Environmental Exposure to Cadmium and Lead Exacerbates Kidney Function in People with Diabetes

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Article

Environmental Exposure to Cadmium and Lead Exacerbates Kidney Function in People with Diabetes

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Abstract

Diabetic kidney disease and hypertension are the leading causes of kidney failure. The reduction in an estimated glomerular filtration rate (eGFR) in response to chronic exposure to low levels of cadmium (Cd) has been causally linked, however, the exact mechanism is poorly understood. We postulate that the toxicity of Cd and lead (Pb) towards kidney tubular cells impairs their function, particularly their ability to clear filtered proteins, notably β2-microglobulin (β2M). As proteins in the glomerular filtrate is concentrated, it becomes toxic to cells, and thus a further reduction in eGFR. In this study we analyzed data from a Thai cohort of 137 persons of which 72 were diagnosed with diabetes. Blood Cd, blood Pb and urinary excretion of Cd (E_{Cd}) were measured to obtain an indication of exposure to these metals. Tubular cell injury and tubular cell function were assessed by measurement of urinary N-acetylglucosaminidase (E_{NAG}) and fractional tubular degradation of β2M ($FrTD_{\beta 2M}$), respectively. $FrTD_{\beta 2M}$ was more strongly associated with eGFR in those with diabetes (β = 0.476) than those without (β = 0.360). Intriguingly, only in those with diabetes, $FrTD_{\beta 2M}$ was inversely associated with E_{Cd} (β = −0.295) as was an association of E_{NAG} with E_{Cd} (R^2 = 0.071). The mediation analysis infers that a falling eGFR was partially linked to a diminished tubular degradation of β2M, caused by Cd-induced tubular cell injury. In conclusion, individuals with diabetes were especially susceptible to tubular cell injury, reductions in both eGFR and the degradation of filtered proteins following Cd/Pb exposure.

Keywords: β2-microglobulin; cadmium; diabetes; eGFR; N-aceylglucosaminidase; lead

1. Introduction

Diabetes, defined as fasting plasma glucose (FPG) levels ≥ 126 mg/dL, is one of the chronic ailments with high prevalence worldwide [1]. Among people with diabetes hypertension and chronic kidney disease (CKD) are common comorbidities [2–5]. Notably, however, hypertension can be both a cause and a consequence of kidney damage [4–6]. The diagnosis of CKD is based on the presence of albuminuria for at least 3 months, and/or a fall of an estimated glomerular filtration rates (eGFR) ≤ 60 mL/min/1.73 m^2 [7–9]. Another notable hallmark of kidney disease is proteinuria, which can predict continued progressive eGFR decline to end-stage kidney disease [10–14], a condition that requires dialysis or a kidney transplant for survival. The associated healthcare costs are substantial.

Due to the widespread contamination of arable soils and staple foods, exposure to cadmium (Cd) and lead (Pb) is inevitable for most people [15,16]. This is reflected by several population studies that reported an association of CKD risk with exposure to the metals [17–21]. A cross-sectional study

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on the U.S. population observed increased risk of CKD by Pb exposure, especially in non-smoking women, aged over 50 years, who had diabetes and body mass index (BMI) higher than 25 kg/m^2 [22]. In a cohort of Swedish women aged 64 years, an increased risk of albuminuria was found only in those with diabetes who had blood Cd within the top quartile [23]. A prospective cohort study from the Netherlands suggested that Cd exposure promoted eGFR deterioration in patients with diabetes [24].

The present study aimed to investigate the mechanisms of the nephrotoxicity of Cd and Pb in people with diabetes, emphasizing the connection between kidney tubular cell injury and the catabolism of filtered proteins, notably β$_2$-microglobulin (β$_2$M). We hypothesize that Cd/Pb exposure and/or diabetes (hyperglycemia) causes injury/damage to kidney tubular cells, which, in turn, diminishes the catabolism of filtered proteins, leading eventually to proteinuria and a declining eGFR.

Our hypothesis was constructed from our previous observations; a decrease in fractional tubular degradation of β$_2$M (FrTD$_{β2M}$) by diabetes [25], and increased risks of hyperglycemia, eGFR reduction, and albuminuria by Cd/Pb exposure [26]. Studies by others have found that risks of hypertension, kidney damage and diabetes-related mortality may rise with an increment of plasma β$_2$M levels [27–29]. Through the *SH2B3*-β$_2$M axis of blood pressure control, β$_2$M has been causally linked to hypertension development and kidney damage [30–32]. For these reasons, FrTD$_{β2M}$ was our main focus.

2. Materials and Methods

2.1. Data Sourcing

Study subjects were chosen from a cohort of 88 individuals diagnosed with diabetes and 88 individuals without diabetes. The recruitment criteria for the participants in the pre-exiting cohort and findings have been reported previously [26]. In brief, the inclusion criteria for cases were residents of the Pak Poon municipality, Nakhon Si Thammarat Province, Thailand, aged at least 40 years, who attended annual health checkups, and who were diagnosed with type 2 diabetes. For the control group, exclusion criteria were non-resident status, pregnancy and/or breastfeeding, and hospital records or a physician's diagnosis of an advanced chronic disease, including heart disease, stroke, and cancer.

