1.036 (1.007, 1.067) | 0.016 | 1.062 (1.027, 1.098) | <0.001 |
| BMI, kg/m² | 448 | 0.971 (0.919, 1.025) | 0.284 | 0.958 (0.896, 1.023) | 0.203 |
| Log[($E_{Cd}/C_{cr}$)×$10^5$], μg/L filtrate | 448 | 1.940 (1.344, 2.802) | <0.001 | 3.343 (2.036, 5.488) | <0.001 |
| Gender (male) | 114 | 1.406 (0.835, 2.367) | 0.200 | 1.299 (0.687, 2.458) | 0.421 |
| Smoking | 140 | 1.067 (0.645, 1.765) | 0.801 | 1.388 (0.763, 2.522) | 0.282 |
| Diabetes | 69 | 5.294 (2.526, 11.09) | <0.001 | 11.52 (5.004, 26.50) | <0.001 |
| Hypertension | 218 | 1.066 (0.714, 1.592) | 0.753 | 1.535 (0.942, 2.501) | 0.085 |

POR, prevalence odds ratio; CI, confidence interval. The units of ($E_{\beta 2M}/C_{cr}$) × 100 and log[($E_{Cd}/C_{cr}$)×$10^5$] are μg/L filtrate; Data were generated from logistic regression analyses relating POR for excessive $\beta_2$M excretion to seven independent variables (first column). For all tests, *p*-values ≤ 0.05 indicate a statistically significant association of POR with a given independent variable.

Among seven independent variables, the prevalence odds ratios (POR) for ($E_{\beta 2M}/C_{cr}$) ×100 ≥ 300–999, and ≥ 1000 μg/L filtrate were increased with age, log[($E_{Cd}/C_{cr}$)×$10^5$], and diabetes. All other 4 independent variables did not show a significant association with abnormal $\beta_2$M excretion. For every 10-fold rise of $E_{Cd}/C_{cr}$ the POR for ($E_{\beta 2M}/C_{cr}$) ×100 of ≥ 300 and ≥ 1000 μg/L were increased by 1.94-fold and 3.34-fold, respectively.

*3.5. Effects of Cadmium Exposure on eGFR*

Similarly, we assessed the effects of Cd exposure on the estimated glomerular filtration rate (eGFR) by multiple linear regression and logistic regression analyses, where $[Cd]_b$ and $E_{Cd}$ were incorporated as the independent variables together with age, BMI, smoking, diabetes, and hypertension (Table 5).

**Table 5.** Associations of eGFR with cadmium exposure measures and other variables.

| Independent. Variables/Factors | eGFR, mL/min/1.73m² | | | | | |
|---|---|---|---|---|---|---|
| | All subjects | | Women | | Men | |
| | β | *p* | β | *p* | β | *p* |
| Age, years | −0.517 | <0.001 | −0.511 | <0.001 | −0.506 | <0.001 |
| BMI, kg/m² | −0.064 | 0.136 | −0.048 | 0.327 | −0.149 | 0.095 |
| Log($[Cd]_b$×$10^3$), μg/L filtrate | 0.053 | 0.420 | 0.102 | 0.182 | −0.153 | 0.291 |
| Log[($E_{Cd}/C_{cr}$)×$10^5$], μg/L filtrate | −0.148 | 0.026 | −0.185 | 0.018 | 0.011 | 0.933 |
| Gender (male) | −0.001 | 0.977 | – | – | – | – |
| Smoking | 0.025 | 0.610 | 0.018 | 0.717 | 0.071 | 0.438 |
| Diabetes | −0.109 | 0.023 | −0.128 | 0.021 | −0.055 | 0.593 |
| Hypertension | −0.079 | 0.055 | −0.050 | 0.295 | −0.212 | 0.014 |
| Adjusted $R^2$ | 0.279 | <0.001 | 0.281 | <0.001 | 0.249 | <0.001 |

eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted $R^2$, coefficient of determination; β indicates strength of association of eGFR with independent variables (first column). Adjusted $R^2$ indicates a fractional variation of eGFR explained by all independent variables. For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of an independent variable to eGFR variability.