Participants were provided with the study objectives, study procedures, potential risks, and benefits, and they gave written informed consent prior to participation. Structured interview questionnaires were used to collect sociodemographic data, educational attainment, occupation, health status, family history of diabetes, use of dietary supplements, alcohol consumption, and smoking status. After individuals with missing data were excluded, a total of 137 individuals (72 with diabetes and 65 without diabetes) were analyzed in the present study. The % of hypertension in subjects with diabetes and without were 64.6 and 44.9, respectively

2.2. Procurement and Storage of Blood and Urine Samples

Collection of blood and urine samples was undertaken at the Pak Poon health center on the morning after an overnight fast. Morning voided urine samples were collected in acid-washed polypropylene collection cups. Blood samples for the glucose assay were collected in tubes containing heparin as an anticoagulant and fluoride as an inhibitor of glycolysis. Blood samples for Cd and Pb analysis were collected in tubes containing ethylene diamine tetra acetic acid as an anticoagulant.

Blood and urine samples were kept on ice and transported within one hour to the laboratory at Walailak University, where aliquots of plasma and serum were prepared. To prevent the degradation of β$_2$M in acidic conditions, an alkaline (NaOH) solution was added to adjust the pH of urine aliquots to >6 before storage. Aliquots of urine, whole blood, serum, and plasma were stored at −80 °C for later analysis.

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2.3. Quantification of Metals and Biomarkers of Kidney Effects

We used the human beta-2 microglobulin/β_2M ELISA pair set (Sino Biological Inc., Wayne, PA, USA) to determine urine and serum concentration of β_2M, with a lower limit of detection of 3.13 pg/mL. The plasma glucose assay was based on the oxidase–peroxidase method (Glu Colorimetric Assay Kit, Elabscience, Catalog No: E-BC-K234-M, Houston, TX, USA) [33]. The urinary NAG assay was based on colorimetry, using 4-nitrophenyl N-acetyl-β-D-glucosaminide as a substrate (Merck KGaA, Darmstadt, Germany). Assays of creatinine in urine and plasma were based on Jaffe's alkaline picrate method [34]. The coefficients of variation (CV) for all blood and urine assays were within acceptable clinical chemistry standards.

Urinary and whole blood Cd and Pb concentrations were determined with GBC System 5000 graphite furnace atomic absorption spectrometry (AAS) (GBC Scientific Equipment, Hampshire, IL, USA) [35]. Standards with As, Be, Cd, Cr (VI), Hg, Ni, Pb, Se, and Tl were used to calibrate the instrument (Merck KGaA, Darmstadt, Germany). Reference urine metal levels 1, 2, and 3 (Lyphocheck, Bio-Rad, Hercules, CA, USA) were used for quality control, accuracy, and precision assurance purposes. When urinary and blood concentrations of Cd and Pb were less than their detection limits, the concentration assigned was the detection limit value divided by the square root of 2 [36].

2.4. Normalization of E_{Cd}, $E_{\beta 2M}$ and E_{NAG}

Given that urine samples were collected at a single time point (voided urine), a correction for interindividual differences in the urine volume was required. Thus, we normalized urinary excretion of Cd, β_2M and NAG (E_{Cd}, $E_{\beta 2M}$ and E_{NAG}) to creatinine clearance (C_{cr}), using the equation; $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd, β_2M or NAG; $[x]_u$ = 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 an amount of x excreted per volume of the glomerular filtrate [37]. This C_{cr}-normalization simultaneously corrects for differences in urine volume and the number of functioning nephrons, and it is not affected by the variation in muscle mass.

For comparison purposes, we provided also data on E_{Cd}, $E_{\beta 2M}$ and E_{NAG} normalized to creatinine excretion (E_{cr}), using the equation; $E_x/E_{cr} = [x]_u/[cr]_u$, where x = Cd, β_2M or NAG; $[x]_u$ = urine concentration of x (mass/volume) and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/E_{cr} was expressed as an amount of x excreted per g of creatinine.

E_{cr}-normalization corrects for difference in urine volume only. Notably, however, it is affected by a large variation in E_{cr} due to differences in muscle mass, especially between men and women. E_{cr}-adjustment can create non-differential errors/imprecisions that bias the dose-response relationships toward the null [38]. A dose-response relationship could not be established between E_{Cd} and risk of abnormal $E_{\beta 2M}$ values when E_{Cd} and $E_{\beta 2M}$ were adjusted to E_{cr} [39].

2.5. Computation of eGFR, $F\beta_2M$, $TD_{\beta 2M}$ and $FrTD_{\beta 2M}$

Estimated GFR (eGFR) was computed with Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations [40]. CKD stages 1, 2, 3, 4, and 5 corresponded to eGFR of 90–119, 60–89, 30–59, 15–29, and <15 mL/min/1.73 m^2, respectively.

The parameters used to describe kidney handling of β_2M were; (i) rate of glomerular filtration of β_2M ($F\beta_2M$), mg/d; (ii) amount of β_2M undergoing tubular degradation per volume of glomerular filtrate ($TD_{\beta 2M}/C_{cr}$), mg/L; and (iii) fractional tubular degradation of filtered β_2M ($FrTD_{\beta 2M}$), decimal fraction. These parameters were computed with below equations [25].

Equation L: $F\beta_2M = eGFR[\beta_2M]_s$

Equation II: $TD_{\beta 2M}/C_{cr} = [\beta_2M]_s - E_{\beta 2M}/C_{cr}$

Equation III: $FrTD_{\beta 2M} = TD_{\beta 2M}/C_{cr})/[\beta_2M]_s$

2.6. Analysis for Cause-Effect Relationship

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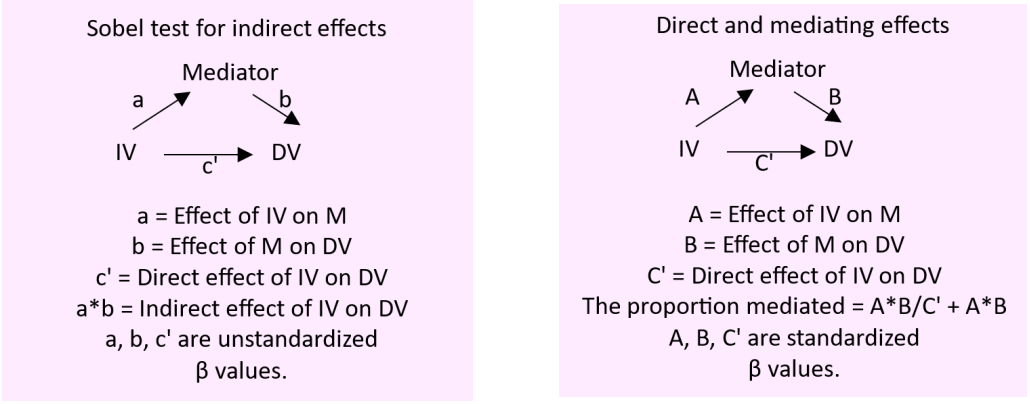
To gain insight into the mediators of effects of Cd/Pb exposure on kidney tubular and glomerular functions, we employed the Baron and Kenny method [41–43], and their generic simple mediation models are depicted together with statistics parameters (β coefficients) describing the direct and indirect effects of the independent variable tested. (Scheme 1).



Scheme 1. A simple mediation model for cause-effect relationship inference. The unstandardized β coefficients and the Sobel test for the indirect effect of the independent variable (**a**), the standardized β coefficients for the direct and mediating effects of the independent variable (**b**). IV, independent variable; DV, dependent variable.

2.7. Statistical Analysis

Data were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The variation in any continuous variable and differences in percentages across the Cd/Pb exposure levels were assessed by the Kruskal–Wallis's test and the Pearson chi-squared test, respectively. Spearman's rank correlation analysis was employed to produce the correlation matrices of nine variables: $FrTD_{\beta 2M}$, age, BMI, eGFR, FPG, E_{Cd}/C_{cr}, E_{NAG}/C_{cr}, and Cd/Pb exposure levels.

The one-sample Kolmogorov–Smirnov test was used to assess departure from a normal distribution of any continuous variable. Logarithmic transformation was applied to E_{Cd}/C_{cr}, $E_{\beta 2M}/C_{cr}$ and E_{NAG}/C_{cr} that showed rightward skewing before they were subjected to parametric statistics analyses, scatterplots, and linear regressions.

Multiple linear regression modeling was employed to identify the variables contributing to the variability of $FrTD_{\beta 2M}$ among subjects with/without diabetes.

3. Results

3.1. Cd/Pb Exposure Categorization

We used the mean blood Cd (0.3 μg/L) and the median blood Pb (2.12 μg/dL) to describe Cd/Pb exposure levels among study subjects. Those with blood Cd and blood Pb levels ≤ medians, blood Cd or blood Pb ≥ the median, and blood Cd and blood Pb both ≥ medians were assigned to Cd/Pb exposure levels 1, 2 and 3, respectively. The corresponding numbers of subjects in the Cd/Pb exposure levels 1, 2 and 3 were 44, 54, and 39. Characteristics of Cd/Pb categorical exposure groups can be found in Table 1.

Table 1. Descriptive data on study subjects according to Cd and Pb exposure levels.

Variables	All Subjects $n = 137$	Cd/Pb Exposure Levels			p
		Level 1 $n = 44$	Level 2 $n = 54$	Level 3 $n = 39$	
Women, %	78.1	90.9	72.2	71.8	0.045
Smoking, %	10.2	0	9.3	23.1	0.002
Diagnosed diabetes, %	47.4	34.1	46.3	64.1	0.023
Hypertension, %	54.5	51.2	44.4	71.8	0.029

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Age (range), years	59.7 (41–80)	59.6 (42–80)	59.8 (45–78)	59.5 (41–78)	0.994
BMI, kg/m^2	25.6 (4.8)	25.6 (4.2)	25.4 (4.0)	25.9 (6.2)	0.805
FPG, mg/dL	129 (61)	115 (40)	131 (73)	143 (59)	0.099
FPG ≥ 110 mg/dL, %	48.9	38.6	48.1	61.5	0.115
FPG ≥ 126 mg/dL, %	39.4	29.5	35.2	56.4	0.031
eGFR, mL/min/1.73 m^2	79 (16)	81 (15)	77 (16)	80 (18)	0.461
[a] Low eGFR, %	12.4	9.1	16.7	10.3	0.469
$[\beta_2M]_s$, mg/L	5.98 (3.29)	5.01 (2.47)	6.16 (3.12)	6.83 (4.06)	0.099
$[Pb]_b$, mg/dL	4.49 (4.78)	2.12 (0.00)	4.65 (5.63)	6.95 (5.00)	<0.001
$[Cd]_b$, µg/L	0.57 (0.70)	0.05 (0.05)	0.60 (0.68)	1.10 (0.73)	<0.001
$[Cd]_u$, µg/L	0.65 (1.11)	0.27 (0.69)	0.59 (1.09)	1.17 (1.33)	<0.001
E_{cr}-normalized data					
E_{Cd}/E_{cr}, µg/g creatinine	0.98 (1.86)	0.33 (0.72)	0.99 (2.06)	1.70 (2.20)	0.006
$E_{\beta2M}/E_{cr}$, µg/g creatinine	108 (118)	60 (58)	116 (128)	151 (136)	0.002
E_{NAG}/E_{cr}, U/g creatinine	10.6 (14.5)	6.67 (4.93)	11.6 (16.7)	13.4 (17.6)	0.288
C_{cr}-normalized data					
E_{Cd}/C_{cr}, (µg/L filtrate) × 100	0.86 (1.68)	0.28 (0.62)	0.86 (1.78)	1.50 (2.11)	0.001
$E_{\beta2M}/C_{cr}$, (µg/L filtrate) × 100	99 (115)	52 (56)	108 (123)	139 (134)	0.001
E_{NAG}/C_{cr}, (U/L filtrate) × 100	10.2 (17.4)	5.31 (4.03)	10.6 (16.0)	14.6 (24.5)	0.154