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In all subjects, eGFR was inversely associated with age (β = −0.517), $E_{Cd}$ (β = −0.148) and diabetes (β = −0.109). In subgroup analysis, inverse associations of eGFR with these three independent variables (age, $E_{Cd}$ and diabetes) were seen only in the women group. In men, eGFR was not associated with $E_{Cd}$, but this parameter showed inverse associations with age (β = −0.506) and hypertension (β = −0.212).

In logistic regression of a reduced eGFR (eGFR ≤60 mL/min/1.73m$^2$), age, BMI, log[($E_{Cd}/C_{cr}$) ×10$^5$], gender, smoking, diabetes, and hypertension were incorporated as independent variables (Table 6).

**Table 6.** Prevalence odds for a reduced eGFR in relation to cadmium excretion and other variables.

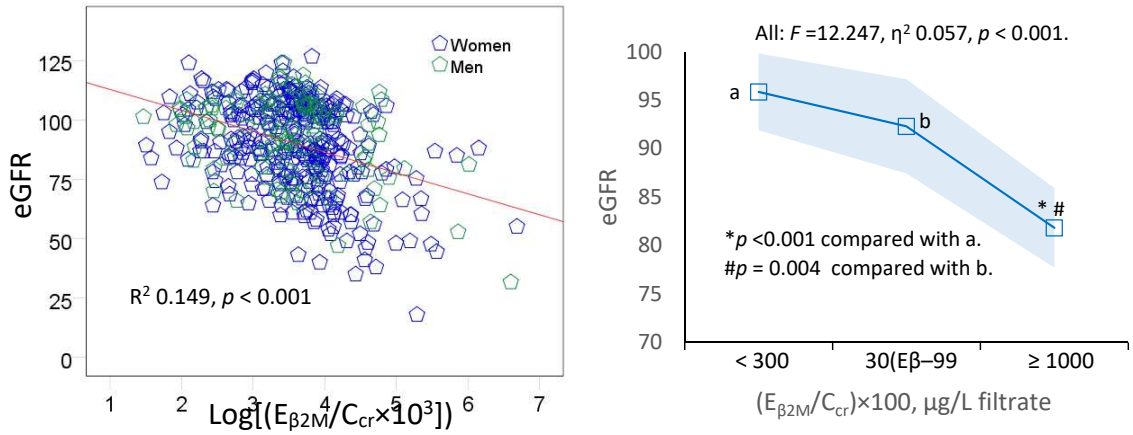
| Independent Variables/ Factors | Reduced eGFR [a] | | | | |
|---|---|---|---|---|---|
| | β Coefficients (SE) | POR | 95% CI | | *p* |
| | | | Lower | Upper | |
| Age, years | 0.136 (0.027) | 1.146 | 1.086 | 1.209 | <0.001 |
| BMI, kg/m$^2$ | 0.020 (0.051) | 1.021 | 0.923 | 1.128 | 0.688 |
| Log[($E_{Cd}/C_{cr}$)×10$^5$], μg/L filtrate | 1.154 (0.358) | 3.172 | 1.572 | 6.402 | 0.001 |
| Gender (male) | −0.542 (0.649) | 0.582 | 0.163 | 2.075 | 0.404 |
| Smoking | −0.228 (0.583) | 0.796 | 0.254 | 2.493 | 0.695 |
| Diabetes | 1.439 (0.524) | 4.217 | 1.510 | 11.78 | 0.006 |
| Hypertension | 0.115 (0.427) | 1.122 | 0.486 | 2.591 | 0.787 |

[a] Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73m$^2$; β, regression coefficient; POR, prevalence odds ratio; S.E., standard error of mean; CI, confidence interval. Data were generated from logistic regression relating POR for a reduced eGFR to seven independent variables (first column). For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of individual independent variables to the POR for a reduced eGFR.

The POR values for a reduced eGFR were increased with age, log[($E_{Cd}/C_{cr}$)×10$^5$], and diabetes. For every 10-fold rise of $E_{Cd}/C_{cr}$, the POR for a reduced eGFR was increased by 3.2-fold. There was a 4.2-fold increase in the POR for a reduced eGFR among the diabetic.

### *3.6. Inverse Relationship of β$_2$M Excretion and eGFR*

Figure 3 provides scatterplots relating eGFR to $E_{β2M}$ among study subjects together with covariates-adjusted means of eGFR in women and men.




(**a**) (**b**)

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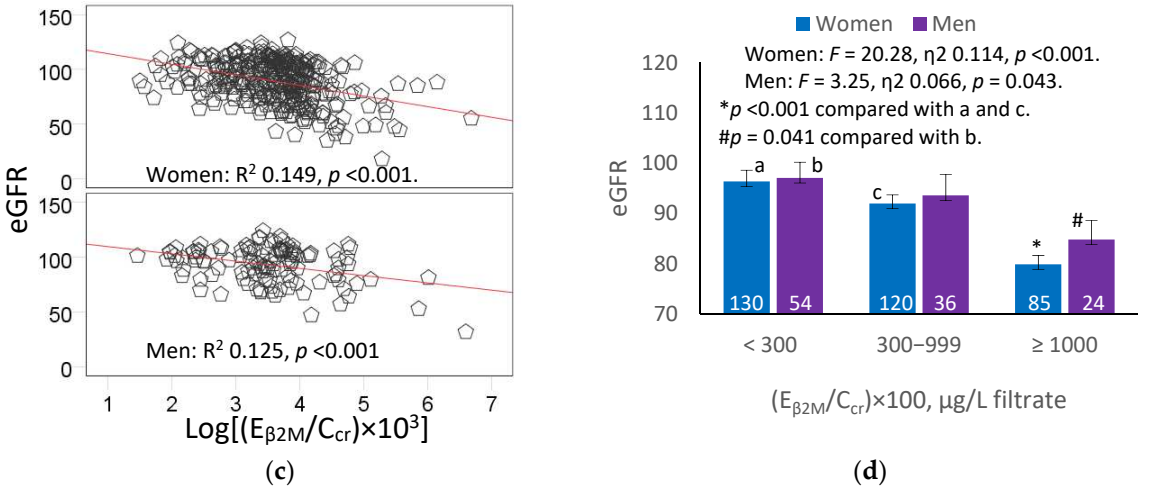


**Figure 3.** An inverse relationship of eGFR with β2-microgloubulin excretion. Scatterplots relate eGFR to log[($E_{\beta M}/C_{cr}$) × $10^3$] in all subjects (**a**), and women and men (**c**). Coefficients of determination ($R^2$) and *p*-values are provided for all scatterplots. The color-coded area graph (**b**) depicts means of eGFR across three $E_{\beta 2M}/C_{cr}$ ranges. Shaded areas indicate variability of means. Bar graph (**d**) depicts means of eGFR in women and men in each $E_{\beta 2M}/C_{cr}$ range. The respective numbers of subjects in ($E_{\beta 2M}/C_{cr}$)×100 <300, 300–999 and ≥1000 µg/L of filtrate are 184, 156 and 109. All means were obtained by univariate analysis with adjustment for covariates and interaction. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

A statistically significant inverse relationship between eGFR and $E_{\beta 2M}$ was seen in all subjects (Figure 3a), as well as in women and men (Figure 3c). In all subjects (Figure 3b), eGFR explained 5.7% of the variation in $E_{\beta 2M}/C_{cr}$ ($F$ =12.247, $p < 0.001$).

For simplicity, the degree of tubulopathy, assessed with $E_{\beta 2M}$, was graded into three levels where levels 1, 2 and 3 of tubulopathy corresponded to ($E_{\beta 2M}/C_{cr}$) ×100 <300, 300–999 and ≥ 1000 µg/L filtrate, respectively.

The covariates-adjusted mean eGFR was 14.0 and 10.5 mL/min/1.73m² lower in subjects with level 3 tubulopathy, compared to those with tubular dysfunction levels 2 and 1, respectively (Figure 3b).

In subgroup analysis (Figure 3d), the $\eta^2$ values indicated a nearly twice larger effect size of eGFR on $E_{\beta 2M}$ in women ($\eta^2$ = 0.114), compared to men ($\eta^2$ = 0.066). In women, those with the tubulopathy of level 3 had the covariates-adjusted mean eGFR 16.5 and 12.0 mL/min/1.73 m² lower, compared to those with tubulopathy levels 1 and 2, respectively. In men, those with tubulopathy of level 3, had the covariates-adjusted mean eGFR 12.3 mL/min/1.73 m² lower, compared to those of tubulopathy level 1. The adjusted mean eGFR values in men of tubulopathy levels 3 and 2 did not differ statistically.