eGFR, estimated glomerular filtration rate; [a] Low eGFR was defined as eGFR values ≤ 60 mL/min/1.73 m^2; FPG, fasting plasma glucose; β$_2$M, β$_2$-microglobulin; Cd; cadmium; Pb, lead; $[x]_p$, plasma concentration of x; $[x]_u$, urine concentration of x; E_{cr}, creatinine excretion; C_{cr}, creatinine clearance; NAG, N-acetyl-β-D-glucosaminidase. Values for continuous variables are mean (standard deviation, SD). Data on NAG were from 102 subjects. All other data were from 137 subjects. The variability of continuous variables across Cd/Pb exposure levels 1,2 and 3 was assessed with the Kruskal-Wallis test. The distribution of categorial variables was assessed with Chi-square test. For all tests, *p*-values ≤ 0.05 indicate statistical significance.

Among 137 study subjects, 107 (78.1%) were women with overall mean age (range) of 59.7 (41–80) years. The group with the Cd/Pb exposure level 3 had the highest percentages of smokers (23.1) diagnosed diabetes (64.1), hypertension (71.8) and fasting plasma glucose (FPG) ≥ 126 mg/dL (56.4).

Nearly half (48.9%) of study subjects had FPG ≥ 110 mg/dL, commensurate with prediabetes status and they seemed to distribute equally; the different % distribution of FPG ≥ 110 mg/dL across Cd/Pb exposure groups did not reach a statistically significant level (p = 0.115). The overall % low eGFR was 12.4, and the prevalences of low eGFR across the Cd/Pb exposure levels did not differ (p = 0.469).

The parameters showing significant variations across the Cd/Pb exposure levels were $[Cd]_b$, $[Cd]_u$, $[Pb]_b$, E_{Cd} and $E_{\beta2M}$. The variations in age, BMI, FPG, eGFR and ENAG across the Cd/Pb exposure levels were not statistically different.

3.2. Cd/Pb Exposure and Kidney Handling of β$_2$M

Figure 1 provides results of an analysis aimed to identify the components of β$_2$M homeostasis that may be affected by Cd/Pb exposure. The tested β$_2$M homeostasis parameters were filtration of β$_2$M ($F_{\beta2M}$), tubular degradation of β$_2$M ($TD_{\beta2M}/C_{cr}$), fractional tubular degradation of β$_2$M ($FrTD_{\beta2M}$) and urinary excretion of β$_2$M ($E_{\beta2M}/C_{cr}$). The calculations of these parameters are described in Section 2.5.

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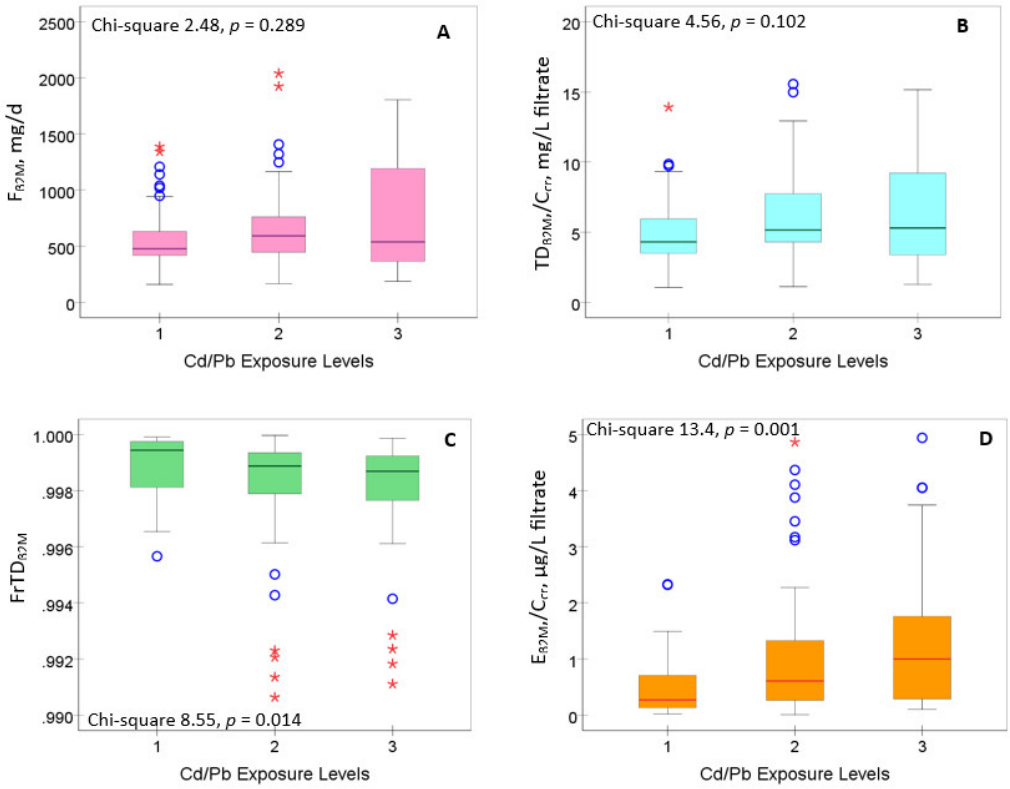

Figure 1. Boxplot distributions of β2M homeostasis parameters in subjects grouped by Cd/Pb exposure levels. Boxplots of data on Fβ2M (**A**), $TD_{\beta 2M}/C_{cr}$ (**B**), $FrTD_{\beta 2M}$ (**C**) and $E_{\beta 2M}/C_{cr}$ (**D**) in subjects with Cd/Pb exposure levels 1, 2 and 3, respectively. Each box represents the 25th and 75th percentile values of the variable indicated on the x-axis. A horizontal line inside each box represents the median. Circles and asterisks represent outliers.

Neither $F_{\beta 2M}$ nor $TD_{\beta 2M}/C_{cr}$ varied with the Cd/Pb exposure levels (Figures 1A,1B). However, $FrTD_{\beta 2M}$ fell as the Cd/Pb exposure levels rose ($p = 0.014$) (Figure 1C), while $E_{\beta 2M}/C_{cr}$ rose with the increment of Cd/Pb exposure levels ($p = 0.001$) (Figure 1D). Thus, Cd/Pb exposure appeared to have a significant effect on β2M homeostasis, reflected by two parameters, $FrTD_{\beta 2M}$ and $E_{\beta 2M}/C_{cr}$.

To gain further mechanistic insights, we conducted the Spearman's rank correlation analysis to examine the interrelationship relationships of indicators/biomarkers of kidney tubular and glomerular functions (Table 2).

Table 2. Bivariate relationship analysis of the fractional tubular degradation of β2-microglobulin.

Variables	Spearman's rank correlation coefficient						
	$FrTD_{\beta 2M}$	Age	BMI	eGFR	FPG	E_{Cd}/C_{cr}	E_{NAG}/C_{cr}
Age	−0.096						
BMI	0.048	−0.262**					
eGFR	0.434**	−0.356**	0.161				
FPG	−0.215*	−0.222**	0.184*	0.089			
E_{Cd}/C_{cr}	−0.527**	0.078	−0.083	−0.227**	0.166		
E_{NAG}/C_{cr}	−0.536**	−0.115	0.002	−0.467**	0.278**	0.328**	
Cd/Pb exposure [a]	−0.249**	0.009	−0.012	−0.013	0.181*	0.301**	0.165

$FrTD_{\beta 2M}$, fractional tubular degradation of β2-microglobulin; BMI, body mass index; eGFR, estimated glomerular filtration rate FPG, fasting plasma glucose concentration. [a] Cd/Pb exposure was categorized as Cd/Pb exposure level 1, 2 or 3 using the median blood Cd of 0.3 µg/L and median blood Pb of 2.12 µg/dL. Subjects with blood Cd or blood Pb below median, blood Cd or blood Pb above its median, and blood Cd and blood Pb levels above medians were classed as Cd/Pb exposure level 1, 2 and 3, respectively. * Significant correlation at the 0.05 level. ** Significant correlation at the 0.01 level.

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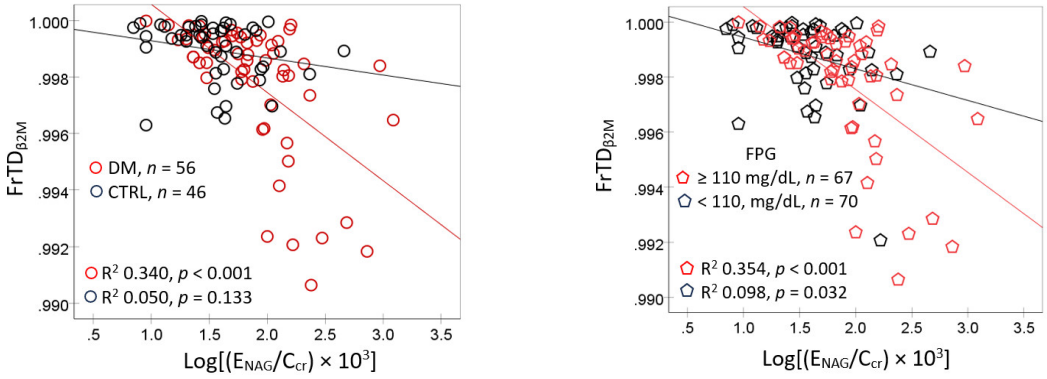
$FrTD_{\beta 2M}$ varied directly with eGFR (r = 0.434), and inversely with FPG (r = −0.215), E_{Cd}/C_{cr} (r =−0.527), E_{NAG}/C_{cr} (r =−0.536) and Cd/Pb exposure (r = −0.249). The correlation between $FrTD_{\beta 2M}$ and E_{Cd}/C_{cr} was particularly strong as was for the correction between $FrTD_{\beta 2M}$ and E_{NAG}/C_{cr}.

The potential tubulo-glomerular effects of Cd/Pb exposure were evident from the eGFR vs. E_{NAG}/C_{cr} (r = −0.467) and E_{Cd}/C_{cr} vs. E_{NAG}/C_{cr} (r = 0.328) and Cd/Pb exposure levels vs. E_{Cd}/C_{cr} (r = 0.301) correlations.