In another logistic regression, a relative contribution of Cd exposure and tubular dysfunction levels to the prevalence of a reduced eGFR was determined. $E_{Cd}$ was entered as a continuous variable, while $E_{\beta 2M}$ was categorized into levels 1, 2 and 3, as previously stated. Table 7 provides results of such analysis.

**Table 7.** Prevalence odds of a reduced eGFR in relation to cadmium and β2M excretion rates normalized to $C_{cr}$.

| Independent Variables/ Factors | Reduced eGFR [a] | | | | |
|---|---|---|---|---|---|
| | Number of subjects | POR | 95% CI | | *p* |
| | | | Lower | Upper | |
| Age, years | 448 | 1.140 | 1.072 | 1.211 | <0.001 |
| BMI, kg/m² | 448 | 1.066 | 0.950 | 1.198 | 0.278 |
| Log[($E_{Cd}/C_{cr}$) ×$10^5$], µg/L filtrate | 448 | 2.251 | 1.043 | 4.858 | 0.039 |

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| | | | | | |
|---|---|---|---|---|---|
| Gender (male) | 114 | 0.674 | 0.176 | 2.583 | 0.565 |
| Smoking | 140 | 0.885 | 0.270 | 2.899 | 0.840 |
| Diabetes | 69 | 1.216 | 0.394 | 3.753 | 0.734 |
| Hypertension | 218 | 1.199 | 0.487 | 2.957 | 0.693 |
| ($E_{\beta 2M}/C_{cr}$) ×100, μg/L filtrate | | | | | |
| < 300 | 184 | Referent | | | |
| 300–999 | 156 | 8.310 | 2.655 | 26.01 | <0.001 |
| ≥ 1000 | 108 | 33.731 | 4.193 | 271.3 | 0.001 |

[a], Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73m$^2$; SE, POR, prevalence odds ratio; CI, confidence interval. Data were generated from multivariable logistic regression analyses relating the POR for a reduced eGFR to eight independent variables (first column). *p*-values < 0.05 indicate a statistically significant increase in the POR for a reduced eGFR.

The POR values for a reduced eGFR rose with age (POR = 1.14), $E_{Cd}/C_{cr}$ (POR = 2.25), tubulopathy level 2 (POR = 8.31), and tubulopathy level 3 (POR = 33.7). All other independent variables, such as diabetes and hypertension, did not show a significant association with the POR for reduced eGFR.

An equivalent logistic regression was conducted using $E_{cr}$-normalized $E_{Cd}$ and $E_{\beta 2M}$ data (Table 8).

**Table 8.** Prevalence odds of a reduced eGFR in relation to cadmium and β$_2$M excretion rates normalized to Ecr.

| Independent Variables/ Factors | Reduced eGFR [a] | | | | |
|---|---|---|---|---|---|
| | Number of subjects | POR | 95% CI | | *p* |
| | | | Lower | Upper | |
| Age, years | 448 | 1.101 | 1.045 | 1.160 | <0.001 |
| BMI, kg/m$^2$ | 448 | 1.032 | 0.929 | 1.147 | 0.555 |
| Log[($E_{Cd}/E_{cr}$) ×10$^3$], μg/g creatinine | 448 | 1.278 | 0.630 | 2.593 | 0.497 |
| Gender (male) | 114 | 0.671 | 0.181 | 2.484 | 0.550 |
| Smoking | 140 | 0.762 | 0.240 | 2.418 | 0.644 |
| Diabetes | 69 | 1.614 | 0.581 | 4.484 | 0.359 |
| Hypertension | 218 | 1.041 | 0.449 | 2.415 | 0.926 |
| ($E_{\beta 2M}/C_{cr}$)×100, μg/g creatinine | | | | | |
| <300 | 160 | | | | |
| 300–999 | 155 | 3.204 | 1.226 | 8.375 | 0.018 |
| ≥1000 | 133 | 19.042 | 2.387 | 151.907 | 0.005 |

[a], Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73m$^2$; SE, POR, prevalence odds ratio; CI, confidence interval. Data were generated from multivariable logistic regression analyses relating the POR for a reduced eGFR to eight independent variables (first column). *p*-values < 0.05 indicate a statistically significant increase in the POR for a reduced eGFR.

The POR values for a reduced eGFR increased with age (POR = 1.10), and the levels of tubular dysfunction; $E_{\beta 2M}/E_{cr}$ 300–999 μg/g creatinine (POR = 3.20) and $E_{\beta 2M}/E_{cr}$ ≥100 μg/g creatinine (POR = 19.0). Associations of POR for a reduced eGFR with $E_{Cd}/E_{cr}$ and all other variables were statistically insignificant.

## 4. Discussion

This study used a cross-sectional analysis of kidney dysfunction, tubular proteinuria and eGFR decline, to determine the differential impact of Cd exposure in men and women. Whereas many previous studies focused primarily on Cd-induced tubulopathy in women, we investigated these health outcomes in both men and women along with confounding risk factors, smoking, diabetes and hypertension. The excretion rate of Cd and β$_2$M ($E_{Cd}$ and $E_{\beta 2M}$) were normalized to the surrogate measure of GFR, creatinine clearance ($C_{cr}$). This $C_{cr}$-normalization of $E_{Cd}$ and $E_{\beta 2M}$, as $E_{Cd}/C_{cr}$ and

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$E_{\beta 2M}/C_{cr}$, depict excretion rates per functional nephron, thereby it corrects for differences in the number of functioning nephrons among study subjects [7]. This $C_{cr}$-normalized excretion rate also corrects for urine dilution, but it is unaffected by creatinine excretion ($E_{cr}$). Thus, $E_{Cd}/C_{cr}$ and $E_{\beta 2M}/C_{cr}$ provide an accurate quantification of the kidney burden of Cd and its toxicity to kidney tubular cells.

We selected subjects from two population-based studies, undertaken in an area with endemic Cd contamination in Mae Sot district, Tak province [8] and a control, non-contamination area in Nakhon-Si-Thammarat province of Thailand [9,10]. The Cd content of the paddy soil samples from the Mae Sot district exceeded the standard of 0.15 mg/kg, and rice samples collected from households contained 4 times the amount of the permissible Cd level of 0.1 mg/kg [16].

### *4.1. Exposure Levels of Cadmium in Women versus Men*

Men and women in this cohort carry the same body burden of Cd, evident from a nearly identical mean ($E_{Cd}/C_{cr}$) ×100 values of 3.12 vs 3.21 μg/L filtrate. The sources of Cd could be differentiated through an analysis of blood-urine Cd relationships.

$[Cd]_b$ and $E_{Cd}/C_{cr}$ correlated strongly with each other in women ($R^2$ =0.624) and men ($R^2$ = 0.661) (Figure 1a), and the covariate-adjusted means $[Cd]_b$ showed a stepwise increase through $E_{Cd}/C_{cr}$ tertiles in both genders. Of note, $E_{Cd}/C_{cr}$ explained a larger fraction of the variation in $[Cd]_b$ in men than it did in women ($\eta^2$ 0.407 vs. 0.105) (Figure 1b). The variability in $[Cd]_b$ was associated mostly with $E_{Cd}/C_{cr}$ in both genders while smoking explained a larger fraction of the $[Cd]_b$ variability among men than among women (5.5% vs 2.8%). This result was expected, given the high % of smokers in the men group (68.4% vs. 18.6%) and the higher mean $[Cd]_b$ in men than that of women (3.25 vs. 4.36 μg/L) In men only, $[Cd]_b$ variation was associated with diabetes.

### *4.2. The Toxic Manifestation of Cadmium Exposure in Women versus Men*

An independent health survey reported that the prevalence of chronic kidney disease (CKD), defined as the eGFR ≤ 60 mL/min/1.73 m², among Mae Sot residents was 16.1%, while the prevalence of tubulopathy, referred to as tubular proteinuria was 36.1% [17]. This reported tubular proteinuria was based on the cut-off value of $E_{\beta 2M}/E_{cr}$ at 300 μg/g creatinine [6], which equates $E_{\beta 2M}/C_{cr}$ of 2–3 μg/ L filtrate or ($E_{\beta 2M}/C_{cr}$) ×100 of 200–300 μg/ L filtrate. Notably, the cut-off value for $E_{\beta 2M}/E_{cr}$ at 300 μg/g creatinine was used as the critical effect of exposure to Cd in the human diet [6].