Scatterplots and regression analysis were used to examines the correlations of $FrTD_{\beta 2M}$ with E_{NAG}/C_{cr} in subgroups (Figure 2).

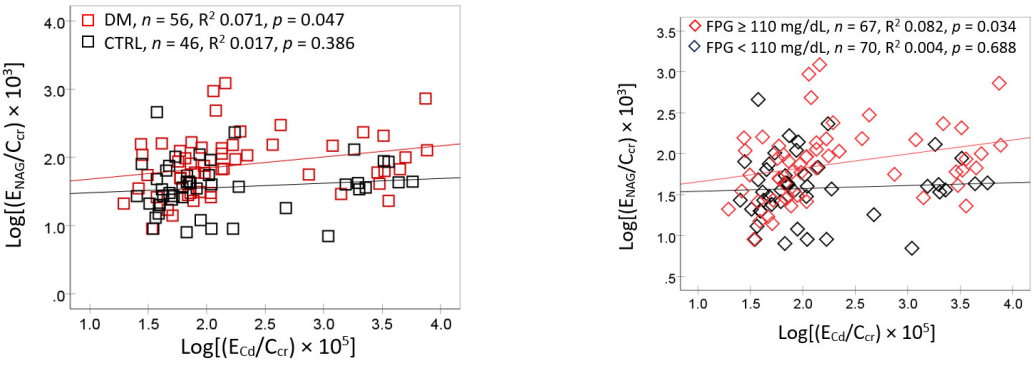


(**a**) (**b**)

Figure 2. Reduction in fractional tubular degradation of β2M as a function of NAG excretion rates. Scatterplots related $FrTD_{\beta 2M}$ to E_{NAG}/C_{cr} in subjects in subjects with and without diagnosed diabetes (**a**) those with FPG <110 and ≥ 110 mg/dL (**b**). $FrTD_{\beta 2M}$; fractional tubular degradation of β2M; NAG, N-acetly- β-D-glucosaminidase; E_{NAG}, urinary excretion of NAG; FPG, fasting plasma glucose concentration; Cd, cadmium; E_{Cd}, urinary excretion of Cd.

In subjects with diagnosed diabetes, $FrTD_{\beta 2M}$ rose linearly with E_{NAG}/C_{cr} (R^2 = 0.340, p < 0.001), but not in those without diabetes (R^2 = 0.050, p = 0.133) (Figure 2a). In subjects with FPG ≥ 110 mg/dL, $FrTD_{\beta 2M}$ was more closely associated E_{NAG}/C_{cr} (R^2 = 0.354, p < 0.001), compared to those who had FPG <110 mg/dL (R^2 = 0.098, p = 0.032) (Figure 2b).

Additional scatterplots and regression analysis were conducted based on correlation between E_{NAG}/C_{cr} and $FrTD_{\beta 2M}$ together with FPG and E_{Cd}/C_{cr}, identified in Table 2. Results are reported in Figure 3.



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

Figure 3. NAG excretion rates as a function of Cd excretion rates. Scatterplots related E_{NAG}/C_{cr} to E_{Cd}/C_{cr} in subjects with and without diabetes (**a**) those with FPG <110 and ≥ 110 mg/dL (**b**). NAG, N-acetly- β-D-glucosaminidase; E_{NAG}, urinary excretion of NAG; FPG, fasting plasma glucose concentration; Cd, cadmium; E_{Cd}, urinary excretion of Cd.

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In subjects with diagnosed diabetes, E_{NAG}/Ccr rose linearly with E_{Cd}/C_{cr} ($R^2 = 0.071$, $p = 0.047$), but not in controls (R^2 0.017, $p = 0.386$) (Figure 3a). In contrast, a significant association between E_{NAG}/C_{cr} and E_{Cd}/C_{cr} was found only in subjects with FPG levels commensurate with prediabetes (R^2 0.082, $p = 0.034$) (Figure 3b).

3.3. Multiple Linear Regression Models for $FrTD_{\beta 2M}$ in Diabetes vs. Controls

To confirm results of $FrTD_{\beta 2M}$ bivariate and regression analyses, multiple regression model analysis was conducted to adjusted for effects of covariates. The independent variables incorporated in each regression model were for age, years BMI, eGFR, E_{Cd}/C_{cr}, diagnosed diabetes, gender, smoking hypertension and FPG (Table 3).

Table 3. Multiple linear regression of $FrTD_{\beta 2M}$ in subjects with and without diabetes.

Independent Variables	$FrTD_{\beta 2M}$					
	All, $n = 137$		Diabetes, $n = 72$		Controls, $n = 65$	
	β	*p*	β	*p*	β	*p*
Age, years	−0.009	0.913	0.095	0.483	−0.079	0.513
BMI, kg/m²	0.082	0.300	0.123	0.300	0.082	0.471
eGFR, mL/min/1.73 m²	0.372	<0.001	0.476	<0.001	0.360	0.003
(E_{Cd}/C_{cr}) ×100, µg/L filtrate	−0.189	0.013	−0.295	0.009	−0.102	0.383
Diagnosed diabetes	−0.222	0.021	–	–	–	–
Gender	0.057	0.544	0.036	0.790	0.140	0.337
Smoking	−0.016	0.866	0.021	0.877	−0.085	0.559
Hypertension	0.052	0.492	0.172	0.113	−0.239	0.053
FPG, mg/dL	−0.258	0.007	−0.220	0.055	0.247	0.033
Adjusted R^2	0.311	<0.001	0.301	<0.001	0.215	0.003

$FrTD_{\beta 2M}$, fractional tubular degradation of β2M; BMI, body mass index; eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted R^2, coefficient of determination. β indicates strength of association of $FrTD_{\beta 2M}$ with nine independent variables (first column). Adjusted R^2 indicates the proportion of $FrTD_{\beta 2M}$ variability explained by all independent variables. For all tests, *p*-values ≤ 0.05 indicate a statistically significant association of $FrTD_{\beta 2M}$ with an individual independent variable.