In this cohort, tubular proteinuria affected more than half of women (61.1%) and men (52.6%). One of four women had severe tubular impairment [($E_{\beta 2M}/C_{cr}$) ×100 ≥ 1000 μg/L filtrate], whereas one of five men had this abnormality.

In women, $E_{\beta 2M}/C_{cr}$ showed a moderate positive association with age (β = 0.131), and an equally strong association with $E_{Cd}/C_{cr}$ (β = 0.306) and diabetes (β = 0.349) (Table 3). In men, $E_{\beta 2M}/C_{cr}$ did not show a significant association with age or $E_{Cd}/C_{cr}$, but this tubular defect was associated with diabetes only (β = 0.279). In covariance analysis, the contribution of $E_{Cd}/C_{cr}$ to the variability of $E_{\beta 2M}/C_{cr}$ in women was demonstrable together with a dose-effect relationship after adjustment of covariates and interactions (Figure 2d). In contrast, the contribution of $E_{Cd}/C_{cr}$ to the variation of $E_{\beta 2M}/C_{cr}$ in men was statistically insignificant (Figure 2d). An association of the marker of tubular dysfunction ($E_{\beta M}$) and diabetes seen in both men and women is in line with the current knowledge that diabetes adversely affects both glomerular (GFR) and tubular function, termed diabetic tubulopathy [18,19].

The overall mean eGFR was 90 mL/min/1.73 m² and the overall prevalence of a reduced in eGFR in this cohort was 6.9% (Table 1). The difference in the % of a reduced eGFR in women and men (8.1% vs. 3.5%) did not reach a statistical significance level ($p$ = 0.097) nor did the difference in mean eGFR in women and men ($p$ = 0.145). The weaker effect of Cd exposure on eGFR in men, compared to women, remains to be confirmed with sufficiently large sample group of men. However, the regression analysis also indicated gender differences in susceptibility to the nephrotoxicity of Cd (Table 5). In women, the eGFR was inversely associated with age (β = −0.511), $E_{Cd}/C_{cr}$ (β =−0.185) and diabetes (β = −0.128). In comparison, the eGFR in men was not associated with $E_{Cd}/C_{cr}$, while showing an inverse association with age (β = −0.506) and hypertension (β = −0.212). Adverse effects of hypertension and diabetes on the eGFR have been noted in a cross-sectional study of the U.S. general

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population, where Cd-induced GFR reduction was more pronounced in those who had diabetes and/or hypertension [20].

We speculate that gender differences in levels of some protective factors, notably body status of nutritionally essential metals such as iron and zinc may contribute to the increased susceptibility to Cd nephrotoxicity seen in women. Similarly, environmental Cd exposure has been linked to a reduction eGFR among participants in various cycles of the U.S. National Health and Nutrition Examination Survey (NHANES) undertaken over 18 years (1999 to 2016) [20–22]. Lin et al. (2014) reported that the risk for a reduced eGFR was higher in those with lower serum zinc (OR 3.38), compared to those with similar Cd exposure levels and serum zinc > 74 μg/dL (OR 2.04) [22].

### *4.3. Increment of β2M Excretion as GFR Falls*

The protein β2M with the molecular weight of 11,800 Da is filtered freely by the glomeruli, and is reabsorbed almost completely by the kidney's tubular epithelial cells [13]. Thus, defective tubular re-absorption of β2M will result in an enhanced excretion rate of β2M [23–26]. Loss of nephrons also raises the excretion β2M for the following reasons [23,26]. When the reabsorption rate of β2M per nephron remains constant, its excretion will vary directly with its production. If the production and reabsorption per nephron remain constant as nephrons are lost, the excretion of β2M will rise [27].