All independent variables explained 31.1, 30.1 and 21.5% of the $FrTD_{\beta 2M}$ variability in all subjects ($p < 0.001$), the diagnosed diabetes group ($p < 0.001$) and controls ($p = 0.003$). $FrTD_{\beta 2M}$ showed a strong positive association with eGFR in both diabetes ($\beta = 0.476$, $p < 0.001$) and controls ($\beta = 0.360$, $p = 0.033$).

Intriguingly, an inverse association of $FrTD_{\beta 2M}$ and E_{Cd}/C_{cr} was found only in the diabetes group ($\beta = -0.295$, $p = 0.009$). A moderate positive association of $FrTD_{\beta 2M}$ and FPG was evident in the control group only ($\beta = 0.247$, $p = 0.033$).

3.4. Mediation Analysis of Effects of Cd/Pb Exposure on $FrTD_{\beta 2M}$

To investigate the mediating role of tubular injury, indicated by E_{NAG}/C_{cr}, a simple mediation model with a single mediator was employed. As depicted in Figure 4, the independent variable, E_{Cd}/C_{cr} was indicative of Cd/Pb exposure, given a significant correlation between E_{Cd}/C_{cr} and Cd/Pb exposure levels, revealed in Table 2.

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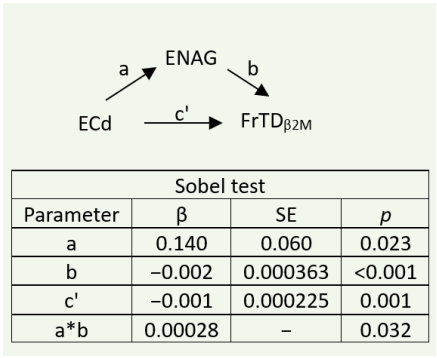
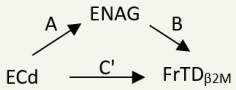


Sobel test			
Parameter	β	SE	*p*
a	0.140	0.060	0.023
b	−0.002	0.000363	<0.001
c'	−0.001	0.000225	0.001
a*b	0.00028	–	0.032

(**a**)



A = 0.226, *p* = 0.023
B = −0.468, p <0.001
C' = −0.290, *p* = 0.001
The proportion mediated = A*B/C' + A*B
= 25.95%

(**b**)

Figure 4. Kidney tubular cell injury as the mediator of Cd effect on β2M degradation. A mediating effect of Cd on $FrTD_{\beta 2M}$ by E_{NAG} and the Sobel test (**a**) the proportion of the effect of Cd mediated by ENAG (**b**). E_{NAG}, excretion of NAG; $FrTD_{\beta 2M}$, fractional tubular degradation of β2M.

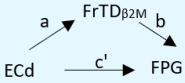
As indicated by the statistical significance of the indirect (a*b) and the direct (c') effects (Figure 4a), tubular injury (E_{NAG}/C_{cr}) mediated about 26% of the total effect of E_{Cd}/C_{cr} on $FrTD_{\beta 2M}$ (Figure 4b).

3.5. Mediation Analysis of Effects of Cd/Pb Exposure on eGFR and FPG

Two additional simple mediation models were conducted with eGFR and FPG were the independent variables to investigate the mediating role of reduced tubular degradation of β2M, reflected by $FrTD_{\beta 2M}$ (Figure 5). Similarly, E_{Cd}/C_{cr} was the independent variable that reflected also a Pb co-exposure.

Sobel test			
Parameter	β	SE	*p*
a	−0.001	0.000228	<0.001
b	2854	675	<0.001
c'	−1.068	1.896	0.574
a*b	2.854	–	0.002

(**a**)



Sobel test			
Parameter	β	SE	*p*
a	−0.001	0.000228	<0.001
b	−24.33	6.748	<0.001
c'	0.012	0.019	0.516
a*b	0.024	–	0.005

(**b**)

Figure 5. Kidney tubular cell dysfunction as the mediator of Cd effects. The mediating effect of Cd on eGFR (**a**) and FPG (**b**) together with the Sobel test. $FrTD_{\beta 2M}$, fractional tubular degradation of β2M; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose fractional tubular degradation of β2M.

As indicated by statistically significant levels of the indirect effects (a*b) at $p < 0.001$ (Figure 5a) and $p = 0.005$ (Figure 5b), the effects of E_{Cd}/C_{cr} on eGFR and FPG both were fully mediated by a reduction in β2M degradation ($FrTD_{\beta 2M}$).

4. Discussion

Low-level environmental exposure to Cd and Pb experienced by subjects in the present study was indicated by median values for blood Pb, blood Cd and urinary Cd excretion rate (E_{Cd}/E_{cr}) of 2.12 μg/dL, 0.3 μg/L and 0.12 μg/g creatinine, respectively. These Cd/Pb exposure levels were less than the levels found to be associated with increased risk of diabetes reported in the studies from U.S. [44,45] and Korea [46]. In the Korean study, a correlation was observed between E_{Cd} and FPG levels together with the increases in prevalence of diabetes by 81% and 39% in men and women who had E_{Cd}/E_{cr} levels ≥ 2 and ≥ 3 μg/g creatinine, respectively [46].

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As can be expected from low E_{Cd} levels and modest sample size (n = 137), the correlation between FPG and urinary Cd exposure levels in our subjects (mean 0.98 μg/g creatinine) did not reach statistically significant levels (r = 0.166, p = 0.053) (Table 2). Notably, however, E_{Cd}/C_{cr} showed a positive correlation with a tubular injury biomarker (E_{NAG}) (r = 0.328, p = 0.001). In subgroup analysis (Figure 3), a significant correlation between E_{NAG} and E_{Cd} was found only in those with diabetes (R^2 0.071, p = 0.047), and those with FPG ≥ 110 mg/dL (R^2 0.082, p = 0.034). Thus, an observed tubular injury could be attributed to the Cd/Pb exposure plus the presence of diabetes and/or hyperglycemia (FPG ≥110 mg/dL). Hyperglycemia and diabetes are known to have an impact on kidney tubular and glomerular functions [2].

Evidence for a significant impact of co-exposure to low levels of Cd and Pb on the function of tubular cells comes from an analysis of tubular degradation of the filtered protein, β2M (Figure 1). The protein β2M, by virtue of its small mass, readily passes through the glomerular membrane to tubular lumen, and is reabsorbed and degraded totally by proximal tubular cells [47]. In conventional Cd toxic risk assessment, an increased urinary excretion of β2M ($E_{β2M}/E_{cr}$) has been used as an indicator of tubular dysfunction. A recent analysis on β2M homeostasis, however, has recommended the use of fractional tubular degradation of β2M ($FrTD_{β2M}$) instead of $E_{β2M}$ [25].

Herein, we observed that $FrTD_{β2M}$ dropped as the Cd/Pb exposure levels rose (p = 0.014) (Figure 1C). The excretion of β2M ($E_{β2M}/C_{cr}$) rose with the increment of Cd/Pb exposure levels (p = 0.001) (Figure 1D). Thus, Cd/Pb co-exposure even in low doses appeared to have a significant effect on tubular cell function, when $FrTD_{β2M}$ was employed an effect indicator. Also, we observed that $FrTD_{β2M}$ varied directly with E_{NAG}/C_{cr} in subjects with diabetes (R^2 = 0.340, p < 0.001) (Figure 2a) and those with hyperglycemia (R^2 = 0.354, p < 0.001) (Figure 2b). Results from the mediation analysis (Figure 4) inferred that tubular injury (E_{NAG}/C_{cr}) mediated about 26% of the reduction in tubular degradation of β2M induced by Cd with Pb co-exposure.

In multiple regression modelling (Table 3), we observed a more closely association of $FrTD_{β2M}$ with eGFR in subjects with diabetes (β = 0.476, p <0.001) than controls (β = 0.360, p = 0.033). In comparison, however, $FrTD_{β2M}$ inversely associated with E_{Cd}/C_{cr} only in the diabetes group (β = −0.295, p = 0.009), while it showed a moderate positive association with FPG in the control group only (β = 0.247, p = 0.033). These may reflect increased susceptibility to the nephrotoxicity of a low-dose Cd among people with diabetes. A similar observation was made in the Cadmibel study [48] and a Swedish cohort study [23]. By mediation analysis (Figure 5), effects of Cd on eGFR and FPG both were mediated fully through tubular functional impairment (a reduced $FrTD_{β2M}$).

The above results suggested that the toxicity of Cd/Pb to the kidney tubular cells impairs their function, assessed with the degradation of β2M (Figure 4), leading to a reduction in eGFR and hyperglycemia (Figure 5). In benchmark dose modeling study [49], urinary Cd excretion rates of 0.0536 and 0.1140 μg/g creatinine were identified to be Cd exposure levels associated with increased protein excretion rates by 5 and 10%, respectively. Because the respective median E_{Cd}/E_{cr} and mean E_{Cd}/E_{cr} in subjects of the present study were 0.12 and 0.98 μg/g creatinine, we speculate that the 5 and 10% increases in protein excretion rates could also be the results of a deceased in tubular protein degradation caused by the metal Cd.

5. Conclusions

In people with hyperglycemia/diabetes, chronic exposure to Cd and Pb, even in low doses, can cause tubular cell injury, accompanied by a reduction in tubular cell function, especially the degradation of filtered proteins, notably β2M. Hyperglycemia, eGFR reduction may follow such kidney tubular functional impairment. It remains to be investigated if a diminished tubular degradation of β2M following Cd/Pb exposure increases plasma β2M and consequential rising blood pressure, given that kidney damage can cause hypertension [4–6].

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Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

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

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Abbreviations

The following abbreviations are used in this manuscript:

GFR	glomerular filtration rate
eGFR	estimated GFR
Cd	cadmium
E_{Cd}	urinary excretion rate of Cd
Pb	Lead
cr	creatinine
C_{cr}	Creatinine clearance
β_2M	β_2-microglobulin
$F_{\beta2M}$	rate of glomerular filtration of β_2M
$E_{\beta2M}$	urinary excretion rate of β_2M
$TD_{\beta2M}$	rate of tubular degradation of β_2M
$FrTD_{\beta2M}$	fractional tubular degradation of filtered β_2M

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