It can thus be expected that the excretion of β2M will increase, when GFR falls for any causes. Indeed, $E_{\beta 2M}/C_{cr}$ was inversely associated with eGFR in both women and men (Figure 3c) although the causes of their eGFR decreases seemed to be different. In a quantitative analysis (Figure 3d), the $\eta^2$ value describing the effect size of $E_{\beta 2M}/C_{cr}$ on eGFR variability among women was 1.7-fold larger (0.114 vs. 0.066). In both women and men, eGFR was lowest in those who had ($E_{\beta 2M}/C_{cr}$) ×100 ≥1000 μg/L filtrate, indicative of severely impaired tubular function.

Of note, the POR for a reduced eGFR was increased by 8.3-fold and 33.7-fold those with ($E_{\beta 2M}/C_{cr}$) ×100 of 300–999, and ≥1000 μg/L filtrate, respectively (Table 7), compared to those with $E_{\beta 2M}/C_{cr}$) ×100 <300 μg/L filtrate. A substantial loss of functioning nephrons was a likely cause of massive increases in $E_{\beta 2M}/C_{cr}$ seen in those who had eGFR below 60 mL/min/1.73 m$^2$.

### *4.4. The Pitfall of Adjusting Excretion Rate to Ecr and Implication for Health Risk Estimation*

The $C_{cr}$-normalized data indicate that women and men shared the same burden of Cd (Table 1). The data indicate also that the % of women and men across three categories of tubulopathy were all statistically different, thereby linking Cd exposure to the severity of tubulopathy in both genders. The logistic regression data (Table 7) show that the likelihood of having a reduced eGFR was increased by 8.3-fold and 33.7-fold in those who had ($E_{\beta 2M}/C_{cr}$) ×100 of 300–999 and ≥1000 μg/L filtrate, compared to those with ($E_{\beta 2M}/C_{cr}$) ×100 <300 μg/L filtrate.

The $E_{cr}$-normalized data indicated that the mean $E_{Cd}/E_{cr}$ in women was statistically higher than that of men (4.26 vs. 3.30 μg/g creatinine). They also indicated that the difference in % of women across the three tubulopathy categories was minuscule, and that the % distribution of men across tubulopathy categories was statistically significant. These data suggest an association of Cd exposure and the severity of tubulopathy in men only. The logistic regression data (Table 8) show that the likelihood of having a reduced eGFR was increased by 3.2-fold and 19-fold in those who had $E_{\beta 2M}/E_{cr}$ of 300–999 and ≥1000 μg/g creatinine, compared to those with $E_{\beta 2M}/E_{cr} < E_{\beta 2M}/E_{cr}$.

Previously, $E_{\beta 2M}/E_{cr}$ of 100–299, 300–999, and ≥1000 μg/g creatinine were found to be associated with 4.7-, 6.2- and 10.5-fold increases in the prevalence odds of a reduced eGFR [28]. Similarly, a rise in $E_{\beta 2M}/E_{cr}$ to levels not higher than 100 μg/g creatinine was associated with an increased risk of hypertension in the Japanese general population [29], while a prospective cohort data showed that $E_{\beta 2M}/E_{cr}$ was associated with a 79% increase in the likelihood of having a large decline in eGFR (10 mL/min/1.73 m$^2$) over a five-year period [30]. Thus, a cut-off value for $E_{\beta 2M}/E_{cr}$ above 300 μg/g creatinine does not reflect an early warning sign of the nephrotoxicity of Cd. The utility of this $E_{\beta 2M}/E_{cr}$ value as a toxicity criterion to derive a toxicity threshold level for Cd is inappropriate.

In summary, adjusting $E_{Cd}$ and $E_{\beta 2M}$ to $E_{cr}$ produces an erroneous interpretation of the effect of Cd exposure on the eGFR, while underestimating the severity of Cd-induced tubulopathy. These data

calls into question the utility of $E_{\beta 2M}/E_{cr}$ of 300 μg/g creatinine to represent the critical effect of exposure to Cd in the human diet. New health guidance values need to be established for this toxic metal as well as public measures to minimize the Cd contamination of food chains.

## 5. Conclusions

Excretion of $\beta_2$M above 300 μg/g creatinine (≈ 2–3 μg/L filtrate) and reduced glomerular function, indicated by eGFR below 60 mL/min/1.73 $m^2$ are the manifestations of severe kidney toxicities of chronic exposure to Cd that are more prevalent and more severe in women than men of the same body burden.

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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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